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Ethiopia National Food and Nutrition Survey to inform the Ethiopian Food and Nutrition Strategy: A study protocol

Journal:	BMJ Open
Manuscript ID	bmjopen-2022-067641
Article Type:	Protocol
Date Submitted by the Author:	25-Aug-2022
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Keywords:	EPIDEMIOLOGY, NUTRITION & DIETETICS, PUBLIC HEALTH
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SCHOLARONE[™] Manuscripts

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22 ABSTRACT

Introduction Ethiopia has made significant progress in reducing malnutrition in the past two decades. Despite such improvements, a substantial segment of the country's population remains chronically undernourished and suffers from not only micronutrient deficiencies but also from increasing diet-related non-communicable diseases. This survey aims to assess the anthropometric status, dietary intake and micronutrient status of Ethiopian children, women, and adolescent girls. The study will also assess the coverage of direct and indirect nutrition-related interventions, and map agricultural soil nutrients. The survey will serve as a baseline for the recently developed Ethiopian food system transformation plan and will inform the implementation of the National Food and Nutrition Strategy.

Methods and analysis Using a population-based cross-sectional survey, the study will collect data in the ten regions and two city administrations of Ethiopia. The study population will be women of reproductive age, children aged 0-59 months, school-aged children and adolescent girls. A total of 16,596 households will be surveyed, allowing us to generate national and regional estimates for selected indicators. A two-stage stratified cluster sampling procedure will be used to select households. In the first stage, 639 enumeration areas will be selected using probability-proportional to size allocation. In the second stage 26 eligible households will be selected within each enumeration area using systematic random selection. Primary outcomes include coverage of direct and indirect nutrition interventions, infant and young child feeding practices, food insecurity, dietary intakes for women, mental health of women and children, anthropometric status, micronutrient status, and soil nutrient status.

41 Ethics and dissemination The study protocol was approved by the Institutional Review Board of the
42 Ethiopian Public Health Institute (protocol no: EPHI-IRB-317-2020). The finding of this survey will be
43 disseminated in scientific forums, national research conference and stakeholder meetings; and will be
44 submitted for publication in peer reviewed journals.

- 35 45 Strengths and limitations of this study
 - To the best of our knowledge, this will be among -if not-the first study to simultaneously collect data on anthropometric status, 24hr recall quantitative dietary intakes and the determination of micronutrient status in the same participants or household, while at the same time capturing data on the food system.
 - Previously, nutrition programs in Ethiopia have relied on data from small-scale studies and population-based surveys such as the Ethiopia Demographic and Health Surveys. The study will help to improve understanding of nutritional problem across multiple facets—from agricultural soil to people to the environment.
 - Inherent to the cross-sectional design of the study, the findings of this study cannot be used to
 establish cause and effect.
 - The study design prevents us from considering seasonal differences in nutritional outcomes and determinants.

60 INTRODUCTION

Globally, one in three are affected by one of more forms of malnutrition.¹ Women and children are particularly vulnerable to malnutrition due to increased physiological nutrient needs required to support fetal and child growth.² Nutritional deprivation during early life impairs growth and development, leading to poor school performance, reduced productivity, and loss of earnings in later life.³ Consequently, the first 1000 days of life, from conception to the child's second year of life, was recognized as a critical window of opportunity to effectively prevent malnutrition.^{3 4} Adolescence is also identified as a second window of opportunity to correct nutritional inadequacies and adversities faced in early life, but little is known about this life stage.

Despite significant progress over the past two decades, the burden of malnutrition in Ethiopia remains high.⁵⁻⁷ Nationally, 37% of Ethiopian children under five years of age are stunted⁷, and 22% of women of reproductive age (WRA) are chronically undernourished (Body Mass Index (BMI) < 18.5 Kg/m²).⁵ Only 14% of children under two years of age consumed the minimum number of recommended food groups.⁵ Furthermore, micronutrient deficiencies co-exist with chronic energy deficiency.⁸ This along with the ongoing nutrition transition, characterized by shifts in diets9, is further complicating the nutrition landscape by increasing the prevalence of overweight and non-communicable diseases.⁵ Nearly a fifth (16%) of Ethiopian adults are estimated to be hypertensive, and 3% are diabetic.¹⁰ Therefore, addressing not only undernutrition but all forms of malnutrition is critical.

The Sustainable Development Goals (SDGs) recognize the importance of nutrition, primarily driven by the need to mitigate its detrimental consequences. Further, the 2012 World Health Assembly (WHA) identified global targets to be achieved by 2025 that aim to reduce stunting, anemia, low birth weight, and childhood obesity. These targets are used to track progress in SDG goal 2: Zero hunger.¹¹Recognizing the importance of good nutrition, the Government of Ethiopia has made ending malnutrition a national priority. Ethiopia started implementing its first National Nutrition Program in 2008.¹² The second phase of this program (2011-2016) was a multisectoral program aimed at accelerating progress in reducing malnutrition.¹³ Moreover, Ethiopia's first Food and Nutrition Policy was endorsed in 2018¹⁴, followed the National Food and Nutrition Strategy (FNS)¹⁵ which was launched in 2021 to provide a framework for the operationalization of the policy. Acceleration of progress in the reduction of malnutrition requires the design and implementation of direct and indirect nutrition interventions that can be implemented at scale. To this end, understanding the various factors contributing to the different forms of malnutrition is critical.

Multiple factors operating at the immediate, underlying, and basic levels contribute to malnutrition.² Inadequate dietary intake and poor health are immediate determinants.² Household food security, child care practices, access to health services, and healthy environments are underlying determinants.¹⁶ Structural and contextual factors such as economic structures, political, environmental, social and cultural factors are the basic determinants of malnutrition.² The contribution of these factors varies across different contexts, and target groups, but studies capturing all these factors in a single survey are scant. The lack of timely and comprehensive information on nutritional status across critical life stages and their determinants is a bottleneck that is preventing Ethiopia from designing effective interventions. Up to date and comprehensive data on the coverage of direct and indirect nutrition

interventions delivered across various implementing sectors of the food and nutrition strategy is not
 available yet. This is unfortunate as such data could inform the implementation of the food and nutrition
 strategy, but also serve as a baseline to which progress can be tracked against.

Therefore, this study aimed to provide the first ever comprehensive information on the
 104 nutritional status of different populations in Ethiopia to support evidence-based implementation of the
 105 national food and nutrition strategy.

12 106 **Objectives of the survey**

The overall goal of this study is to produce nationally and regionally representative estimates on anthropometric status, coverage of nutrition interventions, dietary intakes, and micronutrient status for children, adolescent girls, and women of reproductive age in Ethiopia.

- 19 110 Specific objectives include;
 - 1. Assess the coverage of nutrition-specific and nutrition-sensitive interventions.
 - Assess food consumption patterns and nutrients intakes of children aged 6–59 months, and
 women of reproductive age.
 - Assess the micronutrient status of children (vitamin A, anemia, iron, iodine and zinc), adolescent girls, and women of reproductive age (vitamin A, vitamin D, anemia, iron, iodine, zinc, folate, vitamin B₁₂)
 - 4. Assess the anthropometric status of under-5 children, school-age children (6-12 years),
 adolescent girls, and women of reproductive age.
 - 119 5. Assess the geographical distribution of soil micronutrient status in Ethiopian agricultural soil.

33 120 METHODS AND ANALYSIS

35 121 Study design

This study is a nationally and sub-nationally (regionally) representative cross-sectional survey that will characterize dietary intake, micronutrient status, and access to nutrition-related services for different target populations. Given that soil nutrient content can influence micronutrient content of foods and hence affect nutrient intake, the soil nutrient composition will also be analyzed. The study will have four main components. The first component will assess nutrition-specific and nutrition-sensitive indicators (NSS) for all target groups (children aged 0-59 months and WRA, school-age children, adolescent girls)using semi-structured questionnaires. The second component will measure quantitative dietary intake for children aged 6-59 months and WRA (15-49 years). The third component of the survey will collect biomarker samples from all children (6-59 months), school-age children (6-12 years), adolescent girls (10-19 years), and WRA (15-49 years). The final component of the study will measure micronutrients in agricultural soils. The study data will be collected from July 2021 to December 2022.

49 133 Study setting

Ethiopia has an estimated population size of 120 million and is the second most populous country in Africa.¹⁷ The majority of its population resides in rural areas (70%).¹⁷ Agriculture accounts for 40% of the country's gross domestic product.¹⁷ Children aged 15 years and younger make up 40% of the Ethiopian population in 2021.¹⁸ Ethiopia is administratively divided into 10 regions and two city administrations. This study will be conducted in all of the regions and city administrations of the country. Figure 1 provides a geographic representation of the study areas.

Study participants

The target population of this study are i) women of reproductive age (WRA) aged 15-49 years ii) children
aged 0-59 months iii) school age children aged 6-12 years, and iv) adolescent girls aged 10-19 years, and
V) household head.

9 144 Sample size calculations

Sample size was estimated to guarantee adequate precision to generate national and regional estimates for selected indicators for each study target group. Indicators used for each target group are shown in Table 1S in supplemental materials. The required number of households and target groups was calculated using a single population proportion formula at the regional level. We used region-specific prevalence estimates for indicators, a 5% margin-of-error, a design effect of 1.5, a household response rate of 95%, and an individual response rate of 80%. The initial sample size was then adjusted for region-specific average household size and percentage of the target population from the total population. An indicator that provides the maximum number of households was used to estimate the final sample size for each region. Regional sample sizes were summed up to derive the total (national) sample size. Based on these calculations, the total sample size for the overall survey was 16,596 households (Supplemental material Table 2S).

For WRA, dietary and biomarker data will be collected in half of the selected households within each Enumeration Area (EA). This selection will yield a total sample size of 7,386 WRA (50% of the expected 14,772 WRA). The sample size needed to assess dietary intakes and micronutrient status of WRA was calculated using the prevalence of inadequate zinc intake, which yielded the largest sample size.8 19

32
33161Sampling procedures

A two-stage stratified cluster sampling procedure will be used to select households. In the first stage, 639 EAs, 257 urban and 382 rural will be selected using probability-proportional to-size allocation. We will use the 2018 Ethiopia Population and Housing Census enumeration areas sampling frame to select EAs (the Primary Sampling Units (PSUs)). The Central Statistical Agency (CSA) prepared the enumeration areas sampling frame. An EA typically contains 100-150 households. EA maps will be used to delineate the boundaries of the selected EA. In the second stage of sampling to identify eligible households, all households with the EA will be listed. A household will be eligible for selection if at least one of the study target groups are residents (de jure) or stayed at the household the night before the interview (de factor).

Twenty-six (26) eligible households will be selected within each EA using systematic random selection. All target groups will be eligible for the NSS interview in the selected households. All children aged 6-59 months will also be eligible for dietary assessment. Women residing in 13 households (Out of 26 households) who will be selected randomly will be eligible for dietary assessment. Biomarker samples will be collected for all children under 5 years of age, school-age children, and adolescents in the selected households. Similar to dietary assessment, biomarker samples will be collected for women residing in half of the selected households.

- 55 178

³ 179 **Outcomes**

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180 Coverage of direct and indirect nutrition interventions

6 181 A structured questionnaire will be used to determine the coverage of direct and indirect nutrition 7 182 interventions provided to children aged 6-59 months, WRA, and adolescent girls. Direct nutrition 8 183 interventions included vitamin A supplementation, iron supplementation, zinc supplementation, growth 9 184 monitoring and promotion, nutrition counseling services, and food fortification. Water, sanitation and 10 11 185 hygiene, coverage of food or cash assistance program, women empowerment, and mental health will be 12 186 some nutrition-sensitive indicators considered in this study. We will use standard indicator definitions 13 187 proposed by the Data for Decisions to Expand Nutrition Transformation project (DataDENT) to assess 14 188 coverage of nutrition programs. 15

1617189Anthropometric status

18 190 Using standardized procedures, anthropometric measurements, including weight, height/length, and 19 191 mid-upper arm circumference, will be taken for all study target populations.²⁰ Anthropometric indices 20 (weight-for-height z-scores, length/height-for-age z-scores, weight-for-age z-scores, BMI-for-age z-scores) 192 21 22 193 will be calculated using the WHO 2006 child growth standards and the WHO 2007 child growth reference 23 194 data. Stunting (length/height-for-age z-scores below -2 SD), wasting (weight-for-height z-scores below -2 24 195 SD), underweight (weight-for-age z-scores below -2 SD), thinness (BMI-for-age z-scores below -2 SD) and 25 196 BMI will be the primary anthropometric outcomes of interest. 26

28 197 Infant and young child feeding practices

Infant and young child feeding practices will be assessed using the new World Health Organization (WHO)
 and United Nations Children's Fund (UNICEF) recommended 17 indicators to evaluate IYCF practices.²¹

33 200 Food insecurity

The Food Insecurity Experience Scale (FIES) will be used to assess household food security.²² The FIES consists of eight questions that assess household experience related to adequate food access. Experience questions range from worrying about getting enough food to not eating for a whole day.

39204In addition to these outcome indicators, information on the sociodemographic characteristic of40205households, child health, maternal health, employment status, and household agricultural practices will41206be collected using structured questionnaires.

4344 207 Mental health of women

Common mental health disorders will be assessed using the WHO Self-reporting questionnaire which
consists of 20 questions. Women will be classified as having a common mental health disorder if row
score was greater or equal to 6 out of 20.²³

Assessment of dietary intakes of children and WRA 51

We will measure dietary intake for children aged 6-59 months and WRA. A one-day quantitative multiple pass 24-hour recall will be conducted to assess dietary intakes. The interactive multiple-pass 24-hour
 recall interview consists of four steps designed to enhance memory.²⁴ All days of the week will be
 proportionately represented during the dietary survey to account for the day of the week effects on food

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216 intake. To account for the day-to-day variability of dietary intake within individuals, a second non-217 consecutive day 24-hr recall (repeat) will be collected (within 2 to 10 days of the first recall) on a 218 randomly selected sub-sample of WRA and children. The number of repeats needed is determined by 219 allocating for each region 50 repeats, which is then multiplied by a design effect of 1.5 and a 10% non-220 response rate. The number of repeats was rounded up to 1244 recalls for each target group to ensure 221 that the minimum number of repeats (n=83) needed from each region would be collected. Detailed non-222 standard recipe ingredient data will be collected for all mixed dishes that were prepared at home.

Dietary assessment pre-survey work: We carried out pre-survey work to aid dietary data collection following recommendation set by the Intake: Center for Dietary Assessment.^{25 26} An initial step was developing a food and ingredient list containing a comprehensive list of food items, mixed dishes, and ingredients expected to be consumed by the study target groups. The food list was generated using data from the first 2011 Ethiopian National Food Consumption Survey.¹⁹ Other common foods consumed across the regions in Ethiopia were derived from the 2016 Household Income and Expenditure Surveys,²⁷ the Ethiopian Food Composition Tables, and dietary intake data from other recent dietary assessment surveys conducted by the Ethiopian Public Health Institute (EPHI). Portion size estimation methods suitable for large-scale studies were pre-selected for use in the survey following Intake recommendations.²⁸ The selected methods were direct measurement of actual foods consumed, standard unit: size and number, proxy measurement using play dough, water, rice, and maize flour, and finally using food price to estimate the amount of food consumed. Portion size estimation methods were assigned for all foods included in the food list.

31 236 Assessment of micronutrient status:32

Biological specimens will be collected from the study population to determine serum retinol, ferritin, soluble transferrin receptor (sTfR), zinc, folate, vitamin B₁₂, and red blood cell (RBC) folate. Additionally, markers of inflammation, alpha(1)-acid glycoprotein (AGP), high-sensitivity C-reactive protein (hsCRP) will also be measured. We will also analyze parasites from stool specimens. All laboratory analyses will be performed at the EPHI Clinical chemistry, and Food Science and Nutrition Laboratories. Both laboratories participate in an external quality assessment scheme and are accredited by the Ethiopian National Accreditation Office (ENAO). Collection, storage, and analytical procedures for blood, urine, stool, and salt samples are described below.

Blood sample collection and analysis: Venous blood samples (5-7 mL) will be collected using vacutainer tubes following standard operating procedures.²⁹ Trace mineral-free vacutainer tubes will be used to collect blood for trace metal analysis. After collection, blood samples will be allowed to clot for 30 minutes in cold boxes (<8 °c). Samples will then be centrifuged at 3000 rpm (revolution per minute) for 10 minutes. The separated serum will be aliquoted and stored in -20°c portable freezers in the field. Samples will then be transported to EPHI and stored at -80°c until analysis. Hemoglobin will be measured in the field using Hemocue[®] (Hb 301, Hemocue AB, Angelholm, Sweden)^{30,30} If the hemoglobin values are below WHO cutoff point(11g/dl), the phlebotomist will send whole blood samples to the EPHI laboratory to identify hemoglobinopathies using electrophoresis method.³¹ Malaria test will be conducted on-site using Bioline[™] Malaria Ag P.f rapid diagnostic test kits (RDT) for P. falciparum and P. vivax).³² Serum

soluble transferrin receptor (sTfR), AGP, hsCRP, folate, red blood cell (RBC) folate, vitamin B₁₂, and ferritin will be measured using Cobas 6000 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Serum retinol will be measured using high-performance liquid chromatography (HPLC) method³², and serum zinc and selenium will be measured using a microwave plasma atomic emission spectrometers (MP-AES) analyzer.

Stool and urine sample collection and analysis: Stool samples will be collected using stool cups and stored in 10 % formalin to preserve the parasite until analysis.³³ A portion of each stool sample will be used to detect direct ova, larvae and cysts of intestine parasites using formal ether concentration technique.³⁴Urine samples will be collected from WRA and school-age children using 60 ml urine cup containers. Samples will be stored at -20°c. Urinary iodine excretion will be assessed by Sandell Kolthoff reaction at EPHI Laboratory using Shimadzu 1800 UV-Vis spectroscopy.³⁵

Salt collection and analysis: Salt samples will be collected from households with WRA for whom dietary
 data will be collected. At least 25 grams (one coffee cup) of salt will be collected to determine iodine
 content using the iodometric titration method.³⁶

23 269 Assessment of nutrients in the soil 24

Soil samples will be collected from three households in each EA. Zig-zag or cross sampling method will be used to collect 10 to 20 subsamples (0-30 cm depth) constituting one composite sample. Subsamples will be collected at a separation distance of five meters. After thoroughly mixing composite samples, 1 kg soil sample will be transferred to polyethylene bags. The collected soil samples will be air-dried in wooden trays and disaggregated using a ceramic mortar and pestle (soil grinder) at the EPHI soil laboratory. Samples will then pass through a 6 mm sieve of stainless-steel screens to remove debris and homogenize the soil sample. The sieved fraction will be further pulverized to pass through a 1 mm sieve for the micronutrient analysis. Soil zinc, iron, copper, and manganese will be determined following standard procedures.³⁷ Micronutrient content will be determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES) after extraction with diethylene triamine penta acetic acid (DPTA). Additional variables that affect the mobility of micronutrients in the soil and their uptake into crops will also be measured. These variables include soil reaction (pH), electrical conductivity, organic matter, total nitrogen, and soil organic carbon content. Data collectors will also record topography, slope, cropping history, type, and fertilizer application information. Table 1 provides a summary of procedures for each of the four components of the survey by study target groups.

45 285 Data quality assurance and analysis 46

Training of trainers on components of the survey was held before training the data collectors and supervisors. After fifteen days of training on methodological procedures, questionnaires and quality assurance, the questionnaires were tested in a pilot group (in EAs not included in the actual survey), and adapted based on the received feedback from the survey team. The questionnaires (including the food list) were translated into local languages (Amharic, Oromifa, Tigrigna, Somali, and Afar) and back-translated to English to ensure the quality of the translation. The data collectors' measurements were standardized to ensure that the inter-observer variability was within tolerable limits. Supervisors received additional training on teamwork and on monitoring and supervising the data collection process. All data

collection tools are programmed using open-source software (ODK). Data quality checks were included during ODK programming to prevent data recording errors. These include restricted responses, filter insert choices, skip patterns, and defaults. During data collection daily data tracking forms will be completed to track completed surveys for each study components to prevent missing data. Before the start of the survey high frequency checks were identified and error tracking forms were designed to track data quality in real-time. These checks included completeness checks, target group tracking, and duplicate ID check. Random field-supervision visits will also be made to check data quality. Every day, collected data will be sent to the EPHI central server and imported into statistical software programs as comma-separated values (CSV) files. For laboratory analysis, a quality control chart will be used to ensure the internal and external quality control materials are in the acceptable range.

The primary data analysis will focus on computing frequencies and percentages for categorical variables and summary statistics (like means, medians SD, IQR) for summarizing continuous variables. Sample weights will be constructed based on the selection probabilities of EAs, eligible households, and non-response rates. All analyses will also be adjusted for the survey design. Additional subgroup analysis will be computed for variables with adequate sample sizes for each category. The Biomarkers Reflecting Inflammation and Nutrition Determinants of Anemia (BRINDA) Working Group's regression correction approach will be used to account for inflammation in the study of all micronutrients status using the biomarkers C-reactive protein (CRP) and AGP. Geostatistical analyses will be employed to determine the spatial patterns of micronutrient distribution in the soil and blood samples. The wealth index will be constructed using principal component analysis (PCA).³⁸ The Rasch model will be used to construct the Food Insecurity Experience Scale (FIES). ²² All analysis will be done using STATA 16 and ArcGIS/QGIS.

- Patient and public involvement statement
- No patient involved.
- Dissemination

The study's findings will be disseminated through several communication channels, including stakeholder workshops, various local and International conferences and technical report. Additionally, the findings will be submitted as special issue to peer-reviewed journals.

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DISCUSSION

This comprehensive, nationally representative survey will for the first time characterize simultaneously the dietary intake and micronutrient status of Ethiopian children, adolescent girls and WRA. Besides, the study assesses key drivers of malnutrition including soil nutrient composition, as well as coverage of direct and indirect nutrition interventions. The survey will provide key insights informing the implementation of Ethiopia's National Food and Nutrition Strategy.

High-quality and timely data is critical to assess the burden of nutritional problems, identify vulnerable populations and priority actions, track the implementation of nutrition programs, and assess impact.^{39 40} Ethiopia conducted its first-ever food consumption survey in 2011.¹⁹ and its micronutrient survey in 2015.⁴¹ Both surveys were collected at different times, which made it difficult to link the two surveys. Besides, the causes and solutions of malnutrition are complex and multisectoral; hence, requiring data on multiple indicators from various sectors spanning from soil nutrient, diets,

micronutrient status, to access and exposure to direct and indirect nutrition interventions. In this regard, this survey is uniquely positioned to integrate data from multiple domains to support evidence-based decision making for improved diets, nutrition, and overall wellbeing.

This study will allow us to evaluate progress relative to the last food consumption and micronutrient surveys, but more importantly will serve as a baseline to which future progress related to the implementation of the food and nutrition strategy will be evaluated. Furthermore, the current survey will also serve as a baseline for the Ethiopian Food System Transformation Plan by capturing majority indicators used for monitoring food systems related progress. Thus, filling information gaps that could have impeded successful implementation of the National Food and Nutrition Strategy (FNS). By establishing 13 strategic objectives, the FNS is intended to be aligned with the strategic directions of the Food and Nutrition Policy (FNP). Each strategy direction includes initiatives, actions, and key performance indicators, as well as leading and collaborating sectors. The key performance indicators should be evaluated to determine the progress of each implementing sector's achievement. The current survey will provide up to date national and subnational information on the current food and nutrition situation in Ethiopia for different target populations as well as provide comprehensive list of indicators that are pertinent to the implementation of the policy.³⁹ In addition, this study will provide information on context-specific determinants for prioritizing direct and indirect actions that can be implemented across sectors taking into account the specific needs of different target populations.

Additionally, effective multisectoral interventions that address the immediate and underlying determinants of malnutrition must be implemented in order to accelerate the reduction of malnutrition in its all form.³⁹ These interventions need to address context-specific determinants to reduce malnutrition effectively.³⁹ The lack of timely and disaggregated information on the determinants of malnutrition is a bottleneck to preventing malnutrition, particularly among the most vulnerable target populations. This study will also provide information on the coverage and quality of interventions which can be used to contextualize National Food and Nutrition Strategy monitoring frameworks, monitor implementation and track progress towards global and local targets.

Although this study will provide regionally and nationally representative estimates for key indicators and critical life stages, it has several limitations. Inherent to the cross-sectional design of the study, the findings of this study cannot be used to establish cause and effect. Additionally, the design prevents us from considering seasonal differences in nutritional outcomes and determinants. This study also relies on self-reported data, which is subject to recall bias. Notwithstanding the above-mentioned limitations, this study is uniquely designed to combine the assessment of anthropometric status, 24hr recall quantitative dietary intakes and the determination of micronutrient status in the same participants, while at the same time capturing data on the food system. Additionally, the study will be evaluating micronutrients in the agricultural soil, which will expand our understanding of factors that influence nutrition. To the best of our knowledge, this will be among -if not-the first study to simultaneously collect these variables from the same household. This could contribute to a better understanding of nutritional problem across multiple facets—from soil to people to the environment. In the past, nutrition programs implemented in Ethiopia have relied on information provided from small-scale studies and population-based surveys such as the Ethiopia Demographic and Health Surveys.^{5-7 42 43} Although these data sources

provide some information to track progress and tailor interventions, they only provide data on a limited number of nutrition indicators and do not measure dietary intakes and assess biomarker status. This study will fill these data gaps by providing information on comprehensive indicators that show the burden and spatial distribution of micronutrient deficiencies and shifts of dietary patterns. Additionally, this study will provide information on emerging determinants such as mental health and intake of nutrients such as folate and B₁₂ that have not been included in previous studies. Finally, the inclusion of . L All prov Aded in oth Age of direct in A in the agriculture, W. B, this study will provio stions for the reduction of main. adolescent girls, and school-age children, will provide vital information on nutritional indicators for these target groups, which are often not included in other nationally representative surveys. This survey will also provide information on the coverage of direct interventions implemented in the health sectors and indirect interventions implemented in the agriculture, WASH, education and social protection sectors for whom scant data exists. Hence, this study will provide valuable information that will guide the implementation of strategic actions for the reduction of malnutrition in Ethiopia.

Acknowledgments:

Contributions: MT, AL, SC, MW, AP, AS, and MG conceived the study and drafted the original protocol. All authors participated in refining of the protocol. AH, MW, MG and MT played a major role in the statistical consideration. KB, AL, SC, GT, MH, LT and MZ supervised manuscript preparation. All authors took responsibility for reviewing, final editing and approval of the manuscript.

- Funding: This work was supported by Power of Nutrition, BMGF and World Bank through UNICEF, NI, and GAIN
- **Competing interests:** The authors declare that they have no conflicts of interest.
- Patient consent for publication: Not required
- Ethical approval:

The study protocol is approved by the Institutional Review Board of the Ethiopian Public Health Institute (protocol no: EPHI-IRB-317-2020). Written informed consent will be obtained from each respondent. Confidentiality of all collected data will be given high priority during each stage of data handling. Individual names and personal information of respondents will be kept confidential and data sets will be kept anonymous for analysis.

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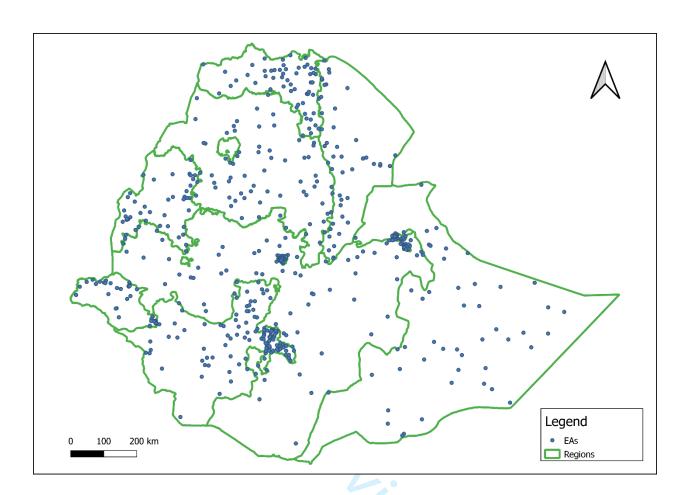
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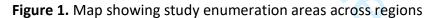
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530	Figure 1.Map showing	g study enumeration	areas across regions

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	Child	Child	Child	School	Adolescent	WRA	Household
	0-5	6-23	24-59	children	girls	15-49	
	months	months	months	6-12 years	10-19 years	years	
Nutrition specific and nutrition sensitive indicators (NSS)							
Infant and young child feeding practices	Х	Х					
Nutritional information for adolescent girls					х		
Food insecurity							Х
Water, sanitation and hygiene practices							Х
Coverage of food fortification							Х
Agricultural practices							Х
Mental health						Х	
Anthropometric status	Х	х	X		х	Х	
Dietary assessment			N.				
24-hr recall quantitative dietary intake		Х	Х			Х	
Assessment of biomarker status				N			
Blood sample		Х	Х	Х	Х	Х	
Urine sample				Х		Х	
Stool sample		Х	Х	Х	x	Х	
Salt sample collection							Х
Assessment of micronutrients in the Soil							
Soil micronutrient assessment							Х

Target Group	Key indicators used to estimate sample size
	Vitamin A deficiency
	Total goiter rate
Children under 5 years of age (0-59	Stunting
months)	Any anemia
	Zinc deficiency Prevalence of inadequate intake of zinc
	Vitamin A deficiency
	Total goiter rate
	Any anemia
	Zinc deficiency
	, RBC folate
Women of reproductive age (15-49 years)	Serum folate
	Vitamin B ₁₂
	lodized salt use
	Prevalence of inadequate intake of iron
	Prevalence of inadequate intake of zinc
	Prevalence of inadequate intake vitamin A
	Vitamin A deficiency
	Total goiter rate
School age children (6 to 12 years)	Any anemia
	lodine deficiency
	Zinc deficiency
Adolescent girls (10 to 19 years)	Any anemia

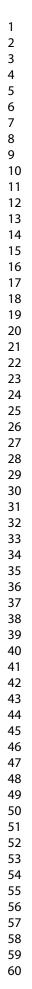


Table 2S. Sample size determination and allocation

Region	Indicator used to estimate sample size	Number of EA	Number of HH	Expected number Under Children	of 5	Expected number of WRA	Expected number of 6-59 months children	Expected number o adolescent girls
Tigray	Any anemia	55	1,432	590		1,236	753	516
Afar	Stunting	51	1,328	539		1,096	695	406
Amhara	Stunting	61	1,585	619		1,253	843	531
Oromia	IDD	62	1,622	891		1,539	1,111	739
Somali	IDD	55	1,424	855		1,268	1,000	492
Benishangul- Gumuz	Stunting	49	1,282	555		1,127	732	475
SNNPR	Any anemia	59	1,528	818		1,492	1,000	692
Gambela	Any anemia	47	1,211	428		1,018	568	373
Harari	Any anemia	45	1,164	375		978	499	348
Addis Ababa	IDD/ TGR	54	1,411	413		1,274	405	262
Dire Dawa	Stunting	47	1,215	382		1,128	480	360
Sidama	Any anemia	54	1,395	747		1,363	914	632
Total sample size		639	16,596	7,213		14,772	9,001	5,824

<u>16,596</u> 7,213 <u>14,772</u> 9,001

STROBE Statement—checklist of items that should be included in reports of observational studies

	ltem No	Recommendation	Reported on page #
Title and	1	(a) Indicate the study's design with a commonly used term in the	Title p.1; Abstract p.2
abstract		title or the abstract	3
		(b) Provide in the abstract an informative and balanced summary of	
		what was done and what was found	
Introduction			1.1
Introduction	4	Explain the scientific background and rationale for the investigation being reported	Introduction
/rationale			<u>p.3-4</u>
Objectives	5	State specific objectives, including any prespecified hypotheses	Background p.5 last statements
Methods		O,	
Study design	5	Present key elements of study design early in the paper	Methods (data source) p.4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Methods (data source) p.5
Participants	5	(a) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	Methods (participants) p.5-8
		Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants	
		 (b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed Case-control study—For matched studies, give matching criteria and the number of controls per case 	
Variables	5	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Methods (all outcome and exposure variables are listed) p.6-8
Data sources/ measurement	NA	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Methods (methods of measurement indicated in) p.5-8
Bias	10	Describe any efforts to address potential sources of bias	Methods (data quality indicated) p.10-11
Study size	5-6	Explain how the study size was arrived at	Methods (data source,statistical

		analysis) p.5
Quantitative 10 variables	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Methods (measureme and statistic analysis sections) p. 9
Statistical 10 methods	(<i>a</i>) Describe all statistical methods, including those used to control for confounding	Methods (analysis section) p. 8-
	(b) Describe any methods used to examine subgroups and interactions	
	(c) Explain how missing data were addressed	
	(d) Cohort study—If applicable, explain how loss to follow-up was addressed	
	Case-control study—If applicable, explain how matching of cases and controls was addressed	
	Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
	(<u>e</u>) Describe any sensitivity analyses	

Participants	5*	(a) Report numbers of individuals at each stage of study—eg numbers	NA
Participants	5	potentially eligible, examined for eligibility, confirmed eligible,	1111
		included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive	14*	(a) Give characteristics of study participants (eg demographic, clinical,	NA
data		social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable	
		of interest	
		(c) Cohort study—Summarise follow-up time (eg, average and total	
		amount)	NT 4
Outcome data	15*	Cohort study—Report numbers of outcome events or summary	NA
		measures over time	
		Case-control study—Report numbers in each exposure category, or	
		summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or	
		summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted	NA
		estimates and their precision (eg, 95% confidence interval). Make	
		clear which confounders were adjusted for and why they were	
		included	
		(b) Report category boundaries when continuous variables were	
		categorized	
		(c) If relevant, consider translating estimates of relative risk into	
		absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and	NA
		interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	p. 9-10
Limitations	19	Discuss limitations of the study, taking into account sources of	Discussion
Linitations	19	potential bias or imprecision. Discuss both direction and magnitude of	(strengths
		any potential bias	and
		any potential bias	weaknesses
			of the
			study) p.10-
			<u>11</u>
Interpretation	20	Give a cautious overall interpretation of results considering	Discussion
		objectives, limitations, multiplicity of analyses, results from similar	(interpretatio
		studies, and other relevant evidence	of findings in
			the context o
			existing
			research,
			meaning of
			the study:

20		ымэ Орен	
			p.9-11 implication
Generalisability	21	Discuss the generalisability (external validity) of the study results	Discussion (strengths an weakness of study) p.10-2
Other informatio	on		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Funding p.12
		arately for cases and controls in case-control studies and, if applicable, for ohort and cross-sectional studies.	exposed and
		g/, and Epidemiology at http://www.epidem.com/). Information on the ST pe-statement.org.	ROBE Initiative is



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Date

4TC EPHL 6.13 233 Ref. No 01 -04- 2021

EPHI-IRB Certificate of Approval

EPHI-IRB MM No.: 076 Protocol Number: EPHI-IRB-317-2020

	Protocol Title: Ethiopian Food and Nutrition Programs Baseline Survey.				
	Primary Investigator	Dr Masresha Tessema			
Institute: EPHI					
Study site/s Ethiopia					
Elements Reviewed (EPHI-IRB AF 01-008/02.0):		Attached	✓ Not attached		
	Mode of Review		Expedited	✓ Full Board	
	Decision of the meeting		 ✓ Approved 		

I. Elements approved: 1. Protocol Version No.: 03

2. Protocol Version Date: 19 Mar 2021

- 3. ICF Version No.: 03
- 4. ICF Version Date: 19 Mar 2021

II. . Obligations of the PI:

1. Should comply with the standard international & national scientific and ethical guidelines

- 2. All amendments and changes made in protocol and consent form needs IRB approval
- 3. The PI should report SAE within 48 hours of the event
- 4. This approval certificate is valid for only one year (specified below). The PI should Submit continuation request before expire date of approval, if project is to continue.
- 5. Final report/Thesis should be submitted to the IRB secretariat office (SERO) within two months following completion of the study, and Articles as soon as published
- Institutional Review Board Approval Date: 16 Jan 2021 Approval Period: From 16 Jan 2021 to 15 Jan 2022

Follow up report expected in:

6 months	9 months	one year	×/	
EPHI-IRB Chairperson	,	EPHI Director	or General	
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Ref: NUT/005/2021 Date: February 15, 2021

To: Ethiopian Public Health Institute (EPHI)

Addis Ababa

As part of the Investment Project Financing (IPF) support by World Bank and signed Memorandum of Understanding with MOH and UNICEF; the National Nutrition program II end line survey which will be served as a baseline for the National Food and Nutrition Strategy will be conducted by EPHI.

The objective of the baseline survey is to produce information on anthropometric status, dietary intakes, and micronutrient status of different population groups in Ethiopia, and assess the coverage of nutrition-sensitive and specific interventions.

UNICEF will be providing financial support (\$ 917,335) to EPHI to conduct Ethiopian Food and Nutrition Strategy Baseline Survey in ten regions and two city administrations and considering of raising additional mobilization of funds for the purpose.

We look forward to continuing to work with your organization as this survey moves forward.

Sincerely Yours,

stanley chitekwe

Stanley Chitekwe, Chief of Nutrition UNICEF Ethiopia

Active in more than 190 countries and territories through country programmes and National Committees. We are UNICEF, the United Nations Children's Fund.

UNICEF Ethiopia: Telephone +251 115 184000 Fax +251 115 511628 Email <u>ethcommunication@unicef.org</u> Website <u>www.unicef.org/ethiopia</u>



Date: <u>15 February 2021</u> Ref. №: <u>01799/GAIN/ET</u>

EPHI, IRB Office Addis Ababa

Subject: Letter of confirmation

This letter is in response to your request on financial and technical support concerning Food and Nutrition program baseline survey. We would like to confirm that GAIN is happy to support this important initiative.

GAIN's support on Food and Nutrition program bassline survey includes the following:

- Financing field supplies for the biomarkers collection.
- Hiring a food consumption survey assistant and
- Assigning two experts from Global and local GAIN office to provide technical support.

Looking forward to a continued fruitful collaboration.

Kind regards,

Thomas Haverkort Country Director



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BMJ Open

Ethiopia National Food and Nutrition Survey to inform the Ethiopian Food and Nutrition Strategy: A study protocol

Journal:	BMJ Open
Manuscript ID	bmjopen-2022-067641.R1
Article Type:	Protocol
Date Submitted by the Author:	23-Feb-2023
Complete List of Authors:	Woldeyohannes, Meseret ; Ethiopian Public Health Institute Girma, Meron; Ethiopian Public Health Institute, National Information Platforms for Nutrition Petros, Alemnesh; Ethiopian Public Health Institute Hussen, Alemayehu ; Ethiopian Public Health Institute Samuel, Aregash; Ethiopian Public Health Institute Dinssa, Danial ; Ethiopian Public Health Institute, Environmental and Public Health Research Challa, Feyissa; Ethiopian Public Health Institute Laillou, Arnaud; UNICEF, Dakar, Senegal Chitekwe, Stanley; UNICEF Ethiopia Baye, Kaleab ; Addis Ababa University, Noor , Ramadhani; UNICEF Ethiopia Donze, Anne Sophie; UNICEF Ethiopia Tollera, Getachew; Ethiopian Public Health Institute Dangiso, Mesay; Ethiopian Public Health Institute Tadesse, Lia; Ethiopia Ministry of Health Zelalem, Meseret; Ethiopia Ministry of Health Tessema, Masresha; Ethiopian Public Health Institute,
Primary Subject Heading :	Public health
Secondary Subject Heading:	Epidemiology
Keywords:	EPIDEMIOLOGY, NUTRITION & DIETETICS, PUBLIC HEALTH

SCHOLARONE[™] Manuscripts

1 2		
3	1	Ethiopia National Food and Nutrition Survey to Inform the Ethiopian Food and Nutrition
4 5	2	Strategy: A Study Protocol
6		
7 8	3	Meseret Woldeyohannes ¹ , Meron Girma ¹ , Alemnesh Petros ¹ , Alemayehu Hussen ¹ , Aregash Samuel ¹ ,
9	4	Daniel Abera Dinssa ¹ , Feyissa Challa ¹ , Arnaud Laillou ² , Stanley Chitekwe ³ , Kaleab Baye ⁴ , Ramadhani Noor ³ ,
10 11	5	Anne Sophie Donze ³ , Getachew Tollera ¹ , Mesay Hailu Dangiso ¹ , Lia Tadesse ⁵ , Meseret Zelalem ⁵ , Masresha
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22 ABSTRACT

Introduction Ethiopia has made significant progress in reducing malnutrition in the past two decades. Despite such improvements, a substantial segment of the country's population remains chronically undernourished and suffers from not only micronutrient deficiencies but also from increasing diet-related non-communicable diseases such as diabetics, hypertension and cancer. This survey aims to assess the anthropometric status, dietary intake and micronutrient status of Ethiopian children, women, and adolescent girls. The study will also assess the coverage of direct and indirect nutrition-related interventions, and map agricultural soil nutrients. The survey will serve as a baseline for the recently developed Ethiopian food system transformation plan and will inform the implementation of the National Food and Nutrition Strategy.

Methods and analysis A population-based cross-sectional survey, the study will collect data in the ten regions and two city administrations of Ethiopia. The study population will be women of reproductive age, children aged 0-59 months, school-aged children and adolescent girls. A total of 16,596 households will be surveyed, allowing us to generate national and regional estimates for selected indicators. A two-stage stratified cluster sampling procedure will be used to select households. In the first stage, 639 enumeration areas will be selected using probability-proportional to size allocation. In the second stage 26 eligible households will be selected within each enumeration area using systematic random selection. Primary outcomes include coverage of direct and indirect nutrition interventions, infant and young child feeding practices, food insecurity, dietary intakes, mental health of women and children, anthropometric status, micronutrient status, and soil nutrient status.

Ethics and dissemination A protocol was full reviewed and approved by the Institutional Review Board of
 the Ethiopian Public Health Institute (protocol no: EPHI-IRB-317-2020). The finding of this survey will be
 disseminated in scientific forums, national research conference, and will be submitted for publication in
 peer reviewed journals.

46 Strengths and limitations of this study
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- The survey covered a large geographic area and collect data on anthropometric status, 24hr recall quantitative dietary intakes and the determination of micronutrient status in the same participants or household, while at the same time capturing data on the food system in Ethiopia.
 - The study will help to improve understanding of nutritional problem across multiple facets—from agricultural soil to people to the environment in Ethiopia.
 - Inherent to the cross-sectional design of the study, the findings of this study cannot be used to
 establish cause and effect.
- The study design prevents us from considering seasonal differences in nutritional outcomes and determinants.

59 INTRODUCTION

Globally, one in every three population are affected by one of more forms of malnutrition.¹ Women and children are particularly vulnerable to malnutrition due to increased physiological nutrient needs required to support fetal and child growth.² Nutritional deprivation during early life impairs growth and development, leading to poor school performance, reduced productivity, and loss of earnings in later life.³ Consequently, the first 1000 days of life, from conception to the child's second year of life, was recognized as a critical window of opportunity to effectively prevent malnutrition.^{3 4} Adolescence is also identified as a second window of opportunity to correct nutritional inadequacies and adversities faced in early life, but little is known about this life stage.

Despite significant progress over the past two decades, the burden of malnutrition in Ethiopia remains high.⁵⁻⁷ Nationally, 37% of Ethiopian children under five years of age are stunted⁷, and 22% of women of reproductive age (WRA) are chronically undernourished (Body Mass Index (BMI) < 18.5 Kg/m²).⁵ Only 14% of children under two years of age consumed the minimum number of recommended food groups.⁵ Furthermore, micronutrient deficiencies co-exist with chronic energy deficiency.⁸ This along with the ongoing nutrition transition, characterized by shifts in diets9, is further complicating the nutrition landscape by increasing the prevalence of overweight and non-communicable diseases.⁵ Nearly a fifth (16%) of Ethiopian adults are estimated to be hypertensive, and 3% are diabetic.¹⁰ Therefore, addressing not only undernutrition but all forms of malnutrition is critical.

The Sustainable Development Goals (SDGs) recognize the importance of nutrition, primarily driven by the need to mitigate its detrimental consequences. Further, the 2012 World Health Assembly (WHA) identified global targets to be achieved by 2025 that aim to reduce stunting, anemia, low birth weight, and childhood obesity. These targets are used to track progress in SDG goal 2: Zero hunger.¹¹Recognizing the importance of good nutrition, the Government of Ethiopia has made ending malnutrition a national priority. Ethiopia started implementing its first National Nutrition Program in 2008.¹² The second phase of this program (2011-2016) was a multisectoral program aimed at accelerating progress in reducing malnutrition.¹³ Moreover, Ethiopia's first Food and Nutrition Policy was endorsed in 2018¹⁴, followed the National Food and Nutrition Strategy (FNS)¹⁵ which was launched in 2021 to provide a framework for the operationalization of the policy. Acceleration of progress in the reduction of malnutrition requires the design and implementation of direct and indirect nutrition interventions that can be implemented at scale. To this end, understanding the various factors contributing to the different forms of malnutrition is critical.

Multiple factors operating at the immediate, underlying, and basic levels contribute to malnutrition.² Inadequate dietary intake and poor health are immediate determinants.² Household food security, child care practices, access to health services, and healthy environments are underlying determinants.¹⁶ Structural and contextual factors such as economic structures, political, environmental, social and cultural factors are the basic determinants of malnutrition.² The contribution of these factors varies across different contexts, and target groups, but studies capturing all these factors in a single survey are scant. The lack of timely and comprehensive information on nutritional status across critical life stages and their determinants is a bottleneck that is preventing Ethiopia from designing effective interventions. Up to date and comprehensive data on the coverage of direct and indirect nutrition

interventions delivered across various implementing sectors of the food and nutrition strategy is not available yet. This is unfortunate as such data could inform the implementation of the food and nutrition strategy, but also serve as a baseline to which progress can be tracked against.

Therefore, this study aimed to provide the first ever comprehensive information on the nutritional status of different populations in Ethiopia to support evidence-based implementation of the national food and nutrition strategy.

Objectives of the survey

The overall goal of this study is to produce nationally and regionally representative estimates on anthropometric status, coverage of nutrition interventions, dietary intakes, and micronutrient status for children, adolescent girls, and women of reproductive age in Ethiopia.

- Specific objectives include;
 - 1. Assess the coverage of direct and indirect nutrition interventions.
 - 2. Assess food consumption patterns and nutrients intakes of children aged 6–59 months, and women of reproductive age.
 - 3. Assess the micronutrient status of children (vitamin A, anemia, iron, iodine and zinc), adolescent girls, and women of reproductive age (vitamin A, vitamin D, anemia, iron, iodine, zinc, folate, vitamin B_{12})
 - 4. Assess the anthropometric status of under-5 children, school-age children (6-12 years), adolescent girls, and women of reproductive age.
 - 5. Assess the geographical distribution of soil micronutrient status in Ethiopian agricultural soil.

METHODS AND ANALYSIS

Study design

This study is a nationally and sub-nationally (regionally) representative cross-sectional survey that will characterize dietary intake, micronutrient status, and access to nutrition-related services for different target populations. Given that soil nutrient content can influence micronutrient content of foods and hence affect nutrient intake, the soil nutrient composition will also be analyzed. The study will have four main components. The first component will assess nutrition-specific and nutrition-sensitive indicators (NSS) for all target groups (children aged 0-59 months and WRA, school-age children, adolescent girls)using semi-structured questionnaires. The second component will measure quantitative dietary intake for children aged 6-59 months and WRA (15-49 years). The third component of the survey will collect biomarker samples from all children (6-59 months), school-age children (6-12 years), adolescent girls (10-19 years), and WRA (15-49 years). The final component of the study will measure micronutrients in agricultural soils. The study data will be collected from July 2021 to December 2022.

Study setting

Ethiopia has an estimated population size of 120 million and is the second most populous country in Africa.¹⁷ The majority of its population resides in rural areas (70%).¹⁷ Agriculture accounts for 40% of the country's gross domestic product.¹⁷ Children aged 15 years and younger make up 40% of the Ethiopian population in 2021.¹⁸ Ethiopia is administratively divided into 10 regions and two city administrations. This study will be conducted in all of the regions and city administrations of the country. Figure 1 provides a geographic representation of the study areas.

³ 139 **Study participants**

The target population of this study are i) women of reproductive age (WRA) aged 15-49 years ii) children
aged 0-59 months iii) school age children aged 6-12 years, and iv) adolescent girls aged 10-19 years, and
V) household head.

143 Sample size calculations

Sample size was estimated to guarantee adequate precision to generate national and regional estimates for selected indicators for each study target group. Indicators used for each target group are shown in Table 1S in supplemental materials. The required number of households and target groups was calculated using a single population proportion formula at the regional level. We used region-specific prevalence estimates for indicators, a 5% margin-of-error, a design effect of 1.5, a household response rate of 95%, and an individual response rate of 80%. The initial sample size was then adjusted for region-specific average household size and percentage of the target population from the total population. An indicator that provides the maximum number of households was used to estimate the final sample size for each region. Regional sample sizes were summed up to derive the total (national) sample size. Based on these calculations, the total sample size for the overall survey was 16,596 households (Supplemental table 2S).

For WRA, dietary and biomarker data will be collected in half of the selected households within each Enumeration Area (EA). This selection will yield a total sample size of 7,386 WRA (50% of the expected 14,772 WRA). The sample size needed to assess dietary intakes and micronutrient status of WRA was calculated using the prevalence of inadequate zinc intake, which yielded the largest sample size.8 19

31 159 Sampling procedures32

A two-stage stratified cluster sampling procedure will be used to select households. In the first stage, 639 EAs, 257 urban and 382 rural will be selected using probability-proportional to-size allocation. We will use the 2018 Ethiopia Population and Housing Census enumeration areas sampling frame to select EAs (the Primary Sampling Units (PSUs)). The Central Statistical Agency (CSA) prepared the enumeration areas sampling frame. An EA typically contains 100-150 households. EA maps will be used to delineate the boundaries of the selected EA. In the second stage of sampling to identify eligible households, all households with the EA will be listed. A household will be eligible for selection if at least one of the study target groups are residents (de jure) or stayed at the household the night before the interview (de factor).

Twenty-six (26) eligible households will be selected within each EA using systematic random selection. All target groups will be eligible for the NSS interview in the selected households. All children aged 6-59 months will also be eligible for dietary assessment. Women residing in 13 households (Out of 26 households) who will be selected randomly will be eligible for dietary assessment. Biomarker samples will be collected for all children under 5 years of age, school-age children, and adolescents in the selected households. Similar to dietary assessment, biomarker samples will be collected for women residing in half of the selected households (figure 2).

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³ 178 **Outcomes**

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179 Coverage of direct and indirect nutrition interventions

6 180 A structured questionnaire will be used to determine the coverage of direct and indirect nutrition 7 181 interventions provided to children aged 6-59 months, WRA, and adolescent girls. Direct nutrition 8 182 interventions included vitamin A supplementation, iron supplementation, zinc supplementation, growth 9 183 monitoring and promotion, nutrition counseling services, and food fortification. Water, sanitation and 10 11 184 hygiene, coverage of food or cash assistance program, women empowerment, and mental health will be 12 185 some nutrition-sensitive indicators considered in this study (table 1). We will use standard indicator 13 186 definitions proposed by the Data for Decisions to Expand Nutrition Transformation project (DataDENT) to 14 187 assess coverage of nutrition programs. 15

1617188Anthropometric status

18 189 Using standardized procedures, anthropometric measurements, including weight, height/length, and 19 190 mid-upper arm circumference, will be taken for all study target populations.²⁰ Anthropometric indices 20 (weight-for-height z-scores, length/height-for-age z-scores, weight-for-age z-scores, BMI-for-age z-scores) 191 21 22 192 will be calculated using the WHO 2006 child growth standards and the WHO 2007 child growth reference 23 193 data. Stunting (length/height-for-age z-scores below -2 SD), wasting (weight-for-height z-scores below -2 24 194 SD), underweight (weight-for-age z-scores below -2 SD), thinness (BMI-for-age z-scores below -2 SD) and 25 195 BMI will be the primary anthropometric outcomes of interest. 26

28 196 Infant and young child feeding practices

Infant and young child feeding practices will be assessed using the new World Health Organization (WHO)
 and United Nations Children's Fund (UNICEF) recommended 17 indicators to evaluate IYCF practices.²¹

33 199 Food insecurity

The Food Insecurity Experience Scale (FIES) will be used to assess household food security.²² The FIES consists of eight questions that assess household experience related to adequate food access. Experience questions range from worrying about getting enough food to not eating for a whole day.

In addition to these outcome indicators, information on the sociodemographic characteristic of
 households, child health, maternal health, employment status, and household agricultural practices will
 be collected using structured questionnaires.

44 206 Mental health of women

Common mental health disorders will be assessed using the WHO Self-reporting questionnaire which
consists of 20 questions. Women will be classified as having a common mental health disorder if row
score was greater or equal to 6 out of 20.²³

50 210 Assessment of dietary intakes of children and WRA

We will measure dietary intake for children aged 6-59 months and WRA. A one-day quantitative multiple pass 24-hour recall will be conducted to assess dietary intakes. The interactive multiple-pass 24-hour
 recall interview consists of four steps designed to enhance memory.²⁴ All days of the week will be
 proportionately represented during the dietary survey to account for the day of the week effects on food

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intake. To account for the day-to-day variability of dietary intake within individuals, a second nonconsecutive day 24-hr recall (repeat) will be collected (within 2 to 10 days of the first recall) on a randomly selected sub-sample of WRA and children. The number of repeats needed is determined by allocating for each region 50 repeats, which is then multiplied by a design effect of 1.5 and a 10% nonresponse rate. The number of repeats was rounded up to 1244 recalls for each target group to ensure that the minimum number of repeats (n=83) needed from each region would be collected. Detailed nonstandard recipe ingredient data will be collected for all mixed dishes that were prepared at home.

We will use 15 food groups to assess dietary intakes of women (15-49 months) and children aged 24-59 months. These food groups were: 1) Cereals and their products, 2) Starchy Roots and tubers, and their products, 3) Pulses, and their products, 4) Vegetables and their products, 5) Fruits and their products, 6) Meat, and poultry their products, 7) Eggs and their products, 8) Fish, shellfish and their products, 9) Milk and milk products, 10) Fats and oils, 11) Nuts and seeds, 12) Sugar and sweetened products, 13) Beverages, 14) Spices and condiments, and 15) Miscellaneous. For children aged 6-23 months we will use the updated WHO, UNICEF food groups: 1) Breastmilk, 2) Grains, roots and tubers, 3) Pulses, nuts and seeds, 4) Dairy products, 5) Flesh foods (meats, fish, poultry, organ meats), 6) Eggs 7) Vitamin-A rich fruits and vegetables, and 8) other fruits and vegetables.

Dietary assessment pre-survey work: We carried out pre-survey work to aid dietary data collection following recommendation set by the Intake: Center for Dietary Assessment.^{25 26} An initial step was developing a food and ingredient list containing a comprehensive list of food items, mixed dishes, and ingredients expected to be consumed by the study target groups. The food list was generated using data from the first 2011 Ethiopian National Food Consumption Survey.¹⁹ Other common foods consumed across the regions in Ethiopia were derived from the 2016 Household Income and Expenditure Surveys,²⁷ the Ethiopian Food Composition Tables, and dietary intake data from other recent dietary assessment surveys conducted by the Ethiopian Public Health Institute (EPHI). Portion size estimation methods suitable for large-scale studies were pre-selected for use in the survey following Intake recommendations.²⁸ The selected methods were direct measurement of actual foods consumed, standard unit: size and number, proxy measurement using play dough, water, rice, and maize flour, and finally using food price to estimate the amount of food consumed. Portion size estimation methods were assigned for all foods included in the food list.

 Table 1. Nutrition-direct and nutrition-indirect interventions coverage

No	Indicator	Target population					
	Nutrition indirect intervention coverage						
	Child interventions						
1.	Children received iron tablets/syrup in the last 12 months	Children aged 6-59 months					
2.	Children received vitamin A supplements in past 6 months	Children aged 6-59 months					
3.	Children received deworming tablets in the past 6 months	Children aged 24-59 months					
4.	All 8 basic vaccinations: one dose of BCG, three doses of DPT, three doses of the polio vaccine,	Children aged 9-59 months					

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		1
	and one dose of the measles vaccine	
5.	No Vaccination	Children aged 0-59 months
	Growth monitoring	
6.	Weight measured in the last 3 months	Children aged 0-23 months
7.	Height measured in the last 3 months (Optional)	Children aged 0-23 months
8.	MUAC measured in the last 3 months (Optional)	Children aged 0-23 months
	Infant and young child feeding (IYCF) counselling	1
9.	Mothers with children 6-23 months received any IYCF counseling	Children aged 6-23 months
10.	Mothers with children 6-23 months received age- appropriate IYCF counseling	Children aged 6-23 months
	Early breast-feeding counseling	
11.	Women received breastfeeding counseling with	Women aged 15-49 years with a
	observation during the first two days after birth	live birth in the past 5 years for the most recent birth
12.	Women received breastfeeding counseling during the first month after birth	Women aged 15-49 years with a live birth in the past 5 years for the most recent birth
	Coverage of Nutritional Interventions during pregnancy/ant	
13.	Percentage of women who had 4 or more ANC	Women aged 15-49 years with a
15.	visits for the most recent birth	birth in the last 5 years
14.	Percentage of women who received counseling about healthy eating during pregnancy	Women aged 15-49 years who received antenatal care for their most recent birth
15.	Percentage of women whose weight gain was monitored during pregnancy	Women aged 15-49 years who received antenatal care for their most recent birth
16.	Women received food or cash assistance during pregnancy	Women aged 15-49 years with a birth in the last 5 years
17.	Women took 90+ iron/folate tablets during pregnancy	Women aged 15-49 years with a live birth in the past 5 years for the most recent birth
18.	Women received deworming tablets during pregnancy	Women aged 15-49 years with a live birth in the past 5 years for the most recent birth
	Nutrition indirect intervention coverage	1
19.	Basic water services	Household
20.	Basic hygiene services	Household
21.	Basic Sanitation services	Household
22.	Food insecurity (not a service hence no coverage)	Household
23.	Women received food or cash assistance during pregnancy	Women aged 15-49 years with a live birth in the past 5 years for the most recent birth
24.	Basic water services	Household
25.	Basic hygiene services	Household
26.	Basic Sanitation services	Household
	Presence of common mental health disorders in	Women aged 15-49 years

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	the past month	
28.	Women empowerment	Women aged 15-49 years
29.	Livestock ownership	Household
30	Agricultural productivity by food group	Household

245 Assessment of micronutrient status:

Blood specimens will be collected from the study population to determine serum retinol, ferritin, soluble transferrin receptor (sTfR), zinc, folate, vitamin B12, red blood cell (RBC) folate and 25-hydroxyvitamin D. Additionally, markers of inflammation, alpha(1)-acid glycoprotein (AGP), high-sensitivity C-reactive protein (hsCRP) will also be measured. We will also analyze parasites from stool specimens. All laboratory analyses will be performed at the EPHI Clinical chemistry, and Food Science and Nutrition Laboratories. Both laboratories participate in an external quality assessment scheme and are accredited by the Ethiopian National Accreditation Office (ENAO). Collection, storage, and analytical procedures for blood, urine, stool, and salt samples are described below. The details of each biomarker analysis are described in Supplemental Material 1-11.

Blood sample collection and analysis: Venous blood samples (5-7 mL) will be collected using vacutainer tubes following standard operating procedures.²⁹ Trace mineral-free vacutainer tubes will be used to collect blood for trace metal analysis. After collection, blood samples will be allowed to clot for 30 minutes in cold boxes (<8 °c). Samples will then be centrifuged at 3000 rpm (revolution per minute) for 10 minutes. The separated serum will be aliquoted and stored in -20°c portable freezers in the field. Samples will then be transported to EPHI and stored at -80°c until analysis. Hemoglobin will be measured in the field using Hemocue[®] (Hb 301, Hemocue AB, Angelholm, Sweden)³⁰.³⁰ If the hemoglobin values are below WHO cutoff point(11g/dl), the phlebotomist will send whole blood samples to the EPHI laboratory to identify hemoglobinopathies using electrophoresis method.³¹ Malaria test will be conducted on-site using Bioline[™] Malaria Ag P.f rapid diagnostic test kits (RDT) for P. falciparum and P. vivax).³² Serum soluble transferrin receptor (sTfR), AGP, hsCRP, folate, red blood cell (RBC) folate, vitamin B₁₂, and ferritin will be measured using Cobas 6000 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Serum retinol will be measured using high-performance liquid chromatography (HPLC) method³², and serum zinc and selenium will be measured using a microwave plasma atomic emission spectrometers (MP-AES) analyzer.

Stool and urine sample collection and analysis: Stool samples will be collected using stool cups and stored in 10 % formalin to preserve the parasite until analysis.³³ A portion of each stool sample will be used to detect direct ova, larvae and cysts of intestine parasites using formal ether concentration technique.³⁴Urine samples will be collected from WRA and school-age children using 60 ml urine cup containers. Samples will be stored at -20°c. Urinary iodine excretion will be assessed by Sandell Kolthoff reaction at EPHI Laboratory using Shimadzu 1800 UV-Vis spectroscopy.³⁵

Salt collection and analysis: Salt samples will be collected from households with WRA for whom dietary
 data will be collected. At least 25 grams (one coffee cup) of salt will be collected to determine iodine
 content using the iodometric titration method.³⁶

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Assessment of nutrients in the soil

Soil samples will be collected from three households in each EA. Zig-zag or cross sampling method will be used to collect 10 to 20 subsamples (0-30 cm depth) constituting one composite sample. Subsamples will be collected at a separation distance of five meters. After thoroughly mixing composite samples, 1 kg soil sample will be transferred to polyethylene bags. The collected soil samples will be air-dried in wooden trays and disaggregated using a ceramic mortar and pestle (soil grinder) at the EPHI soil laboratory. Samples will then pass through a 6 mm sieve of stainless-steel screens to remove debris and homogenize the soil sample. The sieved fraction will be further pulverized to pass through a 1 mm sieve for the micronutrient analysis. Soil zinc, iron, copper, and manganese will be determined following standard procedures.³⁷ Micronutrient content will be determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES) after extraction with diethylene triamine penta acetic acid (DPTA). Additional variables that affect the mobility of micronutrients in the soil and their uptake into crops will also be measured. These variables include soil reaction (pH), electrical conductivity, organic matter, total nitrogen, and soil organic carbon content. Data collectors will also record topography, slope, cropping .en. informat. , study target gro... history, type, and fertilizer application information. Table 2 provides a summary of procedures for each of the four components of the survey by study target groups.

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		Child	Child	Child	School	Adolescent	WRA	Household
		0-5	6-23	24-59	children	girls	15-49	
		months	months	months	6-12 years	10-19 years	years	
-	Nutrition direct and nutrition indirect inter indicators	rventions						
-	Infant and young child feeding practices	Х	X					
	Nutritional information for adolescent girls					Х		
	Food insecurity							Х
	Water, sanitation and hygiene practices							Х
	Coverage of food fortification							Х
	Agricultural practices							Х
	Mental health						Х	
	Anthropometric status	Х	х	Х		х	Х	
_	Dietary assessment		4	A.				
_	24-hr recall quantitative dietary intake		Х	Х			Х	
_	Assessment of biomarker status				0			
	Blood sample		Х	Х	x	Х	Х	
	Urine sample				x		Х	
	Stool sample		Х	Х	х	X	Х	
_	Salt sample collection					<u> </u>		Х
_	Assessment of micronutrients in the Soil							
	Soil micronutrient assessment							Х

Data quality assurance and analysis

Training of trainers on components of the survey was held before training the data collectors and supervisors. After fifteen days of training on methodological procedures, questionnaires and quality assurance, the questionnaires were tested in a pilot group (in EAs not included in the actual survey), and adapted based on the received feedback from the survey team. The questionnaires (including the food list) were translated into local languages (Amharic, Oromifa, Tigrigna, Somali, and Afar) and backtranslated to English to ensure the quality of the translation. The data collectors' measurements were standardized to ensure that the inter-observer variability was within tolerable limits. Supervisors received additional training on teamwork and on monitoring and supervising the data collection process. All data collection tools are programmed using open-source software (ODK) (Supplemental Material 12). Data quality checks were included during ODK programming to prevent data recording errors. These include restricted responses, filter insert choices, skip patterns, and defaults. During data collection daily data tracking forms will be completed to track completed surveys for each study components to prevent missing data. Before the start of the survey high frequency checks were identified and error tracking forms were designed to track data quality in real-time. These checks included completeness checks, target group tracking, and duplicate ID check. Random field-supervision visits will also be made to check data quality. Every day, collected data will be sent to the EPHI central server and imported into statistical software programs as comma-separated values (CSV) files. For laboratory analysis, a quality control chart will be used to ensure the internal and external quality control materials are in the acceptable range.

The primary data analysis will focus on computing frequencies and percentages for categorical variables and summary statistics (like means, medians SD, IQR) for summarizing continuous variables. Sample weights will be constructed based on the selection probabilities of EAs, eligible households, and non-response rates. All analyses will also be adjusted for the survey design. Additional subgroup analysis will be computed for variables with adequate sample sizes for each category. The Biomarkers Reflecting Inflammation and Nutrition Determinants of Anemia (BRINDA) Working Group's regression correction approach will be used to account for inflammation in the study of all micronutrients status using the biomarkers C-reactive protein (CRP) and AGP. Geostatistical analyses will be employed to determine the spatial patterns of micronutrient distribution in the soil and blood samples. The wealth index will be constructed using principal component analysis (PCA).³⁸ The Rasch model will be used to construct the Food Insecurity Experience Scale (FIES). ²² All analysis will be done using STATA 16 and ArcGIS/QGIS. Anthropometric indices will be calculated using the WHO Anthro software for under five children and WHO AnthroPlus software for adolescent.

Patient and public involvement statement

None

Ethics and dissemination

The study protocol is approved by the Institutional Review Board of the Ethiopian Public Health Institute (protocol no: EPHI-IRB-317-2020). Written informed consent will be obtained from each respondent and participants may withdraw at any time (supplemental Material 13).Confidentiality of all

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collected data will be given high priority during each stage of data handling. Individual names and personal information of respondents will be kept confidential and data sets will be kept anonymous for analysis.

The study's findings will be disseminated through several communication channels, including stakeholder workshops, various local and International conferences and technical report. Additionally, the findings will be submitted as special issue to peer-reviewed journals.

DISCUSSION

This comprehensive, nationally representative survey will for the first time characterize simultaneously the dietary intake and micronutrient status of Ethiopian children, adolescent girls and WRA. Besides, the study assesses key drivers of malnutrition including soil nutrient composition, as well as coverage of direct and indirect nutrition interventions. The survey will provide key insights informing the implementation of Ethiopia's National Food and Nutrition Strategy.

High-quality and timely data is critical to assess the burden of nutritional problems, identify vulnerable populations and priority actions, track the implementation of nutrition programs, and assess impact.^{39 40} Ethiopia conducted its first-ever food consumption survey in 2011.¹⁹ and its micronutrient survey in 2015.⁴¹ Both surveys were collected at different times, which made it difficult to link the two surveys. Besides, the causes and solutions of malnutrition are complex and multisectoral; hence, requiring data on multiple indicators from various sectors spanning from soil nutrient, diets, micronutrient status, to access and exposure to direct and indirect nutrition interventions. In this regard, this survey is uniquely positioned to integrate data from multiple domains to support evidence-based decision making for improved diets, nutrition, and overall wellbeing.

This study will allow us to evaluate progress relative to the last food consumption and micronutrient surveys, but more importantly will serve as a baseline to which future progress related to the implementation of the food and nutrition strategy will be evaluated. Furthermore, the current survey will also serve as a baseline for the Ethiopian Food System Transformation Plan by capturing majority indicators used for monitoring food systems related progress. Thus, filling information gaps that could have impeded successful implementation of the National Food and Nutrition Strategy (FNS). By establishing 13 strategic objectives, the FNS is intended to be aligned with the strategic directions of the Food and Nutrition Policy (FNP). Each strategy direction includes initiatives, actions, and key performance indicators, as well as leading and collaborating sectors. The key performance indicators should be evaluated to determine the progress of each implementing sector's achievement. The current survey will provide up to date national and subnational information on the current food and nutrition situation in Ethiopia for different target populations as well as provide comprehensive list of indicators that are pertinent to the implementation of the policy.³⁹ In addition, this study will provide information on context-specific determinants for prioritizing direct and indirect actions that can be implemented across sectors taking into account the specific needs of different target populations.

Additionally, effective multisectoral interventions that address the immediate and underlying determinants of malnutrition must be implemented in order to accelerate the reduction of malnutrition in its all form.³⁹ These interventions need to address context-specific determinants to reduce

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malnutrition effectively.³⁹ The lack of timely and disaggregated information on the determinants of malnutrition is a bottleneck to preventing malnutrition, particularly among the most vulnerable target populations. This study will also provide information on the coverage and quality of interventions which can be used to contextualize National Food and Nutrition Strategy monitoring frameworks, monitor implementation and track progress towards global and local targets.

Although this study will provide regionally and nationally representative estimates for key indicators and critical life stages, it has several limitations. Inherent to the cross-sectional design of the study, the findings of this study cannot be used to establish cause and effect. Additionally, the design prevents us from considering seasonal differences in nutritional outcomes and determinants. This study also relies on self-reported data, which is subject to recall bias. Notwithstanding the above-mentioned limitations, this study is uniquely designed to combine the assessment of anthropometric status, 24hr recall quantitative dietary intakes and the determination of micronutrient status in the same participants, while at the same time capturing data on the food system. Additionally, the study will be evaluating micronutrients in the agricultural soil, which will expand our understanding of factors that influence nutrition. To the best of our knowledge, this will be among -if not-the first study to simultaneously collect these variables from the same household. This could contribute to a better understanding of nutritional problem across multiple facets—from soil to people to the environment. In the past, nutrition programs implemented in Ethiopia have relied on information provided from smallscale studies and population-based surveys such as the Ethiopia Demographic and Health Surveys. 5-7 42 43 Although these data sources provide some information to track progress and tailor interventions, they only provide data on a limited number of nutrition indicators and do not measure dietary intakes and assess biomarker status. This study will fill these data gaps by providing information on comprehensive indicators that show the burden and spatial distribution of micronutrient deficiencies and shifts of dietary patterns. Additionally, this study will provide information on emerging determinants such as mental health and intake of nutrients such as folate and B₁₂ that have not been included in previous studies. Finally, the inclusion of adolescent girls, and school-age children, will provide vital information on nutritional indicators for these target groups, which are often not included in other nationally representative surveys. This survey will also provide information on the coverage of direct interventions implemented in the health sectors and indirect interventions implemented in the agriculture, WASH, education and social protection sectors for whom scant data exists. Hence, this study will provide valuable information that will guide the implementation of strategic actions for the reduction of malnutrition in Ethiopia.

Acknowledgments:

Contributions: MT, AL, SC, MW, AP, AS, and MG conceived the study and drafted the original protocol. All authors participated in refining of the protocol. AH, MW, MG and MT played a major role in the statistical consideration. DAD, FC, MG, RN, and ASD helped to write the draft protocol and made a critical contribution to the content. KB, AL, GT, MHD, LT and MZ supervised manuscript preparation. All authors took responsibility for reviewing, final editing and approval of the manuscript.

Funding: This work was supported by Power of Nutrition, BMGF and World Bank through UNICEF; Nutrition International, and Global Alliance for Improved Nutrition.

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Competing interests: The authors declare that they have no conflicts of interest.

Patient consent for publication: Not required

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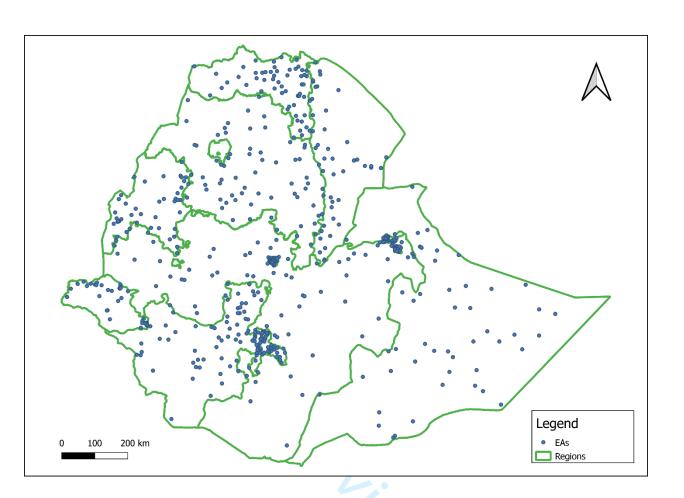
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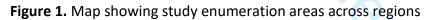
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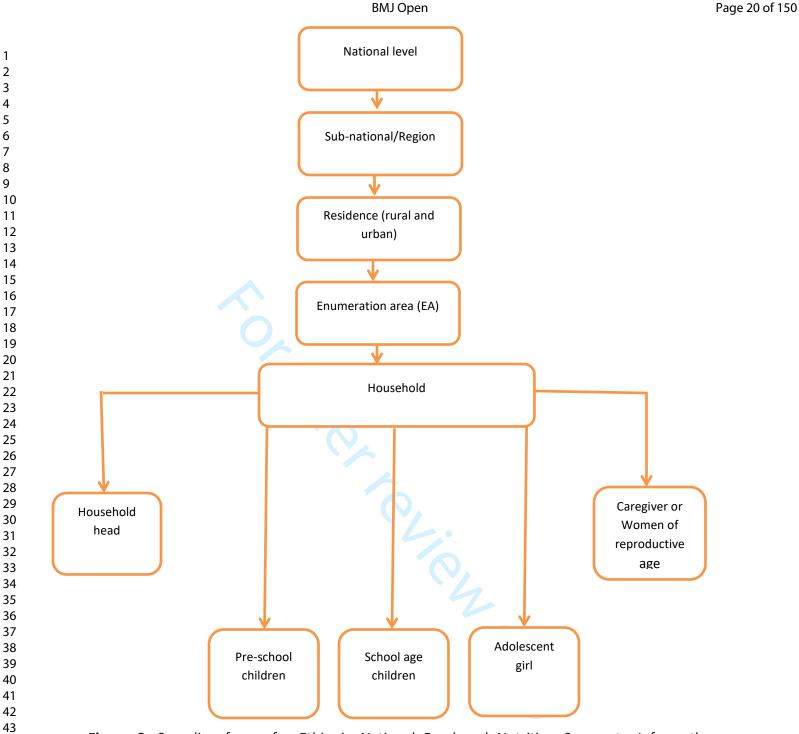


Figure 2. Sampling frame for Ethiopia National Food and Nutrition Survey to Inform the Ethiopian Food and Nutrition Strategy

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	Key indicators used to estimate sample size
	Vitamin A deficiency
	Total goiter prevalence
, e .	Stunting
months)	Any anemia
	Zinc deficiency
	Prevalence of inadequate intake of zinc
	Vitamin A deficiency
	Total goiter prevalence
	Any anemia
	Zinc deficiency
	RBC folate deficiency
Women of reproductive age (15-49 years)	Serum folate deficiency
	Vitamin B ₁₂ deficiency
	Iodized salt coverage
	Prevalence of inadequate intake of iron
	Prevalence of inadequate intake of zinc
	Prevalence of inadequate intake vitamin A
	Vitamin A deficiency
	Total goiter prevalence
School age children (6 to 12 years)	Any anemia
0 (, , ,	Iodine deficiency
	Zinc deficiency
	Any anemia
Adolescent girls (10 to 19 years)	

Table 2S. Sample size determination and allocation

Region	Indicator used to estimate sample size	Number of EA	Number of HH	Expected number pre- school children (0-59 months)	Expected number of WRA (15- 49 years)	Expected number school age children (6-12 years)	Expected number of adolescent girls (10-19 years)	Total population (2019 year projection)
Tigray	Any anemia	55	1,432	590	1,236	753	516	5,443,095
Afar	Stunting	51	1,328	539	1,096	695	406	1,901,863
Amhara	Stunting	61	1,585	619	1,253	843	531	21,842,548
Oromia	IDD	62	1,622	891	1,539	1,111	739	37,267,225
Somali	IDD	55	1,424	855	1,268	1,000	492	6,050,851
Benishangul-Gumuz	Stunting	49	1,282	555	1,127	732	475	1,126,656
SNNPR	Any anemia	59	1,528	818	1,492	1,000	692	15,763,484
Gambela	Any anemia	47	1,211	428	1,018	568	373	463,203
Harari	Any anemia	45	1,164	375	978	499	348	257,362
Addis Ababa	IDD/ TGR	54	1,411	413	1,274	405	262	3,685,684
Dire Dawa	Stunting	47	1,215	382	1,128	480	360	492,819
Sidama	Any anemia	54	1,395	747	1,363	914	632	4,322,685
Total sample size		639	16,596	7,213	14,772	9,001	5,824	98,617,475

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1. Purpose

This procedure used to determine the amount of iodine content in the form of potassium

iodate (KIO₃) by iodometric titration from iodized salt.

2. Abbreviations:

g	gram	KI	Potassium Iodide
ppm	Parts Per million	H_2SO_4	sulfuric acid
Μ	Molarity	KIO ₃	potassium iodate
ml	mili liter	IDD	Iodine deficiency disorder
Ν	Normality	Na ₂ S ₂ O	3.5H₂O sodium thiosulfate penta hydrate

3. Principle:

Iodine released from potassium iodate by the action of sulphuric acid and the released iodine trapped with potassium iodide and titrate with sodium thiosulphate.

4. Material and methods

4.1 Reagents

- ➢ 0.005M Na₂S₂O₃.5H₂O
- ➢ 2N H₂SO₄
- ≻ 10% KI
- ▶ 1% Starch

4.2 Reagents preparation:

- <u>1%starch</u>: Dissolve 1 g of soluble starch in 100ml boiled distilled water heat the solution till starch dissolve completely.
- > <u>10% KI</u>; Dissolve 10gm of KI in 100ml deionized water.

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- 2N H₂SO₄; Slowly add 6 ml of concentrated H₂SO₄ to 90 ml of deionized water make the final volume 100 ml.
- ➢ <u>0.005M Na₂S₂O₃</u>; Dissolve 1.24gm of Na₂S₂O₃.5H₂O in 1000 ml of deionized water

4.3 Reagents stability and storage:

- ▶ Na₂S₂O3.5H₂O & 10% KI reagents store in a cool & dark place for six months.
- \blacktriangleright H₂SO₄ store at room temperature it stable indefinitely.
- Starch should be prepared daily.

5. Supplies and Equipment

- > Balance (Four-beam pan): Sensitivity = 0.01g, Capacity = 410g
- Flask, volumetric, 1000mL, 100mL
- Measuring cylinder, 10mL, 100mL
- Beakers (Pyrex)
- > Flasks, Erlenmeyer (conical) with stopper, 250mL
- Pipette, volumetric, 1mL, 5mL
- Burette w/straight stopcock 10mL
- Burette stand
- Laboratory safety glasses
- > Parafilm, for covering beakers
- ➢ Glass bottles with stoppers, for reagents, 250mL
- Spatula Lab single blade 150mm SS length
- Dropper bottle, glass 25-60mL
- ➢ Hot plate

6. Sample

- Sample type: salt
- > Amount required: 50-100g
- > Transport and storage: At room temperature avoid exposure to direct sunlight

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)	Stability: At room temperature for 3 more			·
10		1015.		
12 7. Spe	cial Safety and Precautions			
• •	While titrate the sample wear eye goog	e.		
15 16	The reaction mixture should be kept in t	he dark before titration	on because a side reaction	can
17	occur when the solution is exposed to li	ght that causes iodide	ions to be oxidized to ioc	line.
18 19	Inaccurate results may occur if starch se	olution is used while	still warm.	
20 21	If starch indicator is added too early,	a strong iodine-starc	h complex is formed, w	hich
22 23	reacts slowly, and gives falsely elevated	l results.		
24	The reaction should be performed at m	ild room temperature	e (<30 ⁰ C), since the iodin	ne is
25 26	volatile, and the indicator solution loses	sensitivity when exp	bosed to high temperature	s.
27 8 P r	ocedure			
²⁸ ²⁹ 8		50mL Erlenmever fl	ask with a stopper.	
30 31 8				
32 33 8			I T	
34 &		uld turn yellow if iod	ine is present.	
35 o 36 8			•	
37 38	(cupboard or drawer).	0		
39 40 8		$a_2S_2O_3$, and adjust lev	vel to zero.	
⁴¹ 8				
42	titration burette Until the solution turn	,		
14 15 8		1 0	olution should turn dark	
46	purple) and continue titrating with 0.0			
47 48	and finally colorless.			7
49 50 8	9 Record the level of thiosulfate in the b	urette and convert to	parts per million (ppm)	
51		diette and convert to	parts per minion (ppm)	
52 53				
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9. Quality control:

- **Control material**: KIO₃
- Level of iodine: 59.3-60.3ppm
- > Stability: stable at room temperature for long period of time.
- **Frequency**: per batch
- **10. Quality control preparation**: Prepare 0.0047M KIO₃ in 100ml. By weighing 0.10058g in 100ml deionized water. From this solution Pipette out 1 ml into conical flask and follow the procedure proceed from step 2 as described above.

11. Calculation

Iodine(ppm) = **10.6*** V Na₂S₂O₃(ml)

Where: VNa₂S₂O_{3:} Volume of sodium thiosulphate takes to titrate iodine in salt

12. Result Interpretation

- Sppm to indicate salt with no added iodine
- > 5-14.9 ppm to indicate inadequately iodized salt
- > 15-39.9 ppm to indicate salt is adequately iodized
- > >40 ppm of iodine is not recommended.

13. Other Records

- ➢ Data Log sheet
- ➢ QC chart

14. References

- De Maeyer, Lowenstein & Thilly, 1979; World Health Organization (WHO), United Nations Children Fund (UNICEF) & ICCIDD, 2007.
- ➢ AOAC 925.56 2016

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15. Annex

Conversion chart for iodine in fortified salt in the form of KIO₃ (PPM)

Volume		Volume									
Thiosulphate	lodine	Thiosulphate	lodin								
(mL)	(ppm)	(mL)	(ppn								
0.1	1.1	2.0	21.2	3.9	41.3	5.8	61.5	7.7	81.6	9.6	101.
0.2	2.1	2.1	22.2	4.0	42.4	5.9	62.5	7.8	82.7	9.7	102.
0.3	3.2	2.2	23.3	4.1	43.5	6.0	63.6	7.9	83.4	9.8	103.
0.4	4.2	2.3	24.4	4.2	44.5	6.1	64.7	8.0	84.8	9.9	104.
0.5	5.3	2.4	25.4	4.3	45.6	6.2	65.7	8.1	85.9	10.0	106.
0.6	6.4	2.5	26.5	4.4	46.4	6.3	66.8	8.2	86.9	10.1	107.
0.7	7.4	2.6	27.6	4.5	47.7	6.4	67.8	8.3	88.0	10.2	108.
0.8	8.5	2.7	28.6	4.6	48.8	6.5	68.9	8.4	89.0	10.3	109.
0.9	9.4	2.8	29.7	4.7	49.8	6.6	70.0	8.5	90.1	10.4	110.
1.0	10.6	2.9	30.7	4.8	50.9	6.7	71.0	8.6	91.2	10.5	111.
1.1	11.7	3.0	31.8	4.9	51.9	6.8	72.1	8.7	92.2	10.6	112.
1.2	12.2	3.1	32.9	5.0	53.0	6.9	73.1	8.8	93.3	10.7	113.
1.3	13.8	3.2	33.9	5.1	54.1	7.0	74.2	8.9	94.3	10.8	114.
1.4	14.8	3.3	35.0	5.2	55.1	7.1	75.3	9.0	95.4	10.9	115.
1.5	15.9	3.4	36.0	5.3	56.2	7.2	76.3	9.1	96.5	11.0	116.
1.6	17.0	3.5	37.1	5.4	57.2	7.3	77.4	9.2	97.5	11.1	117.
1.7	18.0	3.6	38.2	5.5	58.3	7.4	78.4	9.3	98.6	11.2	118.
1.8	19.1	3.7	39.2	5.6	59.4	7.5	79.5	9.4	99.7	11.3	119.
1.9	20.1	3.8	40.3	5.7	60.4	7.6	80.6	9.5	100.7	11.4	120.

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12	Document	Change History			
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14	Revision	No. Date approved	Nature of revision		
15 16	1	February, 2019	Initial release		
17 18	2	November, 2020	Typographical adjustment		
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50 59	checked against	the electronic version prior to use.			
60		For peer review only - http	p://bmjopen.bmj.com/site/about/g	guidelines.xhtml	

Determination of serum retinol using hexane as the serum sample extraction procedure

Contents

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2. Instrument (HPLC) parameters – we have Shimadzu prominence HPLC.	2
3. Sample extraction procedure	2
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1. Reagents and materials

- Methanol (HPLC grade)
- Hexane (HPLC grade)
- Ethanol (HPLC grade/AR grade)
- Normal saline solution (0.9% NaCl)
- Retinol acetate (as internal standard)
- Retinol (as external standard)
- CRM (Certified Reference Material) for serum retinol
- 15 ml centrifuge tube
- Vortex mixer
- Centrifuge (up to 4000 rpm capacity)
- Centrifuge tube rack
- Micropipettes (10-100µl and 100-1000µl)
- Micropipettes tips for both types
- Stopwatch
- HPLC Vials (1ml)

2. Instrument (HPLC) parameters – we have Shimadzu prominence HPLC.

- Detector SPD-10A UV/VIS at 325 nm
- Column SUPELCOSILTMLC-NH₂-NP, 25cm × 4.6mm, 5µm
- Mobile phase HPLC grade methanol
- Elution system isocratic

3. Sample extraction procedure

- Add 200 µl serum sample to 15ml plastic test tube.
- Add an equal volume of $50 \mu g/dl$ retinol acetate and ethanol.
- Take 200 μ l of a serious of standards (10, 20, 40, 60, 75 μ g/dl) to the 15 ml centrifuge tube and add the same volume of retinol acetate and normal saline solution.
- Mix the standard and sample solutions with a vortex mixer
- Add 1ml of HPLC grade hexane and mix for 45 seconds
- Centrifuge the solution at 4000rpm for 7 minutes and transfer the supernatant to other test tubes
- The solution has to be extract twice by adding 1 ml hexane.

- Dry the hexane using nitrogen gas and reconstitute by 500µl HPLC grade methanol
- It has to be mix for 30 minutes using a vortex mixer and transfer to 1 ml HPLC vials
- Analyze the extracted solutions using reversed-phase HPLC by isocratic elution system, at the flow rate of 1.5 ml/min, and injection volume 30µl

N: B: - Extract and analyse the CRM using the sample extraction procedure.

4. Calculation

- Plot the calibration curve using area ratio of retinol (standard) and retinol acetate (internal standard) vs concentration ratio (retinol: retinol acetate).
- From the linear equation formula, Y = mx + b, the concentration of serum retinol can be calculated.

Where Y - is area ratio (retinol: retinol acetate) of sample

- M is slope and b is Y intercept
- X is the concentration of serum retinol in $\mu g/dl$

5. Reference

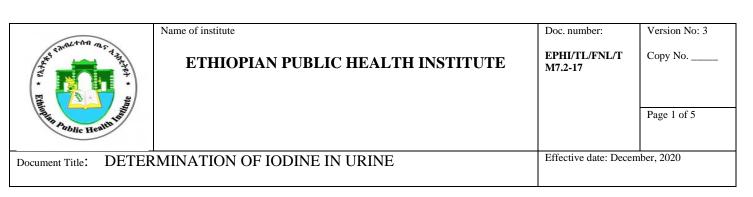
Quadro, Y.-K. K. and L. (2013). Reverse-Phase High-Performance Liquid Chromatography (HPLC) Analysis of Retinol and Retinyl Esters in Mouse Serum and Tissues. *Methods Mol Biol. 2010*; 652: 263–275. Doi:10.1007/978-1-60327-325-1_15., (12), 1–10. https://doi.org/10.1007/978-1-60327-325-1

von Lucke, A., Russell, R. M., Stephensen, C. B., Gannon, B. M., Craft, N. E., Haskell, M. J.,
... Raiten, D. J. (2016). Biomarkers of Nutrition for Development (BOND)—Vitamin A
Review. *The Journal of Nutrition*, 1816s-1848s.
https://doi.org/10.3945/jn.115.229708.FIGURE

Prepared by: Nahom Tefera (September 2020)

Reviewed by: Meseret W/yohannis

Approved by: Dr. Masresha Tessema



1 Scope

This test method is applicable for the determination of iodine from a urine sample.

2 Purpose

This procedure provides methods to control the amount of iodine and it's toxicity by ammonium persulfate digestion with spectrophotometric detection of the Sandell-Kolthoff Reaction method from urine samples.

Principle

Urine is digested with ammonium persulfate. Iodide is the catalyst in the reduction of ceric ammonium sulfate (yellow) to cerous form (colorless) and is detected by the rate of color disappearance (Sandell-Kolthoff reaction).

4 Chemicals and Apparatus

a) Chemicals

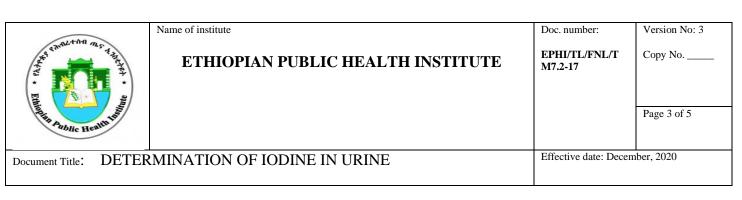
- Ammonium persulphate
- Arsenic trioxide
- Ceric ammonium sulphate
- Potassium iodate
- Deionized water
- Sodium chloride

b) Apparatus

- Hot plate
- Oven
- UV-Vis spectrophotometer
- Volumetric flasks (100 2000 ml)
- Beakers (100- 1000 ml)



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Document Title: DETERMINATION OF IODINE IN URINE	Effective date: Decer	mber, 2020
• Micropipette (100 – 1000 μl)		
• Pipette (5 – 10 ml)		
• Vortex mixer		
• Measuring cylinder (100 – 1000 ml)		
• Glass test tubes (13*100 mm)		
• Analytical balance (nearest to the 0.0001 g)		
 Erlenmeyer flask (2000 ml) 		
5 Reagents preparation		
a) 1 M Ammonium persulphate		
• Dissolve 114.1 g H ₂ N ₂ O ₈ S ₂ in deionized water and makeup to 5	00 ml with H ₂ O.	Store away
from light. Stable for at least one month.		
b) 5 N H ₂ SO ₄		
• Slowly add 139 ml concentrated (36 N) H ₂ SO ₄ to about 700 ml de	eionized water (ca	areful - this
generates heat!). When cool, adjust with deionized water to a final	volume of 1 liter.	
c) 3.5 N H ₂ SO ₄		
• Slowly add 97 ml concentrated (36 N) H ₂ SO ₄ to about 800 ml de	eionized water (ca	areful - this
generates heat!), and when cool, adjusting with deionized water to		
d) Ceric ammonium sulphate solution		
 Dissolve 48 g ceric ammonium sulphate in 1 liter 3.5 N H₂SO₄. Sto 	re in a dark bottle	away from
light at room temperature. The solution is stable for months.		
e) Arsenious acid solution		
 In a 2000 ml Erlenmeyer flask, place 20 g As₂O₃ and 50 g NaCl, 	then clowly add	400 ml 5 N
	•	
H_2SO_4 . Add water to about 1 liter, heat gently to dissolve, cool to r	• ·	
water to 2 liters, filter, store in a dark bottle away from light at roc	m temperature. I	ne solution



6 Standards preparation

a) Stock standard solution (1 mg/ml)

• Dissolve 0.1685 g KIO₃ in deionized water to a final volume of 100 ml (1.68 g KIO₃ contains 1.0 g iodine). KIO₃ is preferred over KI because it is more stable. It may be more convenient to make a more concentrated solution, e.g., 10 or 100 mg iodine/ml.

b) Intermediate standard solution (1 µg/ml)

 Dilute 100 µl stock iodine standard (1mg/ml) to 100 ml deionized water. Store in a dark bottle. The solution is stable for months. Useful standards are 20, 50, 100, 150, 200, and 300 µg/l.

c) Serial standard dilutions

From intermediate standards, prepare 50, 100, 150, 200, and 250 μg/L useful standards for calibration curve purposes.

Note: All standard solutions should store in a dark place. The solutions are stable for months.

7 Procedures

- Mix urine to suspend sediment using a vortex mixer.
- Pipette 250 µl of each urine sample into a 13 x 100 mm test tube.
- Pipette 250 µl each iodine serial standards also into a test tube.
- Add 1 ml 1.0 M ammonium persulfate solution to each tube.
- Vortex all tubes using a vortex mixer
- Heat all tubes for 60 minutes at 100° C in the oven.
- Cool tubes to room temperature in a fume hood.
- Add 2.5 ml arsenious acid solution. Mix by a vortex. Let stand for 15 minutes.
- Add 300 µl of ceric ammonium sulfate solution to each tube (quickly mixing) at 15-30 second intervals between successive tubes. A stopwatch should be used for this. With practice, a 20second interval is convenient.

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• Allow sitting at room temperature. Exactly 30 minutes after the addition of ceric ammonium sulfate to the first tube, read its absorbance by using UV-Vis spectrophotometer at 420 nm. Read successive tubes at the same interval as when adding the ceric ammonium sulfate.

8 Calculation

- Construct a standard curve on graph paper by plotting log absorbance of each concentration versus iodine concentration of each standard.
- Urinary Iodine in µg/l = ((log A-b)/(m)) * 10
 Where log A is log absorbance of sample, b is Y-intercept of the calibration graph, m is the slope of the graph, and 10 is used as a conversion factor when we prepare serials of standards in µg/dl (in this case we multiply the final concentration (µg/ dl) by 10 to get the concentration in µg /l).

9 Quality control and safety precautions

- In each batch, the urine quality control sample (CRM or in-house prepared) should run together with sample and standards.
- While working the urine analysis and reagent preparation wear gloves, lab coat, eye goggle, lab shoe, and mouth cover.

Remember: During the analysis of iodine from urine, you should ensure that the laboratory working environment should free from salt samples (especially iodized salt samples) to avoid contamination. **Limitations:** If the urine sample is analyzed at a high temperature the loss of iodine occurred and the method has to detect very low iodine concentration in urine.

10 References

• ICCIDD, UNICEF, WHO. Dunn JT et al. Methods for measuring iodine in urine. The Netherlands, ICCIDD, 1993.

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19 20	Reviewed by: M	eseret W/yohannes	S			
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AAGP2
Tina-quant α1-Acid Glycoprote

cobas®

Analyzer(s) on which cobas c pack(s) can be used

Roche/Hitachi cobas c 311, cobas c 501/502

tein Gen.2 Order information REF CONTENT 03333795 190 Tina-quant α1-Acid Glycoprotein Gen.2 100 tests System-ID 07 6758 1 11355279 216 Calibrator f.a.s. Proteins (5 x 1 mL) Code 656 Calibrator f.a.s. Proteins (5 x 1 mL, for USA) 11355279 160 Code 656 10557897 122 Code 302 Precinorm Protein (3 x 1 mL)

1055/89/ 122	Precinom Protein (3 x 1 mL)	Code 302	
10557897 160	Precinorm Protein (3 x 1 mL, for USA)	Code 302	
11333127 122	Precipath Protein (3 x 1 mL)	Code 303	
11333127 160	Precipath Protein (3 x 1 mL, for USA)	Code 303	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

For cobas c 311/501 analyzers:

AAGP2: ACN 229

For **cobas c** 502 analyzer:

AAGP2: ACN 8229

Intended use

In vitro test for the quantitative determination of α_1 -acid glycoprotein in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary^{1,2,3,4,5}

 $\begin{array}{rcl} 33 & \alpha_1 - \text{Acid glycoprotein is synthesized in hepatocytes and consists of a} \\ 34 & \text{polypeptide chain having 5 carbohydrate chains N-glycosidically bonded to} \\ 35 & \text{it (molar mass 41000 daltons). Structurally, it belongs to the lipocalin} \\ 36 & \text{retinol-binding protein). } \alpha_1 - \text{Acid glycoprotein promotes fibroblast growth and} \\ 37 & \text{interacts with collagen.} \end{array}$

It is a sensitive acute phase reactant whose concentration can increase by 38 a factor of 3 within 24-48 hours when inflammation occurs. α_1 -Acid 39 glycoprotein can also be used to differentiate between acute phase 40 reactions (elevated serum level) and estrogen effects (normal or decreased serum level) whereas the serum level of other positive reactants such as 41 ceruloplasmin and haptoglobin increases during such reactions. Along with haptoglobin it is perhaps the best protein for identifying slight in vivo hemolysis. An increased α_1 -acid glycoprotein level and normal haptoglobin 42 43 values indicate an acute phase reaction with concomitant slight in vivo 44 hemolysis. Moderate and isolated increases occur when glomerular 45

- filtration is inhibited in the early stages of uremia. The determination is used
 in the assessment of the activity of acute and recurring inflammations as
 well as of tumors with cell necrosis.
- $\begin{array}{lll} \mbox{48} & \mbox{Various assay methods for α_1-acid glycoprotein determination are available} \\ \mbox{49} & \mbox{such as kinetic nephelometry, radial immunodiffusion (RID) and} \\ \mbox{turbidimetry. The Roche α_1-acid glycoprotein assay is based on the} \\ \mbox{50} & \mbox{principle of immunological agglutination.} \end{array}$

51 **Test principle**² 52 Immunoturbidim

Immunoturbidimetric assay.

Anti-α₁-acid glycoprotein antibodies react with antigen in the sample to form
 an antigen/antibody complex. Following agglutination, this is measured
 turbidimetrically.

56 Reagents - working solutions

- 57 R1 TRIS buffer: 50 mmol/L, pH 8.0; NaCI: 300 mmol/L; PEG: 7 %;
 58 preservative; stabilizer
- 59 R2 Polyclonal anti-human α₁-acid glycoprotein antibody (goat):
 60 dependent on titer; TRIS buffer: 13 mmol/L, pH 7.5; NaCl: 198 mmol/L; preservative

R1 is in position B and R2 is in position C.

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

Reagent handling

Ready for use

Storage and stability

AAGP2

Shelf life at 2-8 °C:

See expiration date on **cobas c** pack label.

12 weeks

On-board in use and refrigerated on the analyzer: *Diluent NaCl 9 %* Shelf life at 2-8 °C:

See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the 12 weeks analyzer:

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable. Serum

Plasma: Li-heparin and K₂-EDTA plasma.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:⁶ < 72 hours at 4 °C

6 months at -20 °C

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cobas®

0003333795190c501V10.0 AAGP2

Tina-quant α1-Acid Glycoprotein Gen.2

Tina-quant α1-Acid Glycop	orotein Gen.2								
Materials provided						Sample	Diluent (NaCl)		
See "Reagents – working solutions" section for reagents.			Normal	12 µL	9 µL	180 μL			
Materials required (but not provided)				Decreased	12 µL	4 μL	122 µL		
 See "Order information" section 				Increased	12 µL	18 µL	180 µL		
 General laboratory equips 	ment			Calibration					
Assay				Calibrators S1: H ₂ O					
For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.				Calibratoro	S2-S6: C.f.a.s. Proteins				
The performance of applications not validated by Roche is not warranted and must be defined by the user.					Multiply the lot-specific C.f.a.s. Proteins calibrator value by the factors below to determine the standard concentrations for the 6-point calibration curve:				
Application for serum and plasma									
cobas c 311 test definition					S2: 0.140	S5: 1	.40		
Assay type	2-Point End				S3: 0.280	S6: 2			
Reaction time/Assay points	10/6-32				S4: 0.700				
Wavelength (sub/main)	660/340 nm			Calibration mode	RCM2				
Reaction direction	Increase			Calibration frequency	Full calibration				
Units	g/L (µmol/L, n	ng/dL)			- after reagent lot cl	nange			
Reagent pipetting		Diluent (H ₂ O)			- as required followi	ng quality co	ontrol		
R1	120 µL	-			procedures				
R2	40 µL	-		Calibration interval may	be extended based or	acceptable	verification of		
Sample volumes	Sample	Sampl	e dilution	calibration by the labora	itory.				
		Sample	Diluent (NaCl)	Traceability: This methor preparation of the IRMN	d has been standardiz	ed against the	ne reference		
Normal	12 µL	9 µL	180 µL	Measurements) BCR47	0/CRM470 (RPPHS - I	Reference Pi	reparation for		
Decreased	12 µL	4 µL	122 µL	Proteins in Human Seru	ım). ⁷				
Increased	12 µL	9 µL	180 µL	Quality control	oontuol motoriala oo lia		udou information"		
cobas c 501 test definition				For quality control, use a section.	control materials as its	led in the O	ruer information		
Assay type	2-Point End			In addition, other suitabl					
Reaction time/Assay points	10/10-48			The control intervals and individual requirements.	d limits should be adap	ted to each	laboratory's		
Wavelength (sub/main)	660/340 nm			limits. Each laboratory s	should establish correc	tive measure	es to be taken if		
Reaction direction	Increase			values fall outside the d			delines for		
Units	g/L (µmol/L, n	ng/dL)		Follow the applicable government regulations and local guidelines for quality control.			Idelines for		
Reagent pipetting		Diluent (H ₂ O)		Calculation					
R1	120 µL	-		Roche/Hitachi cobas c systems automatically calculate the analyte					
R2	40 µL	-		concentration of each sa	ample.				
Sample volumes	Sample	Sampl	e dilution	Conversion factors:	g/L x 25 = μ mol/L	mg/dL x	0.01 = g/L		
		Sample	Diluent (NaCl)		$mg/dL \ge 0.25 = \mu mol/$	L g/Lx10	0 = mg/dL		
Normal	12 µL	9 μL	180 µL	Limitations - interferer	nce				
Decreased	12 µL	4 µL	122 µL	Criterion: Recovery with concentration of 0.5 g/L	10% of initial value (12.5 upped/1.50 mg/d	ie at an α₁-a	cid glycoprotein		
Increased	12 µL	9 µL	180 µL	Icterus: ⁸ No significant i			or conjugated		
cobas c 502 test definition				and unconjugated bilirul bilirubin concentration:	bin (approximate conju	gated and u	nconjugated		
Assay type	2-Point End			Hemolysis: ⁸ No significa	ant interference up to a	n H index of	1000		
Reaction time/Assay points	10/10-48			(approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).					
Wavelength (sub/main)	660/340 nm			Lipemia (Intralipid): ⁸ No significant interference up to an L index of 650. There is poor correlation between the L index (corresponds to turbidity) and					
Reaction direction	Increase			triglycerides concentrati	on.		, , ,		
Units	g/L (µmol/L, n	ng/dL)		Rheumatoid factors up t					
Reagent pipetting		Diluent (H ₂ O)		High dose hook-effect: I concentration of 11 g/L	(275 µmol/L. 1100 mol/	up to an α ₁ -a 'dL).	icia giycoprotein		
R1	120 µL	-		Drugs: No interference	was found at therapeut	-	tions using		
R2	40 µL	-		common drug panels. ^{9, 1}			- I - I		
Sample volumes	Sample	Sampl	e dilution	In very rare cases, gam macroglobulinemia), ma	mopatny, in particular ay cause unreliable res	type igM (Wa ults. ¹¹	aidenstrom's		

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0003333795190c501V10.0 **AAGP2** Tina-quant α1-Acid Glycoprotein Gen.2

cobas[®]

4							
5	For diagnostic purposes, t				y =	1.012x - 0.07	
6	conjunction with the patient's medical history, clinical examination and other findings.				т = 0.973		
7 0					The	sample conc	
8	Special Wash Programm				81.3	3 µmol/L, 48.9	
9 10	when certain test combina cobas c systems. The late		References				
10	found with the NaOHD-SM	IS-SmpCln1+2-SC	CS Method Sheets	s. For further	1	Schmid K. α1	
12	instructions refer to the op	erator's manual. co	obas c 502 analyz	er: All	0	Putnam FW,	
13	special wash programming via the cobas link, manua	I input is not require	ed.		2	Greiling H, G Pathobiocher 1995:236.	
14	Where required, special be implemented prior to	3	Tietz NW, ed				
15 I 16	Limits and ranges	reporting recute			0	PA: WB Saur	
17	Measuring range				4	Ganrot K. Pla	
18	0.1-4.0 g/L (2.5-100 µmol/	L, 10-400 mg/dL)			_	Clin Lab Inve	
19	Determine samples having				5	Lievens M, B Tina-guant as	
20	Dilution of samples via the samples diluted using the					α1-antitrypsir	
21	factor of 1.5.		automatically main	plied by a	6	Wu AHB, ed.	
22	Lower limits of measure	ment				(MO): Saund	
23	Lower detection limit of the	e test			7	Baudner S, E matrix refere	
24	0.1 g/L (2.5 µmol/L, 10 mg			14 human se			
25	The lower detection limit re that can be distinguished f					1993;1-186.	
26 27	3 standard deviations above repeatability, $n = 21$).	ve that of the lowes	st standard (standa	ard $1 + 3$ SD,	8	Glick MR, Ry Interferences	
²⁸	Expected values ¹²				0	1986;32:470-	
29	0.5-1.2 g/L (12.5-30 µmol/	L, 50-120 mg/dL)			9	Breuer J. Rep Methods". Eu	
30 31	Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference					Sonntag O, S	
32	ranges.					recommenda interference	
33	Specific performance da	ta			11	—	
34 35	Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.					assays: mech Clin Chem La	
36	Precision				12	Dati F, Schur	
37 38	Precision was determined using human samples and controls in an internal protocol with repeatability ($n = 21$) and intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:					professional interim refere standardizati	
39			-			470). Eur J C	
40	Repeatability	Mean	SD	CV	13	Bablok W, Pa	
41 42		g/L (µmol/L, mg/dL)	g/L (µmol/L, mg/dL)	%		for method tra for method co Chem Clin Bi	
43	Precinorm Protein	0.724 (18.1, 72.4)	0.00 (0.0, 0.0)	0.6	Δn	oint (period/ste	
44	Precipath Protein	1.21 (30.3, 121)	0.01 (0.3, 1)	0.5	sep	arator to mark	
45	Human serum 1	0.642 (16.1, 64.2)	0.00 (0.0, 0.0)	0.7		ecimal numera	
46	Human serum 2	1.07 (26.8, 107)	0.01 (0.3, 1)	0.7	-	nbols	
47						che Diagnostic se listed in the	
48	Intermediate precision	Mean	SD	CV		s://usdiagnost	
49 50		g/L (µmol/L, mg/dL)	g/L (µmol/L, mg/dL)	%	CO	NTENT	
51	Precinorm Protein	0.710 (17.8, 71.0)	0.007 (0.2, 1.0)	0.9		\rightarrow	
52	Precipath Protein	1.19 (30.0, 119)	0.01 (0.3, 1)	0.9	GTI	N	
53	Human serum 3	0.660 (16.5, 66.0)		1.5			
54	Human sorum /	1 21 (30 3 121)		1.5			

1.21 (30.3, 121) 0.02 (0.5, 2)

Linear regression

112x - 0.070 g/L y = 0.998x - 0.056 g/L 73 r = 0.999

The sample concentrations were between 0.489 and 3.25 g/L (12.2 and 81.3 $\mu mol/L,$ 48.9 and 325 mg/dL).

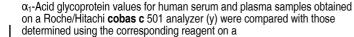
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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

Contents of kit Volume after reconstitution or mixing

Global Trade Item Number



Roche/Hitachi 917 analyzer (x).

Passing/Bablok¹³

Human serum 4

Method comparison

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3/4 For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

2018-08, V 10.0 English

1.5



Tina-quant α1-Acid Glycoprotein Gen.2

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Vitamin D total 25-Hydroxyvitamin D

3 4	25-Hydroxyvitamin D						
5	REF	Σ			SYSTEM		
6					Elecsys 2010		
7					MODULAR ANALYTICS E170		
8	05004012 100	100			cobas e 411		
9	05894913 190	100					
10					cobas e 601		
11					cobas e 602		
12	English		- 0	linguhation	After addition of strents vidin seated micronarticles and		
13 14	English		- Sro vita	amin D (25-O	After addition of streptavidin-coated microparticles and H) labeled with biotin, unbound ruthenium labeled		
14	Intended use	for the quantitative determination of total	vit	amin D ḃindir	ng proteins become occupied. A complex consisting of		
16	25-hydroxyvitamin D in	for the quantitative determination of total human serum and plasma. This assay is to be used ment of vitamin D sufficiency.	the ruthenylated vitamin D binding protein and the biotinylated vitamin D (25-OH) is formed and becomes bound to the solid phase interaction of biotin and streptavidin.				
17		escence binding assay is intended for use on			xture is aspirated into the measuring cell where the		
18 19	Elecsys and cobas e in		mi	croparticles a	are magnetically captured onto the surface of the		
20	Summary	🖸			bund substances are then removed with M. Application of a voltage to the electrode then induces		
21		le steroid hormone precursor that is mainly exposure to sunlight. Vitamin D is biologically inert	ch	emiluminesce	ent emission which is measured by a photomultiplier.		
22	and must undergo two	successive hydroxylations in the liver and kidney to			ermined via a calibration curve which is instrument-		
23	become the biologically	active 1,25-dihydroxyvitamin D.1			erated by 2-point calibration and a master curve provided		
24		t forms of vitamin D are vitamin D_3 (cholecalciferol) ciferol). In contrast to vitamin D_3 , the human body		the reagent			
25		D_2 which is taken up with fortified food or given by	Reagents - working solutions The reagent rackpack (M, R1, R2) and the pretreatment reagents (PT1,				
26	supplements. In human	plasma vitamin D_3 and D_2 are bound to the	PT2) are labeled as VITD-T.				
27		n and transported to the liver where both are amin D (25-OH), i.e. 25-hydroxyvitamin D. It is	PT1 Pretreatment reagent 1 (white cap), 1 bottle, 4 mL:				
28	commonly agreed that	vitamin D (25-OH) is the metabolite to determine the					
29	overall vitamin D status	as it is the major storage form of vitamin D in the ary circulating form of vitamin D is biologically	Dithiothreitol 1 g/L, pH 5.5.				
30	inactive with levels app	roximately 1000-fold greater than the circulating	PT2 Pretreatment reagent 2 (gray cap), 1 bottle, 4 mL:				
31	1,25-dihydroxyvitamin D. The half-life of circulating vitamin D (25-OH) is			Sodium hydro	oxide 55 g/L.		
32	2-3 weeks. Most of the vitamin D (25-OH), measurable in serum, is vitamin D_3 (25-OH) whereas vitamin D_2 (25-OH) reaches measurable levels only in patients			Streptavidin-c	oated microparticles (transparent cap), 1 bottle, 6.5 mL:		
33				Streptavidin-c	oated microparticles 0.72 mg/mL; preservative.		
34 25	taking vitamin D ₂ supple	ements. ^{2,3,4} Vitamin D_2 is considered to be less	R1 Vitamin D binding protein-BPRu (gray cap), 1 bottle, 9 mL:				
35 36	effective. ⁵	a barra ba the la shildren an an da Cairan da sha	Ruthenium labeled vitamin D binding protein 150 µg/L; bis-tris				
37	to bone-malformation, k	or bone health. In children, severe deficiency leads known as rickets. Milder degrees of insufficiency are ced efficiency in the utilization of dietary calcium. ⁶	propane buffer 200 mmol/L; albumin (human) 25 g/L; pH 7.5; preservative.				
38 39	Vitamin D deficiency ca	uses muscle weakness; in elderly, the risk of falling			amin D~biotin (black cap), 1 bottle, 8.5 mL:		
40	deficiency is a common	he effect of vitamin D on muscle function. ⁷ Vitamin D cause of secondary hyperparathyroidism. ^{8,9}					
41	Elevations of PTH level	s, especially in elderly vitamin D deficient adults can		-	itamin D (25-OH) 14 µg/L; bis-tris propane buffer bH 8.6; preservative.		
42	result in osteomalacia, i risk of bone fractures 10	increased bone turnover, reduced bone mass and Low vitamin D (25-OH) concentrations are also					
43	associated with lower b	one mineral density. ¹¹ In conjunction with other		utions and w			
44	clinical data, the results metabolism.	may be used as an aid in the assessment of bone	Exerci	vitro diagnos	al precautions required for handling all laboratory		
45		een shown to affect expression of over 200 different	reage	nts.			
46	genes. Insufficiency has	s been linked to diabetes, different forms of cancer,			te material should be in accordance with local guidelines. available for professional user on request.		
47		, autoimmune diseases and innate immunity. ²			omponents classified as follows in accordance with the		
48		total assay employs a vitamin D binding protein ein to bind vitamin D_3 (25-OH) and	Regul	ation (EC) No	b. 1272/2008:		
49	vitamin D_2 (25-OH).						
50	Test principle		, F	T.			
51		otal duration of assay: 27 minutes.		-			
52		cubating the sample (15 μ L) with pretreatment					
53	reagent 1 and 2, bo	und vitamin D (25-OH) is released from the	Dange	er			
54 55	 vitamin D binding pr 2nd incubation: By in 	otein. ncubating the pretreated sample with the ruthenium	H290	May	/ be corrosive to metals.		
56	labeled vitamin D bi	nding protein, a complex between the	H314	Cau	ises severe skin burns and eye damage.		
57	vitamin D (25-OH) a formed.	nd the ruthenylated vitamin D binding protein is	Preve	ntion:	- -		
58							

- P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.
- Response:

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Vitamin D total

3 4	25-Hydroxyvitamin D							
5 6	P303 + P361 + P353		Remove/Take off immediately all Rinse skin with water/shower.	E E n				
7 8 9 10	P304 + P340 + P310	comfortable for breath	F INHALED: Remove person to fresh air and keep comfortable for breathing. mmediately call a POISON CENTER or doctor/physician.					
11 12 13 14	P305 + P351 + P338 + P310	minutes. Remove cont	F IN EYES: Rinse cautiously with water for several ninutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician.					
15 16		/ labeling primarily follov e: all countries: +49-621	•					
17 18 19 20 21	derived from h donors tested to HCV and H cleared in con List A.	numan blood are prepar individually and shown IV. The testing methods npliance with the Europe	red potentially infectious. All products ed exclusively from the blood of to be free from HBsAg and antibodies applied were FDA-approved or san Directive 98/79/EC, Annex II,	4				
22 23 24	However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed. ^{12,13}							
25 26	calibrators and		and sample types (specimens,					
27 28	Reagent hand The reagents cannot be sep	in the kit have been ass	embled into a ready-for-use unit that	• A				
29 30	All information reagent barco		eration is read in from the respective	C -				
31 32 33 34	Store at 2-8 °C Do not freeze.	Storage and stability Store at 2-8 °C. Do not freeze.						
35 36			in order to ensure complete g automatic mixing prior to use.	•				
37	Stability:							
38	unopened at	2-8 °C	up to the stated expiration date					
39	after opening	at 2-8 °C	56 days (8 weeks)					
40	on Elecsys 20	010 and cobas e 411	21 days (3 weeks)					
41 42	on MODULA	R ANALYTICS E170, and cobas e 602	28 days (4 weeks)	- A				
43 44 45 46	Specimen co Only the spec	llection and preparation imens listed below were ed using standard samp	on tested and found acceptable. ling tubes or tubes containing	∎ F d				
47 48	Li-heparin, K ₂ - and K ₃ -EDTA plasma as well as Li-heparin plasma tubes containing separating gel.							
49 50		Criterion: Method comparison serum versus plasma, slope 0.9-1.1 + intercept within $< \pm 2 \times \text{LoB} + \text{coefficient of correlation} > 0.9.$						
51	for 8 hours at	18-25 °C, 4 days at 2-8	olasma: Vitamin D (25-OH) is stable °C, 24 weeks at -20 °C.	a N F				
52 53 54	assay is in line and mass spe	The stability of vitamin D (25-OH) found with the Elecsys Vitamin D total assay is in line with earlier studies using a vitamin D binding protein assay and mass spectrometry. ¹⁴						
55 56	The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all							

- tubes that were commercially available at the time of testing, i.e. not all 56 available tubes of all manufacturers were tested. Sample collection systems 57 from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary 58 tubes (sample collection systems), follow the instructions of the tube 59 manufacturer 60
 - Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

- REF 05894921190, Vitamin D total CalSet, for 4 x 1 mL
- REF 05618860190, PreciControl Varia, for 2 x 3 mL each of PreciControl Varia 1 and 2
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or cobas e analyzer Accessories for Elecsys 2010 and cobas e 411 analyzers:
- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels

[REF] 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips Accessories for MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M
- Accessories for all analyzers:
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assav

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized against LC-MS/MS¹⁵ which in turn has been standardized to the NIST standard.¹⁶

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

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Vitamin D total

25-Hydroxyvitamin D

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

13 For quality control, use PreciControl Varia.

- 14 In addition, other suitable control material can be used.
- 15 Controls for the various concentration ranges should be run individually at 16 least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.
- The control intervals and limits should be adapted to each laboratory's
 individual requirements. Values obtained should fall within the defined
 limits. Each laboratory should establish corrective measures to be taken if
- limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.
- Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in ng/mL or nmol/L).

ng/mL x 2.50 = nmol/L

 $nmol/L \ge 0.40 = ng/mL$

Limitations - interference

Samples showing visible signs of hemolysis may cause interference.
 Hemoglobin concentrations > 2 g/L (> 0.124 mmol/L) may lead to elevated results.

The assay is unaffected by icterus (bilirubin < 1129 µmol/L or < 66 mg/dL),
 lipemia (Intralipid < 400 mg/dL) and biotin (< 287 nmol/L or < 70 ng/mL).

Criterion: For concentrations from LoQ up to 15 ng/mL, deviation is

 ≤ 1.5 ng/mL; for concentrations > 15 ng/mL, deviation is ≤ 10 %.

Samples should not be taken from patients receiving therapy with high
 biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin
 administration.

- In vitro tests were performed on 17 commonly used pharmaceuticals and 5
 special therapeutic drugs (Bonviva (Ibandronate), EinsAlpha (Alfacalcidol),
 Ecompary (Algorithmate) Pamidron HEYAL (Pamidronate) and Zompta
- Fosamax (Alendronate), Pamidron HEXAL (Pamidronate) and Zometa
 (Zoledronate)). No interference with the assay was found.
- In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These
- 43 effects are minimized by suitable test design.
- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings
- 45 findings. 46 Limits a

Limits and ranges

47 Measuring range

3.00-70.0 ng/mL or 7.50-175 nmol/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 3.00 ng/mL (< 7.50 nmol/L). Values above the measuring range are reported as > 70.0 ng/mL (> 175 nmol/L).

51 Lower limits of measurement

- 52 Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation 53 (LoQ)
- 54 Limit of Blank = 2.00 ng/mL (5.00 nmol/L)
- 55 Limit of Detection = 3.00 ng/mL (7.50 nmol/L)
- 56 Limit of Quantitation = 5.00 ng/mL (12.5 nmol/L) with a total allowable 57 relative error of \leq 30 %
- The Limit of Blank, Limit of Detection and Limit of Quantitation were
- 58 The Limit of Blank, Limit of Detection and Limit of Quantitation were
 determined in accordance with the CLSI (Clinical and Laboratory Standards
 59 Institute) EP17-A requirements.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative error of \leq 30 %.

The total error concept describes the maximum possible error of a test result taking into account the imprecision (SD) and inaccuracy (bias) of the test system. The Total Error (TE) was determined using the RMS (Root Mean Square) model (CLSI EP17-A2). The relative allowable total error refers to the respective concentration of the sample.

Dilution

Samples with vitamin D (25-OH) concentrations above the measuring range can be manually diluted with Diluent Universal or a suitable human serum with a low analyte concentration. The recommended dilution is 1:2. The concentration of the diluted sample must be > 30.0 ng/mL (> 75.0 nmol/L). After manual dilution, multiply the results by the dilution factor 2. The endogenous analyte concentration of the human serum used for dilution has to be taken into account.

Expected values

Due to different standardizations between methods, result variation may arise. Clinical assessment should be taken into consideration when interpreting results.

Health based reference values (recommended for use):

Currently there is no standard definition of the optimal vitamin D status. Many specialists consider the commonly used population based reference values too low. Health based reference values are recommended to replace population based reference values.¹⁷

Most experts agree that vitamin D deficiency should be defined as vitamin D (25-OH) of \leq 20 ng/mL (\leq 50 nmol/L).¹⁸ Vitamin D insufficiency is recognized as 21-29 ng/mL.¹⁸ Similarly, the US National Kidney Foundation considers levels < 30 ng/mL to be insufficient or deficient.¹⁹

The preferred level for vitamin D (25-OH) by many experts is now recommended to be ≥ 30 ng/mL (≥ 75 nmol/L).^{18,20,21,22}

Reference values measured in an apparently healthy population:

It should be taken into consideration that differences in vitamin D (25-OH) levels may exist with respect to gender, age, season, geographical latitude and ethnic groups. 18,20

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Population based reference ranges should not be taken as clinical cutoff to recommend or dissuade from vitamin D supplementation. Guidance for supplementation should be taken from recent literature.^{18,19}

A reference range study was conducted with samples from apparently healthy individuals of Caucasian heritage. The age range was 20-77 years. Samples were collected between November and July in northern Germany. The values given are for information only and may vary from other published data.

	Gender					
	All (n = 453)		Female (n = 252)		Male (n = 201)	
Unit	ng/mL	nmol/L	ng/mL	nmol/L	ng/mL	nmol/L
Mean	20.6	51.5	21.6	54.0	19.4	48.5
2.5 th percentile	5.26	13.2	6.23	15.6	4.92	12.3
97.5 th percentile	47.0	118	49.9	125	42.7	107

A lower recovery may be found in particular clinical cohorts, for example dialysis patients. $^{\rm 23}$

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Vitamin D total

25-Hydroxyvitamin D

Precision

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Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

		Repeatabili			
Sample	Me	ean	S	CV	
	ng/mL	nmol/L	ng/mL	nmol/L	%
HS ^{a)} 1	6.76	16.9	0.525	1.31	7.8
HS 2	15.0	37.5	0.770	1.93	5.1
HS 3	28.0	70.0	0.860	2.15	3.1
HS 4	67.0	168	1.15	2.88	1.7
PC ^{b)} Varia 1	19.9	49.8	0.948	2.37	4.8
PC Varia 2	38.3	95.8	1.05	2.63	2.7

b) PC = PreciControl

Elecsys 2010 and cobas e 411 analyzers						
		Intermediat			e precision	
Sample	Me	Mean		SD		
	ng/mL	nmol/L	ng/mL	nmol/L	%	
HS 1	6.76	16.9	0.724	1.81	10.7	
HS 2	15.0	37.5	1.28	3.20	8.5	
HS 3	28.0	70.0	1.46	3.65	5.2	
HS 4	67.0	168	1.46	3.65	2.2	
PC Varia 1	19.9	49.8	1.23	3.08	6.2	
PC Varia 2	38.3	95.8	1.41	3.53	3.7	

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers

			Re	epeatability	/
Sample	Me	an	S	D	CV
	ng/mL	nmol/L	ng/mL	nmol/L	%
HS 1	8.35	20.9	0.567	1.42	6.8
HS 2	15.8	39.5	0.824	2.06	5.2
HS 3	28.3	70.8	1.11	2.78	3.9
HS 4	69.6	174	1.50	3.75	2.2
PC Varia 1	20.2	50.5	0.924	2.31	4.6
PC Varia 2	39.6	99.0	1.06	2.65	2.7

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers

			Interm	ediate prec	ision
Sample	Me	Mean		SD	
	ng/mL	nmol/L	ng/mL	nmol/L	%
HS 1	8.35	20.9	1.10	2.75	13.1
HS 2	15.8	39.5	1.18	2.95	7.5
HS 3	28.3	70.8	1.83	4.58	6.5
HS 4	69.6	174	2.37	5.93	3.4
PC Varia 1	20.2	50.5	0.954	2.39	4.7
PC Varia 2	39.6	99.0	1.38	3.45	3.5

Method comparison

1) A comparison of the Elecsys Vitamin D total assay (y) using samples measured with LC-MS/MS (x) gave the following correlations (ng/mL):

Number of samples measured: 903

Passing/Bablok ²⁴	y = 1.09x - 0.510
Pearson	r = 0.894

The sample concentrations were between approximately 3 ng/mL (7.5 nmol/L) and 81 ng/mL (203 nmol/L).

2) A comparison of the Elecsys Vitamin D total assay (y) using samples measured with a commercially available vitamin D (25-OH) immunoassay (x) gave the following correlations (ng/mL):

Number of samples measured: 451

Passing/Bablok ²⁴	y = 1.29x + 1.71
Pearson	r = 0.803

The sample concentrations were between approximately 5 ng/mL (12.5 nmol/L) and 81 ng/mL (203 nmol/L).

Analytical specificity

The specificity was assessed at 50 % B_0 and the results are summarized in the following table:

Cross-reactant	Cross-reactivity (%)
25-hydroxyvitamin D_3	100
25-hydroxyvitamin D ₂	92
24,25-dihydroxyvitamin D ₃	149
C3-epimer of 25-hydroxyvitamin D ₃	91
1,25-dihydroxyvitamin D ₃	non detectable
1,25-dihydroxyvitamin D ₂	non detectable
Vitamin D ₃	non detectable
Vitamin D ₂	non detectable

Functional sensitivity

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %. 8 samples with concentrations between 0.722 ng/mL and 10.1 ng/mL were measured on several days. The functional sensitivity was determined to be 4.01 ng/mL (CV 18.5 %).

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Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim www.roche.com

Roche

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
\rightarrow	Volume after reconstitution or mixing
GTIN	Global Trade Item Number
COBAS, COBAS E, ELEC	SYS and PRECICONTROL are trademarks of Roche. INTRALIPID is a trademark of

Fresenius Kabi AB.

All other product names and trademarks are the property of their respective owners.

Additions, deletions or changes are indicated by a change bar in the margin © 2015, Roche Diagnostics

Vitamin **R12** ||

Vitamin B12		
REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
07212771 190	100	cobas e 411
••••••		cobas e 601
		cobas e 602
	I	I
English		Test principle
Intended use		Competition principle. Total duration of assay: 27 minutes.
human serum and	•	 1st incubation: By incubating the sample (15 µL) with the vitamin B12 pretreatment 1 and pretreatment 2, bound vitamin B12 is released.
on Elecsys and c Summary	iluminescence immunoassay "ECLIA" is intended for use obas e immunoassay analyzers. o referred to as cobalamin, is a complex organometallic	 2nd incubation: By incubating the pretreated sample with the ruthenium labeled intrinsic factor, a vitamin B12-binding protein complex is formed, the amount of which is dependent upon the analyte concentration in the sample.
compound in white water-soluble vita synthesized in the origin. Main source	ch a cobalt atom is situated within a corrin ring. It is a unin which is synthesized by microorganisms. It cannot be e human body and is seldom found in products of plant ces of vitamin B12 are meat, fish, eggs and dairy products.	 3rd incubation: After addition of streptavidin-coated microparticles and vitamin B12 labeled with biotin, the still-vacant sites of the ruthenium labeled intrinsic factor become occupied, with formation of a ruthenium labeled intrinsic factor vitamin B12 biotin complex. The entire complex because he used to the solid phene via intervation of biotin and
	ne gastrointestinal tract depends on intrinsic factor, which the gastric parietal cells, and on the "cubam receptor" in	becomes bound to the solid phase via interaction of biotin and streptavidin.
the distal ileum. T	he most frequent cause of severe vitamin B12 deficiency	 The reaction mixture is aspirated into the measuring cell where the
	ic factor due to autoimmune atrophic gastritis. The disease ed "pernicious anemia", even though many patients	microparticles are magnetically captured onto the surface of the
present with main	ly neurologic manifestations. Examples of other causes	electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces
inflammatory bow	eficiency are malabsorption due to gastrectomy, el disease or dietary deficiency, e.g. in strict vegetarians	chemiluminescent emission which is measured by a photomultiplier.
(vegans). ²		 Results are determined via a calibration curve which is instrument- specifically generated by 2-point calibration and a master curve provided
methylmalonyl Co	e cofactor for two enzymes, methionine synthase and of mutase. ^{2,3} Methionine synthase, located in the	via the reagent barcode.
cytoplasm, requir	es vitamin B12 in the form of methylcobalamin and	Reagents - working solutions
catalyzes the con	version of homocysteine to methionine, an essential g this step a methyl group is transferred from	The reagent rackpack (M, R1, R2) and the pretreatment reagents (PT1, PT2) are labeled as B12 II.
methyltetrahydrof	iolate to the amino acid. ³ This enzyme links the	
methylation pathw	vay through synthesis of the methyl donor S-Adenosyl ne pathway in which purine and pyrimidine are synthesized	PT1 Pretreatment reagent 1 (white cap), 1 bottle, 4 mL:
via generation of	tetrahydrofolate.3 In the form of	Dithiothreitol 1.028 g/L; stabilizer, pH 5.5.
5'-deoxyadenosyl	Icobalamin, vitamin B12 is also required for the zyme methylmalonyl CoA mutase, which converts	PT2 Pretreatment reagent 2 (gray cap), 1 bottle, 4 mL:
methylmalonyl Co	oA to succinyl CoA. This is a step in the oxidation of odd-	Sodium hydroxide 40 g/L; sodium cyanide 2.205 g/L.
	and catabolism of ketogenic amino acids. ³ Thus, portant for DNA synthesis, regenerating methionine for	M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
protein synthesis	and methylation, as well as for the development and initial	Streptavidin-coated microparticles 0.72 mg/mL; preservative.
myelination of the of normal CNS fu	e central nervous system (CNS) and for the maintenance	R1 Intrinsic factor \sim Ru(bpy) ²⁺ ₃ (gray cap), 1 bottle, 10 mL:
	iencies are common in wealthier countries principally	Ruthenium labeled recombinant porcine intrinsic factor 4 µg/L;
among the elderly	y and are most prevalent in poorer populations. In general	cobinamide dicyanide 15 μ g/L; stabilizer; human serum albumin;
the prevalence in	creases with age. ^{4,5}	phosphate buffer, pH 5.5; preservative.
Vitamin B12 defic	iency impacts red blood cell synthesis, resulting in emia due to abnormal DNA synthesis. ³ In addition it	R2 Vitamin B12~biotin (black cap), 1 bottle, 8.5 mL:
impairs neurologi	cal function, in particular demyelination of nerves in part	Biotinylated vitamin B12 25 µg/L; biotin 3 µg/L; phosphate buffer,
	methylation, leading to peripheral neuropathy, dementia, rformance, and depression. ³ Other effects of vitamin B12	pH 7.0; preservative.
deficiency or dep	letion are increased risk of neural tube defects,	Precautions and warnings
osteoporosis, cer	ebrovascular and cardiovascular diseases. ³ Early	For in vitro diagnostic use.
risk of permanent	ntial, because of the latent nature of this disorder and the neurological damage. ^{3,5}	Exercise the normal precautions required for handling all laboratory
Generally, the pri	mary test performed to confirm the diagnosis of	reagents. Disposal of all waste material should be in accordance with local guidelines.
vitamin B12 defic	iency is measurement of serum vitamin B12 level. ² Recent	Safety data sheet available for professional user on request.
	est that in addition the following biomarkers should be rove the specificity of diagnosis: folate, methylmalonic acid	This kit contains components classified as follows in accordance with the
(MMA), homocys	teine and holotranscobalamin. ^{2,5,6,7}	Regulation (EC) No. 1272/2008:
The Elecsys Vitar	min B12 II assay employs a competitive test principle using	
	ecific for vitamin B12. Vitamin B12 in the sample competes tamin B12 labeled with biotin for the binding sites on the	

ruthenium-labeled intrinsic factor complex^a).

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)₃²⁺)

Danger

ms_07212771190V1.0 Vitamin R12 II



2	VILA						
3	Vitamin B12						
4 5	H290	May be corrosive to metals.					
6 7	H314	Causes severe skin burns and eye damage.					
8	H412	Harmful to aquatic life with long lasting effects.					
9 10	Prevention:						
11	P234	Keep only in original container.					
12 13	P264	Wash skin thoroughly after handling.					
14	P273	Avoid release to the environment.					
15 16 17	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.					
17	Response:						
19 20	P301 + P330 + P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.					
21 22 23	P303 + P361 + P353	IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.					
24 25	P304 + P340	IF INHALED: Remove person to fresh air and keep comfortable for breathing.					
26 27 28 29	P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.					
30	P310	Immediately call a POISON CENTER or doctor/physician.					
31 32	P363	Wash contaminated clothing before reuse.					
33	P390	Absorb spillage to prevent material damage.					
34 35	Storage:						
36	P405	Store locked up.					
37 38	P406	Store in corrosive resistant stainless steel container with a resistant inner liner.					
39	Disposal:						
40 41	P501	Dispose of contents/container to an approved waste					
42	Dueduet estet	disposal plant.					
43 44		/ labeling primarily follows EU GHS guidance. e: all countries: +49-621-7590					
45	All human ma	terial should be considered potentially infectious. All products					
46	derived from h	numan blood are prepared exclusively from the blood of individually and shown to be free from HBsAg and antibodies					
47	to HCV and H	IV. The testing methods applied were FDA-approved or					
48	cleared in con List A.	npliance with the European Directive 98/79/EC, Annex II,					
49 50	However, as r	no testing method can rule out the potential risk of infection					
50 51	with absolute certainty, the material should be handled with the same level						
52	of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed. ^{8,9}						
53	Avoid foam formation in all reagents and sample types (specimens,						
54	calibrators and	d controls).					
55	Reagent han	•					
56		The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.					
57		n required for correct operation is read in from the respective					
58	reagent barco						
59 60	Storage and Store at 2-8 °C	•					

Do not freeze.

Store the Elecsys reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	84 days (12 weeks)
on the analyzers	35 days (5 weeks) onboard or 60 days when stored alternatively in the refrigerator and on the analyzer, with the total time onboard on the analyzer not exceeding 10 x 8 hours

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable. Serum collected using standard sampling tubes or tubes containing separating gel.

Na-heparin, Li-heparin, K₂-EDTA and K₃-EDTA plasma. Li-heparin plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + intercept within < ± 2x Limit of Blank (LoB) + coefficient of correlation ≥ 0.95 .

Stable for 2 hours at 15-25 °C, 48 hours at 2-8 °C, 56 days at (-15)-(-25) °C. Freeze once only.

Stability of serum obtained with separating tubes: 24 hours at 2-8 °C (note the data provided by the tube manufacturer).

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Avoid hemolysis.

Do not use samples and controls stabilized with azide.

Vitamin B12 determinations should be performed on serum or plasma samples from fasting patients.

Note: Samples with extremely high total protein concentrations (e.g. patients suffering from Waldenström's macroglobulinemia) are not suitable for use in this assay, since they may lead to the formation of protein gel in the assay cup. Processing protein gel may cause a run abort. The critical protein concentration is dependent upon the individual sample composition. The formation of protein gel was seen in samples (spiked with human IgG or human serum albumin) having a total protein concentration > 160 g/L.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 07212780190, Vitamin B12 II CalSet, for 4 x 1 mL
- REF 05618860190, PreciControl Varia, for 2 x 3 mL each of PreciControl Varia 1 and 2
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or cobas e analyzer • Accessories for Elecsys 2010 and cobas e 411 analyzers:
- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution

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REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive

- [REF] 11933159001, Adapter for SysClean
- [REF] 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips Accessories for MODULAR ANALYTICS E170, cobas e 601 and
- cobas e 602 analyzers:
- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- [REF] 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M
- Accessories for all analyzers:
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assav

- 26 For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's 27 28 manual for analyzer-specific assay instructions.
- 29 Resuspension of the microparticles takes place automatically prior to use. 30 Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence 31
- of numbers. 32
- MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers: 33 PreClean M solution is necessary.
- 34 Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system 35 automatically regulates the temperature of the reagents and the 36 opening/closing of the bottles.
- 37

Calibration

- 38 Traceability: This method has been standardized against the Vitamin B12 assay ([REF] 04745736).
- 40 Every Elecsys reagent set has a barcoded label containing specific 41 information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet. 42
- Calibration frequency: Calibration must be performed once per reagent lot 43 using fresh reagent (i.e. not more than 24 hours since the reagent kit was 44 registered on the analyzer). Renewed calibration is recommended as 45 follows:
 - after 1 month (28 days) when using the same reagent lot
 - after 7 days (when using the same reagent kit on the analyzer)
 - as required: e.g. quality control findings outside the defined limits

Quality control

- For quality control, use PreciControl Varia.
- 51 In addition, other suitable control material can be used.
- 52 Controls for the various concentration ranges should be run individually at 53 least once every 24 hours when the test is in use, once per reagent kit, and following each calibration. 54
- The control intervals and limits should be adapted to each laboratory's 55
- individual requirements. Values obtained should fall within the defined 56 limits. Each laboratory should establish corrective measures to be taken if 57 values fall outside the defined limits.
- 58 Follow the applicable government regulations and local guidelines for quality control. 59

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in pmol/L or pg/mL).

pmol/L x 1.36 = pg/mL pg/mL x 0.738 = pmol/L

Limitations - interference

The assay is unaffected by icterus (bilirubin $\leq 1112 \mu mol/L$ or $\leq 65 mg/dL$), hemolysis (Hb ≤ 0.025 mmol/L or ≤ 0.04 g/dL), lipemia (triglycerides \leq 17.1 mmol/L or \leq 1500 mg/dL), biotin (\leq 205 nmol/L or \leq 50 ng/mL), lgG \leq 28 g/L, IgA \leq 16 g/L and IgM \leq 10 g/L.

Criterion: Recovery within ± 10 % of initial value with samples > 200 pg/mL and $\leq \pm 20$ pg/mL with samples ≤ 200 pg/mL.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

Because intrinsic factor is typically used as the binding protein in serum vitamin B12 assays, anti-intrinsic factor antibodies (which are common in pernicious anemia) can lead to elevated vitamin B12 measurement values.^{2,} ^{10,11} The Elecsys Vitamin B12 II assay is designed to avoid interference due to anti-intrinsic factor antibodies.12

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findinas.

Note: The presence of immunoglobulin-vitamin B12 complexes may cause unexpectedly high values of vitamin B12.13,14

Limits and ranges

Measuring range

50.0-2000 pg/mL or 36.9-1476 pmol/L (defined by the Limit of Blank and the maximum of the master curve). Values below the Limit of Blank are reported as < 50.0 pg/mL or < 36.9 pmol/L. Values above the measuring range are reported as > 2000 pg/mL or > 1476 pmol/L.

Lower limits of measurement

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

Limit of Blank = 50 pg/mL (36.9 pmol/L)

Limit of Detection = 100 pg/mL (73.8 pmol/L)

Limit of Quantitation = 150 pg/mL (111 pmol/L) with a allowable imprecision of ≤ 20 %

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from $n \ge 60$ measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a allowable imprecision of ≤ 20 %.

It has been determined using low concentration vitamin B12 samples.

Dilution

Samples with vitamin B12 concentrations above the measuring range can be manually diluted 1:2 with Diluent Universal. The concentration of the diluted sample must be > 738 pmol/L or > 1000 pg/mL. After manual dilution, multiply the results by the dilution factor 2.

Note: Sample-dependent non-linearity upon dilution is seen with samples having analyte levels beyond the measuring range. As Diluent Universal may contain low levels of endogenous vitamin B12, it is recommended that linearity studies be performed using a known low analyte-containing serum

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pool. Samples outside the measuring range can be diluted 1:2 with Diluent Universal; the effect of endogenous vitamin B12 concentration is insignificant at these levels.

Expected values

Because differences may exist with respect to population and dietary status, it is recommended that normal ranges be determined by each laboratory over a suitable period of time and in a statistically significant number of assays before clinical significance is attached to the results of these tests.

The values shown below were performed on samples from an apparently healthy population, using the Elecsys Vitamin B12 II assay. The calculation is based on 135 sera (68 men, 67 women). The age range was between 20 and 78 years. Pregnant women were excluded. The reference population was selected according to normal homocysteine values.

Ν	Median		Range (2.5th-97.5th percentile)	
	pg/mL	pmol/L	pg/mL	pmol/L
135	425	314	197 - 771	145-569

These values should only be used as guidelines.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

Elecsys 2010 and cobas e 411 analyzers						
		Repeata	Repeatability		Intermediate precision	
Sample	Mean	SD	CV	SD	CV	
	pg/mL	pg/mL	%	pg/mL	%	
Human serum 1	176	8.86	5.0	12.7	7.2	
Human serum 2	405	13.0	3.2	17.5	4.3	
Human serum 3	960	19.7	2.1	31.0	3.2	
Human serum 4	1230	27.4	2.2	46.4	3.8	
Human serum 5	1940	40.9	2.1	72.6	3.7	
PreciControl Varia1	447	12.2	2.7	18.6	4.2	
PreciControl Varia2	934	20.2	2.2	38.4	4.1	

Elecsys 2010 and cobas e 411 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean	SD	CV	SD	CV
	pmol/L	pmol/L	%	pmol/L	%
Human serum 1	130	6.54	5.0	9.37	7.2
Human serum 2	299	9.59	3.2	12.9	4.3
Human serum 3	708	14.5	2.1	22.9	3.2
Human serum 4	908	20.2	2.2	34.2	3.8
Human serum 5	1432	30.2	2.1	53.6	3.7
PreciControl Varia1	330	9.00	2.7	13.7	4.2
PreciControl Varia2	689	14.9	2.2	28.3	4.1

MODULAR ANALYTICS E170, 0	cobas e 601 and cobas e 602 analyzers
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· · · · · · · · · · · · · · · · · · ·					
		Repeatability		Intermediate precision	
Sample	Mean	SD	CV	SD	CV
	pg/mL	pg/mL	%	pg/mL	%
Human serum 1	176	5.84	3.3	9.14	5.2
Human serum 2	407	8.24	2.0	12.7	3.1
Human serum 3	1010	13.2	1.3	21.1	2.1
Human serum 4	1230	19.8	1.6	28.8	2.3
Human serum 5	1890	29.8	1.6	41.5	2.2
PreciControl Varia1	448	7.16	1.6	15.3	3.4
PreciControl Varia2	917	12.0	1.3	27.8	3.0

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers

		Repeata	bility	Intermeo precisi	
Sample	Mean	SD	CV	SD	CV
	pmol/L	pmol/L	%	pmol/L	%
Human serum 1	130	4.31	3.3	6.75	5.2
Human serum 2	300	6.08	2.0	9.37	3.1
Human serum 3	745	9.74	1.3	15.6	2.1
Human serum 4	908	14.6	1.6	21.3	2.3
Human serum 5	1395	22.0	1.6	30.6	2.2
PreciControl Varia1	331	5.28	1.6	11.3	3.4
PreciControl Varia2	677	8.86	1.3	20.5	3.0

Method comparison

a) A comparison of the Elecsys Vitamin B12 assay (calibrated with Vitamin B12 CalSet II; x) and the Elecsys Vitamin B12 II assay (calibrated with Vitamin B12 II CalSet; y) using clinical samples gave the following correlations (pg/mL):

Number of samples measured: 100

Passing/Bablok ¹⁵	Linear regression
y = 0.952x + 15.1	y = 0.957x + 11.6
т = 0.977	r = 0.999

The sample concentrations were between 69 and 1890 pg/mL (51 and 1395 pmol/L).

b) A comparison of the Elecsys Vitamin B12 II assay (y) and a commercially available method (x) using clinical samples gave the following correlations (pg/mL):

Number of samples measured: 106

Passing/Bablok ¹⁵	Linear regression
y = 0.923x + 4.90	y = 0.881x + 27.6
т = 0.952	r = 0.993

The sample concentrations were between 182 and 1797 pg/mL (134 and 1326 pmol/L).

c) A comparison of the Elecsys Vitamin B12 II assay on the cobas e 601 analyzer (y) and the Elecsys Vitamin B12 II assay on the cobas e 411 analyzer (x) using clinical samples gave the following correlations (pg/mL): Number of samples measured: 117

Passing/Bablok ¹⁵	Linear regression
y = 1.01x - 2.77	y = 1.01x + 3.22
т = 0.933	r = 0.995

The sample concentrations were between 56 and 1887 pg/mL (41 and 1393 pmol/L).

4/5 For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Vitamin B12 II

Vitamin B12

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Analytical specificity

The following cross-reactivities were found, tested with vitamin B12 concentrations of 129 pg/mL and 550 pg/mL.

Cross-reactant	Maximum concentration tested ng/mL	Cross-reactivity %
Cobinamide dicyanide	210	0.003

	2	Ro	ferences		Œ	
1		1	Thomas L. Clinical Laboratory Diagnostics: Use and Assessme	nt of		
1.			Clinical Laboratory Results; 1st Edition, Frankfurt/Main: TH-Boo		اسد	Roche Diagnostics GmbH, Sandhofer Strasse 116, D-
1			VerlGes.,1998:424-431.			www.roche.com
1		2	Stabler SP. Vitamin B12 deficiency. N Engl J Med 2013;368:14	9-160.		
1 1		3	Allen LH. Vitamin B-12. Adv Nutr 2012;3(1):54-55. doi: 10.3945/an.111.001370. Epub 2012 Jan 5.			
1 2		4	Allen LH. How common is vitamin B-12 deficiency? Am J Clin N 2009;89(2):693S-696S. Epub 2008 Dec 30.	lutr		
2	1	5	Chatthanawaree W. Biomarkers of cobalamin (vitamin B12) de and its application. J Nutr Health Aging 2011 Mar;15(3):227-31			
2 2		6	Yetley EA, Pfeiffer CM, Phinney KW, et al., Biomarkers of vitan status in NHANES: a roundtable summary. Am J Clin Nutr 201	nin B-12		
2 2			Jul;94(1):313S-321S.			
2	6	7	Hvas AM, Nexo E. Diagnosis and treatment of vitamin B12 defi an update. Haematologica 2006;91(11):1506-1512.	ciency -		
2		8	Occupational Safety and Health Standards: bloodborne pathog (29 CFR Part 1910.1030). Fed. Register.	ens.		
2' 3'	0	9	Directive 2000/54/EC of the European Parliament and Council 18 September 2000 on the protection of workers from risks rela exposure to biological agents at work.	ited to		
3 3	2	10	Yang DT, Cook RJ. Spurious elevations of vitamin B12 with pe anemia. N Engl J Med 2012;366:1742-1743.	rnicious		
3.	4	11	Carmel R, Agrawal YP. Failures of cobalamin assays in pernici anemia. N Engl J Med 2012;367:385-386. [Erratum, N Engl J M 2012;367:976.]	ous Ied		
3 3	6 7	12	Schilling KA, Wiesgigl M. The Elecsys® Vitamin B12 assay is r affected by anti-intrinsic factor antibodies. Clin Chem Lab Med Jun 29;51(11):e251-e252.	ot 2013		
3 3 4 4	9 0	13	Jeffery J, Millar H, MacKenzie P, et al. An IgG complexed form vitamin B12 is a common cause of elevated serum concentration Biochem 2010 Jan;43(1-2):82-88. doi: 10.1016/j.clinbiochem.2009.08.022. Epub 2009 Sep 8.	of ons. Clin		
4 4	2 3	14	Bowen RA, Drake SK, Vanjani R, et al. Markedly increased vita concentrations attributable to IgG-IgM-vitamin B12 immune cor Clin Chem 2006;52(11):2107-2114.			
4 4 4 4	5 6	15	Bablok W, Passing H, Bender R, et al. A general regression pro for method transformation. Application of linear regression pro for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.	cedure edures		
4 4 5	9 0	the info	r further information, please refer to the appropriate operator's m a analyzer concerned, the respective application sheets, the prod prmation and the Method Sheets of all necessary components (if ailable in your country).	uct		
5 5 5	2	se	point (period/stop) is always used in this Method Sheet as the deparator to mark the border between the integral and the fractional lecimal numeral. Separators for thousands are not used.			
5 5	5	Ro	mbols che Diagnostics uses the following symbols and signs in additior se listed in the ISO 15223-1 standard.	n to		
5 5			DNTENT Contents of kit			
5	8	SY	STEM Analyzers/Instruments on which reagents can be	e used		
5		RE	EAGENT Reagent			
6	0	CA	ALIBRATOR Calibrator			



Volume after reconstitution or mixing

Global Trade Item Number

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Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim



cobas®

STFF		Descrit										cobas
Order information	ble Transferrin F	Receptor	11									
REF											h	
		ala Tranaf	orrin De		× II/100) to ata)	Cure.		7 7 4 7 0 1	Analyzer(s) on whic		ack(s) can be used
07227841190	Tina-quant Solul			epio	r II(100	(lesis)		tem-ID 0	/ /4/3	cobas c 311, cobas	5 C 501/502	
08753776190	Calibrator sTfR I		L)	-			000	les 697				
08278202190	ControlSet sTfR			-			1.000		150			
	Level I (3 x 1 mL	,		-				el I Code				
04489357190	Level II (3 x 1 m Diluent NaCl 9 %	,					-	el II Code	7 6869 3			
04409007190		₀ (50 mL)					Sys		1 0009 3			
English								R2		ticles coated with mor		human sTfR antibod
System informa									. ,	n glycine buffer; prese		
For cobas c 311									•	B and R2 is in position	- C.	
STFR2: ACN 439										l warnings		
For cobas c 502 STFR2: ACN 843								Exerci	vitro diagno se the norr	ostic use. nal precautions requir	ed for handli	ng all laboratory
Intended use								reager	its.			
	e quantitative det	ermination	n of solu	ible tra	ansferri	n recer	otor			aste material should be t available for professi		
(sTfR) in human	serum and plasm									components classified		•
Summary ^{1,2,3,4,5,}								Regula	ation (EC) I	No. 1272/2008:		
The transferrin re	eceptor is an integ of 190 kilodalton	ral memb	rane gly	/copro	tein ha	ving a		Contai	ns 2-methy	/I-2H-isothiazol-3-one	hydrochloric	le
	y disulfide bridges						Da	EUH 2	08 M	ay produce an allergic	reaction.	
	onent which can b to the soluble for							Produc	t safety la	beling follows EU GHS	S guidance.	
plasma, the solul	ble transferrin rec	eptor is pr	esent ir	n the fo	orm of a	a comp	lex	Reage	nt handlin	Ig	-	
with transferrin h	aving a molecular	weight of	approx	imatel	y 320 k	(D. The	of		for use	0		
the receptor on t	tion of sTfR is dire he membrane.	ecuy propo	ortional	to the	concer	itration	01			eagent container seve	ral times pric	or to use to ensure th
The uptake of iro	n by the body's c								•	oonents are mixed.		
	tor (TfR). If the int a ferritin concent								e and sta	bility		
TfR is expressed	. The affinity of th	e transfer	rin rece	ptor to	o transfe	errin		STFR	2			
depends on the I	atter's loading sta calized on erythro	te. As 80-	95 % of	the tr	ansferr	in rece	ptor	Shelf I	fe at 2-8 °0	C:		See expiration dat
hence also the s	TfR concentration) reflects t	the iron	requir	rement	of these	е					on cobas c pack label.
cells. When iron	deficiency exists, nemoglobin conce	the sTfR (concent	ration	in seru	m rises	5	On ha		and refrigerated on the		
sTfR concentration	on can therefore c	lescribe th	ne functi	ional ii	ron stat	tus whil	le			and refrigerated on the	e analyzer:	26 weeks
	e iron storage sta				ent of t	he iron			t NaCl 9 %			•
(= sTfR concentr	tained by determin ation/log ferritin co	oncentrati	on).	3X				Shelf I	fe at 2-8 °(D:		See expiration date on cobas c pack
As - in contrast to	o ferritin - the cond	centration	of sTfR	l is not	t affecte	əd by						label.
acute-phase read	ctions, acute liver erentiate betweer	tunction d	lisorders	s or ma	alignan	t tumor	rs, it d	On-ho	ard in use :	and refrigerated on the	e analvzer:	12 weeks
iron deficiency a	nemia (IDA). Eleva	ated sTfR	values	are als	so foùn	nd in				ction and preparation	-	
polycythemia, he	molytic anemia, tl a, megaloblastic a	nalassemi	a, hereo	ditary s	spheroo	cytosis, ome ar	d			lection and preparation		uitable tubes or
vitamin B ₁₂ defici	ency. Elevated sT	fR conce	ntration	s occu	ir during	g		collect	ion contain	iers.		
pregnancy when	there is a deficier	ncy of fund	ctional ir	ron.				Only th Serum		ens listed below were t	ested and fo	und acceptable.
Parameter	Change	IDA	ACD		IDA +	ACD				(Li-, Na-, NH4+-) plasi	ma	
Ferritin	iron stores	\downarrow	Ť	—	or	1				s listed were tested wi		
TIBC/TRSF	iron status	↑	↓	↑	or	_				ommercially available f all manufacturers we		
Serum iron	iron status	\downarrow	↓		↓			from v	arious man	ufacturers may contai	in differing m	naterials which could
sTfR	functional iron	Ť	_		Ť			affect t	ne test res	ults in some cases. W llection systems), follo	hen process	ing samples in prima
	deficiency								acturer.			
	increased, - uno	changed								es containing precipita	-	• •
Test principle ⁸										is and interferences se	ection for def	ails about possible
	d immunoturbidim		•	L 1					e interferen	ices.		
	ansferrin receptor						ea	Stabilit	y:	6	6 days at 15-	25 °C
				. 1.001	r nato le	-				1	15 days at 2-	8 °C
determined photo	ometrically.										· · ·) · · ·	

C(O)has[®]

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See "Reagents - working solutions" section for reagents.

Tina-quant Soluble Transferrin Receptor II

Materials required (but not provided)

- See "Order information" section
- General laboratory equipment

Assav

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 311 test definition

23	Assay type	2-Point End			
24	Reaction time/Assay points	10 / 8-21			
25	Wavelength (sub/main)	800/570 nm			
26 27	Reaction direction	Increase			
27 28	Unit	mg/L (mg/dL,	nmol/L	\	
28 29		mg/⊑ (mg/u⊑,			
30	Reagent pipetting		Diluer	nt (H ₂ O)	
31	R1	100 µL	-		
32	R2	40 µL	-		
33	Sample volumes	Sample		Sample	dilution
34			Samp	ole	Diluent (NaCl)
35	Normal	2 µL	-		-
36	Decreased	2 µL	25 µL		75 μL
37	Increased	2 µL			
38					
39	cobas c 501 test definition				
40	Assay type	2-Point End			
41	Reaction time/Assay points	10 / 13-30			
42	Wavelength (sub/main)	800/570 nm			
43 44	Reaction direction	Increase			
44	Unit	mg/L (mg/dL,	nmol/L)	
46	Reagent pipetting		Diluer	nt (H₂O)	
47	R1	100 µL	-		
48	R2	40 μL	-		
49	Sample volumes	Sample		Sample	dilution
50	Campio Volamoo	Campio	Samp	-	Diluent (NaCl)
51	Normal	2 µL	- Oump	nc	
52		-	-		75
53	Decreased	2 µL	25 µL		75 µL
54	Increased	2 µL			
55 56	cobas c 502 test definition				
56 57	Assay type	2-Point End			
58	Reaction time/Assay points	10 / 13-30			
50 59	Wavelength (sub/main)	800/570 nm			
60	Reaction direction	Increase			
	Unit	mg/L (mg/dL,	nmol/l	\	
	Unit	my/⊏ (my/u∟,	IIII0I/L)	

Reagent pipetting		Diluent (H ₂ O)		
R1	100 µL	-			
R2	40 µL	-			
Sample volumes	Sample	S	ample	dilution	
		Sample		Diluent (NaCl)	
Normal	2 µL	-		-	
Decreased	2 µL	25 µL		75 µL	
Increased	2 µL				
Calibration					
Calibrators	S1: H ₂ O				
	S2-S6: Calibra	tor sTfR	II		
Calibration mode	Non-linear				
Calibration frequency	Full calibration	l			
	 after reagent 	lot chang	je		
	after 12 week				
		is when u	sing a	single reagent	
	lot	allowing o	u olitu	aantral	
	 as required following quality control procedures 				
Calibration interval may be ex calibration by the laboratory.		on accept	able ve	erification of	
Traceability: This method has	been standard	ized agaiı	nst an	in-house	

einou nas been standardized against an in-house reference preparation.

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

cobas c systems automatically calculate the analyte concentration of each sample in the unit mg/L (mg/dL, nmol/L)

Conversion factors:

mg/L x 11.8 = nmol/L^{9,a)} $mg/L \ge 0.1 = mg/dL$

a) Based on a molecular mass of 85 kDa for circulating transferrin receptor.

Limitations – interference

Criterion: Recovery within \pm 0.2 mg/L (2.36 nmol/L) of initial values of samples $\leq 2 \text{ mg/L}$ (23.6 nmol/L) and within $\pm 10 \%$ for samples > 2 mg/L. Icterus:¹⁰ No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:¹⁰ No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 622 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):¹⁰ No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference from rheumatoid factors up to a concentration of 1200 IU/mL.

High dose hook-effect: No false result occurs up to an sTfR concentration of 80 mg/L (944 nmol/L).

The antibodies are specific for sTfR. There is no cross-reactivity with diferrotransferrin, apotransferrin or ferritin under the assay conditions. Drugs: No interference was found at therapeutic concentrations using common drug panels.11,12

4 5

Tina-quant Soluble Transferrin Receptor II

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.1

6 7 In very rare cases, patient samples may contain particle agglutinating proteins (e.g. heterophilic antibodies or antibodies due to abnormal 8 immunoglobulin synthesis, such as gammopathies like MGUS* or 9 Waldenström's macroglobulinemia) which may lead to incorrect low or high 10 results with this assay. Correct results cannot be obtained by sample dilution and these samples should be analyzed by an alternative method. 11 noclonal Gammopathy of unknown significance

12 For diagnostic purposes, the results should always be assessed in 13 conjunction with the patient's medical history, clinical examination and other 14 findings

ACTION REQUIRED 15

Special Wash Programming: The use of special wash steps is mandatory 16 when certain test combinations are run together on cobas c systems. The 17 latest version of the carry-over evasion list can be found with the NaOHD-18 SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. cobas c 502 analyzer: All special wash programming 19 necessary for avoiding carry-over is available via the cobas link, manual 20 input is required in certain cases.

21 Where required, special wash/carry-over evasion programming must 22 be implemented prior to reporting results with this test.

23 Limits and ranges

24 Measuring range

25 0.50-20.0 mg/L (5.9-236 nmol/L)

Determine samples having higher concentrations via the rerun function. 26 Dilution of samples via the rerun function is a 1:4 dilution. Results from 27 samples diluted using the rerun function are automatically multiplied by a 28 factor of 4 29

Lower limits of measurement

20	
30	
50	
	Limit of Riank Limit of Detection and Limit of Duantitation
	Limit of Blank, Limit of Detection and Limit of Quantitation
21	

31	LITTIL OF DIATIK, LITTIL O	Delection and Linni of Quantit
•	Limit of Blank	= 0.25 mg/L (2.95 nmol/L)
32	Limit of Data sting	

33	Limit of Detection	= 0.40 mg/L (4.72 nmol/L)

34 = 0.50 mg/L (5.90 nmol/L)Limit of Quantitation

35 The Limit of Blank and Limit of Detection were determined in accordance 36 with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements. 37

- The Limit of Blank is the 95th percentile value from $n \ge 60$ measurements of 38 analyte-free samples over several independent series. The Limit of Blank 39 corresponds to the concentration below which analyte-free samples are found with a probability of 95 %. 40
- The Limit of Detection is determined based on the Limit of Blank and the 41 standard deviation of low concentration samples. 42
- The Limit of Detection corresponds to the lowest analyte concentration 43 which can be detected (value above the Limit of Blank with a probability of 44 95 %).
- 45 The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 20 %. It has been determined 46 using low concentration sTfR samples. 47

Expected values

- 48 The values shown below were performed on samples from an apparently 49 healthy population, using the Tina-guant Soluble Transferrin Receptor II assay (STFR2). The calculation is based on 165 sera (101 men, 50 64 women). The age range was between 22 and 83 years. The analysis of the data with the 2.5 % and the 97.5 % percentile gave a soluble transferrin receptor (sTfR) range from 1.71 mg/L (20.2 nmol/L) to 4.13 mg/L 51 52 53 (48.7 nmol/L). 54
- Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference 55 ranges. 56

Specific performance data

57 Representative performance data on the analyzers are given below. 58 Results obtained in individual laboratories may differ. 59

Precision

60

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute)

EP5-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days) The following results were obtained:

Popostability Moon

Repeatability	Mean	SD	CV
	mg/L	mg/L	%
Control Set sTfR II L 1	2.56	0.0389	1.5
Control Set sTfR II L 2	6.91	0.0626	0.9
Human serum 1	1.21	0.0375	3.1
Human serum 2	2.00	0.0438	2.2
Human serum 3	5.27	0.0526	1.0
Human serum 4	9.23	0.108	1.2
Human serum 5	17.7	0.157	0.9
Intermediate precision	Mean	SD	CV
Intermediate precision	Mean mg/L	SD mg/L	CV %
Intermediate precision Control Set sTfR II L 1		-	• •
	mg/L	mg/L	%
Control Set sTfR II L 1	<i>mg/L</i> 2.56	<i>mg/L</i> 0.0444	% 1.7
Control Set sTfR II L 1 Control Set sTfR II L 2	<i>mg/L</i> 2.56 6.91	<i>mg/L</i> 0.0444 0.0732	% 1.7 1.1
Control Set sTfR II L 1 Control Set sTfR II L 2 Human serum 1	<i>mg/L</i> 2.56 6.91 1.21	mg/L 0.0444 0.0732 0.0388	% 1.7 1.1 3.2
Control Set sTfR II L 1 Control Set sTfR II L 2 Human serum 1 Human serum 2	<i>mg/L</i> 2.56 6.91 1.21 2.00	<i>mg/L</i> 0.0444 0.0732 0.0388 0.0475	% 1.7 1.1 3.2 2.4

Method comparison

sTfR values for human serum and plasma samples obtained on a cobas c 501 analyzer (y) were compared with those determined using the Soluble Transferrin Receptor assay (STFR) on a cobas c 501 analyzer(x). Sample size (n) = 87

Passing/Bablok ¹⁴	Linear regression		
y = 0.987x + 0.0347 mg/L	y = 0.989x + 0.0264 mg/L		
т = 0.939	r = 0.996		

The sample concentrations were between 0.660 and 19.1 mg/L.

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0107227841190c501V1.0 STFR2

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit	
\rightarrow	Volume for reconstitution	
GTIN	Global Trade Item Number	
All other product names and	-QUANT are trademarks of Roche. trademarks are the property of their respective owners. es are indicated by a change bar in the margin.	2
Roche Diagnostic www.roche.com	s GmbH, Sandhofer Strasse 116, D-68305 Mannheim	Roche

Ferritin

cobas®

4 5	REF	Σ		SYSTEM		
6		V				
7				Elecsys 2010		
8				MODULAR AN	ALYTICS E170	
9	03737551 190	100		cobas e 411		
10				cobas e 601		
11 J				cobas e 602		
12				I		
13	English		 The reaction mixt 	ture is aspirated i	nto the measuring cell where the	
14	Intended use		microparticles are	e magnetically ca and substances a	ptured onto the surface of the re then removed with	
15 16	Immunoassay for the in serum and plasma.	vitro quantitative determination of ferritin in human	ProCell/ProCell N	 Application of a 	a voltage to the electrode then induces is measured by a photomultiplier.	
17		escence immunoassay "ECLIA" is intended for use e immunoassay analyzers.			ration curve which is instrument- alibration and a master curve provided	
18	Summary		via the reagent b		alibration and a master curve provided	
19	-	cule with a molecular weight of at least 440 kDa	a) Tris(2,2'-bipyridyl)ruther		ppy) ₃ ²⁺)	
20	(depending on the iron	content) and consists of a protein shell (apoferritin)	Reagents - working	solutions		
21	of 24 subunits and an in 2500 Fe ³⁺ ions (in liver	on core containing an average of approx.	The reagent rackpac	k is labeled as Fl	ERR.	
22	,	gomers, and when it is present in excess in the cells	M Streptavidin-co	ated microparticle	es (transparent cap), 1 bottle, 6.5 mL:	
23 24	of the storage organs th	ere is a tendency for condensation to	-	-	es 0.72 mg/mL; preservative.	
25		lerin to occur in the lysosomes.	·		0	
26	At least 20 isoterritins c	an be distinguished with the aid of isoelectric terogeneity is due to differences in the contents of), 1 bottle, 10 mL:	
27	the acidic H and weakly	basic L subunits. The basic isoferritins are	Biotinylated monoclonal anti-ferritin antibody (mouse) 3.0 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.			
28	responsible for the long	-term iron storage function, and are found mainly in				
29	the liver, spleen, and bo		R2 Anti-ferritin-Ab~	-Ru(bpy) $_3^{2+}$ (black	cap), 1 bottle, 10 mL:	
30	tissue. They have a low	und mainly in the myocardium, placenta, and tumor er iron content and presumably function as		-	(mouse) labeled with ruthenium	
31	intermediaries for the tr	ansfer of iron in various syntheses.4,5,6		g/L; phosphate bu	uffer 100 mmol/L, pH 7.2;	
32		ritin is a suitable method for ascertaining the iron	preservative.			
33		etermination of ferritin at the beginning of therapy we measure of the body's iron reserves. A storage	Precautions and wa	arnings		
34	deficiency in the reticulo	p-endothelial system (RES) can be detected at a	For in vitro diagnosti		ing the second is a set to be a set on a	
35	very early stage. ⁷		reagents.	precautions requ	ired for handling all laboratory	
36	Clinically, a threshold va	alue of 20 µg/L (ng/mL) has proved useful in the on deficiency. This value provides a reliable	Disposal of all waste	material should	be in accordance with local guidelines.	
37		of the iron reserves that can be mobilized for			sional user on request.	
38		atent iron deficiency is defined as a fall below the	Avoid foam formation calibrators and contr		and sample types (specimens,	
39		threshold. These two values necessitate no further even when the blood picture is still morphologically		013).		
40	normal. If the depresse	d ferritin level is accompanied by hypochromic,	Reagent handling	kit have been ass	embled into a ready-for-use unit that	
41	•	manifest iron deficiency is present.1	cannot be separated		chibica into a ready for use and that	
42	When the ferritin level is disorder can be ruled or	elevated and the possibility of a distribution ut, this is a manifestation of iron overloading in the		ed for correct op	eration is read in from the respective	
43 44	body. 400 µg/L (ng/mL)	ferritin is used as the threshold value. Elevated	reagent barcodes.			
44 45	ferritin values are also e	encountered with the following tumors: acute	Storage and stabili	ty		
45 46	prostate. The determina	ease and carcinoma of the lung, colon, liver and tion of ferritin has proved to be of value in liver	Store at 2-8 °C.			
47	metastasis. Studies ind	cate that 76 % of all patients with liver metastasis	Do not freeze.			
48	nave territin values abo	ve 400 µg/L (ng/mL). Reasons for the elevated crosis, blocked erythropoiesis or increased synthesis	Store the Elecsys rea	agent kit upright proparticles during	in order to ensure complete a automatic mixing prior to use.	
49	in tumor tissue.		-		, actionate many pror to door	
50		antibodies - M-4.184 and M-3.170 - are used to	Stability:			
51	form the sandwich com	plex in the assay.	unopened at 2-8 °C		up to the stated expiration date	
52	Test principle		after opening at 2-8	°C	12 weeks	
53		al duration of assay: 18 minutes.	on the analyzers		6 weeks	
54		of sample, a biotinylated monoclonal ody, and a monoclonal ferritin-specific antibody	Specimen collectio	n and preparatio	Dn	
55	labeled with a ruthe	nium complex ^{a)} form a sandwich complex.			e tested and found acceptable.	
56		r addition of streptavidin-coated microparticles, the	Serum collected usir			
57	complex becomes b	ound to the solid phase via interaction of biotin and	Li-, Na-heparin, K ₃ -E	-	-	
58	streptavidin.		When sodium citrate	is used, the resu	Its must be corrected by + 10 %.	
59 60			Criterion: Recovery	within 90-110 % c	f serum value or slope	
60			0.9-1.1 + intercept w correlation > 0.95.	$trin < \pm 2x$ analy	tical sensitivity (LDL) + coefficient of	
			Stable for 7 days at 2	2-8 °C, 12 month	s at -20 °C.8	

ms 03737551190V14.0 Ferritin

Do not use heat-inactivated samples.

The sample types listed were tested with a selection of sample collection

available tubes of all manufacturers were tested. Sample collection systems

tubes that were commercially available at the time of testing, i.e. not all

from various manufacturers may contain differing materials which could

Centrifuge samples containing precipitates before performing the assay.

tubes (sample collection systems), follow the instructions of the tube

affect the test results in some cases. When processing samples in primary

Ferritin

manufacturer.

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Calibration

Traceability: The Ferritin assay ([REF] 03737551) has been standardized against the Ferritin assay ([REF] 11820982). The Ferritin assay (REF 11820982) has been standardized against the Enzymun-Test Ferritin method. This in turn has been standardized against the 1st International Standard (IS) NIBSC (National Institute for Biological Standards and Control) "Reagent for Ferritin (human liver)" 80/602.

Recovery studies, including a published study,⁹ to assess traceability of the Elecsys Ferritin assay to more recent international standards (2nd IS 80/578 and 3rd IS 94/572) have been conducted, with results showing very good agreement.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. guality control findings outside the defined limits

Quality control

For quality control, use PreciControl Tumor Marker or PreciControl Varia. In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in µg/L or ng/mL).

Limitations - interference

The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), hemolysis (Hb < 0.31 mmol/L or < 0.5 g/dL), lipemia (Intralipid < 3300 mg/dL) and biotin (< 205 nmol/L or < 50 ng/mL).

Criterion: Recovery within ± 10 % of initial value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 2500 IU/mL

There is no high-dose hook effect at ferritin concentrations up to 100000 µg/L (ng/mL).

In vitro tests were performed on 19 commonly used pharmaceuticals. No interference with the assay was found.

Iron²⁺⁻ and iron³⁺⁻ions at therapeutic concentrations do not interfere with the Elecsys Ferritin assay.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings

Limits and ranges

Measuring range

0.500-2000 µg/L (ng/mL) (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.500 µg/L (ng/mL). Values above the measuring range are reported as > 2000 μ g/L (ng/mL) (or up to 100000 μ g/L (ng/mL) for 50-fold diluted samples).

Do not use samples and controls stabilized with azide. Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours. Materials provided See "Reagents - working solutions" section for reagents. Materials required (but not provided) [REF] 03737586190, Ferritin CalSet, 4 x 1 mL REF 11776452122, PreciControl Tumor Marker, for 2 x 3 mL each of PreciControl Tumor Marker 1 and 2 or REF 05618860190, PreciControl Varia, for 2 x 3 mL each of PreciControl Varia 1 and 2 REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent General laboratory equipment Elecsys 2010, MODULAR ANALYTICS E170 or cobas e analyzer . Accessories for Elecsys 2010 and cobas e 411 analyzers: REF 11662988122, ProCell, 6 x 380 mL system buffer REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive REF 11933159001, Adapter for SysClean REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips Accessories for MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers: REF 04880340190, ProCell M, 2 x 2 L system buffer . REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change [REF] 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags [REF] 03023150001, WasteLiner, waste bags . REF 03027651001, SysClean Adapter M Accessories for all analyzers: REF 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution Assav For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions. Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers Bring the cooled reagents to approx. 20 °C and place on the reagent disk °C) of the analyzer. Avoid foam formation. The system automatically (20 regulates the temperature of the reagents and the opening/closing of the bottles 2/4

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Lower limits of measurement

Lower detection limit of the test

Lower detection limit: 0.50 µg/L (ng/mL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, 10 standard 1 + 2 SD, repeatability study, n = 21). 11

Dilution

12 Samples with ferritin concentrations above the measuring range can be 13 diluted with Diluent Universal. The recommended dilution is 1:50 (either 14 automatically by the MODULAR ANALYTICS E170, Elecsys 2010 or cobas e analyzers or manually). The concentration of the diluted sample 15 must be > 40 μ g/L (ng/mL).

16 After manual dilution, multiply the result by the dilution factor.

17 After dilution by the analyzers, the MODULAR ANALYTICS E170, 18 Elecsys 2010 and cobas e software automatically takes the dilution into 19 account when calculating the sample concentration.

20 Expected values

21 Results of a study with the Enzymun-Test Ferritin method on samples from 224 healthy test subjects (104 women - mainly premenopausal - and 22 120 men) are given below. The values correspond to the 5th and 95th percentiles.¹⁰ 23

24 Men, 20-60 years: 30-400 µg/L (ng/mL)

25 Women, 17-60 years: 13-150 µg/L (ng/mL)

26 Each laboratory should investigate the transferability of the expected values 27 to its own patient population and if necessary determine its own reference ranges. 28

Specific performance data

Representative performance data on the analyzers are given below. 30 Results obtained in individual laboratories may differ. 31

Precision

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32 Precision was determined using Elecsys reagents, pooled human sera and 33 controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory 34 Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained: 35

Elecsys 2010 and cobas e 411 analyzers						
		Repea	Intermediate preci- sion			
Sample	Mean µg/L (ng/mL)	SD µg/L (ng/mL)	CV %	SD μg/L (ng/mL)	CV %	
Human serum 1	1.45	0.101	7.0	0.168	11.6	
Human serum 2	11.9	0.411	3.5	0.798	6.7	
Human serum 3	19.2	0.780	4.1	1.47	7.7	
Human serum 4	376	10.8	2.9	17.2	4.6	
Human serum 5	1361	26.5	1.9	84.4	6.2	
PreciControl Varia 1	134	1.96	1.5	2.75	2.1	
PreciControl Varia 2	858	15.1	1.8	21.7	2.5	

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers

,						
R	Repeatability			Intermediate preci- sion		
Mean µg/L (ng/mL)	SD µg/L (ng/mL)	CV %	SD μg/L (ng/mL)	CV %		
1.12	0.139	12.4	0.263	23.4		
12.3	0.467	3.8	0.789	6.4		
20.5	0.837	4.1	1.67	8.1		
392	8.14	2.1	16.9	4.3		
-	Mean μg/L (ng/mL) 1.12 12.3 20.5	Mean μg/L (ng/mL) SD μg/L (ng/mL) 1.12 0.139 12.3 0.467 20.5 0.837	Mean μg/L (ng/mL) SD μg/L (ng/mL) CV % 1.12 0.139 12.4 12.3 0.467 3.8 20.5 0.837 4.1	Mean μg/L (ng/mL) SD μg/L (ng/mL) CV μg/L % (ng/mL) SD μg/L (ng/mL) 1.12 0.139 12.4 0.263 12.3 0.467 3.8 0.789 20.5 0.837 4.1 1.67		

cobas ®

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers

	Repeatability			Intermediat sior	-
Sample	Mean µg/L (ng/mL)	SD µg/L (ng/mL)	CV %	SD μg/L (ng/mL)	CV %
Human serum 5	1449	35.6	2.5	92.8	6.4
PreciControl Varia 1	140	2.31	1.7	3.53	2.5
PreciControl Varia 2	900	14.4	1.6	25.0	2.8

Method comparison

A comparison of the Ferritin assay, REF 03737551 (y) with the Ferritin assay, REF 11820982 (x) using clinical samples gave the following correlations:

Number of samples measured: 134

Passing/Bablok ¹¹	Linear regression
y = 1.00x + 0.72	y = 0.99x + 4.11
т = 0.984	r = 0.999

The sample concentrations were between approximately 2.68 and 1891 µg/L (ng/mL).

Analytical specificity

Human liver ferritin: 100 % recovery

Human spleen ferritin: 85 % recovery

Human heart ferritin: 1 % recovery

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

3/4 For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

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Ferritin

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
\rightarrow	Volume after reconstitution or mixing

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CE

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Roche



2015-11, V 14.0 English

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Elecsys Folate RBC

cobas®

	REF		E	SYSTEM
I	05944295190	05944295500	100	cobas e 411 cobas e 601 cobas e 602

English

System information

For cobas e 411 analyzer: test number 1210

For cobas e 601 and cobas e 602 analyzers: Application Code

14 Number 272

Intended use This assay is used for the in vitro quantitative determination of folate in erythrocytes (red blood cells, RBC). The electrochemiluminescence binding assay is intended for use on the Elecsys and cobas e immunoassay

- analyzers.
- Summary
 Folate belongs to the family of B-group vitamins composed of an aromatic pteridine ring linked through a methylene group to p-aminobenzoic acid and a glutamate residue. Folate (folic acid) is vital for normal cellular functions and plays an essential role in nucleic acid synthesis, methionine regeneration, shuttling and redox reactions of one-carbon units required for normal metabolism and regulation.^{1,2}
- 25 The folate metabolism can be exemplified as a cycle, where folate 26 facilitates the transfer of one-carbon units from one molecule to another required in various biochemical reactions: for example, tetrahydrofolate 27 (THF) accepts a single carbon unit from serine, which is reduced in a number of steps to 5-methyltetrahydrofolate (5-MTHF). 5-MTHF gives its 28 29 methyl group to homocysteine, which is - with involvement of methionine synthase and vitamin B12 - enzymatically converted to methionine. The 30 resulting THF starts again the cycle of methyl group synthesis. From 31 methionine, the methyl groups are transferred to S-adenosylmethionine 32 (SAM).³ SAM serves as a methyl group donor in several methylation
- reactions, like DNA, RNA and protein methylation.¹
 The methionine cycle is highly sensitive to folate deficiency: with a low folate status, the ability of the cell to reamethylate homeoversine is important.
- folate status, the ability of the cell to re-methylate homocysteine is impaired and this results in increased homocysteine concentrations in plasma.²
 Solate also plays an accential rate in the surthesis of purine and multiplicities.
- Folate also plays an essential role in the synthesis of purine and pyrimidine
 precursors of nucleic acids. Altered distribution of methyl groups and
 impaired DNA synthesis play an essential role in the development of
 cancers. Abnormal folate status has also been linked with the development
 diseases like cardiovascular diseases, neural tube defects, cleft lip and
 palate, late pregnancy complications, neurodegenerative and psychiatric
 disorders.^{1,2}
- Folate belongs to the group of essential vitamins, i.e. it cannot be
 synthesized by the human organism and therefore must be absorbed from
 diet. Primary sources of folates are green and leafy vegetables, sprouts,
 fruits, brewer's yeast and liver.^{1,2}
- Folate deficiency can be caused by decreased nutritional intake, poor absorption of ingested folate in the intestine or increased demand of folate, for example during physical activity or pregnancy. Deficiency of folate can also be a result of liver diseases or impaired folate metabolism due to genetic defects or drug interactions.²
- A clinical manifestation of both folate and vitamin B12 deficiency is the so
 called megaloblastic (macrocytic) anemia: due to the affected DNA
 synthesis and cell maturation, especially involving the cells of
- synthesis and centinaturation, especially involving the cells of
 erythropoiesis, the total count of erythrocytes is significantly reduced. The
 hemoglobin synthesis capacity however is normal, which leads to
 abnormally large erythrocyte precursors ("macrocytes" or "megaloblasts"),
- which have an elevated hemoglobin content ("hyperchromic anemia").^{3,4}
 Serum folate concentrations may be affected by recent folate intakes,
- Serum totate concentrations may be attected by recent folate intakes, whereas red blood cell (RBC) folate is a measure of the folate intake across the 90-120 days lifespan of erythrocytes. Thus, folate concentrations in RBC give a more accurate picture of a patient's underlying folate status than serum folate concentrations, and are considered by experts as the better measure for folate status.⁵
- Because vitamin B12 and folate are closely interrelated in the cellular
 one-carbon unit metabolism, and also hematologic and clinical consequences of the two vitamin deficiency states might be similar, it is

advisable to determine both parameters simultaneously in patients with the relevant symptoms of vitamin deficiency. $^{\rm 3,4}$

Test principle

Competition principle. Total duration of assay: 27 minutes.

Whole blood treated with anticoagulants (heparin or EDTA) is mixed with ascorbic acid solution and incubated for approximately 90 minutes at 20-25 °C. Lysis of the erythrocytes takes place, with liberation and stabilization of the intracellular folate. The resulting hemolysate sample is then used for subsequent measurement.

- 1st incubation: By incubating 25 µL of hemolysate sample with the folate pretreatment reagents 1 and 2, bound folate is released from endogenous folate binding proteins.
- 2nd incubation: By incubating the pretreated sample with the ruthenium labeled folate binding protein, a folate complex is formed, the amount of which is dependent upon the analyte concentration in the sample.
- 3rd incubation: After addition of streptavidin-coated microparticles and folate labeled with biotin, the unbound sites of the ruthenium labeled folate binding protein become occupied, with formation of a ruthenium labeled folate binding protein-folate biotin complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrumentspecifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

Reagents - working solutions

The reagent rackpack (M, R1, R2) is labeled as RBC-FOL.

- PT1 Pretreatment reagent 1 (white cap), 1 bottle, 4 mL: Sodium 2-mercaptoethanesulfonate (MESNA) 40 g/L, pH 5.5.
- PT2 Pretreatment reagent 2 (gray cap), 1 bottle, 5 mL: Sodium hydroxide 25 g/L.
- M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Folate binding protein~Ru(bpy)₃²⁺ (gray cap), 1 bottle, 9 mL: Ruthenium labeled folate binding protein 75 μg/L; human serum albumin (stabilizer); borate/phosphate/citrate buffer 70 mmol/L, pH 5.5; preservative.
- R2 Folate~biotin (black cap), 1 bottle, 8 mL:

Biotinylated folate 17 $\mu g/L;$ biotin 120 $\mu g/L;$ human serum albumin (stabilizer); borate buffer 100 mmol/L, pH 9.0; preservative.

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

2-methyl-2H-isothiazol-3-one hydrochloride

EUH 208 May produce an allergic reaction.

Elecsys Folate RBC



- For the determination of folate in RBC: Determine hematocrit in whole blood samples and record the value.
- Preparation of the hemolysate sample
- Mix 3.0 mL of Folate RBC Hemolyzing Reagent (ascorbic acid solution, 0.2 %) and 100 μL of well-mixed whole blood, avoiding foam formation. Incubate with closed caps for 90 \pm 15 minutes at 20-25 °C.

Stability:

Whole blood: 2 hours at 20-25 °C8, 24 hours at 2-8 °C, 1 month at -20 °C (± 5 °C) (only EDTA-blood).

Hemolysate sample: 1 month at -20 °C (± 5 °C), freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Samples should not subsequently be altered with additives (biocides, anti-oxidants or substances possibly changing the pH of the sample) in order to avoid erroneous folate recovery.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

If measurements cannot be carried out within 2 hours please store the hemolysate sample at -20 °C (± 5 °C).

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

- REF 05944309190, Folate RBC CalSet, for 4 x 1.0 mL
- REF 05944317190, Folate RBC Hemolyzing Reagent kit for 4 x 200 mL, contains ascorbic acid
- General laboratory equipment

cobas e analvzer

Additional materials for the cobas e 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- [REF] 11706799001, AssayTip, 30 x 120 pipette tips
- [REF] 11800507001, Clean-Liner

Additional materials for cobas e 601 and cobas e 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REFI 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Additional materials for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution
- Danger H290 May be corrosive to metals. H314 Causes severe skin burns and eye damage. Prevention. P280 Wear protective gloves/ protective clothing/ eye protection/ face protection. **Response:** P301 + P330 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. + P331 P303 + P361 IF ON SKIN (or hair): Take off immediately all contaminated + P353 clothing. Rinse skin with water. P304 + P340 IF INHALED: Remove person to fresh air and keep + P310 comfortable for breathing. Immediately call a POISON CENTER/ doctor. P305 + P351 IF IN EYES: Rinse cautiously with water for several + P338 minutes. Remove contact lenses, if present and easy to do. + P310 Continue rinsing. Immediately call a POISON CENTER/ doctor. P390 Absorb spillage to prevent material damage. Product safety labeling follows EU GHS guidance. Contact phone: all countries: +49-621-7590 All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.^{6,7} Avoid foam formation in all reagents and sample types (specimens, calibrators and controls). **Reagent handling** The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated. 46 All information required for correct operation is read in from the respective 47 reagent barcodes. 48 Storage and stability 49 Store at 2-8 °C. 50 Do not freeze. 51 Store the Elecsys reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use. 52 53 Stability: 54 unopened at 2-8 °C up to the stated expiration date 55 after opening at 2-8 °C 8 weeks 56 on the analyzers 2 weeks 57 58 Specimen collection and preparation Only the specimens listed below were tested and found acceptable.
- 59 Hemolysate prepared from whole blood treated with anticoagulants 60 (Na-heparin or K₃-EDTA).

2 3 4

Elecsys Folate RBC

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5 Assay 6 The well-mixed hemolysate sample is placed in the sample zone of the analyzer and recorded by entering the sample identification data. Complete 7 determinations on the analyzer within 2 hours after finalizing the preparation 8 of the hemolysate sample. 9 For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's 10 manual for analyzer-specific assay instructions. 11 Resuspension of the microparticles takes place automatically prior to use. 12 Read in the test-specific parameters via the reagent barcode. If in 13 exceptional cases the barcode cannot be read, enter the 15-digit sequence 14 of numbers. 15 cobas e 601 and cobas e 602 analyzers: PreClean M solution is necessary. 16 Bring the cooled reagents to approximately 20 °C and place on the reagent 17 disk (20 °C) of the analyzer. Avoid foam formation. The system 18 automatically regulates the temperature of the reagents and the 19 opening/closing of the bottles. 20 Calibration 21 Traceability: This method has been standardized against the Elecsys Folate III assay (REF] 04476433190)/RBC application. 22 23 The standardization of the Elecsys Folate RBC assay includes the volume correction to account for the preparation of hemolysate sample 24 (1:31 vol/vol). 25 Every Elecsys reagent set has a barcoded label containing specific 26 information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet. 27 Calibration frequency: Calibration must be performed once per reagent lot 28 using fresh reagent (i.e. not more than 24 hours since the reagent kit was 29 registered on the analyzer). 30 Calibration interval may be extended based on acceptable verification of 31 calibration by the laboratory. 32 Renewed calibration is recommended as follows: 33 after 1 month (28 days) when using the same reagent lot 34 after 7 days (when using the same reagent kit on the analyzer) 35 as required: e.g. quality control findings outside the defined limits 36 Quality control 37 For quality control, use commercially available whole blood control material. 38 Controls for the various concentration ranges should be run individually at 39 least once every 24 hours when the test is in use, once per reagent kit, and following each calibration. 40 41 The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined 42 limits. Each laboratory should establish corrective measures to be taken if 43 values fall outside the defined limits. 44 If necessary, repeat the measurement of the samples concerned. 45 Follow the applicable government regulations and local guidelines for 46 quality control. 47 Calculation 48 1. Whole blood folate (from hemolysate sample) 49 The standardization of the Elecsys Folate RBC assay includes the volume correction to account for the preparation of hemolysate sample 50 (1:31 vol/vol). 51 The analyzer automatically calculates the analyte concentration of each 52 sample in nmol/L or ng/mL. 53 Conversion factors: $nmol/L \ge 0.44 = ng/mL$ 54 ng/mL x 2.27 = nmol/L55 56 2. RBC folate 57 To calculate the folate concentration in the erythrocyte fraction of the sample (RBC folate), the predetermined sample specific hematocrit value 58 must be taken into account using the following equation: 59 60 analyzer result RBC folate = × 100 % hematocrit

Limitations - interference

The assay is unaffected by icterus (bilirubin < 564 μ mol/L or < 33 mg/dL), lipemia (Intralipid < 1500 mg/dL), biotin (< 86.1 nmol/L or < 21 ng/mL), lgG < 16 g/L and lgA < 4.0 g/L.

Criterion: Recovery within \pm 10 % of initial value with samples > 155 ng/mL and $\leq \pm$ 15.5 ng/mL with samples \leq 155 ng/mL.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1000 $\mbox{IU/mL}.$

In vitro tests were performed on 16 commonly used pharmaceuticals and in addition on human erythropoietin. No interference with the assay was found.

It is contraindicated to measure samples of patients receiving therapy with certain pharmaceuticals, e.g. methotrexate or leucovorin, because of the cross-reactivity of folate binding protein with these compounds.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

In rare cases, samples with low erythrocyte folate concentration, but high serum folate concentration can occur. In these cases, a correction of the folate concentration in erythrocytes by the serum folate concentration with the following equation is recommended*:

* expected values can be used as an indicator for high serum folate concentration

Corrected RBC folate concentration =

, serum folate		100 - % hematocrit	,
(concentration	Х	% hematocrit)

concentration Example

RBC folate

RBC folate concentration: 241 (ng/mL RBC);

serum folate concentration: 10.5 (ng/mL S);

hematocrit measured (%) = 45

Corrected RBC folate concentration =

241 ng/mL RBC	- (10.5 ng/mL S x	<u>100 - 45</u> 45) = 228 ng/mL RBC
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Limits and ranges

Measuring range

120-620 ng/mL or 272-1407 nmol/L (defined by the Limit of Quantitation and the maximum of the master curve). Values below the Limit of Quantitation are reported as < 120 ng/mL (< 272 nmol/L). Values above the measuring range are reported as > 620 ng/mL (> 1407 nmol/L). Values are not corrected for the sample hematocrit.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation:

Limit of Blank = 20.0 ng/mL

Limit of Detection = 46.5 ng/mL

Limit of Quantitation = 120 ng/mL

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from n \geq 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative

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Elecsys Folate RBC

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error of \leq 30 %. It has been determined using low concentration folate samples.

Dilution

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Hemolysate samples with folate concentrations above the measuring range can be diluted manually with Elecsys Folate RBC Hemolyzing Reagent (ascorbic acid solution, 0.2 %). The recommended dilution is 1:2. The concentration of the diluted sample must be > 265 ng/mL or > 602 nmol/L. After manual dilution, multiply the results by the dilution factor 2.

Expected values

The values shown below were measured on samples from an apparently healthy population, using the Elecsys Folate III/RBC application. The values can be applied for the Elecsys Folate RBC assay on all Elecsys and **cobas e** analyzers. The calculation is based on 290 sera (96 men, 104 merce) applied applied on the elecsys application.

194 women) from an European population. The age range was between 18 and 65 years. Pregnant or lactating women were excluded. The reference population was selected according to normal homocysteine values. The following values were obtained:

Whole blood folate (from hemolysate samples)							
	Ν	Med	Median 2.5 th -97.5 th percentile				
		nmol/L ng/mL nmol/L ng/m					
Europe 290 673 296 481-1212 212-534							

The measured hematocrit value in this study showed a range from 37.1-46.1 %.

RBC folate (folate in erythrocyte fraction)							
	N Median 2.5 th -97.5 th percentile						
		nmol/L	ng/mL	nmol/L	ng/mL		
Europe 290 1657 730 1187-2854 523-1257							

If pathologically low hematocrit values are considered for calculation of RBC folate in the erythrocyte fraction, elevated RBC folate concentrations may be observed. No medical conclusion should be based on the calculation considering hematocrit values in such cases. Instead, whole blood folate results (from hemolysate samples) and suitable expected values may be used.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents and hemolysate samples in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). Results are given as whole blood folate (from hemolysate sample). The following results were obtained:

cobas e 411 analyzer									
			Repeatability Intermed				ermediat recision		
Sample	Me	ean	S	D	CV	SD		CV	
	nmol/L	ng/mL	nmol/L	ng/mL	%	nmol/L	ng/mL	%	
HL ^{a)} 1	154	68.0	11.7	5.17	7.6	21.9	9.65	14.2	
HL 2	352	155	17.5	7.73	5.0	27.7	12.2	7.9	
HL 3	618	272	25.4	11.2	4.1	38.4	16.9	6.2	
HL 4	1195	527	38.8	17.1	3.3	56.3	24.8	4.7	

a) HL = Hemolysate

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cobas e 601 and cobas e 602 analyzers								
			Repeatability			Inte pr	e	
Sample	Me	an	SD CV		SD		CV	
	nmol/L	ng/mL	nmol/L	ng/mL	%	nmol/L	ng/mL	%
HL 1	138	61.0	12.1	5.31	8.8	14.3	6.32	10.4
HL 2	434	191	26.1	11.5	6.0	28.4	12.5	6.5
HL 3	586	258	32.0	14.1	5.5	34.3	15.1	5.9
HL 4	1317	580	29.1	12.8	2.2	44.7	19.7	3.4

Method comparison

a) A comparison of the Elecsys Folate RBC assay (calibrated with Folate RBC CalSet; y) and the Elecsys Folate III/RBC application (calibrated with Folate III CalSet; x) using hemolyzed clinical samples gave the following correlations (ng/mL). Results are given as whole blood folate (from hemolysate sample).

Number of samples measured: 187

Passing/Bablok9	Linear regression
y = 1.02x - 14.1	y = 1.00x - 12.0
т = 0.869	r = 0.985

The sample concentrations were between 151 and 551 ng/mL (343 and 1251 nmol/L).

b) A comparison of the Elecsys Folate RBC assay on the MODULAR ANALYTICS E170 analyzer (y) with the Elecsys Folate RBC assay on the Elecsys 2010 analyzer (x) (both tests have been calibrated with Folate RBC CalSet) using hemolyzed clinical samples gave the following correlations (ng/mL). Results are given as whole blood folate (from hemolysate sample).

Number of samples measured: 187

Passing/Bablok	Linear regression		
y = 1.04x + 1.94	y = 1.02x + 8.07		
т = 0.814	r = 0.970		

The sample concentrations were between 137 and 557 ng/mL (311 and 1264 nmol/L).

References

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- 9 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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1 2	Flece	sys Folate RBC	
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5 6	For further inform	ation, please refer to the appropriate operator's manual for cerned, the respective application sheets, the product	
7	information and t	he Method Sheets of all necessary components (if	
8	available in your	country). op) is always used in this Method Sheet as the decimal	
9	separator to mar	the border between the integral and the fractional parts of	
10 11		al. Separators for thousands are not used.	
12	Symbols Roche Diagnostic	cs uses the following symbols and signs in addition to	
13	those listed in the definition of symb	e ISO 15223-1 standard (for USA: see dialog.roche.com for	
14 15	·		
15 16	CONTENT SYSTEM	Contents of kit Analyzers/Instruments on which reagents can be used	
17	REAGENT	Reagent	
18		Calibrator	
19 20		Volume after reconstitution or mixing	
20 21	GTIN	Global Trade Item Number	
22			
23	COBAS, COBAS E, ELEC Fresenius Kabi AB.	CSYS and PRECICONTROL are trademarks of Roche. INTRALIPID is a trademark of	
24 25		nd trademarks are the property of their respective owners.	
25 26	© 2020, Roche Diagnosti		
27	CE		
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29 30	Roche Diagno www.roche.co	m Sandhofer Strasse 116, D-68305 Mannheim Roche	
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2021-03, V 8.0 English

cobas®





Cardiac C-Reactive Protein (Latex) High Sensitive

Order information

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REF	CONTENT		Analyzer(s) on which cobas c pack(s) can be used
04628918 190	Cardiac C-Reactive Protein (Latex) High Sensitive (300 tests)	System-ID 07 6866 9	Roche/Hitachi cobas c 311, cobas c 501/502
11355279 216	Calibrator f.a.s. Proteins (5 x 1 mL)	Code 656	
11355279 160	Calibrator f.a.s. Proteins (5 x 1 mL, for USA)	Code 656	
20766321 322	CRP T Control N (5 x 0.5 mL)	Code 235	
10557897 122	Precinorm Protein (3 x 1 mL)	Code 302	
10557897 160	Precinorm Protein (3 x 1 mL, for USA)	Code 302	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information 21

For cobas c 311/501 analyzers: 22

23 CRPHS: ACN 217

For cobas c 502 analyzer: 24

CRPHS: ACN 8217 25

Intended use

In vitro test for the quantitative determination of C-reactive protein (CRP) in 27 human serum and plasma on Roche/Hitachi cobas c systems. Measurement of CRP is of use for the detection and evaluation of 28 29 inflammatory disorders and associated diseases, infection and tissue injury. Highly sensitive measurement of CRP may also be used as an aid in the 30 assessment of the risk of future coronary heart disease. When used as an 31 adjunct to other laboratory evaluation methods of acute coronary 32 syndromes, it may also be an additional independent indicator of recurrent 33 event prognosis in patients with stable coronary disease or acute coronary syndrome. 34

Summary1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21

35 C-reactive protein is the classic acute phase protein in inflammatory 36 reactions. It is synthesized by the liver and consists of five identical 37 polypeptide chains that form a five-member ring having a molecular weight of 105000 daltons. CRP is the most sensitive of the acute phase reactants 38 and its concentration increases rapidly during inflammatory processes. 39 Complexed CRP activates the complement system beginning with C1q. CRP then initiates opsonization and phagocytosis of invading cells, but its 40

main function is to bind and detoxify endogenous toxic substances 41 produced as a result of tissue damage. 42

CRP assays are used to detect systemic inflammatory processes (apart 43 from certain types of inflammation such as SLE and Colitis ulcerosa); to assess treatment of bacterial infections with antibiotics; to detect 44 intrauterine infections with concomitant premature amniorrhexis; to 45 differentiate between active and inactive forms of disease with concurrent 46 infection, e.g. in patients suffering from SLE or Colitis ulcerosa; to therapeutically monitor rheumatic disease and assess anti-inflammatory therapy; to determine the presence of post-operative complications at an early stage, such as infected wounds, thrombosis and pneumonia, and to 47 48 49 distinguish between infection and bone marrow transplant rejection.

50 Sensitive CRP measurements have been used and discussed for early 51 detection of infection in pediatrics and risk assessment of coronary heart disease. Several studies came to the conclusion that the highly sensitive 52 measurement of CRP could be used as a marker to predict the risk of 53 coronary heart disease in apparently healthy persons and as an indicator of recurrent event prognosis. Increases in CRP values are non-specific and should not be interpreted without a complete clinical history. The American Heart Association and the Centers for Disease Control and Prevention have 54 55 56 made several recommendations concerning the use of high sensitivity C-Reactive Protein (hsCRP) in cardiovascular risk assessment.²¹ Testing for any risk assessment should not be performed while there is an indication of infection, systemic inflammation or trauma. Patients with persistently unexplained hsCRP levels above 10 mg/L (95.2 nmol/L) should 57 58 59 60 be evaluated for non-cardiovascular etiologies. When using hsCRP to assess the risk of coronary heart disease, measurements should be made on metabolically stable patients and compared to previous values.

Optimally, the average of hsCRP results repeated two weeks apart should be used for risk assessment. Screening the entire adult population for hsCRP is not recommended, and hsCRP is not a substitute for traditional cardiovascular risk factors. Acute coronary syndrome management should not depend solely on hsCRP measurements. Similarly, application of secondary prevention measures should be based on global risk assessment and not solely on hsCRP measurements. Serial measurements of hsCRP should not be used to monitor treatment.

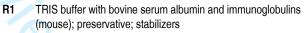
Various assay methods are available for CRP determination, such as nephelometry and turbidimetry. The Roche CRP assay is based on the principle of particle-enhanced immunological agglutination.

Test principle^{22,23}

Particle enhanced immunoturbidimetric assay.

Human CRP applutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically.

Reagents - working solutions



- R2 Latex particles coated with anti-CRP (mouse) in glycine buffer; preservative; stabilizers
- R1 is in position B and R2 is in position C.

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

Reagent handling

Ready for use

Mix **cobas c** pack well before placing on the analyzer.

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

Storage and stability

CRPHS	
Shelf life at 2-8 °C:	See expiration date on cobas c pack label.
On-board in use and refrigerated on the analyzer:	12 weeks
Diluent NaCl 9 %	
Shelf life at 2-8 °C:	See expiration date on cobas c pack label.

On-board in use and refrigerated on the analyzer: 12 weeks

2 3

0004628918190c501V12.0 Cardiac C-Reactive Protein (Latex) High Sensitive

5	Specimen collection and pr	enaration			Reaction direction	Increase		
6	For specimen collection and preparation only use suitable tubes or			Units	mg/L (nmol/L, mg/dL)			
7	collection containers.			Reagent pipetting	Diluent (H ₂ O)			
8	Only the specimens listed below were tested and found acceptable. Serum.							
9	Plasma: Li-heparin and K_2 -EDTA plasma			R1	82 µL	42 µL		
10	The sample types listed were	-	selection of san	nple collection	R2	28 µL	20 µL	
11	tubes that were commercially							
12	available tubes of all manufaction from various manufacturers m	cturers were te nav contain diff	sted. Sample c fering materials	which could	Sample volumes	Sample	Sam	ple dilution
13	affect the test results in some	cases. When	processing san	nples in primary			Sample	Diluent (NaCl)
14 15	tubes (sample collection system manufacturer.	ems), follow the	e instructions o	f the tube	Normal	6 µL	-	_
15 16	Centrifuge samples containing	a precipitates h	nefore nerformi	ng the assay	Decreased	6 µL	10 µL	140 μL
17	See the limitations and interfe	••••	•	• •	Increased	6 µL	_	-
18	sample interferences.							
19	Sample stability claims were	established by	experimental c	lata by the	cobas c 502 test defini	tion		
20	manufacturer or based on refeteemeratures/time frames as				Assay type	Rate A		
21	responsibility of the individual	laboratory to u	use all available	e references	Reaction time / Assay p	oints 10/12-70		
22	and/or its own studies to dete laboratory.	rmine specific	stability criteria	for its	Wavelength (sub/main)	– /546 nm		
23	-				Reaction direction	Increase		
24	Stability: ²⁴	11 da	ys at 15-25 °C		Units	mg/L (nmol/	L, mg/dL)	
25		2 mor	nths at 2-8 °C		Reagent pipetting	0 (Diluent (H ₂	C)
26		3 yea	rs at (-15)-(-25)	O° (R1	82 µL	42 µL	- /
27	Materials provided				R2	02 μL	42 μ⊑ 20 μL	
28	See "Reagents - working solu	utions" section	for reagents.		TTZ	20 µĽ	20 µL	
29 30	Materials required (but not	provided)			0	0	0	
30	 See "Order information" set 				Sample volumes	Sample		ple dilution
32	 General laboratory equipn 	nent					Sample	Diluent (NaCl)
33	Assay				Normal	6 µL	-	-
34	For optimum performance of	the assay follo	w the directions	s given in this	Decreased	6 µL	10 µL	140 µL
35 36	document for the analyzer co manual for analyzer-specific a	assay instructio	ons.			12 µL	-	-
37	The performance of application and must be defined by the use	ons not validate ser.	ed by Roche is	not warranted	Calibration Calibrators	S1: H₂O		
38	Application for serum and p	olasma				S2: C.f.a.s. Prote	ins	
39	cobas c 311 test definition							Proteins calibrator
40 41	Assay type	Rate A				value by the facto		
41	Reaction time / Assay points	10/7-57						6-point calibration
43	Wavelength (sub/main)	– /546 nm				curve:		
44	Reaction direction	Increase				S2: 0.0125	S5: 0.1	00
45	Units	mg/L (nmol/L	ma/dL)			S3: 0.0250	S6: 0.2	200
46	Reagent pipetting		Diluent (H ₂ O)	1		S4: 0.0500		
47	R1	82 µL	42 μL	1	Calibration mode	Line Graph		
48		-	-		Calibration frequency	Full calibration		
49 50	R2	28 µL	20 µL			after reagent lot		
50 51	0	0	0			• as required follo		
52	Sample volumes	Sample	· · ·	le dilution	Calibration interval may calibration by the labora		d on acceptabl	e verification of
53			Sample	Diluent (NaCl)	Traceability: This metho		rdized against	the reference
54	Normal	6 µL	-	-	preparation of the IRMN	I (Institute for Refe	rence Material	s and
55	Decreased	6 µL	10 µL	140 μL	Measurements) BCR47(Proteins in Human Seru)/CRM470 (RPPH) m) ²⁵	S - Reference	Preparation for
56	Increased	6 µL	-	-	Quality control			
57 58	cobas c 501 test definition				For quality control, use of	control materials as	s listed in the "	Order information"
59	Assay type	Rate A			section. In addition, other suitable control material can be used.			
60	Reaction time / Assay points	10/12-70			The control intervals and	d limits should be a	adapted to eac	h laboratory's
	Wavelength (sub/main)	– /546 nm			individual requirements.	Values obtained s	hould fall withi	n the defined

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limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits. Follow the applicable government regulations and local guidelines for quality control.

Calculation

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Roche/Hitachi cobas c systems automatically calculate the analyte concentration of each sample.

12	Conversion factors:	mg/L x 9.52 = nmol/L
13		$mg/L \ge 0.1 = mg/dL$

Limitations - interference

- 15 Criterion: Recovery within ± 10 % of initial values at CRP levels of 1.0 mg/L.
- 16 Icterus:²⁶ No significant interference up to an Lindex of 60 for conjugated 17 bilirubin and unconjugated bilirubin (approximate conjugated and 18 unconjugated bilirubin concentration: 60 mg/dL or 1026 µmol/L).
- 19
- Hemolysis:²⁶ No significant interference up to an H index of 1000 (approximate hemoglobin concentration: $622 \mu mol/L$ or 1000 mg/dL). 20
- Lipemia (Intralipid):²⁶ No significant interference up to an L index of 600. 21 There is poor correlation between the L index (corresponds to turbidity) and 22 triglycerides concentration.
- 23 Rheumatoid factors: No significant interference from rheumatoid factors up 24 to a concentration of 1200 IU/mL.
- Drugs: No interference was found at therapeutic concentrations using 25 common drug panels.27,28 26
- Therapeutic drugs: Significantly decreased CRP values may be obtained 27 from samples taken from patients who have been treated with 28 carboxypenicillins.
- 29 High dose hook-effect: No false result occurs up to a CRP concentration of 30 1000 mg/L.
- 31 In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.² 32
- Although measures were taken to minimize interference caused by human 33 anti-mouse antibodies, erroneous findings may be obtained from samples 34 taken from patients who have been treated with monoclonal mouse 35 antibodies or have received them for diagnostic purposes.
- For diagnostic purposes, the results should always be assessed in 36
- conjunction with the patient's medical history, clinical examination and other 37 findinas.

38 ACTION REQUIRED

- 39 Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi 40 cobas c systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further 41
- 42 instructions refer to the operator's manual. cobas c 502 analyzer: All 43 special wash programming necessary for avoiding carry-over is available
- via the cobas link, manual input is required in certain cases. 44

Where required, special wash/carry-over evasion programming must 45 be implemented prior to reporting results with this test. 46

Limits and ranges 47

Measuring range

- 48 0.15-20.0 mg/L (1.43-190 nmol/L, 0.015-2.0 mg/dL)
- 49 Determine samples having higher concentrations via the rerun function. 50 Dilution of samples via the rerun function is a 1:15 dilution. Results from 51 samples diluted using the rerun function are automatically multiplied by a factor of 15 52

Lower limits of measurement 53

Lower detection limit of the test 54

0.15 mg/L (1.43 nmol/L, 0.015 mg/dL) 55

- The lower detection limit represents the lowest measurable analyte level 56 that can be distinguished from zero. It is calculated as the value lying
- 57 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, 58 repeatability, n = 21).
- 59 Functional sensitivity
- 60 0.3 mg/L (2.96 nmol/L, 0.03 mg/dL)

The functional sensitivity is the lowest CRP concentration that can be reproducibly measured with an inter-assay coefficient of variation of < 10 %. Consensus reference interval for adults:30

IFCC/CRM 470

mg/dL	mg/L	nmol/L
< 0.5	< 5.0	< 47.6

The CDC/AHA recommended the following hsCRP cut-off points (tertiles) for CVD risk assessment:21,31

hsCRP level (mg/L)	hsCRP level (nmol/L)	Relative risk
< 1.0	< 9.52	low
1.0-3.0	9.52-28.6	average
> 3.0	> 28.6	high

Patients with higher hsCRP concentrations are more likely to develop myocardial infarction and severe peripheral vascular disease. 5-95 % reference intervals of neonates and children:32

Neonates (0-3 weeks): 0.1-4.1 mg/L (0.95-39.0 nmol/L)

Children (2 months-15 years): 0.1-2.8 mg/L (0.95-26.7 nmol/L)

It is important to monitor the CRP concentration during the acute phase of the illness.

Roche has not evaluated reference ranges in a pediatric population. Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Increases in CRP values are non-specific and should not be interpreted without a complete clinical history.

When using hsCRP to assess the risk of coronary heart disease, measurements should be made on metabolically stable patients and compared to previous values. Optimally, the average of hsCRP results repeated two weeks apart should be used for risk assessment. Measurements should be compared to previous values. When the results are being used for risk assessment, patients with persistently unexplained hsCRP levels of above 10 mg/L (95.2 nmol/L) should be evaluated for non-cardiovascular origins. Testing for any risk assessment should not be performed while there is indication of infection, systemic inflammation or trauma.21

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	Mean	SD	CV
	mg/L (nmol/L, mg/dL)	mg/L (nmol/L, mg/dL)	%
Precinorm Protein	9.00 (85.7, 0.900)	0.10 (1.0, 0.010)	1.2
CRP T Control N	4.34 (41.3, 0.434)	0.04 (0.4, 0.004)	1.0
Human serum 1	15.9 (151, 1.59)	0.1 (1, 0.01)	0.4
Human serum 2	0.54 (5.14, 0.054)	0.01 (0.10, 0.001)	1.6
Intermediate	Mean	SD	CV
Intermediate precision	Mean mg/L (nmol/L, mg/dL)	SD mg/L (nmol/L, mg/dL)	CV %
precision	mg/L (nmol/L, mg/dL)	mg/L (nmol/L, mg/dL)	%
precision Precinorm Protein	mg/L (nmol/L, mg/dL) 9.06 (86.3, 0.906)	mg/L (nmol/L, mg/dL) 0.11 (1.1, 0.011)	% 1.3

Method comparison

CRP values for human serum and plasma samples obtained on a Roche/Hitachi cobas c 501 analyzer (y) were compared with those 0004628918190c501V12.0

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ana	ermined using the correspo alyzer (x). nple size (n) = 192	nding reagent on a Roche/Hitachi	917 2	20	Lindahl B, Toss H, and Inflammation in Coronary Artery Dis
	ssing/Bablok ³³	Linoar rogrossion		21	Pearson TA, Mens
	0.992x + 0.254 mg/L	Linear regression y = 0.946x + 0.514 mg/L			Inflammation and C Public Health Pract
-	0.944	y = 0.940x + 0.514 mg/L r = 0.996			the Centers for Dis Heart Association.
The	e sample concentrations we 7 mg/L (4.76 and 188 nmol/	re between 0.500 and	2	22	Price CP, Trull AK, particle-enhanced t
Re	ferences		,	22	Immunol Methods
1 2	Methods. Vol II. Philadelpi	gnosis and Management by Labora nia, Pa: WB Saunders 1979. eds. Lehrbuch der Klinischen Cher	atory	20	Microparticle-Enha Superior Features i Lab Anal 1998;12:1
2		Stuttgart/New York: Schattauer Ver		24	Use of Anticoagula Publication WHO/E
3	Entzündung. Lab med 199		-	25	Baudner S, Bienve matrix reference m 14 human serum p
4	Pathology 1991;23:118-12	ps AW. C-reactive protein: A critica	ai leview.		1993;1-186.
5	WB Saunders Co; 1995.	o Laboratory Tests. 3rd ed. Philade		26	Glick MR, Ryder KV Interferences in Cli 1986;32:470-475.
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7		allimore JR, et al. The prognostic v n amyloid A protein in severe unsta 4:331:417-424.		28	Sonntag O, Schole recommendation of interference studies
8		en J, et al. Relation of c-reactive p the MRFIT nested case control stu		29	Bakker AJ, Mücke assays: mechanisr Clin Chem Lab Me
9	Ridker PM, Glynn RJ, Her to the Predictive Value of	nekens CH, et al. C-Reactive Prot Total and HDL Cholesterol in Dete	rmining	30	Dati F, Schumann professional societ
10	Ridker PM, Cushman M, S	farction. Circulation 1998;97:2007- Stampfer MJ, et al. Plasma Concer sk of Developing Peripheral Vascu	ntration of Ilar		interim reference ra standardization aga 470). Eur J Clin Ch
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	Other Markers of Inflamma Disease in Women. N Eng	ation in the Prediction of Cardiovas I J Med 2000;342(12):836-843.	cular	Αp	for method compar Chem Clin Biocher oint (period/stop) is
14		saty BM, et al. Relationship of C-R ascular Disease in the Elderly. Arte ?:1121-1127.	erioscler	sep a de	aratör to mark the b ecimal numeral. Sep
15	Reactive Protein Levels an	A, Hakanen M, et al. Elevated Seru nd Early Arterial Changes in Health omb Vasc Biol, (August) 2002;1323	nn C- ny I R-1328 t	Roc	nbols che Diagnostics use se listed in the ISO
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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

Contents of kit

Volume after reconstitution or mixing



Cardiac C-Reactive Protein (Latex) High Sensitive

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES

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Roche Diagnostics, Indianapolis, IN US Customer Technical Support 1-800-428-2336 Roche





Elecsys Folate III

cobas®

		\sim	Σ	
	REF		\swarrow	SYSTEM
				cobas e 411
	07559992190	07559992500	100	cobas e 601
				cobas e 602
	English		 1st incubation: By 	y incubating 25 μ L of sample with the folate
	System information			gents 1 and 2, bound folate is released from
	For cobas e 411 analyzer: test nu	imber 1520	5	e binding proteins.
I	For cobas e 601 and cobas e 602 Number 721	2 analyzers: Application Code	labeled folate bin	Ay incubating the pretreated sample with the ruther ding protein, a folate complex is formed, the amout ant upon the analyte concentration in the sample.
	Intended use			fter addition of streptavidin-coated microparticles a
	Binding assay for the in vitro quan serum and plasma.	titative determination of folate in human	folate labeled with	h biotin, the unbound sites of the ruthenium labele
	The binding assay is intended for	use on Flecsus and cohas e		tein become occupied, with formation of a rutheniu ding protein-folate biotin complex. The entire comp
	immunoassay analyzers.	allo on Elousys and Cobas C	becomes bound t	to the solid phase via interaction of biotin and
	Summary		streptavidin.	
	Folate belongs to the family of B-g	group vitamins composed of an aromatic	 The reaction mixt 	ure is aspirated into the measuring cell where the
	pteridine ring linked through a met	thylene group to p-aminobenzoic acid and		e magnetically captured onto the surface of the nd substances are then removed with
	a glutamate residue. Folate (folic a and plays an essential role in nucl	acid) is vital for normal cellular functions		1. Application of a voltage to the electrode then inc
	regeneration, shuttling and redox	reactions of one-carbon units required for		t emission which is measured by a photomultiplier
	normal metabolism and regulation			mined via a calibration curve which is instrument-
	The folate metabolism can be exe	mplified as a cycle, where folate on units from one molecule to another	via the readent b	ated by 2-point calibration and a master curve pro arcode or e-barcode.
	required in various biochemical re-	actions: for example, tetrahydrofolate	Reagents - working	
	(THF) accepts a single carbon uni	t from serine, which is reduced in a		k (M, R1, R2) and the pretreatment reagents (PT1
	methyl group to homocysteine wh	ydrofolate (5-MTHF). 5-MTHF gives its nich is - with involvement of methionine	PT2) are labeled as l	Fol III.
	synthase and vitamin B12 - enzym	natically converted to methionine. The	PT1 Pretreatment n	eagent 1 (white cap), 1 bottle, 4 mL:
	resulting THF starts again the cycl	le of methyl group synthesis.From 🛛 🦯		captoethanesulfonate (MESNA) 40 g/L, pH 5.5.
		e transferred to S-adenosylmethionine group donor in several methylation		
	reactions, like DNA, RNA and prot			eagent 2 (gray cap), 1 bottle, 5 mL:
		nsitive to folate deficiency: with a low	Sodium hydrox	-
	and this results in increased homo	to re-methylate homocysteine is impaired ocysteine concentrations in plasma. ²	M Streptavidin-co 6.5 mL:	pated microparticles (transparent cap), 1 bottle,
	precursors of nucleic acids. Altere	in the synthesis of purine and pyrimidine d distribution of methyl groups and	Streptavidin-co	pated microparticles 0.72 mg/mL; preservative.
	impaired DNA synthesis play an e cancers. Abnormal folate status ba	ssential role in the development of as also been linked with the development	R1 Folate binding	protein~Ru(bpy) $_{3}^{2+}$ (gray cap), 1 bottle, 9 mL:
	of diseases like cardiovascular dis	eases, neural tube defects, cleft lip and		eled folate binding protein 75 µg/L; human serum
	palate, late pregnancy complication disorders. ^{1,2}	ons, neurodegenerative and psychiatric	albumin (stabil	izer); borate/phosphate/citrate buffer 70 mmol/L,
	Folate belongs to the group of ess	sential vitamins, i.e. it cannot be	pH 5.5; preser	vative.
	synthesized by the human organis	sm and therefore must be absorbed from	R2 Folate~biotin (black cap), 1 bottle, 8 mL:
	diet. Primary sources of folates are fruits, brewer's yeast and liver. ^{1,2}	e green and leafy vegetables, sprouts,	Biotinylated fol	ate 17 μg/L; biotin 120 μg/L; human serum albumi
	-	by decreased nutritional intake, poor		rate buffer 100 mmol/L, pH 9.0; preservative.
	absorption of ingested folate in the	e intestine or increased demand of folate,	Precautions and wa	arninas
	for example during physical activit	y or pregnancy. Deficiency of folate can	For in vitro diagnosti	-
	also be a result of liver diseases o genetic defects or drug interaction	r impaired folate metabolism due to s. ²	Exercise the normal	precautions required for handling all laboratory
	• •	ate and vitamin B12 deficiency is the so	reagents. Disposal of all waste	material should be in accordance with local guide
	called megaloblastic (macrocytic)	anemia: due to the affected DNA	Safety data sheet av	ailable for professional user on request.
	synthesis and cell maturation, esp	ecially involving the cells of rythrocytes is significantly reduced. The		ponents classified as follows in accordance with the
	hemoglobin synthesis capacity ho	wever is normal, which leads to	Regulation (EC) No.	
	abnormally large erythrocyte preci	ursors ("macrocytes" or "megaloblasts"),	2-methyl-2H-isothiaz	ol-3-one hydrochloride
	•	bin content ("hyperchromic anemia"). ^{3,4}	EUH 208 May p	produce an allergic reaction.
	one-carbon unit metabolism, and a	re closely interrelated in the cellular also hematologic and clinical	^	
	consequences of the two vitamin of	deficiency states might be similar, it is	Par	
	advisable to determine both paran relevant symptoms of vitamin defin	neters simultaneously in patients with the	<u>₩</u> ₹	
			$\mathbf{\vee}$	
	Test principle		Dangar	
	Competition principle. Total duration	on of assay: 27 minutes	Danger	

Elecsys Folate III

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H314 Prevention:	Causes severe skin bu	irns and eye damage.			
P280	Wear protective gloves face protection.	s/ protective clothing/ eye protection/			
Response:	·				
P301 + P330 + P331	IF SWALLOWED: Rins	se mouth. Do NOT induce vomiting.			
P303 + P361 + P353	IF ON SKIN (or hair): 1 clothing. Rinse skin wit	Fake off immediately all contaminated th water.			
P304 + P340 IF INHALED: Remove person to fresh air and keep P310 comfortable for breathing.					
Immediately call a POISON CENTER/ doctor. P305 + P351 IF IN EYES: Rinse cautiously with water for several + P338 minutes. Remove contact lenses, if present and easy to c Continue rinsing. Immediately call a POISON CENTER/ doctor.					
P390	Absorb spillage to prev	vent material damage.			
	/ labeling follows EU GH				
Contact phone	e: all countries: +49-621	-7590			
All human ma	terial should be conside	red potentially infectious. All products			
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All human ma derived from h donors tested to HCV and H	terial should be conside numan blood are prepare individually and shown IV. The testing methods	ed exclusively from the blood of to be free from HBsAg and antibodies used assays approved by the FDA or			
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Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin plasma. Li-heparin plasma tubes containing separating gel can be used.

Criterion: Method comparison serum versus Li-heparin plasma, slope 0.9-1.1 + intercept within < \pm 2x Limit of Blank (LoB), coefficient of correlation \ge 0.95.

Serum: Stable for 2 hours at 15-25 °C, 48 hours at 2-8 °C, 28 days at -20 °C (\pm 5 °C). Freeze only once. Protect from light. Store the samples at 2-8 °C if they cannot be measured immediately.

Li-heparin plasma: Stable for 2 hours at 15-25 °C, 48 hours at 2-8 °C, 28 days at -20 °C (\pm 5 °C). Freeze only once. Protect from light. Store the samples at 2-8 °C if they cannot be measured immediately.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Samples should not subsequently be altered with additives (biocides, anti-oxidants or substances possibly changing the pH of the sample) in order to avoid erroneous folate recovery.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples.

Ensure the samples, calibrators and controls are at 20-25 $^\circ\mathrm{C}$ prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Note: Hemolysis may significantly increase folate values due to high concentrations of folate in red blood cells. Therefore, hemolyzed samples are not suitable for use in this assay. Samples for folate determinations should be collected from fasting persons.

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

- REF 07560001190, Folate III CalSet, for 4 x 1.0 mL
- REF 05618860190, PreciControl Varia, for 4 x 3.0 mL
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment

cobas e analyzer

- Additional materials for the cobas e 411 analyzer:
- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials for cobas e 601 and cobas e 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M
- Additional materials for all analyzers:

Elecsys Folate III



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6	 REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution
7	Assay
8	For optimum performance of the assay follow the directions given in this
9	document for the analyzer concerned. Refer to the appropriate operator's
10 I	manual for analyzer-specific assay instructions.
11	Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in
12	exceptional cases the barcode cannot be read, enter the 15-digit sequence
13	of numbers.
14	cobas e 601 and cobas e 602 analyzers: PreClean M solution is necessary.
15	Bring the cooled reagents to approximately 20 °C and place on the reagent
16	disk (20 °C) of the analyzer. Avoid foam formation. The system
17 18	automatically regulates the temperature of the reagents and the opening/closing of the bottles.
18	Calibration
20	Traceability: This method has been standardized against the WHO
21	International Standard NIBSC code: 03/178.
22	Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined
23	master curve is adapted to the analyzer using the relevant CalSet.
24	Calibration frequency: Calibration must be performed once per reagent lot
25	using fresh reagent (i.e. not more than 24 hours since the reagent kit was
26	registered on the analyzer). Calibration interval may be extended based on acceptable verification of
27	calibration by the laboratory.
28	Renewed calibration is recommended as follows:
29	 after 1 month (28 days) when using the same reagent lot
30	 after 7 days (when using the same reagent kit on the analyzer)
31	 as required: e.g. quality control findings outside the defined limits
32 33	Quality control
33 34	For quality control, use PreciControl Varia.
35	In addition, other suitable control material can be used.
36	Controls for the various concentration ranges should be run individually at
37	least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.
38	The control intervals and limits should be adapted to each laboratory's
39	individual requirements. Values obtained should fall within the defined
40	limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.
41	If necessary, repeat the measurement of the samples concerned.
42	Follow the applicable government regulations and local guidelines for
43	quality control.
44	Calculation
45	The analyzer automatically calculates the analyte concentration of each
46 47	sample (either in nmol/L or ng/mL).
47 48	Conversion factors: nmol/L x 0.44 = ng/mL
48 49	ng/mL x 2.27 = nmol/L
50	Limitations - interference
51	The assay is unaffected by icterus (bilirubin \leq 496 µmol/L or \leq 29 mg/dL),
52	lipemia (Intralipid \leq 1500 mg/dL), biotin (\leq 86.1 nmol/L or \leq 21 ng/mL), IgG
53	\leq 16 g/L, IgA \leq 4.0 g/L and IgM \leq 10 g/L. Criterion: Recovery within \pm 10 % of initial value with samples > 4 ng/mL
54	and $\leq \pm 0.4$ ng/mL with samples ≤ 4 ng/mL.
55	Hemolysis may significantly increase folate values due to high
56	concentrations of folate in red blood cells. Therefore, hemolyzed samples are not suitable for use in this assay.
57	Samples should not be taken from patients receiving therapy with high
58	biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin
59	administration.
60	No interference was observed from rheumatoid factors up to a concentration of 1000 IU/mL.

In vitro tests were performed on 16 commonly used pharmaceuticals and in addition on human erythropoietin. No interference with the assay was found.

It is contraindicated to measure samples of patients receiving therapy with certain pharmaceuticals, e.g. methotrexate or leucovorin, because of the cross-reactivity of folate binding protein with these compounds.

Samples with extremely high total protein concentrations (hyperproteinemia) are not suitable for use in this assay. Hyperproteinemia may be caused by, but not limited to, the following conditions: Lymphoma^{7,8}, bone marrow disorders such as multiple myeloma, monoclonal gammopathy of undetermined significance (MGUS), Waldenström macroglobulinemia, plasmocytoma^{7,8,9,10,11,12,13}, Amyloidosis^{13,14}. Respective samples may lead to the formation of protein gel in the assay cup, which may cause a run abort. The critical total protein concentration is dependent upon the individual sample composition.

In rare cases, interference due to extremely high titers of antibodies to streptavidin and ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with RBC folate, the patient's medical history, clinical examination, and other findings.

Limits and ranges

Measuring range

0.6-20.0 ng/mL or 1.36-45.4 nmol/L (defined by the Limit of Blank and the maximum of the master curve). Values below the Limit of Blank are reported as < 0.6 ng/mL (< 1.36 nmol/L). Values above the measuring range are reported as > 20.0 ng/mL (> 45.4 nmol/L).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.6 ng/mL (1.36 nmol/L)

Limit of Detection = 1.2 ng/mL (2.72 nmol/L)

Limit of Quantitation = 2.0 ng/mL (4.54 nmol/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from $n \ge 60$ measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative error of ≤ 20 %.

It has been determined using low concentration folate samples.

Dilution

Samples with folate concentrations above the measuring range can be diluted manually with Diluent Universal. The recommended dilution is 1:2. The concentration of the diluted sample must be > 8.5 ng/mL or > 19.3 nmol/L. After manual dilution, multiply the results by the dilution factor 2.

Expected values

Referring to "The American Journal of Clinical Nutrition"¹⁵ serum folate (folic acid) values were found as follows:

Sex	Age	N	Median		2.5th-97.5th percentile	
	years		ng/mL nmol/L		ng/mL	nmol/L
Both	all	23345	13.0	29.5	4.6-34.8	10.4-78.9
Male	all	11387	12.3	27.9	4.5-32.2	10.2-73.0
Female	all	11958	13.6	30.1	4.8-37.3	10.9-84.5
Both	4-11	3595	17.2	39.0	8.6-37.7	19.5-85.4
Both	12-19	6390	12.1	27.4	5.0-27.2	11.3-61.6
Both	20-59	8689	11.6	26.3	4.4-31.0	10.0-70.2

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Sex	Age	Ν	Med	dian	2.5 th -97.5 th	percentile		
	years		ng/mL nmol/L		ng/mL	nmol/L		
Both	≥ 60	4671	16.6	37.6	5.6-45.8	12.7-103.8		
These values were obtained in the USA during the National Health and								

Nutrition Examination Survey (NHANES), 1999-2004.

The values shown below were performed on samples from an apparently healthy population, using the Elecsys Folate III assay.

The calculation is based on 404 sera (177 men, 227 women). The age range was between 20 and 65 years. Pregnant or lactating women were excluded. The reference population was selected according to normal homocysteine values.

Ν	Mee	dian	2.5 th -97.5 th percentile		
	ng/mL nmol/L		ng/mL	nmol/L	
404	8.94 20.3		3.89-26.8	8.83-60.8	

Please note: These values should only be used as a guideline.

It should be taken into consideration that differences in the expected values may exist with respect to population and dietary status.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Folate deficient sample values

25 samples considered to be deficient^{a)} in serum folate concentration were assessed using the Elecsys Folate III assay. All samples were found to be below the 2.5th percentile as given in the table above.

a) Folate deficiency was assessed by measurement of serum folate by two commercially available folate assays.

Specific performance data

Representative performance data on the analyzers are given below.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84).

cobas e 411 analyzer							
		Repeatability		Interme precis			
Sample	Mean	SD	CV	SD	CV		
	ng/mL	ng/mL	%	ng/mL	%		
Human serum 1	1.88	0.150	8.0	0.205	10.9		
Human serum 2	3.92	0.200	5.1	0.318	8.1		
Human serum 3	11.9	0.346	2.9	0.571	4.8		
Human serum 4	13.4	0.301	2.2	0.574	4.3		
Human serum 5	17.8	0.440	2.5	0.666	3.7		
PreciControl Varia1	3.24	0.215	6.6	0.309	9.5		
PreciControl Varia2	11.6	0.314	2.7	0.566	4.9		

cobas e 411 analyzer								
		Repeata	bility	Interme precis				
Sample	Mean	SD	CV	SD	CV			
	nmol/L	nmol/L	%	nmol/L	%			
Human serum 1	4.27	0.341	8.0	0.465	10.9			
Human serum 2	8.90	0.454	5.1	0.722	8.1			
Human serum 3	27.0	0.785	2.9	1.30	4.8			
Human serum 4	30.4	0.683	2.2	1.30	4.3			

cobas e 411 analyzer							
		Repeata	ability	Interme precis			
Sample	Mean	SD	CV	SD	CV		
	nmol/L	nmol/L	%	nmol/L	%		
Human serum 5	40.4	0.999	2.5	1.51	3.7		
PreciControl Varia1	7.35	0.488	6.6	0.701	9.5		
PreciControl Varia2	26.3	0.713	2.7	1.28	4.9		

cobas e 601 and cobas e 602 analyzers								
		Repeatability		Intermediate precision				
Sample	Mean	SD	CV	SD	CV			
	ng/mL	ng/mL	%	ng/mL	%			
Human serum 1	1.66	0.255	15.4	0.268	16.1			
Human serum 2	4.10	0.219	5.4	0.303	7.4			
Human serum 3	11.1	0.449	4.1	0.503	4.6			
Human serum 4	12.2	0.454	3.7	0.467	3.8			
Human serum 5	16.4	0.502	3.1	0.625	3.8			
PreciControl Varia1	2.34	0.189	8.1	0.228	9.8			
PreciControl Varia2	10.1	0.443	4.4	0.489	4.9			

cobas e 601 and cobas e 602 analyzers

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			Intermediate precision			
Mean	SD	CV	SD	CV		
nmol/L	nmol/L	%	nmol/L	%		
3.77	0.579	15.4	0.608	16.1		
9.31	0.497	5.4	0.688	7.4		
25.2	1.02	4.1	1.14	4.6		
27.7	1.03	3.7	1.06	3.8		
37.2	1.14	3.1	1.42	3.8		
5.31	0.429	8.1	0.518	9.8		
22.9	1.01	4.4	1.11	4.9		
	nmol/L 3.77 9.31 25.2 27.7 37.2 5.31	Mean SD nmol/L nmol/L 3.77 0.579 9.31 0.497 25.2 1.02 27.7 1.03 37.2 1.14 5.31 0.429	nmol/L nmol/L % 3.77 0.579 15.4 9.31 0.497 5.4 25.2 1.02 4.1 27.7 1.03 3.7 37.2 1.14 3.1 5.31 0.429 8.1	Mean SD CV SD nmol/L nmol/L % nmol/L 3.77 0.579 15.4 0.608 9.31 0.497 5.4 0.688 25.2 1.02 4.1 1.14 27.7 1.03 3.7 1.06 37.2 1.14 3.1 1.42 5.31 0.429 8.1 0.518		

Method comparison

a) A comparison of the Elecsys Folate III assay (traceable to WHO IS 03/178; y) and the Elecsys Folate III assay prior to standardization against WHO IS 03/178 (x) using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 113

Passing/Bablok ¹⁶	Linear regression
y = 1.14x - 1.97	y = 1.11x - 1.77
$\tau = 0.939$	r = 0.994

The sample concentrations were between 2.1 and 18 ng/mL (4.8 and 41 nmol/L).

b) A comparison of the Elecsys Folate III assay (y) with a commercially available method (x) using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 106

Passing/Bablok ¹⁶	Linear regression
y = 0.980x - 0.095	y = 1.09x - 0.659
т = 0.924	r = 0.984
The sample concentrations were bet	veen 1 9 and 17 ng/ml (4 3

concentrations were between 1.9 and 17 ng/mL (4.3 and 39 nmol/Ĺ).

Results obtained in individual laboratories may differ. The following results were obtained:

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Elecsys Folate III

c) A comparison of the Elecsys Folate III assay on the cobas e 601 analyzer (y) with the Elecsys Folate III assay on the cobas e 411 analyzer (x) using clinical samples gave the following correlations (ng/mL): Number of samples measured: 105

Passing/Bablok ¹⁶	Linear regression
y = 1.05x - 0.303	y = 0.981x + 0.143
т = 0.868	r = 0.982

The sample concentrations were between 1.6 and 19 ng/mL (3.6 and 43 nmol/L).

Analytical specificity

The following cross-reactivities were found, tested with folate concentrations of approximately 3.5 ng/mL, 10 ng/mL and 19 ng/mL.

Cross-reactant	Concentration tested ng/mL	Cross-reactivity %
Amethopterin	750	2.5
Aminopterin	750	4.4
Folinic acid	750	0.7

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- For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
\longrightarrow	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

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Data collection tool Household Listing Form

FNS/1

FNS/1							
FNS EA no							
Serial no of	Address	Residence	Serial no	Name of HH	Occupie	Serial no	Remark(mark "X" fo
structure	description of		House/HH	head	d	occupied	selected HH)
	structure	(Y/N)	in the		(Y/N)	EA	
			structure				
					-		
					-		
			\mathbf{n}				
	1	1		1		1	1

Annex 2: Nutrition direct and indirect interventions/indicators assessment questionnaire <u>Nutrition direct and indirect Interventions Questionnaire</u>

Module 1: Household identifier, characteristics and socio-demographic status

Househo	old identifier and character	istics						
101	Region Code							
102	Woreda Code							
103	Kebele Code	_ _						
104	Gote Code							
105	Household Code					GPS Coordinates		
			<u>_ </u> _	1	1			
106	Unique Household Code							
		Region Code	Woreda Code	Kebele Code	EA Code	Household Code		
107	Residence	1 = Urban 2 = Rural						
108	Do you own this house?	1 = Yes 0 = No						
109	What is the main material of the walls? Observe	1 = No walls 2 = Natural r 3 = Stone wi 4 = Stone/br						
		99 = Other						
110	What is the main floor material? Observe	1 = Natural floor (earth/sand/dung) 2 = Rudimentary floor (wood/palm/bamboo)3=Finished floor (polished wood/ vinyl/3=Finished floor (polished wood/ vinyl/tiles/cement/carpet)99 = Other						
111	What is the main material of the roof? Observe	1 = Thatch/g 2 = Iron shee 99 = Other						
112		1 = Dung 2 = Firewood 3 = Charcoal 4 = Kerosene 5 = Gas (met 6 = Electricit 99 = Other						
113	Is the house connected	1 = Yes						
11/	to electricity? In total, how many of the	0 = No A kerosene lamp/pressure lamp						
	following items are	Mobile phor		eiailip				
	owned by residents of	Cart						
110	•	Bicycle						
117								
118	Add the household total	Motorcycle al Radio _						
119	for each item	Television						
120								

121		Car/tractor/Bajaj	
122	Does this household own	1 = Yes	
	any livestock, herds,	0 = No (Go to→131)	
	other farm animals, or		
	poultry?		
123	In total, how many of the	Milk cows, oxen or bulls?	
124	following animals are	Other cattle?	
125		Horses, donkeys, or mules?	
126	this household?	Camels	
127	Add the household total	Goats?	
128	for each item	Sheep?	
129		Chickens or other poultry?	
130		Beehives?	
131	Does any member of this	1 = Yes	
	household own any	0 = No	
	agricultural land?		
132	How often does anyone	1 = Daily	
	smoke inside your	2 = Weekly	
	house?	3 = Monthly	
		4 = Less once monthly	
	Would you say daily,	5 = Never	
	weekly, monthly, less		
	often than once a month,	4	
	•		
	or never?		
ouseh	or never? old head socio-demographi	c status	
		c status	
133	old head socio-demographi	c status 1. Single	
133	old head socio-demographi Age in years	1. Single 2. Married	
133	old head socio-demographi Age in years	1. Single 2. Married 3. Divorced	
133	old head socio-demographi Age in years	1. Single 2. Married	
133	old head socio-demographi Age in years	1. Single 2. Married 3. Divorced	
133 134	old head socio-demographi Age in years	 Single Married Divorced Separated 	
133 134	old head socio-demographi Age in years Marital status	1. Single 2. Married 3. Divorced 4. Separated 5. Widowed 1. None	
133 134	old head socio-demographi Age in years Marital status What is the highest level	 Single Married Divorced Separated Widowed None Primary Secondary 	
133 134	old head socio-demographi Age in years Marital status What is the highest level of school the head of the	1. Single 2. Married 3. Divorced 4. Separated 5. Widowed 1. None 2. Primary	
133 134	old head socio-demographi Age in years Marital status What is the highest level of school the head of the	 Single Married Divorced Separated Widowed None Primary Secondary Technical/vocational certificate Higher / university/ college 	
133 134	old head socio-demographi Age in years Marital status What is the highest level of school the head of the	 Single Married Divorced Separated Widowed None Primary Secondary Technical/vocational certificate 	
133 134 135	old head socio-demographi Age in years Marital status What is the highest level of school the head of the household completed?	 Single Married Divorced Separated Widowed None Primary Secondary Technical/vocational certificate Higher / university/ college 98.Don't know 99.Other (specify) 	
133 134 135	old head socio-demographi Age in years Marital status What is the highest level of school the head of the	 Single Married Divorced Separated Widowed None Primary Secondary Technical/vocational certificate Higher / university/ college 98.Don't know 99.Other (specify) 	
133 134 135	old head socio-demographi Age in years Marital status What is the highest level of school the head of the household completed?	 Single Married Divorced Separated Widowed None Primary Secondary Technical/vocational certificate Higher / university/ college 98.Don't know 99.Other (specify) 	
133 134 135	old head socio-demographi Age in years Marital status What is the highest level of school the head of the household completed? What is the religion of	 Single Married Divorced Separated Widowed None Primary Secondary Technical/vocational certificate Higher / university/ college 98.Don't know 99.Other (specify) Orthodox 	
133 134 135	old head socio-demographi Age in years Marital status What is the highest level of school the head of the household completed? What is the religion of	 Single Married Divorced Separated Widowed None Primary Secondary Technical/vocational certificate Higher / university/ college 98.Don't know 99.Other (specify) Orthodox Protestant 	
133 134 135	old head socio-demographi Age in years Marital status What is the highest level of school the head of the household completed? What is the religion of	 Single Married Divorced Separated Widowed None Primary Secondary Technical/vocational certificate Higher / university/ college 98.Don't know 99.Other (specify) Orthodox Protestant Catholic/ other Christian Muslim No religion 	
133 134 135	old head socio-demographi Age in years Marital status What is the highest level of school the head of the household completed? What is the religion of	 Single Married Divorced Separated Widowed None Primary Secondary Technical/vocational certificate Higher / university/ college 98.Don't know 99.Other (specify) Orthodox Protestant Catholic/ other Christian Muslim 	
133 134 135	old head socio-demographi Age in years Marital status What is the highest level of school the head of the household completed? What is the religion of	 Single Married Divorced Separated Widowed None Primary Secondary Technical/vocational certificate Higher / university/ college 98.Don't know 99.Other (specify) Orthodox Protestant Catholic/ other Christian Muslim No religion 	

about	each separately, starting with the youngest.		
201	Child's code		1
202	Mother's name	Mother's given name	
203	Mother's age		
204	Mother's education level	1=None	
-•·		2=Primary	
		3=Secondary	
		4=Technical/vocational certificate	
		5=Higher / university/ college	
		98=Don't know	
		99. Other (specify)	
205	Mother's marital status	1=Single	
		2 =Married	
		3=Divorced	
		4 =Separated	
		5=Widowed	
206	Mother's religion	1=Orthodox	
200		2=Protestant	
		3=Catholic/ other Christian	
		4=Muslim	
		5=No religion	
		98=Don't know	
		99=Other religion (specify)	
207	Mother's ethnicity	Specify	
208	Child's name	Child's given name	
209	Child (NAME) sex	1 = Boy	Ι.
		2 = Girl	
210	Child (NAME) age?	Age in months or age at the time of	
		the child's death	
211	In the last six months, was (NAME) given	1 = Yes	
	any vitamin A supplement?	0 = No	
		98 = Don't know	
212	When was the child (NAME) given the vitamin A supplement?	Specify	
213	In the last 12 months, was (NAME) given any iron	1 = Yes	
-	tablet or syrup or supplement?	0 = No	'
	- · / · F - · · F F - · · · F	98 = Don't know	
214	In the last 6 months, was (name) given any	1 = Yes	
-	medicine for intestinal worms?	0 = No	1
		98 = Don't know	'
215	In the last 3 months, has any healthcare provider	1 = Yes, 0 = No, 98 = Don't know	1
-	measured?	Weight	1
		Height/length	<u> </u>
		MUAC	<u> -</u>
216	Has (name) had diarrhea in the last 2 weeks?	1 = Yes	'
210		0 = No (Go to 224)	

		98 = Don't know	
217	Now I would like to know how much was the child	1 = Much less	
	given to drink during diarrhea, including breast	2 = Somewhat less	
	milk. Was the child given less than usual to drink,	3 = About the same	
	about the same amount, or more than usual to	4 = More	
	drink?	5 = Nothing to drink	
		98 = Don't know	
218	When the child had diarrhea, was he/she given	1 = Much less	
	less than usual to eat, about the same amount,	2 = Somewhat less	
	more than usual, or nothing to eat?	3 = About the same	
		4 = More	
		5 = Nothing to drink	
		98 = Don't know	
219	Did you seek advice or treatment for the diarrhea	1 = Yes	
-	from any source?	0 = No (Go to 221)	
220	Where did you seek advice or treatment?	1= Government hospital	
	Anywhere else?	2= Government health center	
		3= Government health post	
	Probe to identify the type of source.	4 = Mobile clinic	
	If unable to determine if public, private, or	5 = Community health worker/	
	NGO sector, record '21' and write the name	fieldworker	
	Of the place(s).	6 = Other public sector (specify)	
	Of the place(s).	7 = Private hospital	
		8 = Private clinic	
		9 = Pharmacy 10 = Private doctor	
		10 = Private doctor 11 = Mobile clinic	1
		12 = Community health worker/fieldworker	
		13 = Other private medical sector	
		(specify)	
		14 = NGO hospital	
		15 = NGO clinic	
		16 = Other NGO medical sector	
		(specify)	
		17 = Shop	
		18 = Traditional practitioner	
		19 = Market	
		20 = Itinerant drug seller	
		99 = Other (specify)	
	Was (name) given any of the following at any time	1 = Yes, 0 = No, 98 = Don't know	1
221	since (name) started having diarrhea:	Fluid from ORS packet	
222		Zinc	
223		Homemade fluid	
224	Has (name) been ill with a fever at any time in the	1 = Yes	
	last 2 weeks?	0 = No (Go to 226)	
		98 = Don't know	
225	Where did you seek advice or treatment for	1= Government hospital	

	fever?	2= Government health center	
	Anywhere else?	3= Government health post	
	,	4 = Mobile clinic	
	Probe to identify the type of source.	5 = Community health worker/	
	If unable to determine if public, private, or	fieldworker	
	NGO sector, record '21' and write the name	6 = Other public sector (specify)	
	Of the place(s).	7 = Private hospital	
		8 = Private clinic	1 1 11
		9 = Pharmacy	'''
		10 = Private doctor	
		11 = Mobile clinic	
		12 = Community health	
		worker/fieldworker	
		13 = Other private medical sector	
		(specify)	
		14 = NGO hospital	
		15 = NGO clinic	
		16 = Other NGO medical sector	
		(specify)	
		17 = Shop	
		18 = Traditional practitioner	
		19 = Market	
		20 = Itinerant drug seller	
		99 = Other (specify)	
226	Was Child (Name) ever breastfed?	1 = Yes	
		0 = No (Go 228)	
227	How many months the child (NAME) was breastfee		
Anthr	opometric and clinical nutrition assessment		
228	Weight	9	_
229	Height/length		
230	MUAC		
231	Presence of bilateral oedema for children 6-59	1 = Yes	<u> </u>
	months	0 = No	·
232	Bitot spot	1 = Yes	
	· ·	0 = No	

	hildren 0-23 months		<u> </u>
	module is to be administered to the mother/care	-	
	with respondents. Verify that the respondent yo		giver of the child
301	Was Child (Name) ever breastfed?	1 = Yes	
		0 = No (Go to 304)	
202		98 = Don't know	
302	Was Child (NAME) given the first milk	1 = Yes	
	(colostrum) after birth?	0 = No	
		98 = Don't know	
303	How long after birth did you first put (NAME)	1 = Immediately after birth, or	
	to the breast, even if your breast milk did not	within 1 hour	
	arrive?	2 = Between 1 and 24 hours	
		3 = More than 24 hours after	''
		delivery	
		98 = Don't know	
304	Child (NAME) alive now?	1 = Yes	
		0 = No (Go to 401)	
305	Was (NAME) breastfed yesterday from	1 = Yes	
	sunrise until today sunrise?	0 = No (Go to 307)	
	NB: Breastfeeding could be by the mother	98 = Don't know	
	herself or by wet mother.		
306	Sometimes babies are fed breast milk in	1 = Yes	
	different ways, for example by spoon, cup or	0 = No	
	bottle. This can happen when the mother	98 = Don't know	
	cannot always be with her baby. Sometimes		
	babies are breastfed by another woman, or		
	given breast milk from another woman by		
	spoon, cup or bottle or some other way. This		
	can happen if a mother cannot breastfeed her		!
	own baby.		
	Did (NAME) consume breast milk in any of		
	these ways yesterday from sunrise until today		
	sunrise?		
307	Now I would like to ask you about some	1 = Yes	
	medicines and vitamins that are sometimes	0 = No	
	given to infants.	98 = Don't know	
	Was (NAME) given any vitamin drops or other		
	medicines as drops yesterday from sunrise		
202	until today sunrise?		
308	Was (NAME) given Lemlem or ORS in the last	1 = Yes	
	two weeks?	0 = No	
		98 = Don't know	1

	In the last 6 months, did any healthcare	1 = Yes	
	provider or community health worker talk	0 = No	
	with you about how and what to feed your	98 = Don't know	
	child?		
310	Now, I would like to ask you about some	Did (NAME) have any (item from	
	liquids that (NAME) may have had yesterday	list)?	
	from sunrise until today sunrise?	1 = Yes	
		0 = No (Go to 321)	''
	If yes to Q310, read the list of liquids starting	98 = Don't know	
311	with 'plain water'. Plain water	1 = Yes	
311		0 = No	II
		98 = Don't know	
312	Infant formula such as S-26?	1 = Yes	
712		0 = No (Go to 314)	''
		98 = Don't know	
313	How many times infant		
515	formula such as S-26?		''
314	Milk such as tinned,	1 = Yes	
	powdered, or fresh animal	0 = No (Go to 316)	''
	milk?	98 = Don't know	
315	How many times milk drink?		
316	Yogurt drink?	1 = Yes	
-		0 = No	''
		98 = Don't know	
317	Chocolate flavored drink?	1 = Yes	
	6	0 = No	
		98 = Don't know	
318	Sodas, malt drinks or energy	1 = Yes	
	drinks?	0 = No	
		98 = Don't know	
319	Clear broth or clear soup?	1 = Yes	_
		0 = No	
		98 = Don't know	
320	Any other liquids?	1 = Yes	
		0 = No	
	would like to ask you about foods that (NAME) h	98 = Don't know	1

			0 = No (Go to 342) 98 = Don't know
322	Yogurt, other than yogurt drink?		
	How many times did child (NAME) eat yogurt?		
324	Injera, bread, rice, noodles, pasta, macaroni, porridge	, or other foods made from	
325	grains such as tef, oats, maize, barley? Any commercially fortified baby food like Fafa, Hilina, Choice?	Cerilak, Cerifam, Mother	
	Pumpkin, carrots, squash, or sweet potatoes that are	yellow or orange inside?	
327	White potatoes, white yams, bulla, kocho, manioc, ca made from roots?		
	Any dark green leafy vegetables (kale, dark green lett	uce, moringa)?	
	Any other vegetable?	,	<u> </u>
	Ripe mangoes, ripe papayas (insert other local vitamir	n a-rich fruits)?	
	Any other fruit?		
	Liver, kidney, heart, or other organ meats?		
	Any meat, such as beef, pork, lamb, goat, chicken?		
34	Egg?		
35	Fresh or dried fish, shellfish, or seafood?		
36	Any foods made from beans, peas, lentils, nuts, or see	eds?	
37	Cheese or other food made from milk?		
	Any sugary foods such as chocolates, sweets, candies,		
	Any savory junk foods, such as crisps/chips/salted bise	cuits/instant noodles?	
	Any other solid, semi-solid, or soft food?		
	How many times did (NAME) eat solid, semi-solid,	Fill in the number of times.	
	or soft foods other than liquids yesterday during the	98 = Don't know	
	day or at night?		
	Did (NAME) drink anything from a bottle with a	1 = Yes	
	nipple yesterday during the day or night?	0 = No 98 = Don't know	

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Module 4: KAP of mothers or caregivers on children's care and feeding

-	oing to read you some knowledge question questions.	ons about breastfeeding. Please tell me y	our answers on
401	How long after birth should a baby	1 = Immediately, within 1 hour of	1 11 1
401	start breastfeeding?	delivery	IIII
	start breastreeung:	2 = Some hours later but within 24	
		hours	
		3 = After 1 day	
		4 = After 2 days	
		5 = After >3 days	
		6 = Does not think a baby should be	
		breastfed	
		98 = Don't know	
402	How long should a baby receive	1 = From birth to six months	
.02	nothing other than breast milk?	2 = Other	''
	nothing other than breast mik.	98 = Don't know	
403	How often should a baby younger	1 = On-demand, whenever the baby	
100	than six months be breastfed or fed	wants	··
	with breast milk?	2 = Other	
		98 = Don't know	
404	How much should a child be fed when	1 = Less frequent than usual	
	he/she is sick?	2 = Same as usual	''
		3 = More than usual	
		98 = Don't know	
405	How often should a child be fed when	1 = Less frequently than usual	
.00	he/she is sick?	2 = Same as usual	''
		3 = More frequently than usual	
		98 = Don't know	
406	At what age should a baby first start	Months of age (Specify)	
	to receive foods in addition to breast	98= Don't know	'''
	milk?		
407	At what age should children begin	Years of age (Specify)	
	observing fasting days if that is their	98=Don't know/remember	
	culture or religion?		
	(If <2 years, enter age in months.)		
408	Have you ever heard of child stunting?	1 = Yes	
		0 = No	
		98 = Don't know	
409	What age are children at the highest	Years of age (Specify)	
	risk of becoming stunted?	Months of age (Specify)	
		98 = Don't know/remember	
410	What are the consequences of	1 = Higher risk of severe infectious	
	stunting for young children?	diseases	
	Mark all that are mentioned by the	2 = Poor educational performance	
	respondent	3 = Weaker immune system	
		4 = Low adult wages	
		5 = Lost productivity	
		6 = Excessive weight gain in later life	

		 7 = Increased risk of nutrition-related chronic diseases in adult life 8 = Increased mortality rate 98= Don't know 99 = Other 	
411	Poor diet during pregnancy and the first two years of child age can cause child stunting	1 = Agree 2 = Do not agree 98 = Don't know	
mothe	oing to read you some statements about ers who live in a community like yours. Ple are no correct answers! I would like to kn	ease tell me if you agree with these state	
412	The colostrum (the "first yellowish milk") is not good for the baby and should be discarded	1 = Strongly disagree 2 = Disagree 3 = Agree somewhat 4 = Agree 5 = Strongly agree 98 = Don't know	
413	It is good to exclusively breastfeed give a baby only breast milk and no other foods or liquids for the first six months	 1 = Strongly disagree 2 = Disagree 3 = Agree somewhat 4 = Agree 5 = Strongly agree 98 = Don't know 	
414	If a child is sick (for example has fever/diarrhea) breastfeeding must be continued	1 = Strongly disagree 2 = Disagree 3 = Agree somewhat 4 = Agree 5 = Strongly agree 98 = Don't know	
415	A child should eat eggs, cow milk, or meat even on fasting days	1 = Strongly disagree 2 = Disagree 3 = Agree somewhat 4 = Agree 5 = Strongly agree 98 = Don't know	1111
416	Eating a meal from different food groups is not necessary until children are old enough to go to school	 1 = Strongly disagree 2 = Disagree 3 = Agree somewhat 4 = Agree 5 = Strongly agree 98 = Don't know 	IIII
417	It is good to feed a two years child at least four times each day	 1 = Strongly disagree 2 = Disagree 3 = Agree somewhat 4 = Agree 5 = Strongly agree 98 = Don't know 	
418	A mother should eat nutritious food	1 = Strongly disagree	

	(four) times daily from the time of pregnancy	2 = Disagree 3 = Agree somewhat 4 = Agree	
		5 = Strongly agree 98 = Don't know	
419	A mother should take iron folic acid tablets during pregnancy	 1 = Strongly disagree 2 = Disagree 3 = Agree somewhat 4 = Agree 5 = Strongly agree 98 = Don't know 	IIII
420	A mother should take iodized salt during pregnancy	 1 = Strongly disagree 2 = Disagree 3 = Agree somewhat 4 = Agree 5 = Strongly agree 98 = Don't know 	IIII
421	A mother should take de-worming medicines during pregnancy	 1 = Strongly disagree 2 = Disagree 3 = Agree somewhat 4 = Agree 5 = Strongly agree 98 = Don't know 	1111

4 - Agree 5 = Strongly agree 98 = Don't know

501	ssent (adolescent girls) form Girl's code	I	
502	Girl's name	Given name	
503	Girl's age		
504	Girl's education level	1=None	
		1=Primary	
		2=Secondary	
		3=Technical/vocational	
		certificate	
		98=Don't know	
		99=Other (specify)	
505	Girl's marital status	1 = Single	
		2 = Married	
		3 = Divorced	
		4 = Separated	
500	Cirl's religion	5 = Widowed 1=Orthodox	
506	Girl's religion		
		2=Protestant 3=Catholic/ other	
		Christian	
		4=Muslim	
		5=No religion	
		98=Don't know	
		Other religion (specify)	
507	Girl's ethnicity	specify	
508	Are you currently a student	1 = Yes	
500		0 = No	
509	Were you given any iron/foliate tablets at school or out of	1 = Yes	
	school? (<i>show the tablet</i>)	0 = No (Go to 511)	''
510	How many weeks per month have you taken the iron	Weeks per month	
	tablets?	(specify)	'''
		98 = don't know	
511	Were you given any drug for intestinal worms at school or	1 = Yes	
	out of school in the last six months?	0 = No	
512	Have you received any nutrition counseling in the last six	1 = Yes	
	months?	0 = No	
513	Did you receive nutritional assessment services in health	1 = Yes	
	facilities when you went for any kind of health service?	0 = No	
514	Is there any food taboo for adolescent girls in your	1 = Yes	II
	community?	0 = No (Go to 516)	
515	Mention types of food taboo?		
Anthro	ppometry and clinical nutrition assessment		
516	Weight (in kg)		
517	Height (in CM)		
518	Waist circumference (in CM)		

Module 5: Adolescent girls (10-19 Years)

519 Goite	<u>r</u>	1 = Yes, 0 = No	
		14	

Module 6: Reproductive age women (15-49 Years)
--

601	ently pregnant women Woman's code		
602	Woman's name	Given name	
603	Woman's age		
604	Woman's education level	1=None	
		2=Primary	
		3=Secondary	
		4=Technical/vocational certificate	
		5=Higher / university/ college	
		98=Don't know	
		99=Other (specify)	
605	Woman's marital status	1 = Single	
		2 = Married	
		3 = Divorced	
		4 = Separated	
		5 = Widowed	1
606	Woman's religion	1=Orthodox	
		2=Protestant	
		3=Catholic/ other Christian	
		4=Muslim	
		5=No religion	
		98=Don't know	
		99=Other religion (specify)	
607	Woman's ethnicity	Specify	
608	What was your age at first marriage	Year (specify)	
		96=Not applicable	
		98=Don't know	
609	What was your age at your first pregnancy	Year (specify)	
		96=Not applicable	
		98=Don't know	
610	Were you pregnant in the last 3 years?	1= Yes	
		0=No (Go to 629)	
611	Did you see anyone for antenatal care for the	1 = Yes	
	pregnancy?	0 = No (Go to 629)	<u> </u>
612	Whom did you see for antenatal care?	1=Health personnel	
	Probe to identify each type of person and	2 = Doctor	1
	record all	3 = Nurse	1
		4 = Midwife	1
		5= Health officer	
		6 = Health extension worker	
		7 = Other person	
		8 = Traditional birth	
		Attendant	
		99 = Other (specify)	
613	Where did you receive antenatal care for this	1= My home	
	pregnancy?	2 = Her home	
	Anywhere else?	3 = Other home	
		4 = Health center	

614	How many months pregnant were you when you first received antenatal care for this pregnancy?	5 = Government Hospital 6 = Private Clinic or Hospital 7 = Health post 8 = NGO clinic or hospital 99 = Other SPECIFY Months (Specify) 98 = Don't know	
615	How many times did you receive antenatal care during this pregnancy?	Number of times (specify) 98 = Don't know	
616	As part of your antenatal care during this pregnancy, were any of the following done at least once:	1 = Yes 0 = No Was your blood pressure measured?	I_
	least once.	Did you give a urine sample? Did you give a blood sample?	
	0,	Did a health care provider talk with you about which foods to eat while pregnant?	_ _
		Did a health care provider weigh you? Did a health care provider talk with	
		you about your weight? Did a health care provider talk with	_ _
617	During this pregnancy, did you ever receive food or cash assistance from government, an NGO, religious institution or other group?	you about breastfeeding? 1 = Yes 0 = No (Go to 619)	
618	What type of assistance did you receive?	1 = Cash only 2 = Food only 3 = Cash and food mix 99 = Other (specify)	
619	During this pregnancy, were you given or did you buy any iron tablets or iron syrup?	1 = Yes 0 = No (Go to 622) 98 = Don't Know (Go to 622)	
	show tablets/syrup/multiple micronutrient supplement	2/	
620	Where did you get the iron tablet or syrup from?	 1 = Govt. Health Facility 2 = Private Health Facility 3 = Mobile Clinic 4 = CHW 5 = [Mass Distribution Campaign – Add Local Name] 6 = Pharmacy 7 = Shop/Market 8 = School 98 = Don't Know 	1_1
		99= Other	
621	During this pregnancy, for how many days did you take the iron tablets?	Number of days (specify) 98 = Don't Know	

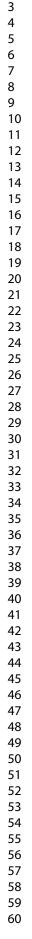
600	approximate number of days.		
622	During this pregnancy, did you take any drug	1 = Yes	_
	for intestinal worms?	0 = No	
		98 = Don't Know	
623	During this pregnancy, did any health care	1 = Yes	
	provider talk with you about breastfeeding?	0 = No	'''
		98 = Don't Know	
624	During this pregnancy, did you practice	1 = Yes	
024	fasting?	0 = No	' ' '
	Tusting:	98 = Don't Know	
625	At your last ANC visit, did the health provider	1 = Yes	
025		0 = No	
	weigh you?		
		98 = Don't Know	
626	During this pregnancy has your health	1 = Yes	
	provider given you information about your	0 = No (Go to 629)	
	weight gain?	98 = Don't Know	
627	During your pregnancy have you been thin for	1 = Yes	
	your height?	0 = No	
		98 = Don't Know	
628	Did you received treatment for malnutrition?	1 = Yes	
		0 = No	
nthrop	oometry		
629	Weight (in kg)		
630	Height (in CM)		
631	MUAC		
632	Waist circumference (in cm)		
633	Goiter	1 = Yes	
000		0 = No	
		0 - 110	

	v I'd like to ask you about foods and drinks that you ate or drank yesterday d	uring the day or night,
	ther you ate it at home or anywhere else.	
	erday, during the day or at night did you eat or drink:	r
S.N	Question	Response
		1 = Yes
		0 = No
701	Woman's code	
702	Woman's name	Given name
	Woman's age	
704	Woman's education level	0=None
		1=Primary
		2=Secondary
		3=Technical/vocationa
		certificate
		4=Higher / university/
		college
		98=Don't know
705		99=Other (specify)
705	Woman's marital status	1= Single
		2= Married
		3= Divorced
		4= Separated 5= Widowed
706	Woman's religion	1=Orthodox
/00		2=Protestant
		3=Catholic/other
		Christian
		4=Muslim
		5=No religion
		98=Don't know
		99=Other religion
		(specify)
707	Woman's ethnicity	Specify
708	Any vegetables or roots that are orange-colored inside, like: pumpkin,	
/00	carrots, squash, or sweet potatoes that are yellow or orange inside	!!
709	Any white roots and tubers or plantains, such as: white potatoes, white	
, 05	yams, manioc/cassava/yucca, cocoyam, taro or any other foods made	' '
	from white-fleshed roots or tubers, or plantains	
710	Any dark green leafy vegetables, such as: [list examples of any medium-to-	
•	dark green leafy vegetables including wild/foraged leaves]	''
711	Any fruits that are dark yellow or orange inside, like: ripe mango, ripe	
_	papaya	''
712	Any other fruits	
713	Any other vegetables	
714	Any meat made from animal organs, such as: liver, kidney, heart or other	
	organ meats or blood-based foods, including from wild game	
715	Any other types of meat or poultry, like: beef, pork, lamb, goat, rabbit,	
115		

716	Any eggs	
717	Any fish or seafood, whether fresh or dried	II
718	Any beans or peas, such as: mature beans or peas (fresh or dried seed),	II
	lentils, or bean/pea products, including hummus, tofu and tempeh	
719	Any nuts or seeds, like tree nut, groundnut/peanut, or certain seeds or nut/seed "butters" or pastes	II
720	Any milk or milk products, such as: milk, cheese, yoghurt or other milk	
/20	products, but not including butter, ice cream, cream or sour cream	II
721	Any oils and fats	I
722	Any savory and fried snacks, such as: crisps and chips, fried dough, other	
	fried snacks	
723	Any sweets, such as: sugary foods, such as chocolates, candies,	
	cookies/sweet biscuits and cakes, sweet pastries or ice cream	
724	Any sugar-sweetened beverages, like: sweetened fruit juices and "juice	
	drinks", soft drinks/fizzy drinks, chocolate drinks, malt drinks, yoghurt	
	drinks, sweet tea or coffee with sugar	
725	Any condiments and seasonings, such as: ingredients used in small	
	quantities for flavour, such as chilies, spices, herbs, fish powder, tomato	
	paste, flavor cubes or seeds	
726	Any other beverages and foods like tela, tej, bordea, arkea, cheka, tselo	_
727	Did you eat anything (meal or snack) OUTSIDE of the home yesterday?	I
728	Did you fast yesterday during the day or night?	

yesterday during the day or night:

No	Question	Answer	
		1 = Yes	
		0 = No	
801	Do you often have headaches?		I_
802	Is your appetite poor?		I
803	Do you sleep badly?		I_
804	Are you easily frightened?		I
805	Do your hands shake?		
806	Do you feel nervous, tense or worried?		
807	Is your digestion poor?		_
808	Do you have trouble thinking clearly?		I_
809	Do you feel unhappy?		I_
810	Do you cry more than usual?		I_
811	Do you find it difficult to enjoy your daily activities?		_
812	Do you find it difficult to make decisions?		
813	Is your daily work suffering?		_
814	Are you unable to play a useful part in life?		
815	Have you lost interest in things?		_
816	Do you feel that you are a worthless person?		_
817	Has the thought of ending your life been on your mind?		
818	Do you feel tired all the time?		I
819	Do you have uncomfortable feelings in your stomach?		
820	Are you easily tired?		



	Module 9: Women empowerme	n	
901	Identify the most senior mother of the mothers who have a		
	selected child. She is the mother who should respond to the		
	rest of this interview from this point.		
902	In the past major growing season (Meher) and minor growing		
	(Belg) season, not including the current season, did you work	1 = Yes	1 1
	on the family farm?	0 = No → Go to 913)	11
903	What sort of work you did on the family farm?	1 = Home (kitchen)	
505		gardening	1 1
904		2 = Fieldwork	
905		3 = Cash crop farming	
906		4 = Dairy processing	
907		5 = Poultry rearing	<u> </u>
907		· ·	
		6 = Raising livestock	
909		7 = Fishpond/ aquaculture	
910		99 = Other (specify)	
911	From the work that you did on the farm did your household	1 = Yes	
	earn any money?	0 = No	
		98 = Don't know	
912	Who usually decides how the money you earn will be used?	1 = Self	
		2 = Husband	
	READ THE LIST.	3 = Self and husband	
		jointly	
	· · · · · · · · · · · · · · · · · · ·	4 = Someone else	
913	Who usually makes decisions about major household	1 = Self	
	purchases/sell such as cattle or livestock?	2 = Husband	
		3 = Self and husband	
	READ THE LIST.	jointly	
		4 = Someone else	
914	Who usually makes decisions about minor household	1 = Self	
	purchases/sell such as spices/oils, soap, utensils, or daily	2 = Husband	
	household needs?	3 = Self and husband	
		jointly	
	READ THE LIST.	4 = Someone else	
915	Who usually makes decisions about health care for your	1 = Self	
	children?	2 = Husband	
		3 = Self and husband	
		jointly	
	READ THE LIST.		
	READ THE LIST.	4 = Someone else	
916		4 = Someone else	
916	READ THE LIST. Do you have husband?	4 = Someone else 1 = Yes	
916		4 = Someone else	
916 917	Do you have husband?	4 = Someone else 1 = Yes	
	Do you have husband? Who usually decides how the money your husband earns will	4 = Someone else 1 = Yes 0 = No (Go to 918)	II
	Do you have husband?	4 = Someone else 1 = Yes 0 = No (Go to 918) 1 = Self 2 = Husband	
	Do you have husband? Who usually decides how the money your husband earns will	4 = Someone else 1 = Yes 0 = No (Go to 918) 1 = Self	

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918	Do you have children?	1 = Yes 0 = No (Go to 923)	
919	Does your husband help you care for the children?	1 = Yes 0 = No (Go to 923)	
920	Does he help care for the children almost every day, at least once a week, or rarely?	1 = Every day 2 = At least once a week 3 = Rarely	
921	Does your husband help you with household chores like cooking, cleaning the house, fetching water, collecting firewood or other domestic work?	1 = Yes 0 = No (Go to 923)	
922	Does he help almost every day, at least once a week, or rarely?	1 = Every day 2 = At least once a week 3 = Rarely	

923	At any time	Use improved seed varieties for any of your	1 = Yes	
	during the	crops?	0 = No	
	previous	\sim	98 = Don't know	
924	major seasons	Keep improved varieties of livestock?	1 = Yes	
	(Meher) and		0 = No	
	minor season		98 = Don't know	
925	(Belg) not	Use animal manure to improve you crops	1 = Yes	
	including the	yield?	0 = No	
	current		98 = Don't know	
926	season,	Use any other source of fertilizer on your	1 = Yes	
		crops?	0 = No	
	Did you:		98 = Don't know	
927		Irrigate your crops?	1 = Yes	
			0 = No	
			98 = Don't know	
928		Rotate your crops from one field to another	1 = Yes	
		when planting?	0 = No	
			98 = Don't know	
929		Harvest water during the rains?	1 = Yes	
			0 = No	
			98 = Don't know	
930		Practice intercropping?	1 = Yes	
			0 = No	
			98 = Don't know	
931	Have you ever t	aken any steps to reduce soil erosion on your	1 = Yes	
	farm?		0 = No (Go to 936)	
			98 = Don't know (Go to	''
			936)	
			For each mentioned: 1=Yes	0=No
932	What steps did	you take to reduce soil erosion?	Plant trees or shrubs	
933			Terracing	

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934		Use drainage system	
935		Other	
936	Have you received any inputs for your farm from a	1 = Yes	
	social/government program?	0 = No ( <b>Go to 1001)</b>	
937	What farm inputs have you received?	For each mentioned: 1=Yes	s 0=No
938		Seeds	_
939		Improved seeds	
940		Livestock or poultry	_
941		Improved varieties of	1 1
		livestock/ poultry	II
942		Aquaculture (fish)	
943		Fertilizer	
944		Other	

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No	Question	10: WASH choices	
	What is the main source of drinking water	1 = Piped into dwelling	1 11
1001	for the household?	2 = Piped to yard/plot	11
	for the household?	3 = Piped to neighbour	
	Do not read list		
	Do not read list	4 = Public tap/standpipe 5 = Tube well or borehole	
		6 = Protected well	
		7 = Unprotected well	
		8 = Protected spring	
		9 = Unprotected spring	
		10 = Rainwater	
		11 = Tanker truck	
		12 = Cart with small tank	
		13 = Surface water (river/dam/	
		Lake/pond/stream/canal/ irrigation	
		channel)	
		14 = Bottled water	
		99 = Other	
1002	Do you do anything to your household	1 = Yes	
	water to make it safer to drink?	0 = No ( <b>Go to 1004</b> )	
		98 = Don't know ( <b>Go to 1004)</b>	
1003	What is the main thing you do to make the	1 = Boil	
	water safer?	2 = Add bleach/chlorine	
		3 = Strain through a cloth	
		4 = Use water filter (ceramic/	
		Sand/composite/etc)	
		5 = Solar disinfection	
		6 = Let it stand and settle	
		7 = Other(specify)	
		98 = Don't know	
1004	What kind of toilet facility do members of	1 = Flush to piped sewer system	1 11
	your household usually use? If not possible	2 = Flush to septic tank	'''
	to determine, ask for Permission to	3 = Flush to pit latrine	
	observe the facility.	4 = Flush to somewhere else	
	······································	5 = Flush, don't know where	
		6 = Ventilated improved pit latrine	
		7 = Pit latrine with slab	
		8 = Pit latrine without slab/open pit	
		9 = Composting toilet	
		10 = Bucket toilet	
		11 = Hanging toilet/hanging latrine	
		12 = No facility/bush/field	
		99 = Other (specify)	
1000	How does your HH primarily dispose of HH	1 = Collected by municipality	1 11
1003	waste?	2 = Buried	'''
	waster	3 = Collected by private establishment	
		4 = Dumped in street/open space	
		Dumpeu in succey open space	1 I

		<ul> <li>5 = Disposed in the compound</li> <li>6 = Dumped in river</li> <li>7 = Burned</li> <li>98 = Other</li> </ul>	
1006	Do you have separate cooking room?	1 = Yes 2 = No <b>(Go to 1008)</b>	
1007	Does the stove or cooking room have a chimney?	1 = yes 2 = No	_
1008	What type of fuel or energy source is used in this cook stove?	<ul> <li>1 = Alcohol/ethanol</li> <li>2 = Gasoline/diesel</li> <li>3 = Kerosene/paraffin</li> <li>4 = Coal/lignite</li> <li>5 = Charcoal</li> <li>6 = Wood</li> <li>7 = Straw/shrubs/grass</li> <li>8 = Agricultural crop</li> <li>9 = Animal dung/waste</li> <li>10 = Processed biomass (pellets) or</li> <li>woodchips</li> <li>11 = Garbage/plastic</li> </ul>	111_
1000		12 = Sawdust 96 = Other (specify)	<u>  </u>
1009	Do you have a confined space (beret/gata) to keep livestock?	1 = Yes 0 = No 96 = Not applicable	11
1010	Do you keep poultry in cages/confined systems (kote)?	1 = Yes 0 = No 96 = Not applicable	11
1011	What do you think are the activities before	For each mentioned: 1=Yes 0=No	
1012	which you should wash your hands with	Before preparing food	
1013	soap?	Before touching or eating food	
1014		Before feeding a child or other person	
1015	DO NOT PROMPT.	Praying	
1016		Don't know	
1017	What do you think are the activities after	For each mentioned: <b>1=Yes 0=No</b>	
1018	which you should wash your hands with	After defecation or urinating	
1019	soap?	After handling animals and their waste	
1020		After housework or fieldwork	
1021	DO NOT PROMPT.	After touching pets or handling animals	
		and their waste	<u> </u>
1022		After blowing nose or coughing	
1023		After cleaning a child's bottom	
1024		None	
1025	What do you think are the reasons to keep poultry and livestock in a confined space?	To keep out of house	
1026	For each mentioned: 1=Yes 0=No	To keep away from water source	

1028 <b>DO NOT PROMPT.</b>	To protect livestock/poultry	
1029	Other	
	26	
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3= Received from food aid/social

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Module 11: Food fortification (household coverage of fortifiable foods)			
SN	Question	Response	
1101	Does your household use cooking oil to prepare foods or add to foods at home?	1 = Yes 0 = No <b>→Go to 1103</b>	
1102	The last time your household get cooking oil, where did you get it from?	<ul> <li>1=Purchased from market/shop/kiosk/wholesaler/street vendor</li> <li>2= Homemade or obtained from local farm or local small factory/processor</li> </ul>	

		<ul> <li>98= Don't know/remember</li> </ul>	
1103	Does your household prepare foods using wheat flour at home, such as bread, kita, injera?	1 = Yes 0 = No <b>→Go to 1105</b>	_
1104	The last time your household get wheat flour, where did you get it from?	<ul> <li>1=Purchased from market/shop/kiosk/wholesaler/street vendor</li> <li>2= Homemade or obtained from local farm or local small factory/processor</li> <li>3= Received from food aid/social protection program</li> <li>4 = Other (specify):</li></ul>	1111
1105	I would like to check whether the salt used in your household is iodized. May I have a sample of the salt used to cook meals in your household? Test salt for iodine	1=lodine present 2= No iodine 3= Household uses salt but there is no salt in household 4= Household does not use salt 5= Salt not tested, specify reason	II
1106	The last time your household get salt, where did you get it from?	<ul> <li>1=Purchased from market/shop/kiosk/wholesaler/street vendor</li> <li>2= Homemade or obtained from local farm or local small factory/processor</li> <li>3= Received from food aid/social protection program</li> <li>98= Don't know/remember</li> </ul>	1111

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3	99 = Other (specify):	
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60	For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml	

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A I		– Agriculture practices	
About	t the household		1
	Does any member of the household	1 = Yes	
1201	own any agricultural land (purchased	0 = No ( <b>Go to 1212)</b>	
	or own?		
1202	,		
	land do members of this household	Enter total number of hectares	
	own?	(If less than 1, Enter in decimals	
		(example 0.5)	
	Note: Convert local land measurement	Enter 9999 if hectares are not	
	unit into hector after discussing with	known	
	agriculture focal person/AEW.		<u> </u>
	In the past major growing season		
	(Meher) and minor growing (Belg)	1 = Yes	
1203	season, not including the current	0 = No ( <b>Go to 1211)</b>	
	season, did you work on the family		
	farm?	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
	What sort of work did you do on the	1 = Yes 0 = 1	NO
	family farm?	1 = Home (kitchen) gardening	I
1204	READ THE LIST	2 = Fieldwork	
1205	READ THE LIST	3 = Cash crop farming	
1206		4 = Producing dairy	<u> </u>
1207		5 = Rearing poultry	<u> </u>
1208		6 = Raising livestock	<u> </u>
1209		7 = Fishpond/ aquaculture	<u> </u>
1210		99 = Other	<u> </u>
1211	Does this household own any	1 = Yes	
	livestock, herds, other farm animals,	0 = No → Go to 1221	
	or poultry?		
	How many of the following animals do	For each: Enter number. If none, e	nter 0
1212	this household own?	Chickens	
1213		Goats	
1214		Sheep	
1215		Donkeys	
1216		Horses	
1217		Mules	
1218		Camels	
1219		Milk cows	
1220		Oxen	

In the past 2 growing seasons (Meher and Belg), not including the current season, please describe all the crops (cereals, legumes, vegetables, fruits, seeds, and other crops) grown on your household farm.

Please also describe all animal source foods (meat, eggs, milk, dairy, fish, other) that you have produced on your household farm in same period.

Write down all crops and animals mentioned by the respondent. When the respondent has finished, probe for crops and animal source foods not mentioned. Then ask about production/yields in the relevant units. Ask the respondent to estimate the amount of the total production that went to sales, food consumption, and storage/losses/other uses.

	Group	Crop	Did HH	Season	Amount	During th		ajor seasons (Meher)
	Group	Стор	cultivate	1=Meher	Amount			elg) not including the
			crop?	2=Belg			current	
			1 = yes	3=Both		Sold	Consumed	Storage, losses,
			0 = No	J-Dotti		5010	consumed	animal feed or othe
			0 - 110					uses
1221	Staples	Maize						
1222		Teff						
1223		Wheat	_					
1224		Barley	_					
1225		Sorghum	_					
1226		Millet						
1227		Rice						
1228		Emmer						
		wheat						
		(oaths)						
1229		Other cereals						
1230	Pulses	Bean			_			
1231	(legumes)	Haricot bean						_  _
1232		Lentil (Miser)					_  _	_  _
1233		Grass pea (guaya)						_  _
1234		Chickpea						
1235		Field pea (Ater)						_  _
1236		Soya bean						_  _

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1237		Other						
		legumes	••		••	''	''	''
1238		Niger seed (Nug)						_  _
1239		Sunflower						
1240		Sesame						
1241		Linseed						
1242	Oil Crops	Rapeseed (Gomenzer)						_  _
1243		Lupine (Gibto)						
1244		Nuts						
1245		Other oil crops	_					
1246	Root	Cassava						
1247	crops/	Enset				<u> _  _ </u>		
1248	tubers/	Irish potato	<b>VZL</b>					
1249	vegetables	Sweet potato	ILN					_  _
1250		Sweet potato - orange flesh	_	1				
1251		Onion						
1252		Pepper		6				
1253		Tomato						
1254		Cabbage						
1255		Other light green leafy vegetables			I_T		_  _	
1256		Kale	_		_			
1257		Other dark green leafy vegetables					1111	
1258		Carrot						
1259		Other roots or tubers						
1260		Other vegetables						
1261	Perennial	Coffee						
1262	crops/	Chat (khat)						
1263	fruits	Banana	<u> _</u>					
1264		Orange				<u> _  </u> _		
1265		Mango						
1266		Нор			_		_	

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2		
3 4		(Gesho)
5	1267	Avocado         I_I         I_IIIII         I_IIIIII         I_IIIIII
6 7	1268	Lemon         I_I         I_I         I_II_I         I_II_I         I_II_I
8	1269 1270	Papaya          _           _           _           _           _            Cuara
9	1270	Guava     II     II     III     IIII       Water      I    I    I
10 11		Mater         II         II <t< th=""></t<>
12	1272	Tirngo fruit
13	1273	Other
14 15		perennial
15		crops
17	1274	Other fruits
18 19		
19 20		
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22 23		
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	Group	Animal source food	Does HH produce?	Amount	During the previous Major seasons (Meher) and Minor season (Belg) not including the current season				
		(unit)	1=yes 0=no		Season 1=Meher	How much?			
			<u>(If no, skip</u> <u>to the</u> <u>next item)</u>		2=Belg 3=Both	Sold	Consumed	Storage, losses, anima feed or others	
1275	All	Chicken eggs							
1276		Chicken meat							
1277		Goat milk		_		_	_	_	
1278		Goat meat	1			_	_	_	
1279		Camel milk				_	_	///	
1280		Sheep meat				_	_	///	
1281		Cow milk		_		_	_	_	
1282		Cow other dairy		<b> _</b>	II			111	
1283		Beef				_	_	_	
1284		Other meat (e.g. camel, horse)			II		_ _	_	
1285		Farmed fish							

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Now wher	I would like to ask you some questions about food. During the la n:	st 12 MONTHS, wa	s there a ti
SN	Questions	Answers	
1301	You or others in your household worried about not having enough food to eat because of a lack of money or other resources?	1=Yes 0= No 98=Don't know 97=Refused	_  _
1302	Still thinking about the last 12 MONTHS, was there a time when you or others in your household were unable to eat healthy and nutritious food because of a lack of money or other resources?	1=Yes 0= No 98=Don't know 97=Refused	_  _
1303	Was there a time when you or others in your household ate only a few kinds of foods because of a lack of money or other resources?	1=Yes 0= No 98=Don't know 97=Refused	_  _
1304	Was there a time when you or others in your household had to skip a meal because there was not enough money or other resources to get food?	1=Yes 0= No 98=Don't know 97=Refused	
1305	Still thinking about the last 12 MONTHS, was there a time when you or others in your household ate less than you thought you should because of a lack of money or other resources?	1=Yes 0= No 98=Don't know 97=Refused	
1306	Was there a time when your household ran out of food because of a lack of money or other resources?	1=Yes 0= No 98=Don't know 97=Refused	
1307	Was there a time when you or others in your household were hungry but did not eat because there was not enough money or other resources for food?	1=Yes 0= No 98=Don't know 97=Refused	
1308	Was there a time when you or others in your household went without eating for a whole day because of a lack of money or other resources?	1=Yes 0= No 98=Don't know 97=Refused	

CN	Module 14: Employment and socia	•	
S.N.	Questions	Response	
1401	Since last year, what has been the main livelihood or	1 = Sale of self-produced	
	income source of the HH?	2 = horticulture crops Sale of	
	/	self-produced field crops	
	(DO NOT READ LIST. PROBE FOR ONE RESPONSE)	3 = Own business (including	
		commerce, livestock rearing)	
		4 = Wage employment	
		5 = Remittances	
		6 = Property income	
		7 = Government	
		transfers/NGO support	
		8 = Pension	
		99 = Other	
1402	Since last year, have there been other livelihood or	1 = Sale of self-produced	
	income sources for the HH?	2 = horticulture crops Sale of	
		self-produced field crops	
	(DO NOT READ LIST. PROBE FOR ALL RESPONSES)	3 = Own business (including	
		commerce, livestock rearing)	
		4 = Wage employment	
		5 = Remittances	
		6 = Property income	
		7 = Government	
		transfers/NGO support	
		8 = Pension	
		99 = Other	
1403	Since last year, did anyone in your HH receive any kind of	1 = Yes	
	food or cash assistance from the government, NGO, or	0 = No	
	other agencies? Clarify: This is not formal employment or	98 = Don't know	
	pension. However, it may or may not be conditional on		
	work.		
1404	Since last year, which members of this HH were targeted	1 = All HH members	_
	to receive this support?	2 = Specific HH members	
		98 = Don't know	
1405	Which specific HH members received food or cash	Link this back to the HH roster	
	assistance?	and have interviewer select	
	Clarify: This includes children whose parents receive cash	names.	
	on their behalf.	98 = Don't know	
1406	Which of these categories apply to the persons who	1 = Pregnant women	
	received food or cash assistance?	2 = Lactating women	
		3 = Children under 5 years	
	(READ RESPONSES ALOUD. SELECT ALL THAT APPLY)	4 = Elderly	
	-	5 = Disabled person	
		6 = None of the above	
1407	Since last year, which food or social assistance program	1 = PSNP	
	did members of the HH receive support from? (DO NOT	2 = Community Care Coalition	'''
	READ LIST ALOUD. PROBE FOR ALL RESPONSES)	3 = Other assistance program	
		98 = Don't know	
1408	Since last year, what was the form of assistance that	1 = Cash only transfer	
- 100	members of your HH received form these programs:	2 = Food only transfer	'''
	food, cash or both food and cash?	3 = Cash and food mix	
		99 = Other	
		98 = Don't know	

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1409	Is this HH currently receiving food or cash?	1 = Yes	
		0 = No	
		98 = Don't know	
1410	Since last year, how has your HH used the food received?	1 = HH consumption	
	(READ RESPONSES ALOUD. SELECT ALL THAT APPLY)	2 = Sold food for cash	
		3 = Other activities	
		98 = Don't know	

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1501.	How do you preserve soil fertility?	1 = Fertilization (Chemicals, animal manure, green manure etc)	
		2 = Crop rotation (Cultivation of a series of dissimilar types of crops in the same area in sequential seasons)	
	[Multiple answer is allowed! Do not read	3=Intercropping (Cultivation of two or more dissimilar types of crops in the same area in the same season)	
	the choices. Listen and mark the one they mention]	4=Tillage	
		99=Other (specify):	
1502.	Do you apply fertilizers?	1= Yes	
		0= No (Go to 1506)	
1503.	Which kinds of fertilizers do you use more often?	1= Chemical fertilizers	
		2 = Organic fertilizers (non-chemicals like animal manure, green manure compost, etc.) (Go to $\rightarrow$ 1505)	
1504.	Which chemical fertilizers are used most often, can you specify the type/name?	1 = UREA	
		2 = DAP	
		3= NPS	
		98= Don't know	
		99= Other (specify):	
1505.	Which organic fertilizers are used more often, can you specify the type?	1 = Livestock manure	
		2 = Poultry manure	
		99=Other (specify)	
1506.	What is the most dominant cereals/crop you produce on your farm?	1 = Wheat	
1500.		2 = Teff	
		3 = Maize	
		99= Other (Specify):	
	[Only one answer is allowed! Do not read		
	the choices. Listen,		
	mark/specify the one they mention]		
1507.	How far is your agricultural land from your house? [In case they own many farms, Consider only the one with the dominant crop]	1= within 500 meter radius	
		2= 500 -1000 meter radius	
		3= 1000 - 3000 meter radius	
		4= More than 3000 meter	

1 2	Module 15: Soil information questionnaire
2 3 4	Observational checklist for soil sampling
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Г		Begion BMJ Open	Page 114 of 15(
	1	Region	. age 114 01 130
1	2	Zone	
2	3	Woreda	
3 – 4	4	Kebele	
5 6	5	Gote Code	
7	6	Household Code	
8 – 9		Sample code	
10- 11	7	Crop history	
12 13	7.1	Last two-year crop (Please, specify the crop harvested in 2011, growing season)	
14	7.2	Last crop (Please, specify the crop harvested from the previous growing season)	
15– 16	7.3	Crop to be planted for the current season	
17 18	7.4	Please write the intended planting dates (Year and Month)	
19	7.5	When was the last time the agricultural field gets tilled?	
20_ 21	,	1 = 0 to 3 months ago	
22- 23		2 = 3 to 6 months ago	
24 25		1 = 6 to 12 months ago	
26		2 = before a year	
27 28	8	Fertilizer utilization	
29 30	8.1	Which fertilizer is applied	
31	0.1	1 = Chemical fertilizer	
32_ 33		2 = Organic fertilizer (Go to 8.4)	
34- 35		3 = Both 1&2	
36 37		4 = Fertilizer not applied (Go to 9)	
38	8.2	Which Chemical fertilizer is applied	
39_ 40	0.2	1 = UREA	
41 42		2 = DAP	
43		3 = NPS	
44_ 45		4 = Other, please specify	
46- 47	8.3	Please write the last date (Year and Month) you applied chemical fertilizer?	
48 49	8.4	Which Organic fertilizer is applied	
50	0.7	1 = Animal Manure	
51_ 52		2 = Green Manure	
53 54		3 = Compost	
54 55 56		4 = Other, please specify	
56_ 57	0 -	Please write the last date (Year and Month) you applied organic fertilizer?	
57 58- 59	8.5	Soil characteristics	
59 60	9		

1	9.1	Observation: What is the colour of the soil you are about to sample
3		1 = Dark brown/Black
4 – 5		2 = Red
6 7		3 = Grey
8		4 = Other, please specify
9 10	9.1	Observation: Field area landscape
11 12		1 = plains/level grounds
13 14		2 = Sloppy/Inclined
15	9.4	Observation: Is there a standing crop on the sampling field or to the nearby farmland.
16- 17		1 = Yes
18 19		2 = No
20	9.3	Observation: Is sampling field tilled/ is it being tilled at the time of sampling.
21 22		1 = Yes
23 24		2 = No
25 26	10	Distance of the farmland to the nearby houses
27		1 = below 100meter
28- 29		2 = 100 to 500meter
30 31		3 = 500 to 1000meter
		4 = More than 1000meter
32 33_ 34		Please take picture for the surrounding environment i.e., plot, houses, anything permanent or even moving
35 36	11	cattle
37 38	12	Please capture GPS for the sampled farmland
38_ 39	13	Name of sample collector
40- 41		LIST ANY ABNORMAL CONDITIONS OR SPECIFIC INFORMATION DESIRED:
42 43		
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Module 16: A Dietary assessment questionnaire

Note for the data collectors: Among the household members, this module questionnaire is to be filled for the child under-five years of age and women of the reproductive age in the household.

Part 3	1: Before	24-hr	recall
--------	-----------	-------	--------

	24-hour c	lietary recall	
	EA code     Household code   _	Line Number   _  Child ID	
l	Jnique ID Woman:   _ _ _ _ _ _  Uni	que ID Child:	II
Intervie	ew Date: Date// Day - 01=Mon 0	2=Tue 03=Wed 04=Thu 05=Fri 06=Sat	07=Sun
	Date of food intake / _ /		
	Question	Coding category	skip
1.	Enumerator Code:		
2.	For which target group is the recall being done?	0. Woman 1. Child	
3.	Recall number	0. Recall 1	
		1. Recall 2	
4.	Name of the woman interviewed	<u></u>	
5.	Age of the woman (in complete years)	Age in years ()	
6.	Name of child		
7.	Date of birth (DOB): Use Ethiopian calendar	_ _ / _ _ /20  _	
8.	Age of the child (in complete month)	months	
9.	Child's sex:	0=Male 1= Female	
10.			
	Food weighing scale number:		
11.	Was yesterday's food intake different from		
	your usual diet?	1=Yes 0=No	No →13
12.		1=Holyday/celebration	
	If yes,	2=I was sick	
		3=Other	
13.	Was [child name] yesterday's food intake	1=Yes	
	different from your usual diet?	0=No	No →15

If yes, Did you take medicine/supplement yesterday? Did [child name] take medicine/supplement yesterday?	1=Holyday/celebration         2=I was sick         3=Other         1=Yes         0=No         If yes, name:         0=No         If yes, name:         0=No         If yes, name:
Did you take medicine/supplement yesterday? Did [child name] take medicine/supplement yesterday?	3=Other         1=Yes         0=No         If yes, name:         0=No         If yes, name:
Did [child name] take medicine/supplement yesterday?	1=Yes         0=No         If yes, name:         1=Yes         0=No         If yes, name:
Did [child name] take medicine/supplement yesterday?	0=No <i>If yes, name:</i>
Did [child name] take medicine/supplement yesterday?	If yes, name:
yesterday?	1=Yes 0=No <i>If yes, name:</i>
yesterday?	0=No <i>If yes, name:</i>
yesterday?	If yes, name:
	<u> </u>

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# Procedures to collect the required information

# 1. Pass 1: list all foods and drinks consumed during the 24-hour period.

Now I would like to ask you about the foods and drinks that [YOU/ YOUR CHILD] consumed yesterday from the time you work up until you went to sleep, sunrise yesterday to sunrise today. Please list all foods or drinks you ate, weather you ate or drank them at home or somewhere else. Please think about snacks and small meals as well as main meals.

- 1) "WHAT WAS THE FIRST THING [you/ your child] ATE YESTERDAY AFTER SUNRISE?"
- 2) "WHEN WAS THAT"
- 3) "DID [you/your child] HAVE ANYTHING WITH THAT?
- 4) "WHAT DID [you/he/she] HAVE?"
- 5) "WHAT IS THE NEXT THING [you/ your child] ATE OR DRANK AND WHEN WAS THAT?"
- 6) REPEAT questions 3-5 until you have a full record for both DAY AND NIGHT
  - a. The reference period is from sunrise yesterday to sunrise this morning. If they wake up at a different time than sunrise, you can use the time from waking up yesterday until waking up today

# 2. Pass 2: get more detail about each food.

- 7) "NOW, PLEASE DESCRIBE EACH FOOD [you/ your child] ATE YESTERDAY"
- 8) "WHAT TYPE WAS IT?"
- 9) "WHERE DID YOU GET IT?"
- 10) "WHAT ARE THE INGREDIENTS?"
  - a. Use standard "probes" (probing questions) to get these details for each food.
- 11) "HOW MUCH DID THIS RECIPE MAKE?" or "WHAT WAS THE TOTAL AMOUNT THIS MADE?"
- 12) "HOW WAS The Recipe PREPARED?"
  - a. Identify the cooking methods used (particularly if raw, fermented, or fried in oil).

# 3. Pass 3: estimate the amount consumed of each food on the list

- 13) "HOW MUCH OF [name the first food] DID [you/ your child] CONSUME?"
  - a. Help the mother remember and **estimate the amount** of each food or recipe that her child ate and that she herself ate.

# 14) "WAS ANY LEFT OVER?"

- a. If any food is leftover from what the mother served to the child, enter that amount.
- 15) "PLEASE HELP ME ESTIMATE THE AMOUNT OF FOOD YOU ARE OR USED IN THE RECIPE"

Use following portion size estimation method to estimate the amount of food/ingredient eaten or used in a recipie 1. Direct weight (g) 2.Proxy weight (g) 3. Water (g) 4. Number 5. Other (specify).

# 4. Pass 4: verify everything consumed

a. Quickly read the information back to the respondent, "HAVE I FORGOTTEN TO ADD ANYTHING?"

 Part 2. Quick list

# Pass 1

Please describe the foods the foods and drinks that [YOU/ YOUR CHILD] consumed yesterday from the time you work up until you went to sleep (sunrise yesterday to sunrise today). Please list all foods or drinks you ate, weather you ate or drank them at home or somewhere else. Please think about snacks and small meals as well as main meals.

Write down all foods and drinks mentioned. When composite dishes are mentioned, ask for the list of ingredients

When the respondent has finished, probe for meals and snacks not mentioned.

Early morning	Mid-morning	Noon	Afternoon	Evening	Late eveni
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	Milestowe the first				Place of	<i>a</i> :	þ		c	<b>Recipe information</b>			n
Foo d No.	What was the first thing [YOU/ YOUR CHILD] ate or drank after sunrise yesterday? Any else?	Time of meal	Please describe this food / beverage/ ingredient:	this prepared ?	preparat ion	How was the food / Ing.	Amount served	Amount left over	Amount eaten	State of each	Cooking method of	Total amount of recipe	Links to
1													
	Ingredient:		Description										
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Time of meal: 1. Early morning 2. Mid-Morning 3. Noon 4. Afternoon 5. Evening 6. Late evening

Place of preparation: 1. Home 2. Outside home

How was it prepared: 1=raw/ no change/ as purchased; 2=fermented; 3=fried; 04=cooked or boiled – wet heat; 5=baked/grilled/ broiled – dry heat; 6=local miller; 7=blanched (dipped in boiling water); 8=other For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

				How was			g		c		Recipe i	informatio	n
Foo d No.	What was the first thing [YOU/ YOUR CHILD] ate or drank after sunrise yesterday? Any else?	Time of meal	Please describe this food / beverage/ ingredient:	this prepared ?	preparat ion	How was the food / Ing.	Amount served	Amount left over	Amount eaten	State of each	Cooking method of	Total amount of recipe	Links to food/
1													
	Ingredient:		Description										
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Wh	at was the first	Time of	Please describe this food /	How was	Place of	чo	4 E	(	۲ ک	ε (	Recipe	informatio	וכ
					NA								
Time o	<b>f meal</b> : 1. Early n	norning 2	. Mid-Morning 3. Noon 4. Afternoo	n 5. Evenin	g 6. Late e	vening							
Place o	of preparation: 1	L. Home 2	. Outside home										
	<b>as it prepared</b> : 1 broiled – dry he	l=raw/ no at; 6=loca	. Outside home change/ as purchased; 2=fermente al miller; 7=blanched (dipped in boil	ed; 3=fried; ling water);	04=cooke 8=other	d or boi	led – we	et heat;	5=bal	ked/			
			For peer review only - htt	p://bmjope	n.bmj.com/	site/abo	ut/guide	lines.xh	tml				

Module 17:	Biomarkers	collection	tools
	Diomainero		

#### **PRESCHOOL AGE CHILDREN (6-59 MONTHS)** ETHIOPIAN FOOD AND NUTRITION STRATEGY BASELINE SURVEY 2020/21 **Biochemical and Health Related Data Collection Tool**

NTIFICATION
HH01. CLUSTER NUMBER:
HH03. RESIDENCE (RURAL=1, URBAN=2):
HH05 CHILD LINE NUMBER
HH07. TEAM LEADER, NAME:
CODE:

# PRESCHOOL CHILDREN 6-59 MONTHS OLD

## **PART I: CHILD HEALTH QUESTIONS**

I would like to ask you some health and food questions about your child. Fill or Circle the correct answer

S.N	Questions	Response	SKIP
5 5 7	What is the birth date of the child? In day/month/ year (How many months old is this child?)	Birth Date:	
8 9 0 1 2 3 4	NOTE FOR INTERVIEWERS (Screening question to verify that the date of birth of the child)	(Day/Month/Year) Age in months	
5 2	Has (child's name) been diagnosed with anemia in the past 6 months?	No0	
7 8		Yes1 Don't know98	

Consent given for: <b>PL01</b> Blood PL02 St (Y OR N)	ool
PL03 Code for Laboratory Technician:	Lab Tech Name
PLO4 BLUE TOP TUBE (METAL FREE)	
Not collected =00.0 Refused = 77.7	ML.
PL05 PURPLE TOP TUBE (EDTA)	
Not collected =00.0	ML.
Refused = 77.7 PL06 RED TOP TUBE (EDTA)	
Not collected =00.0	
Refused = 77.7	
PL07 Date blood sample taken (Ethiopian	Date://
Day/Month/Year)	David Marth / Yaar
PL08 TIME BLOOD DRAW (Ethiopian time)	Day / Month / Year
	Blood draw: Hour N
PL09 When did you eat your most recent meal (food)	
(Ethiopian time)	· · · · ·
	Hour Minute
PL10 MALARIA RESULTS (RDK)	NEGATIVE0
	POSITIVE P FALCIPARUM1
	Positive P VIVAX2
	INVALID3
PL11 FEVER in last 24 HR?	NO0
	YES1
PL12 HEMOGLOBIN RESULTS	g/dL
In order to determine if you have worms in the stool,	
you can provide this now, we appreciate it. If not no	w, we can come back to pick up the sample at a
INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL:	al. We would like the freehest starly server -
<b>For stool</b> : We will return tomorrow to pick up your sto use one cup to collect the first stool you pass.	oi. We would like the freshest stool you can giv
PL13 STOOL COLLECTED?	NO0
	1
PL14 Date stool sample taken (Ethiopian	Date://
Day/Month/Year)	Day / Marth / Mart
PL15 TIME: STOOL COLLECTED (Ethiopian time)	Day / Month / Year
	· ·

YES

	Hour	Minute
<b>PL16</b> TIME: STOOL PASSED, Ethiopian time (as recorded on cup)		:
	Hour	Minute
PL17 Time Blood centrifuged (Ethiopian time)	·:	
	Hour	Minute

Thank you for completing this interview.

## INTERVIEWER'S OBSERVATIONS TO BE FILLED IN AFTER COMPLETING INTERVIEW

_____

COMMENTS ABOUT RESPONDENT:

# SCHOOL AGE CHILDREN 6-12 YEARS ETHIOPIAN FOOD AND NUTRITION STRATEGY BASELINE SURVEY 2020/21 Biochemical and Health Related Data Collection Tool

IDENTIFICATION	
SG01. CLUSTER NUMBER:	
SG02. HH NUMBER:	4
<b>SG03</b> . RESPONDENT LINE NUMBER: (SHOULD BE MOTHER/CAREGIVER)	
SG04 SCHOOL CHILD LINE NUMBER	

In general, for children 6-10 years of age: get parental report (ask the questions of the caretaker and enter the child's name into the parentheses) For children 11-12 years of age who are present and can provide information: get self-report (ask

questions directly of the child and enter "you" or "yourself" into the parentheses)

## PART I: CHILD HEALTH RELATED QUESTIONS

No.	QUESTION	CODING CATEGORIES		SKIP
<b>S1</b>	How old are you/is your child?	Years		
	(Verify that the age is the same age as written on the household listing)			
<b>S2</b>	Have you/ your child ever attended school?	No	00	<b>00 →</b> \$4
		Yes	01	
<b>S3</b>	What is the highest level of school (name	None0		
	of child) completed?			
		Primary1		

# PART II: CHILD BIOCHEMICAL MEASUREMENT

Verbal consent given for: SL01_Blood SL02_Urine	e SLO3 Stool
0= No OR 1= yes	
SLO4 Phlebotomist Code	
SL5 BLUE TOP TUBE (METAL FREE)	
Did not work =00.0	ML.
Refused = 77.7	
SL6 PURPLE TOP TUBE (EDTA)	
Did not work =00.0	ML.
Refused = 77.7	9
SL7 REDTOP TUBE (EDTA)	
Did not work =00.0	ML.
Refused = 77.7	
<b>SL8</b> DATE BLOOD SAMPLE TAKEN (Ethiopian calendar)	Date://
	Day / Month / Year
SL9 TIME BLOOD DRAW (Ethiopian time)	Blood draw::
	Hour Minute
SL10 When did you eat your most recent meal (food)?	Last Meal Eaten : :
(Ethiopian time)	
	Hour Minute
<b>SL11</b> FEVER in last 24 HR? (Since same time yesterday)	No00
	Yes01
SL12 MALARIA RESULTS (RDK)	NEGATIVE
	POSITIVE P falciparum01
	POSITIVE P vivax02
	INVALID03

SL13 HEMOGLOBIN RESULTS	g/dL
<b>SL14</b> Is that finger prick or venous sample taken?	Finger prick00
	Venous01
In order to determine if you have blood in urine or wo sample. If you can provide this now, we appreciate it. at a later time.	
SL15 Urine collected?	No00 yes01
SL16 Blood in urine RESULTS	Negative00 positive01
SL17 Stool collected?	No00 yes01
<b>SL18</b> Date and <b>t</b> ime when stool passed by the respondent (as recorded on cup) (Ethiopian time)	Date:         /         and             Day / Month /Year         Hour         Minute
SL19 Date stool sample taken (Ethiopian calendar)	Date:/ Day / Month / Year
<b>SL20</b> Time when stool collected from the respondent (Ethiopian time)	Hour Minute
SL21 TIME BLOOD centrifuged (Ethiopian time)	Hour Minute

Thank the respondent and tell them that the lab team will be arriving later.

## INTERVIEWER'S OBSERVATIONS TO BE FILLED IN AFTER COMPLETING INTERVIEW

COMMENTS:

# ADELESCENT GIRLS (10-19 YEARS) ETHIOPIAN FOOD AND NUTRITION STRATEGY BASELINE SURVEY 2020/21 Biochemical and Health Related Data Collection Tool

IDENTIFICATION	
HH00. CLUSTER (EA) NAME	HH01. CLUSTER NUMBER:
HH02. HH NUMBER:	HH03. RESIDENCE (RURAL=1, URBAN=2):
HH04. RESPONDENT LINE NUMBER: (SHOULD BE MOTHER/CAREGIVER)	HH05 WOMEN LINE NUMBER
HH06. INTERVIEWER NAME	HH07. TEAM LEADER, NAME:
CODE:	CODE:
HH08. SUPERVISOR NAME:	
CODE	

## PART I: HEALTH RELATED QUESTIONS

I would like to ask you some health and food questions about yourself. Fill or Circle the correct answer

No.	Question	Coding categories	Skip
1	How old are you?		
	(verify that the age is the same age as written on the household listing)	Years	
2	Have you been diagnosed with anemia in the past six months?	No0	
		Yes1 Don't know98	
<u>3</u>	Do you smoke? (do not include the powder and chew type)	No0	
		Yes1	

#### PART II: ADOLESCENT BIOCHEMICAL MEASUREMENT

Consent given for: 0= No or 1= Yes	AG <b>01</b> Blood	AG <b>L02</b> Stool	
AG03 BLUE TOP TUBE Did not work =00.0	(METAL FREE)		ML.

AG04 PURPLE TOP TUBE (EDTA)		
Did not work =00.0	ML.	
Refused = 77.7		
AG05 REDTOP TUBE (EDTA)		
Did not work =00.0	ML.	
Refused = 77.7		
AG06 Date blood sample taken (Ethiopian calendar)	Date:/	
	Day / Month / Year	
AG07 TIME BLOOD DRAW (Ethiopian time)	Blood draw : :	
	Hour Minute	
AG08 When did you eat your most recent meal (food)?		
(Ethiopian date and time)		
	Date /Month/ Year Hour Minute	
AG09 Is it Finger prick or venous blood sample taken?	01 Finger prick	
	02 Venous	
AG09 MALARIA RESULTS (RDT)	NEGATIVE	
	POSITIVE P falciparum	
	POSITIVE P <i>vivax</i>	
	POSITIVE P VIVAX	
	INVALID	
	04	
AG10 HEMOGLOBIN RESULTS		
In order to determine if you have worms in the stool we w	would like to collect a stool comple. If you can provide	
this now, we appreciate it. If not now, we can come back		
INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL:	to pick up the sample at a later time.	
	Mo would like the fresh steel you can give us. Places us	
For stool: We will return tomorrow to pick up your stool.	we would like the fresh stool you can give us. Please us	
and cup to collect the first steel you see		
one cup to collect the first stool you pass.		
· · · ·		
one cup to collect the first stool you pass. AG11 Stool collected?	No	
· · · ·		
AG11 Stool collected?	yes	
AG11 Stool collected?	yes Date:/	
AG11 Stool collected? AG12 Date stool sample taken (Ethiopian calendar)	yes Date:/ Daty / Month / Year	
AG11 Stool collected? AG12 Date stool sample taken (Ethiopian calendar) AG13 Time when stool passed by the respondent (as recor	yes Date:// Day / Month / Year	
AG11 Stool collected? AG12 Date stool sample taken (Ethiopian calendar)	yes Date:/ Date:/ Day / Month / Year ded on cup)	
AG11 Stool collected? AG12 Date stool sample taken (Ethiopian calendar) AG13 Time when stool passed by the respondent (as recor (Ethiopian time)	yes Date:/ Date:/ Day / Month / Year ded on cup) :: Hour Minute	
AG11 Stool collected? AG12 Date stool sample taken (Ethiopian calendar) AG13 Time when stool passed by the respondent (as recor (Ethiopian time) AG14 Time when stool collected from the respondent (Eth	Day / Month / Year ded on cup)	
AG11 Stool collected? AG12 Date stool sample taken (Ethiopian calendar) AG13 Time when stool passed by the respondent (as recor (Ethiopian time)	yes Date:/ Date:/ Day / Month / Year ded on cup) :: Hour Minute	

			He	our	Minute	
AC	G15 ⁻	TIME BLOOD centrifuged (Ethiopian time)				
					:	
			He	our	Minute	
	CON		ERVATIONS AFTER COMPLETING IN		EW	
		WOMEN OF REPRODU ETHIOPIAN FOOD AND NUTRITIO Biochemical and Health		SURVE	•	
DEN	ITIFI	CATION				
1H0	0. CL	USTER (EA) NAME	HH01. CLUSTER NU	MBER:		]
HO	<b>2</b> . HI	HNUMBER:	HH03. RESIDENCE ( URBAN=2):	RURAL	=1,	
		BE MOTHER/CAREGIVER)	HH05 WOMEN LINE	E NUME	BER	
10	6. IN	TERVIEWER NAME	HH07. TEAM LEADE	ER, NAN	ЛЕ:	
	CO	 DE:	CODE:			
HO		IPERVISOR NAME:				
	CO	DE				
	PAR	T I: HEALTH RELATED QUESTIONS				
:	S.N	QUESTION		Re	esponse	SKI
	1	How old are you?				
		(verify that the age is the same age as writte listing)			ears	
		I would like to ask you some questions about				
	-	Have you been diagnosed with anemia in the	e past six months?	l N	0	0
	2	nave you been alagnosed with allernia in th			0	

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		Yes1	
		Don't know98	
2	Have you been ill with malaria in the past 2 weeks?	No0	
		Yes1	
		Don't know98	
3	Do you smoke? (do not include the powder and chew type)	No0	
		Yes1	
4	Are you currently lactating?	No0	
		Yes1	
5	During that last pregnancy (that resulted in a live birth) did you have	No0	
	difficulty with your vision at night ("Dafent" night blindness in local		
	language)?	Yes1	
		Don't	
		know98	
6	In the first two months after delivery, did you receive a	No0	
	vitamin A dose (like this)?	Yes1	
		Don't know98	
	SHOW THE CAPSULE		
<u> </u>		•	
PA	RT II: WOMEN BIOCHEMICAL MEASUREMENT		

If the women is pregr	iant do not collect v	ienous blood	
Consent given for:	WL01 Blood	WL02 Urine	WL03 Stool
0= No or 1= Yes			
WL4 BLUE TOP TUBE	(METAL FREE)		
Did not work =00.0			
Refused = 77.7			
Pregnant = 99.9			
WL5 PURPLE TOP TUE	BE (EDTA)		
Did not work =00.0			ML.
Refused = 77.7			
Pregnant = 99.9			
WL6 REDTOP TUBE (E	DTA)		
Did not work =00.0			
Refused = 77.7			
Pregnant = 99.9			
WL7 Date blood samp	le taken (Ethiopian	calendar)	Date://

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	Day / Month / Year
WL8 TIME BLOOD DRAW (Ethiopian time)	Blood draw::
	Hour Minute
WL9 When did you eat your most recent meal (food)?	
(Ethiopian date and time)	/
	Date /Month/ Year Hour Minute
WL 10 Finger prick or venous sample taken?	01 Finger prick
	02 Venous
WL11 MALARIA RESULTS (RDT)	NEGATIVE
	POSITIVE P falciparum
	POSITIVE P vivax
	POSITIVE FOR BOTH P falciparum and P vivax
	03
	INVALID
	04
WL12 HEMOGLOBIN RESULTS	
stool sample. If you can provide this now, we appreciate at a later time. INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL:	e it. If not now, we can come back to pick up the sam
INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL: For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass.	we would like the fresh stool you can give us. Please
<ul> <li>stool sample. If you can provide this now, we appreciate at a later time.</li> <li>INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL:</li> <li>For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass.</li> <li>For urine: We will return tomorrow to pick up your urine.</li> </ul>	we it. If not now, we can come back to pick up the sam We would like the fresh stool you can give us. Please
stool sample. If you can provide this now, we appreciate at a later time. INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL: For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass.	We would like the fresh stool you can give us. Please
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stool sample. If you can provide this now, we appreciate at a later time. INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL: For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass. For urine: We will return tomorrow to pick up your urine. WL13 Urine collected?	we it. If not now, we can come back to pick up the sam         We would like the fresh stool you can give us. Please         No
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stool sample. If you can provide this now, we appreciate at a later time. INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL: For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass. For urine: We will return tomorrow to pick up your urine. WL13 Urine collected? WL14 RESULTS (blood in urine) Ask the women if she is Menstruating	we it. If not now, we can come back to pick up the sam         We would like the fresh stool you can give us. Please         No
stool sample. If you can provide this now, we appreciate at a later time. INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL: For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass. For urine: We will return tomorrow to pick up your urine. WL13 Urine collected? WL14 RESULTS (blood in urine)	e it. If not now, we can come back to pick up the sam         We would like the fresh stool you can give us. Please         No0       yes        01         Negative         positive
<ul> <li>stool sample. If you can provide this now, we appreciate at a later time.</li> <li>INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL:</li> <li>For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass.</li> <li>For urine: We will return tomorrow to pick up your urine.</li> <li>WL13 Urine collected?</li> <li>WL14 RESULTS (blood in urine)</li> <li>Ask the women if she is Menstruating (Don't test if the women is in Menstruation)</li> </ul>	e it. If not now, we can come back to pick up the sam         We would like the fresh stool you can give us. Please         No01         No01         Negative
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<ul> <li>stool sample. If you can provide this now, we appreciate at a later time.</li> <li>INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL:</li> <li>For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass.</li> <li>For urine: We will return tomorrow to pick up your urine.</li> <li>WL13 Urine collected?</li> <li>WL14 RESULTS (blood in urine)</li> <li>Ask the women if she is Menstruating (Don't test if the women is in Menstruation)</li> <li>WL15 Stool collected?</li> <li>WL16 Date stool sample taken (Ethiopian calendar)</li> <li>WL17 Time when stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent to the stool passed by the respondent (as recompared to the stool passed by the respondent to the stool</li></ul>	e it. If not now, we can come back to pick up the sam         We would like the fresh stool you can give us. Please         No0         0       yes        01         Negative
<ul> <li>stool sample. If you can provide this now, we appreciate at a later time.</li> <li>INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL:</li> <li>For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass.</li> <li>For urine: We will return tomorrow to pick up your urine.</li> <li>WL13 Urine collected?</li> <li>WL14 RESULTS (blood in urine)</li> <li>Ask the women if she is Menstruating</li> <li>(Don't test if the women is in Menstruation)</li> <li>WL15 Stool collected?</li> <li>WL16 Date stool sample taken (Ethiopian calendar)</li> <li>WL17 Time when stool passed by the respondent (as recording cup) (Ethiopian time)</li> </ul>	e it. If not now, we can come back to pick up the sam         We would like the fresh stool you can give us. Please         No0         0       yes        01         Negative
<pre>stool sample. If you can provide this now, we appreciate at a later time. INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL: For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass. For urine: We will return tomorrow to pick up your urine. WL13 Urine collected? WL14 RESULTS (blood in urine) Ask the women if she is Menstruating (Don't test if the women is in Menstruation) WL15 Stool collected? WL16 Date stool sample taken (Ethiopian calendar) WL17 Time when stool passed by the respondent (as record) (Ethiopian time) WL18 Time when stool collected from the respondent (Ethiopian calendar)</pre>	e it. If not now, we can come back to pick up the sam         We would like the fresh stool you can give us. Please         No0         0       yes        01         Negative
<ul> <li>stool sample. If you can provide this now, we appreciate at a later time.</li> <li>INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL:</li> <li>For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass.</li> <li>For urine: We will return tomorrow to pick up your urine.</li> <li>WL13 Urine collected?</li> <li>WL14 RESULTS (blood in urine)</li> <li>Ask the women if she is Menstruating</li> <li>(Don't test if the women is in Menstruation)</li> <li>WL15 Stool collected?</li> <li>WL16 Date stool sample taken (Ethiopian calendar)</li> <li>WL17 Time when stool passed by the respondent (as recording cup) (Ethiopian time)</li> </ul>	e it. If not now, we can come back to pick up the sam         We would like the fresh stool you can give us. Please         No0         0       yes        01         Negative

Ig TIME BLOOD centrifuged (Ethiopian time)	Minute	
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1. Informed Consent Form for Household Head			
Ethiopian Food and Nutrition Strategy (FNS) Baseline Survey			
Investigator(s): Dr.Masresha Tessema (PI), Mese	ret W/Yohannes, Dr. Meron Girma, Alemnesh Petros, D		
Aregash Samuel, Arnaud Laillou, Stanley Chitekwe, Kaleab Baye, Ramadhani Noor, Anne S			
other co-authors You are being invited to take part in this research because you are head of household. There			
characteristics, and socio-economic statu	s of your household. We will also assess dietary intake,		
anthropometric status, nutrition sensitive	and specific indicators and micronutrient status of		
your household member.			
Box 1. Taking part in this research is voluntary			
You can refuse to take part in this study.			
□You can withdraw your participation from the st	tudy at any time		
Information related to the study	¢		
The FNS baseline survey will be conduct	ed in the 12 regions of Ethiopia. The study population		
will be children age 0-59 months having	caregivers/mothers, school-age children (6-12 years),		
adolescent girls (10-19 years), reproduct	ive-age women (15-49 years), pregnant and lactating		
	ors that will be collected for the survey will be dietary		
	sensitive and specific indicators and micronutrient		
status.			
The expected possible adverse effects: there is no a	dverse effect by participating in this study		
	e information on anthropometric status, dietary		
	ent population groups in Ethiopia, and assess the		
coverage of direct and indirect nutrition interventions. Study design: A population-based, cross-sectional design			
			The schedule of the study: The study will be conducted
Foreseeable risks and expected benefits arising from <b>p</b>	articipation in the study		
oreseeable risks	Expected benefits		
lisks to study participants for involvement in the	The findings of the study will help the ministry of		
coverage survey are low. There may be risks associated	health and other stakeholders engaged in nutrition		

vith COVID pandemic. Interviewers will be trained to		to improve and/or design appropriate health a	
ninimize this risk and will use appropriate prevention		nutrition intervention programs in the country.	
leasures.			
ccurrences that may take place during the	study period		
ccurrences	How to man	nage	
		se, we would respect the volunteer's decision to	
		nd also get a clear understanding of the reason for rawal	
At the end of the study, you will not	be receiving	any financial benefits, but will get your results for	
height, weight, mid upper arm and wa	aist circumfe	rence measurements, anemia and goiter status f	
time you spent and participation.			
All data collected from the study will be	e kept confide	ential. If you have any questions related to the stud	
you may contact directly Dr. Masresha	Tessema who	o is the project PI.	
The contact persons 1. Dr. Masresha Tessema Tel. [+251 919782082] E-			
		nail: [masresha88@gmail.com] or	
2. [Mr. Ibrahim Kedir] Tel. [+251 91195]	7161] EPHI's	IRB	
	Certificate o	of Consent	
I have read the foregoing information.	I have an	I confirm that the participant was given ar	
opportunity to ask questions and all my qu		opportunity to ask questions about the study and	
have been answered to my satis	faction. I	all questions have been answered correctly.	
volunteer to give consent to participate in this research study  Printed name of the participant		confirm that the consent has been given	
		voluntarily	
		J.	
		Printed name of the person taking the consent	
Signature of the participant			
Date		Signature of the person taking the consent	
day/month/year		Date	
		day/month/year	

	2. Informed Consent Form for Women of Rep	nouncive Age
	Ethiopian Food and Nutrition Strategy (FNS) Baseli	ne Survey
	Investigator(s): Dr.Masresha Tessema (PI), Mesere	t W/Yohannes, Dr. Meron Girma, Alemnesh Petros, I
	Kaleab Baye, Ramadhani Noor, Anne Sophie Donze ar	
	You are being invited to take part in this research be	ecause you are women of reproductive age. There
	are [16596] households taking part in this research.	We will assess your dietary intake, anthropometric
	status, nutrition sensitive and specific indicators and	d micronutrient status
	Box 1. Taking part in this research is voluntary	
	You can refuse to take part in this study.	
	□You can withdraw your participation from the stud	dy at any time
	Information related to the study	
	The FNS baseline survey will be conducted in the	12 regions of Ethiopia. The study population will be
	children age 0-59 months having caregivers/mothe	rs, school-age children (6-12 years), adolescent girls
	(10-19 years), reproductive-age women (15-49 years)	ars), pregnant and lactating women, and household
	head. The indicators that will be collected for the	survey will be dietary intake, anthropometric status,
	nutrition sensitive and specific indicators and micro	nutrient status.
Т	he expected possible adverse effects: there is no adv	verse effect by participating in this study
	The objective of this research: to produce informat	ion on anthropometric status, dietary intakes, and
	micronutrient status of different population groups	in Ethiopia, and assess the coverage of direct and
	indirect nutrition interventions.	
Stuc	y design: A population-based, cross-sectional design	
The	schedule of the study: The study will be conducted f	rom July, 2021 to April, 2023
Fore	eseeable risks and expected benefits arising from par	ticipation in the study
Fore	eseeable risks	Expected benefits
Risk	s to study participants for involvement in the	The findings of the study will help the ministry of
cove	erage survey are low. There may be risks associated	health and other stakeholders engaged in nutrition
with	COVID pandemic. Interviewers will be trained to	to improve and/or design appropriate health and
mini	imize this risk and will use appropriate prevention	nutrition intervention programs in the country.
mea	isures.	

currences	How to manage
thdrawal of volunteers from the study	In such a case, we would respect the volunteer's decision to withdraw and also get a clear understanding of the reason for their withdrawal
At the end of the study, you will not	be receiving any financial benefits, but will get your results for
height, weight, mid upper arm and w	aist circumference measurements, anemia and goiter status for
time you spent and participation.	
All data collected from the study will b	e kept confidential. If you have any questions related to the study
you may contact directly Dr. Masresha	Tessema who is the project PI.
The contact persons	
1. Dr. Masresha Tessema Tel. [+251 91	9782082] E-mail: [masresha88@gmail.com] or
2. [Mr. Ibrahim Kedir] Tel. [+251 91195	7161] EPHI's IRB
	Certificate of Consent
I have read the foregoing information.	. I have an I confirm that the participant was given an
opportunity to ask questions and all	my quest opportunity to ask questions about the study and
have been answered to my satis	sfaction. I all questions have been answered correctly. I
volunteer to give consent to participate	in this confirm that the consent has been given
research study	voluntarily
Printed name of the participant	
	Printed name of the person taking the consent
Signature of the participant	
Date	Signature of the person taking the consent
day/month/year	Date
	day/month/year

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# 3. Informed Consent Form for Pregnant Women

## Ethiopian Food and Nutrition Strategy (FNS) Baseline Survey

**Investigator(s):** Dr.Masresha Tessema (PI), Meseret W/Yohannes, Dr. Meron Girma, Alemnesh Petros, Dr Aregash Samuel, Arnaud Laillou, Stanley Chitekwe, Kaleab Baye, Ramadhani Noor, Anne Sophie Donze and other co-authors

You are being invited to take part in this research because you are pregnant women. There are [16596] households taking part in this research. We will assess your, anthropometric status, nutrition sensitive and specific indicators and anemia status

#### Box 1. Taking part in this research is voluntary

You **can refuse** to take part in this study.

You **can withdraw** your participation from the study at any time

#### Information related to the study

The FNS baseline survey will be conducted in the 12 regions of Ethiopia. The study population will be children age 0-59 months having caregivers/mothers, school-age children (6-12 years), adolescent girls (10-19 years), reproductive-age women (15-49 years), pregnant and lactating women, and household head. The indicators that will be collected for the survey will be dietary intake, anthropometric status, nutrition sensitive and specific indicators and micronutrient status.

The expected possible adverse effects: There is no adverse effect by participating in this study

**The objective of this research:** to produce information on anthropometric status, dietary intakes, and micronutrient status of different population groups in Ethiopia, and assess the coverage of direct and indirect nutrition interventions.

Study design: a population-based, cross-sectional design	
The schedule of the study: The study will be conducted from July, 2021 to April, 2023	
Foreseeable risks and expected benefits arising from participation in the study	

Foreseeable risks	Expected benefits
Risks to study participants for involvement in the	The findings of the study will help the ministry of
coverage survey are low. There may be risks	health and other stakeholder engaged in nutrition
associated with COVID pandemic. Interviewers	to improve and/or design appropriate health and
will be trained to minimize this risk and will use	nutrition intervention programs in the country.
appropriate prevention measures.	۱ <u>ــــــــــــــــــــــــــــــــــــ</u>

#### Occurrences that may take place during the study period

Occurrences	How to manage
Withdrawal of volunteers from the study	In such a case, we would respect the volunteer's
	decision to withdraw and also get a clear
	understanding of the reason for their withdrawal
At the end of the study, you will not be receiving	g any financial benefits, but will get your results for
height, weight, mid upper arm circumference me	asurements, anemia and goiter status for time you
spent and participation.	
All data collected from the study will be kept confid	dential. If you have any questions related to the study
you may contact directly Dr. Masresha Tessema wh	o is the PI
The contact persons	
1. Dr. Masresha Tessema	
Tel. [+251 919782082] E-mail: [masresha88@gmail	.com]
2. [Mr. Ibrahim Kedir] Tel. [+251 911957161]	
Certificate	of Consent
I have read the foregoing information. I have an	I confirm that the participant was given an
opportunity to ask questions and all my quest	opportunity to ask questions about the study and
have been answered to my satisfaction.	all questions have been answered correctly. I
volunteer to give consent to participate in this	confirm that the consent has been given
research study	voluntarily
Printed name of the participant	0
Signature of the participant	Printed name of the person taking the consent
Date	
day/month/year	Signature of the person taking the consent Date
	day/month/year

# 4. Informed Consent Form for Preschool Child

Ethiopian Food and Nutrition Strategy (FNS) Baseline Survey Investigator(s): Dr.Masresha Tessema (PI), Meseret W/Yohannes, Dr. Meron Girma, Alemnesh Petros, Dr

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Aregash Samuel, Arnaud Laillou, Stanley Chitekwe,	Kaleab Baye, Ramadhani Noor, Anne Sophie Donz		
other co-authors			
You are being invited to take part in this research b	ecause you are either a mother or caregiver who h		
a child under the age of 5 years (0-59 months). The	re are [16596] households taking part in this		
research. We would collect a sample of your child's	dietary information, blood, urine and stool. And, v		
will also measure your child's height/ length, weigh	it, and mid upper arm circumference. Finally we wi		
assess, your child's eye for bitot spot			
Box 1. Taking part in this research is voluntary			
You can refuse to take part in this study.			
□You can withdraw your participation from the stu	idy at any time		
Information related to the study			
The FNS baseline survey will be conducted in the	12 regions of Ethiopia. The study population will		
children age 0-59 months having caregivers/moth	ers, school-age children 6-12 years), adolescent g		
(10-19 years), reproductive-age women (15-49 ye	ars), pregnant and lactating women, and househ		
head. The indicators that will be collected for the	survey will be dietary intake, anthropometric stat		
nutrition sensitive and specific indicators and micro	onutrient status.		
The expected possible adverse effects :there is no	adverse effect by participating in this study		
The objective of this research: to produce information	tion on anthropometric status, dietary intakes, and		
micronutrient status of different population groups	s in Ethiopia, and assess the coverage of direct and		
indirect nutrition interventions.			
Study design: a population-based, cross-sectional des	ign		
The schedule of the study: The study will be conducted	ed from July, 2021 to April, 2023		
Foreseeable risks and expected benefits arising from	participation in the study		
Foreseeable risks	Expected benefits		
Risks to study participants for involvement in the	The findings of the study will help the ministr		
coverage survey are low. There may be risks	health and other stakeholder engaged in nutritio		
associated with COVID pandemic. Interviewers will	improve and/or design appropriate health		
be trained to minimize this risk and will use	nutrition intervention programs in the country.		
appropriate prevention measures.			
Occurrences that may take place during the study pe	riod		
Occurrences	How to manage		

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Withdrawal of volunteers from the study	in such a case, we would respect the volunteer's
	decision to withdraw and also get a clear
	understanding of the
	reason for their withdrawal
At the end of the study, you will not be receiving	g any financial benefits, but will get your results for
height/length, weight, mid upper arm circumferend	ce measurements, and anemia for time you spent and
participation.	
All data collected from the study will be kept confid	dential. If you have any questions related to the study
you may contact directly Dr. Masresha Tessema wh	o is the project principal investigator
The contact persons	
1. Dr. Masresha Tessema	
Tel. [+251 919782082] E-mail: [masresha88@gmail	.com]
2. [Mr. Ibrahim Kedir] Tel. [+251 911957161] EPHI'	s IRB
Certificate	of Consent
I have read the foregoing information. I have an	I confirm that the participant was given an
opportunity to ask questions and all my quest	opportunity to ask questions about the study and
have been answered to my satisfaction. I	all questions have been answered correctly. I
volunteer to give consent to participate in this	confirm that the consent has been given
research study	voluntarily
	0
Printed name of the participant	
Signature of the participant's parent or guardian	Printed name of the person taking the consent
Date	
day/month/year	Signature of the person taking the consent Date
	day/month/year

# 5. Informed Consent Form for School Age Children

## Ethiopian Food and Nutrition Strategy (FNS) Baseline Survey

**Investigator(s):** Dr.Masresha Tessema (PI), Meseret W/Yohannes, Dr. Meron Girma, Alemnesh Petros, Dr Aregash Samuel, Arnaud Laillou, Stanley Chitekwe, Kaleab Baye, Ramadhani Noor, Anne Sophie Donze and other co-authors

You are being invited to take part in this research because you are either a mother or caregiver who has a school-age child. Among children 6 - 12 years, we will collect your child's blood, urine and stool.

## Box 1. Taking part in this research is voluntary

You can refuse to take part in this study.

You can withdraw your participation from the study at any time

#### Information related to the study

The FNS baseline survey will be conducted in the 12 regions of Ethiopia. The study population will be children age 0-59 months having caregivers/mothers, school-age children (6-12 years), adolescent girls (10-19 years), reproductive-age women (15-49 years), pregnant and lactating women, household head. The indicators that will be collected for the survey will be dietary intake, anthropometric status, nutrition sensitive and specific indicators and micronutrient status.

The expected possible adverse effects: there is no adverse effect by participating in this study

**The objective of this research:** to produce information on anthropometric status, dietary intakes, and micronutrient status of different population groups in Ethiopia, and assess the coverage of direct and indirect nutrition interventions.

Study design: a population-based, cross-sectional design

The schedule of the study: The study will be conducted from July, 2021 to April, 2023

Foreseeable risks and expected benefits arising from participation in the study

Foreseeable risks	Expected benefits	
Risks to study participants for involvement in the	The findings of the study will help the ministry of	
coverage survey are low. There may be risks	health and other stakeholder engaged in nutrition	
associated with COVID pandemic. Interviewers	to improve and/or design appropriate health and	
will be trained to minimize this risk and will use	nutrition intervention programs in the country.	
appropriate prevention measures.		
Occurrences that may take place during the study	period	
Occurrences	How to manage	

Withdrawal of volunteers from the study	In such a case, we would respect the volunteer's
	decision to withdraw and also get a clear
	understanding of the reason for their withdrawal
At the end of the study, you will not be receiving an	ny financial benefits, but you will get your <b>anemia</b> and
goiter status for time you spent and participation.	
All data collected from the study will be kept config	dential. If you have any questions related to the study
you may contact directly Dr. Masresha Tessema wh	o is the project's PI
The contact persons	
1. Dr. Masresha Tessema	
Tel. [+251 919782082] E-mail: [masresha88@gmail	.com]
2. [Mr. Ibrahim Kedir] Tel. [+251 911957161] EPHI'	s IRB
Certificate	of Consent
I have read the foregoing information. I have an	I confirm that the participant was given an
opportunity to ask questions and all my quest	opportunity to ask questions about the study and
have been answered to my satisfaction. I	all questions have been answered correctly. I
volunteer to give consent to participate in this	confirm that the consent has been given
research study	voluntarily
Printed name of the participant	C
Signature of the participant's parent or guardian	Printed name of the person taking the consent
Date	Signature of the person taking the consent Date
day/month/year	
	day/month/year

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	6. Assent form for Adolescent Girls (10-19 years)
Et	hiopian Food and Nutrition Strategy (FNS) Baseline Survey
In	vestigator(s): Dr.Masresha Tessema (PI), Meseret W/Yohannes, Dr. Meron Girma, Alemnesh Petros, D
Ar	egash Samuel, Arnaud Laillou, Stanley Chitekwe, Kaleab Baye, Ramadhani Noor, Anne Sophie Donze an
ot	her co-authors
	You are being invited to take part in this research because you are Adolescent girl. There are [16596]
	households taking part in this research. We will measure your dietary information (for those adolescent
	girls aged 15-17 years), information related to nutrition -sensitive and nutrition-specific practices, blood
	and stool, we will also measure your height, weight, and mid upper arm and waist circumference and
	your goiter status
	Box 1. Taking part in this research is voluntary
	You can refuse to take part in this study.
	You can withdraw your participation from the study at any time
	Information related to the study
	The FNS baseline survey will be conducted in the 12 regions of Ethiopia. The study population will be
	children age 0-59 months having caregivers/mothers, school-age children (6-12 years), adolescent girl
	(10-19 years), reproductive-age women (15-49 years), pregnant and lactating women, and household
	head. The indicators that will be collected for the survey will be dietary intake, anthropometric status
	nutrition sensitive and specific indicators and micronutrient status
	The expected possible adverse effects: There is no adverse effect by participating in this study
	The objective of this research: to produce information on anthropometric status, dietary intakes, and
	micronutrient status of different population groups in Ethiopia, and assess the coverage of direct and
	indirect nutrition interventions.
	Study design: a population-based, cross-sectional design
	The schedule of the study: The study will be conducted from July, 2021 to April, 2023
	Foreseeable risks and expected benefits arising from participation in the study
	Foreseeable risks Expected benefits
	Risks to study participants for involvement in the coverage survey are low. There may be risks associated with COVID pandemic. Interviewers will be trained to minimize this risk and will use appropriate prevention measures.
	For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

Occurrences that may take place during the study	period	
Occurrences How to manage		
Withdrawal of volunteers from the study	In such a case, we would respect the volunteer	
	decision to withdraw and also get a cle	
	understanding of the reason for their withdrawal	
At the end of the study, you will not be receiving	g any financial benefits, but will get your results	
height, weight, mid upper arm and waist circumf	erence measurements, anemia and goiter status	
time you spent and participation.		
All data collected from the study will be kept confid	dential. If you have any questions related to the stu	
you may contact directly Dr. Masresha Tessema wh	no is the project's PI	
The contact persons		
1. Dr. Masresha Tessema		
Tel. [+251 919782082] E-mail: [masresha88@gmail	.com]	
2. [Mr. Ibrahim Kedir] Tel. [+251 911957161] EPHI'	's IRB	
Certificate	e of Assent	
have read the foregoing information. I have an	I confirm that the participant was given a	
opportunity to ask questions and all my quest	opportunity to ask questions about the study an	
nave been answered to my satisfaction. I all questions have been answered correct		
volunteer to give assent to participate in this confirm that the assent has been given volun		
research study		
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rinted name of the participant		
	Printed name of the person taking the assent	
nature of the participant		
Date	Signature of the person taking the assent Date	
day/month/year	day/month/year	

STROBE Stater	nent—checklist of items that	should be included in i	reports of observational studies
ornobe otater			

	ltem No	Recommendation	Reported on page #
Title and	1	(a) Indicate the study's design with a commonly used term in the	Title p.1; Abstract p.2
abstract		title or the abstract	3
		(b) Provide in the abstract an informative and balanced summary of	
		what was done and what was found	
Introduction			
Introduction	4	Explain the scientific background and rationale for the investigation being reported	Introduction
/rationale			p.3-4
Objectives	5	State specific objectives, including any prespecified hypotheses	Background p.5 last statements
Methods			
Study design	5	Present key elements of study design early in the paper	Methods (data source) p.4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Methods (data source) p.5
Participants	5	(a) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	Methods (participants) p.5-8
		<ul> <li>(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed</li> <li>Case-control study—For matched studies, give matching criteria and the number of controls per case</li> </ul>	
Variables	5	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Methods (all outcome and exposure variables are listed) p.6-8
Data sources/ measurement	NA	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Methods (methods of measurement indicated in) p.5-8
Bias	10	Describe any efforts to address potential sources of bias	Methods (data quality indicated) p.10-11
Study size	5-6	Explain how the study size was arrived at	Methods (data source,statistical

	• • •	
anal	lysis)	$\mathbf{p}.5$
ana	,,	P.0

Quantitative 10 variables	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Methods (measurement and statistic analysis sections) p. 3 9
Statistical 10 methods	( <i>a</i> ) Describe all statistical methods, including those used to control for confounding	Methods (analysis section) p. 8-
	(b) Describe any methods used to examine subgroups and interactions	
	(c) Explain how missing data were addressed	
	(d) Cohort study—If applicable, explain how loss to follow-up was addressed	
	<i>Case-control study</i> —If applicable, explain how matching of cases	
	and controls was addressed	
	Cross-sectional study—If applicable, describe analytical methods	
	taking account of sampling strategy	
Continued on next page	( <u>e</u> ) Describe any sensitivity analyses	

Participants	5*	(a) Report numbers of individuals at each stage of study—eg numbers	NA
Participants	J		
		potentially eligible, examined for eligibility, confirmed eligible,	
		included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	<b>NT</b> 4
Descriptive	14*	(a) Give characteristics of study participants (eg demographic, clinical,	NA
data		social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable	
		of interest	
		(c) Cohort study—Summarise follow-up time (eg, average and total	
		amount)	
Outcome data	15*	Cohort study—Report numbers of outcome events or summary	NA
		measures over time	
		Case-control study—Report numbers in each exposure category, or	
		summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or	
		summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted	NA
	-	estimates and their precision (eg, 95% confidence interval). Make	
		clear which confounders were adjusted for and why they were	
		included	
		(b) Report category boundaries when continuous variables were	
		categorized	
		(c) If relevant, consider translating estimates of relative risk into	
		absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and	NA
	17	interactions, and sensitivity analyses	1111
Discussion		O	
Key results	18	Summarise key results with reference to study objectives	p. 9-10
Limitations	19	Discuss limitations of the study, taking into account sources of	Discussio
		potential bias or imprecision. Discuss both direction and magnitude of	(strength
		any potential bias	and
			weakness
			of the
			study) p.1
			11
Interpretation	20	Give a cautious overall interpretation of results considering	Discussio
		objectives, limitations, multiplicity of analyses, results from similar	(interpre
		studies, and other relevant evidence	of finding
			the conte
			existing
			research,
			meaning

Discuss the generalisability (external validity) of the study resul	ts Discussion (strengths and
	weakness of the study) p.10-11
Give the source of funding and the role of the funders for the p study and, if applicable, for the original study on which the pres article is based	01
5	study and, if applicable, for the original study on which the pres

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org. **BMJ** Open

# **BMJ Open**

# Ethiopia National Food and Nutrition Survey to inform the Ethiopian National Food and Nutrition Strategy: a study protocol

Journal:	BMJ Open
Manuscript ID	bmjopen-2022-067641.R2
Article Type:	Protocol
Date Submitted by the Author:	28-Mar-2023
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<b>Primary Subject Heading</b> :	Public health
Secondary Subject Heading:	Epidemiology
Keywords:	EPIDEMIOLOGY, NUTRITION & DIETETICS, PUBLIC HEALTH

# SCHOLARONE[™] Manuscripts

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4	1	Ethiopia National Food and Nutrition Survey to inform the Ethiopian National Food and
5 6	2	Nutrition Strategy: a study protocol
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#### ABSTRACT

**Introduction** Ethiopia has made significant progress in reducing malnutrition in the past two decades. Despite such improvements, a substantial segment of the country's population remains chronically undernourished and suffers from not only micronutrient deficiencies but also from increasing diet-related non-communicable diseases such as diabetes, hypertension and cancer. This survey aims to assess anthropometric status, dietary intake and micronutrient status of Ethiopian children, women, and adolescent girls. The study will also assess coverage of direct and indirect nutrition-related interventions and map agricultural soil nutrients. The survey will serve as a baseline for the recently developed Ethiopian Food System Transformation Plan and will inform the implementation of the National Food and Nutrition Strategy. 

Methods and analysis As a population-based, cross-sectional survey, the study will collect data from the ten regions and two city administrations of Ethiopia. The study population will be women of reproductive age, children aged 0-59 months, school-aged children and adolescent girls. A total of 16,596 households will be surveyed, allowing the generation of national and regional estimates. A two-stage stratified cluster sampling procedure will be used to select households. In the first stage, 639 enumeration areas will be selected using probability-proportional-to-size allocation. In the second stage, 26 eligible households will be selected within each enumeration area using systematic random selection. Primary outcomes include coverage of direct and indirect nutrition interventions, infant and young child feeding practices, food insecurity, dietary intakes, mental health, anthropometric status, micronutrient status, and soil nutrient status. 

Ethics and dissemination The protocol was fully reviewed and approved by the Institutional Review Board of the Ethiopian Public Health Institute (protocol no: EPHI-IRB-317-2020). The study is based voluntary participation and written informed consent is required from study participants. The findings will be disseminated via forums and conferences and will be submitted for publication in peer-reviewed journals. 

#### Strengths and limitations of this study

- The survey covers a large geographic area, collecting data on anthropometric status, 24hr recall quantitative dietary intakes and the determination of micronutrient status in the same participants or household, while also capturing data on the food system in Ethiopia.
  - The study aims improve understanding of nutritional problems across multiple facets—from agricultural soil to people to the environment in Ethiopia.
    - Inherent to the cross-sectional design of the study, the findings of this study cannot be used to establish cause and effect.
    - The study design prevents us from considering seasonal differences in nutritional outcomes and • determinants.

# 60 INTRODUCTION

Globally, one in every three population are affected by one of more forms of malnutrition.¹ Women and children are particularly vulnerable to malnutrition due to increased physiological nutrient needs required to support fetal and child growth.² Nutritional deprivation during early life impairs growth and development, leading to poor school performance, reduced productivity, and loss of earnings in later life.³ Consequently, the first 1000 days of life, from conception to the child's second year of life, was recognized as a critical window of opportunity to effectively prevent malnutrition.^{3 4} Adolescence is also identified as a second window of opportunity to correct nutritional inadequacies and adversities faced in early life, but little is known about this life stage.

Despite significant progress over the past two decades, the burden of malnutrition in Ethiopia remains high.⁵⁻⁷ Nationally, 37% of Ethiopian children under five years of age are stunted⁷, and 22% of women of reproductive age (WRA) are chronically undernourished (Body Mass Index (BMI) < 18.5 Kg/m²).⁵ Only 14% of children under two years of age consumed the minimum number of recommended food groups.⁵ Furthermore, micronutrient deficiencies co-exist with chronic energy deficiency.⁸ This along with the ongoing nutrition transition, characterized by shifts in diets⁹, is further complicating the nutrition landscape by increasing the prevalence of overweight and non-communicable diseases.⁵ Nearly a fifth (16%) of Ethiopian adults are estimated to be hypertensive, and 3% are diabetic.¹⁰ Therefore, addressing not only undernutrition but all forms of malnutrition is critical. 

The Sustainable Development Goals (SDGs) recognize the importance of nutrition, primarily driven by the need to mitigate its detrimental consequences. Further, the 2012 World Health Assembly (WHA) identified global targets to be achieved by 2025 that aims to reduce stunting, anemia, low birth weight, and childhood obesity. These targets are used to track progress in SDG goal 2: Zero hunger.¹¹ Recognizing the importance of good nutrition, the Government of Ethiopia has made ending malnutrition a national priority. Ethiopia started implementing its first National Nutrition Program in 2008.¹² The second phase of this program (2011-2016) was a multisectoral program aimed at accelerating progress in reducing malnutrition.¹³ Moreover, Ethiopia's first Food and Nutrition Policy was endorsed in 2018¹⁴, followed the National Food and Nutrition Strategy¹⁵ which was launched in 2021 to provide a framework for the operationalization of the policy. Acceleration of progress in the reduction of malnutrition requires the design and implementation of direct and indirect nutrition interventions that can be implemented at scale. To this end, understanding the various factors contributing to the different forms of malnutrition is critical. 

Multiple factors operating at the immediate, underlying, and basic levels contribute to malnutrition.² Inadequate dietary intake and poor health are immediate determinants.² Household food security, child care practices, access to health services, and healthy environments are underlying determinants.¹⁶ Structural and contextual factors such as economic structures, political, environmental, social and cultural factors are the basic determinants of malnutrition.² The contribution of these factors varies across different contexts, and target groups, but studies capturing all these factors in a single survey are scant. The lack of timely and comprehensive information on nutritional status across critical 

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98 life stages and their determinants is a bottleneck that is preventing Ethiopia from designing effective 99 interventions. Up to date and comprehensive data on the coverage of direct and indirect nutrition 100 interventions delivered across various implementing sectors of the National Food and Nutrition Strategy 101 is not yet available. This is unfortunate as such data could inform the implementation of the Strategy, but 102 it can also serve as a baseline against which progress can be tracked.

10 103 Therefore, this study aims to provide the first ever comprehensive information on the nutritional
 104 status of different populations in Ethiopia to support evidence-based implementation of the National
 105 Food and Nutrition Strategy.

## **Objectives**

The overall goal of this study will be to produce nationally and regionally representative estimates on
 anthropometric status, coverage of nutrition interventions, dietary intakes, and micronutrient status for
 children, adolescent girls, and women of reproductive age in Ethiopia.

# 2122110 Specific objectives include:

- 1. Assess the coverage of direct and indirect nutrition interventions.
- 2. Assess food consumption patterns and nutrients intakes of children aged 6–59 months, and women of reproductive age.
  - Assess the micronutrient status of children (vitamin A, anemia, iron, iodine and zinc), adolescent girls, and women of reproductive age (vitamin A, vitamin D, anemia, iron, iodine, zinc, folate, vitamin B₁₂)
- - 119 5. Assess the geographical distribution of soil micronutrient status in Ethiopian agricultural soil.
- 37 121 METHODS AND ANALYSIS
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# ³⁹ 122 Study design

This study is a nationally and sub-nationally (regionally) representative cross-sectional survey that will characterize dietary intake, micronutrient status, and access to nutrition-related services for different target populations. Given that soil nutrient content can influence micronutrient content of foods and hence affect nutrient intake, the soil nutrient composition will also be analyzed. The study will have four main components. The first component will assess nutrition-specific and nutrition-sensitive indicators (NSS) for all target groups (children aged 0-59 months and WRA, school-age children, and adolescent girls) using semi-structured questionnaires. The second component will measure quantitative dietary intake for children aged 6-59 months and WRA (15-49 years). The third component of the survey will collect biomarker samples from all children (6-59 months), school-age children (6-12 years), adolescent girls (10-19 years), and WRA (15-49 years). The final component of the study will measure micronutrients in agricultural soils. The study data will be collected from July 2021 to December 2023. 

54 134 Setting

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Ethiopia has an estimated population size of 120 million and is the second most populous country in

Africa.¹⁷ The majority of its population resides in rural areas (70%).¹⁷ Agriculture accounts for 40% of the

country's gross domestic product.¹⁷ Children aged 15 years and younger make up 40% of the Ethiopian

population in 2021.¹⁸ Ethiopia is administratively divided into 10 regions and two city administrations.

This study will be conducted in all of the regions and city administrations of the country. Figure 1

# 141 Participants

142 The target population of this study are i) women of reproductive age (WRA) aged 15-49 years ii) children 143 aged 0-59 months iii) school-age children aged 6-12 years, and iv) adolescent girls aged 10-19 years, and 15 144 V) bousehold head

provides a geographic representation of the study areas.

15 144 V) household head.16

# 17 145 Sample size calculations

Sample size was estimated to guarantee adequate precision to generate national and regional estimates for selected indicators for each study target group. Indicators used for each target group are shown in Supplemental Table 1S. The required number of households and target groups was calculated using a single population proportion formula at the regional level. We used region-specific prevalence estimates for indicators, a 5% margin-of-error, a design effect of 1.5, a household response rate of 95%, and an individual response rate of 80%. The initial sample size was then adjusted for region-specific average household size and percentage of the target population from the total population. An indicator that provides the maximum number of households was used to estimate the final sample size for each region. Regional sample sizes were summed up to derive the total (national) sample size. Based on these calculations, the total sample size for the overall survey was 16,596 households (Supplemental Table 2S). 

For WRA, dietary and biomarker data will be collected in half of the selected households within each Enumeration Area (EA). This selection will yield a total sample size of 7,386 WRA (50% of the expected 14,772 WRA). The sample size needed to assess dietary intakes and micronutrient status of WRA was calculated using the prevalence of inadequate zinc intake, which yielded the largest sample size.8 19 

# 39 161 Sampling procedures

A two-stage stratified cluster sampling procedure will be used to select households. In the first stage, 639 EAs, 257 urban and 382 rural will be selected using probability-proportional-to-size allocation. We will use the 2018 Ethiopia Population and Housing Census enumeration areas sampling frame to select EAs (the Primary Sampling Units (PSUs)). The Central Statistical Agency (CSA) prepared the enumeration areas sampling frame. An EA typically contains 100-150 households. EA maps will be used to delineate the boundaries of the selected EA. In the second stage of sampling to identify eligible households, all households with the EA will be listed. A household will be eligible for selection if at least one of the study target groups are residents (de jure) or stayed at the household the night before the interview (de factor). 

51<br/>52171Twenty-six (26) eligible households will be selected within each EA using systematic random53172selection. All target groups will be eligible for the NSS interview in the selected households. All children54173aged 6-59 months will also be eligible for dietary assessment. Women residing in 13 households (Out of5517426 households) who will be selected randomly will be eligible for dietary assessment. Biomarker samples

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will be collected for all children under 5 years of age, school-age children, and adolescents in the selected
households. Similar to dietary assessment, biomarker samples will be collected for women residing in half
of the selected households (Figure 2).

8 178 9 179 **Outcomes** 

# 10 180 Coverage of direct and indirect nutrition interventions

A structured questionnaire will be used to determine the coverage of direct and indirect nutrition interventions provided to children aged 6-59 months, WRA, and adolescent girls. Direct nutrition interventions included vitamin A supplementation, iron supplementation, zinc supplementation, growth monitoring and promotion, nutrition counseling services, and food fortification. Water, sanitation and hygiene, coverage of food or cash assistance program, women empowerment, and mental health will be some nutrition-sensitive indicators considered in this study (Table 1). We will use standard indicator definitions proposed by the Data for Decisions to Expand Nutrition Transformation project (DataDENT) to assess coverage of nutrition programs. 

# 22 189 Anthropometric status 23

Using standardized procedures, anthropometric measurements, including weight, height/length, and mid-upper arm circumference, will be taken for all study target populations.²⁰ Anthropometric indices (weight-for-height z-scores, length/height-for-age z-scores, weight-for-age z-scores, BMI-for-age z-scores) will be calculated using the WHO 2006 child growth standards and the WHO 2007 child growth reference data. Stunting (length/height-for-age z-scores below -2 SD), wasting (weight-for-height z-scores below -2 SD), underweight (weight-for-age z-scores below -2 SD), thinness (BMI-for-age z-scores below -2 SD) and BMI will be the primary anthropometric outcomes of interest. 

# 197 Infant and young child feeding practices

Infant and young child feeding practices will be assessed using the new World Health Organization (WHO)
 and United Nations Children's Fund (UNICEF) recommended 17 indicators to evaluate IYCF practices.²¹

# 3839200 Food insecurity

The Food Insecurity Experience Scale (FIES) will be used to assess household food security.²² The FIES
 consists of eight questions that assess household experience related to adequate food access. Experience
 questions range from worrying about getting enough food to not eating for a whole day.

In addition to these outcome indicators, information on the sociodemographic characteristic of
 households, child health, maternal health, employment status, and household agricultural practices will
 be collected using structured questionnaires.

50 207 *Mental health of women* 

Common mental health disorders will be assessed using the WHO Self-reporting questionnaire which
 consists of 20 questions. Women will be classified as having a common mental health disorder if the row
 score will be greater or equal to 6 out of 20.²³

# Assessment of dietary intakes of children and women of reproductive age

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We will measure dietary intake for children aged 6-59 months and WRA. A one-day quantitative multiple-pass 24-hour recall will be conducted to assess dietary intakes. The interactive multiple-pass 24-hour recall interview consists of four steps designed to enhance memory.²⁴ All days of the week will be proportionately represented during the dietary survey to account for the day of the week effects on food intake. To account for the day-to-day variability of dietary intake within individuals, a second nonconsecutive day 24-hr recall (repeat) will be collected (within 2 to 10 days of the first recall) on a randomly selected sub-sample of WRA and children. The number of repeats needed is determined by allocating for each region 50 repeats, which is then multiplied by a design effect of 1.5 and a 10% non-response rate. The number of repeats will be rounded up to 1244 recalls for each target group to ensure that the minimum number of repeats (n=83) needed from each region would be collected. Detailed non-standard recipe ingredient data will be collected for all mixed dishes that were prepared at home. 

We will use 15 food groups to assess the dietary intakes of women (15-49 months) and children aged 24-59 months. These food groups were: 1) Cereals and their products, 2) Starchy Roots and tubers, and their products, 3) Pulses, and their products, 4) Vegetables and their products, 5) Fruits and their products, 6) Meat, and poultry their products, 7) Eggs and their products, 8) Fish, shellfish and their products, 9) Milk and milk products, 10) Fats and oils, 11) Nuts and seeds, 12) Sugar and sweetened products, 13) Beverages, 14) Spices and condiments, and 15) Miscellaneous. For children aged 6-23 months, we will use the updated WHO, UNICEF food groups: 1) Breastmilk, 2) Grains, roots, and tubers, 3) Pulses, nuts, and seeds, 4) Dairy products, 5) Flesh foods (meats, fish, poultry, organ meats), 6) Eggs 7) Vitamin-A rich fruits and vegetables, and 8) other fruits and vegetables. These food groups were adapted from the FAO/WHO Global Individual Food consumption data Tool (GIFT) food groups.²⁵

Dietary assessment pre-survey work: We carried out pre-survey work to aid dietary data collection following recommendations set by the Intake: Center for Dietary Assessment.^{26 27} An initial step will be developed a food and ingredient list containing a comprehensive list of food items, mixed dishes, and ingredients expected to be consumed by the study target groups. The food list will be generated using data from the first 2011 Ethiopian National Food Consumption Survey.¹⁹ Other common foods consumed across the regions in Ethiopia will be derived from the 2016 Household Income and Expenditure Surveys,²⁸ the Ethiopian Food Composition Tables, and dietary intake data from other recent dietary assessment surveys conducted by the Ethiopian Public Health Institute (EPHI). Portion size estimation methods suitable for large-scale studies will be pre-selected for use in the survey following Intake recommendations.²⁹ The selected methods will be direct measurement of actual foods consumed, standard unit: size and number, proxy measurement using play dough, water, rice, and maize flour, and finally using food price to estimate the amount of food consumed. Portion size estimation methods will be assigned for all foods included in the food list. 

**Table 1.** Nutrition direct and indirect interventions coverage

No	Indicator	Target population				
	Nutrition indirect intervention coverage					
	Child interventions					
1.	Children received iron tablets/syrup in the last 12 months	Children aged 6-59 months				

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2.	Children received vitamin A supplements in the past 6 months	Children aged 6-59 months
3.	Children received deworming tablets in the past 6 months	Children aged 24-59 months
A		Children aged 0 50 menths
4.	All 8 basic vaccinations: one dose of BCG, three	Children aged 9-59 months
	doses of DPT, three doses of the polio vaccine,	
	and one dose of the measles vaccine	
5.	No Vaccination	Children aged 0-59 months
	Growth monitoring	
6.	Weight measured in the last 3 months	Children aged 0-23 months
7.	Height measured in the last 3 months (Optional)	Children aged 0-23 months
8.	MUAC measured in the last 3 months (Optional)	Children aged 0-23 months
	Infant and young child feeding (IYCF) counselling	
9.	Mothers with children 6-23 months received any	Children aged 6-23 months
	IYCF counseling	_
10.	Mothers with children 6-23 months received age-	Children aged 6-23 months
	appropriate IYCF counseling	
	Early breast-feeding counseling	I
11.	Women received breastfeeding counseling with	Women aged 15-49 years with a
	observation during the first two days after birth	live birth in the past 5 years for
		the most recent birth
12.	Women received breastfeeding counseling during	Women aged 15-49 years with a
12.	the first month after birth	live birth in the past 5 years for
		the most recent birth
	Coverage of Nutritional Interventions during pregnancy/ant	
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13.	Percentage of women who had 4 or more ANC	Women aged 15-49 years with a
13.	Percentage of women who had 4 or more ANC visits for the most recent birth	Women aged 15-49 years with a birth in the last 5 years
13. 14.	Percentage of women who had 4 or more ANC visits for the most recent birth Percentage of women who received counseling	Women aged 15-49 years with a birth in the last 5 years Women aged 15-49 years who
	Percentage of women who had 4 or more ANC visits for the most recent birth	Women aged 15-49 years with a birth in the last 5 years Women aged 15-49 years who received antenatal care for their
14.	Percentage of women who had 4 or more ANC visits for the most recent birth Percentage of women who received counseling about healthy eating during pregnancy	Women aged 15-49 years with a birth in the last 5 years Women aged 15-49 years who received antenatal care for their most recent birth
14.	Percentage of women who had 4 or more ANC visits for the most recent birth Percentage of women who received counseling about healthy eating during pregnancy Percentage of women whose weight gain was	Women aged 15-49 years with a birth in the last 5 years Women aged 15-49 years who received antenatal care for their most recent birth Women aged 15-49 years who
14.	Percentage of women who had 4 or more ANC visits for the most recent birth Percentage of women who received counseling about healthy eating during pregnancy	Women aged 15-49 years with a birth in the last 5 years Women aged 15-49 years who received antenatal care for their most recent birth Women aged 15-49 years who received antenatal care for their
14. 15.	Percentage of women who had 4 or more ANC visits for the most recent birth Percentage of women who received counseling about healthy eating during pregnancy Percentage of women whose weight gain was monitored during pregnancy	Women aged 15-49 years with a birth in the last 5 years Women aged 15-49 years who received antenatal care for their most recent birth Women aged 15-49 years who received antenatal care for their most recent birth
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<ul> <li>14.</li> <li>15.</li> <li>16.</li> <li>17.</li> <li>18.</li> <li>19.</li> </ul>	Percentage of women who had 4 or more ANC visits for the most recent birth         Percentage of women who received counseling about healthy eating during pregnancy         Percentage of women whose weight gain was monitored during pregnancy         Women received food or cash assistance during pregnancy         Women took 90+ iron/folate tablets during pregnancy         Women received deworming tablets during pregnancy         Women received necessary         Women took 90+ iron/folate tablets during pregnancy         Women received deworming tablets during pregnancy         Women received deworming tablets during pregnancy	Women aged 15-49 years with a birth in the last 5 years Women aged 15-49 years who received antenatal care for their most recent birth Women aged 15-49 years who received antenatal care for their most recent birth Women aged 15-49 years with a birth in the last 5 years Women aged 15-49 years with a live birth in the past 5 years for the most recent birth Women aged 15-49 years with a live birth in the past 5 years for the most recent birth
14. 15. 16.	Percentage of women who had 4 or more ANC visits for the most recent birth         Percentage of women who received counseling about healthy eating during pregnancy         Percentage of women whose weight gain was monitored during pregnancy         Women received food or cash assistance during pregnancy         Women took 90+ iron/folate tablets during pregnancy         Women received deworming tablets during pregnancy         Women received severation coverage         Basic water services	Women aged 15-49 years with a birth in the last 5 years Women aged 15-49 years who received antenatal care for their most recent birth Women aged 15-49 years who received antenatal care for their most recent birth Women aged 15-49 years with a birth in the last 5 years Women aged 15-49 years with a live birth in the past 5 years for the most recent birth Women aged 15-49 years with a live birth in the past 5 years for the most recent birth Household
<ul> <li>14.</li> <li>15.</li> <li>16.</li> <li>17.</li> <li>18.</li> <li>19.</li> <li>20.</li> </ul>	Percentage of women who had 4 or more ANC visits for the most recent birth         Percentage of women who received counseling about healthy eating during pregnancy         Percentage of women whose weight gain was monitored during pregnancy         Women received food or cash assistance during pregnancy         Women took 90+ iron/folate tablets during pregnancy         Women received deworming tablets during pregnancy         Women received severation coverage         Basic water services         Basic hygiene services	Women aged 15-49 years with a birth in the last 5 years Women aged 15-49 years who received antenatal care for their most recent birth Women aged 15-49 years who received antenatal care for their most recent birth Women aged 15-49 years with a birth in the last 5 years Women aged 15-49 years with a live birth in the past 5 years for the most recent birth Women aged 15-49 years with a live birth in the past 5 years for the most recent birth Household Household

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	pregnancy	live birth in the past 5 years for
		the most recent birth
24.	Basic water services	Household
25.	Basic hygiene services	Household
26.	Basic Sanitation services	Household
27.	Presence of common mental health disorders in the past month	Women aged 15-49 years
28.	Women empowerment	Women aged 15-49 years
29.	Livestock ownership	Household
30	Agricultural productivity by food group	Household

# 247 Assessment of micronutrient status

Blood specimens will be collected from the study population to determine serum retinol, ferritin, soluble transferrin receptor (sTfR), zinc, folate, vitamin B12, red blood cell (RBC) folate and 25-hydroxyvitamin D. Additionally, markers of inflammation, alpha(1)-acid glycoprotein (AGP), high-sensitivity C-reactive protein (hsCRP) will also be measured. We will also analyze parasites from stool specimens. All laboratory analyses will be performed at the EPHI Clinical chemistry, and Food Science and Nutrition Laboratories. Both laboratories participate in an external quality assessment scheme and are accredited by the Ethiopian National Accreditation Office (ENAO). Collection, storage, and analytical procedures for blood, urine, stool, and salt samples are described below. The details of each biomarker analysis are described in Supplemental Materials 1-11. 

Blood sample collection and analysis: Venous blood samples (5-7 mL) will be collected using vacutainer tubes following standard operating procedures.³⁰ Trace mineral-free vacutainer tubes will be used to collect blood for trace metal analysis. After collection, blood samples will be allowed to clot for 30 minutes in cold boxes (<8 °c). Samples will then be centrifuged at 3000 rpm (revolution per minute) for 10 minutes. The separated serum will be aliquoted and stored in -20°c portable freezers in the field. Samples will then be transported to EPHI and stored at -80°c until analysis. Hemoglobin will be measured in the field using Hemocue[®] (Hb 301, Hemocue AB, Angelholm, Sweden)^{31,31} If the hemoglobin values are below WHO cutoff point(11g/dl), the phlebotomist will send whole blood samples to the EPHI laboratory to identify hemoglobinopathies using electrophoresis method.³² Malaria test will be conducted on-site using Bioline[™] Malaria Ag P.f rapid diagnostic test kits (RDT) for P. falciparum and P. vivax).³³ Serum soluble transferrin receptor (sTfR), AGP, hsCRP, folate, red blood cell (RBC) folate, vitamin B₁₂, and ferritin will be measured using Cobas 6000 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Serum retinol will be measured using high-performance liquid chromatography (HPLC) method³³, and serum zinc and selenium will be measured using a microwave plasma atomic emission spectrometers (MP-AES) analyzer. 

50 272 Stool and urine sample collection and analysis: Stool samples will be collected using stool cups and
 51 273 stored in 10 % formalin to preserve the parasite until analysis.³⁴ A portion of each stool sample will be
 52 274 used to detect direct ova, larvae and cysts of intestine parasites using formal ether concentration
 54 275 technique.³⁵ Urine samples will be collected from WRA and school-age children using 60 ml urine cup

containers. Samples will be stored at -20°c. Urinary iodine excretion will be assessed by Sandell Kolthoff reaction at EPHI Laboratory using Shimadzu 1800 UV-Vis spectroscopy.³⁶

Salt collection and analysis: Salt samples will be collected from households with WRA for whom dietary data will be collected. At least 25 grams (one coffee cup) of salt will be collected to determine iodine content using the iodometric titration method.³⁷ 

#### Assessment of nutrients in the soil

Soil samples will be collected from three households in each EA. Zig-zag or cross-sampling method will be used to collect 10 to 20 subsamples (0-30 cm depth) constituting one composite sample. Subsamples will be collected at a separation distance of five meters. After thoroughly mixing composite samples, 1 kg soil sample will be transferred to polyethylene bags. The collected soil samples will be air-dried in wooden trays and disaggregated using a ceramic mortar and pestle (soil grinder) at the EPHI soil laboratory. Samples will then pass through a 6 mm sieve of stainless-steel screens to remove debris and homogenize the soil sample. The sieved fraction will be further pulverized to pass through a 1 mm sieve for the micronutrient analysis. Soil zinc, iron, copper, and manganese will be determined following standard procedures.³⁸ Micronutrient content will be determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES) after extraction with diethylene triamine penta acetic acid (DPTA). Additional variables that affect the mobility of micronutrients in the soil and their uptake into crops will also be measured. These variables include soil reaction (pH), electrical conductivity, organic matter, total nitrogen, and soil organic carbon content. Data collectors will also record topography, slope, cropping history, type, and fertilizer application information. Table 2 provides a summary of procedures for each of the four components of the survey by study target groups. 

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-		Child	Child	Child	School	Adolescent	WRA	Household
		0-5 months	6-23 months	24-59 months	children 6-12 years	girls 10-19 years	15-49 years	
-	Nutrition direct and indirect intervention in	dicators						
-	Infant and young child feeding practices	Х	Х					
	Nutritional information for adolescent girls					Х		
	Food insecurity							х
	Water, sanitation and hygiene practices							Х
	Coverage of food fortification							х
	Agricultural practices							х
	Mental health						Х	
	Anthropometric status	Х	x	Х		Х	Х	
-	Dietary assessment			F				
-	24-hr recall quantitative dietary intake		Х	X			Х	
-	Assessment of biomarker status							
-	Blood sample		Х	Х	X	Х	Х	
	Urine sample				X		Х	
	Stool sample		х	Х	x	Х	Х	
	Salt sample collection							Х
-	Assessment of micronutrients in the Soil					5,		
-	Soil micronutrient assessment							Х
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# Data quality assurance and analysis

Training of trainers on components of the survey will be held before training the data collectors and supervisors. After fifteen days of training on methodological procedures, questionnaires and quality assurance, the questionnaires will be tested in a pilot group (in EAs not included in the actual survey), and adapted based on the received feedback from the survey team. The questionnaires (including the food list) were translated into local languages (Amharic, Oromifa, Tigrigna, Somali, and Afar) and backtranslated to English to ensure the quality of the translation. The data collectors' measurements will be standardized to ensure that the inter-observer variability is within tolerable limits. Supervisors received additional training on teamwork and on monitoring and supervising the data collection process. All data collection tools are programmed using open-source software (ODK) (Supplemental Material 12). Data quality checks will be included during ODK programming to prevent data recording errors. These include restricted responses, filter insert choices, skip patterns, and defaults. During data collection daily data tracking forms will be completed to track completed surveys for each study components to prevent missing data. High frequency checks will be identified prior to the survey's, and error tracking forms will be designed to track data quality in real-time. These checks included completeness checks, target group tracking, and duplicate ID checks. Random field supervision visits will also be made to check data quality. Every day, collected data will be sent to the EPHI central server and imported into statistical software programs as comma-separated values (CSV) files. For laboratory analysis, a quality control chart will be used to ensure the internal and external quality control materials are in the acceptable range.

The primary data analysis will focus on computing frequencies and percentages for categorical variables and summary statistics (like means, medians SD, IQR) for summarizing continuous variables. Sample weights will be constructed based on the selection probabilities of EAs, eligible households, and non-response rates. All analyses will also be adjusted for the survey design. Additional subgroup analysis will be computed for variables with adequate sample sizes for each category. The Biomarkers Reflecting Inflammation and Nutrition Determinants of Anemia (BRINDA) Working Group's regression correction approach will be used to account for inflammation in the study of all micronutrients status using the biomarkers C-reactive protein (CRP) and AGP. Geostatistical analyses will be employed to determine the spatial patterns of micronutrient distribution in the soil and blood samples. The wealth index will be constructed using principal component analysis (PCA).³⁹ The Rasch model will be used to construct the Food Insecurity Experience Scale (FIES). ²² All analyses will be done using STATA 16 and ArcGIS/QGIS. Anthropometric indices will be calculated using the WHO Anthro software for under five children and WHO AnthroPlus software for adolescents.

# Patient and public involvement

None.

# **Ethics and dissemination**

The study protocol is approved by the Institutional Review Board of the Ethiopian Public Health Institute (protocol no: EPHI-IRB-317-2020). Written informed consent will be obtained from each respondent and participants may withdraw at any time (Supplemental Material 13). Confidentiality of all collected data

will be given high priority during each stage of data handling. Individual names and personal information of respondents will be kept confidential and data sets will be kept anonymous for analysis.

The study's findings will be disseminated through several communication channels, including stakeholder workshops, various local and international conferences and technical reports. Additionally, the findings will be submitted for publication in peer-reviewed journals.

## DISCUSSION

This comprehensive, nationally representative survey will for the first time characterize simultaneously the dietary intake and micronutrient status of Ethiopian children, adolescent girls and WRA. Besides, the study assesses key drivers of malnutrition including soil nutrient composition, as well as coverage of direct and indirect nutrition interventions. The survey will provide key insights informing the implementation of Ethiopia's National Food and Nutrition Strategy.

High-quality and timely data is critical to assess the burden of nutritional problems, identify vulnerable populations and priority actions, track the implementation of nutrition programs, and assess impact.^{40 41} Ethiopia conducted its first-ever food consumption survey in 2011.¹⁹ and its micronutrient survey in 2015.⁴² Both surveys were collected at different times, which made it difficult to link the two surveys. Besides, the causes and solutions of malnutrition are complex and multisectoral; hence, requiring data on multiple indicators from various sectors spanning from soil nutrient, diets, and micronutrient status, to access and exposure to direct and indirect nutrition interventions. In this regard, this survey is uniquely positioned to integrate data from multiple domains to support evidence-based decision making for improved diets, nutrition, and overall wellbeing.

This study will allow us to evaluate progress relative to previous food consumption and micronutrient surveys, but, more importantly, will serve as a baseline against which future progress related to the implementation of the National Food and Nutrition Strategy will be evaluated. Furthermore, the current survey will also serve as a baseline for the Ethiopian Food System Transformation Plan by capturing the majority of indicators used for monitoring food systems-related progress, thus filling information gaps that could have impeded successful implementation of the National Food and Nutrition Strategy. By establishing 13 strategic objectives, the National Food and Nutrition Strategy is intended to be aligned with the strategic directions of the Food and Nutrition Policy. Each strategy direction includes initiatives, actions, and key performance indicators, as well as leading and collaborating sectors. The key performance indicators should be evaluated to determine the progress of each implementing sector's achievement. The current survey will provide up to date national and subnational information on the current food and nutrition situation in Ethiopia for different target populations as well as provide comprehensive list of indicators that are pertinent to the implementation of the policy.⁴⁰ In addition, this study will provide information on context-specific determinants for prioritizing direct and indirect actions that can be implemented across sectors taking into account the specific needs of different target populations.

Additionally, effective multisectoral interventions that address the immediate and underlying determinants of malnutrition must be implemented in order to accelerate the reduction of malnutrition in its all form.⁴⁰ These interventions need to address context-specific determinants to reduce

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malnutrition effectively.⁴⁰ The lack of timely and disaggregated information on the determinants of malnutrition is a bottleneck to preventing malnutrition, particularly among the most vulnerable target populations. This study will also provide information on the coverage and quality of interventions which can be used to contextualize National Food and Nutrition Strategy monitoring frameworks, monitor implementation and track progress towards global and local targets.

Although this study will provide regionally and nationally representative estimates for key indicators and critical life stages, it has several limitations. Inherent to the cross-sectional design of the study, the findings of this study cannot be used to establish cause and effect. Additionally, the design prevents us from considering seasonal differences in nutritional outcomes and determinants. This study also relies on self-reported data, which is subject to recall bias. Notwithstanding the above-mentioned limitations, this study is uniquely designed to combine the assessment of anthropometric status, 24hr recall quantitative dietary intakes and the determination of micronutrient status in the same participants, while at the same time capturing data on the food system. Additionally, the study will be evaluating micronutrients in agricultural soil, which will expand our understanding of factors that influence nutrition. To the best of our knowledge, this will be among -if not-the first study to simultaneously collect these variables from the same household. This could contribute to a better understanding of nutritional problems across multiple facets—from soil to people to the environment. In the past, nutrition programs implemented in Ethiopia have relied on information provided from smallscale studies and population-based surveys such as the Ethiopia Demographic and Health Surveys. 5-7 43 44 Although these data sources provide some information to track progress and tailor interventions, they only provide data on a limited number of nutrition indicators and do not measure dietary intakes and assess biomarker status. This study will fill these data gaps by providing information on comprehensive indicators that show the burden and spatial distribution of micronutrient deficiencies and shifts in dietary patterns. Additionally, this study will provide information on emerging determinants such as mental health and intake of nutrients such as folate and B₁₂ that have not been included in previous studies. Finally, the inclusion of adolescent girls, and school-age children, will provide vital information on nutritional indicators for these target groups, which are often not included in other nationally representative surveys. This survey will also provide information on the coverage of direct interventions implemented in the health sectors and indirect interventions implemented in the agriculture, WASH, education and social protection sectors for whom scant data exists. Hence, this study will provide valuable information that will guide the implementation of strategic actions for the reduction of malnutrition in Ethiopia.

Contributors: MT, AL, SC, MW, AP, AS, and MG conceived the study and drafted the original protocol. All authors participated in refining of the protocol. AH, MW, MG and MT played a major role in the , kg, k , kg, k, , iewing, fina. . orted by Power c . iobal Alliance for Impu. . authors declare that they hav. . ication: Not applicable. statistical consideration. DAD, FC, MG, RN, and ASD helped to write the draft protocol and made a critical contribution to the content. KB, AL, GT, MHD, LT and MZ supervised manuscript preparation. All authors took responsibility for reviewing, final editing and approval of the manuscript.

Funding: This work was supported by Power of Nutrition, BMGF and World Bank through UNICEF; Nutrition International, and Global Alliance for Improved Nutrition.

**Competing interests:** The authors declare that they have no conflicts of interest.

Patient consent for publication: Not applicable.

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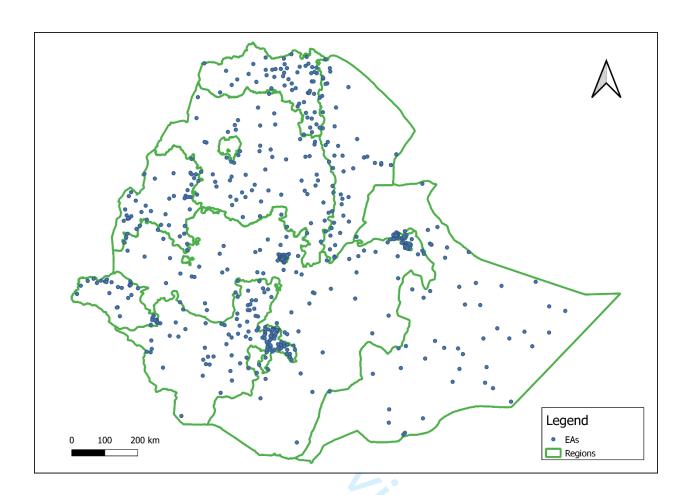
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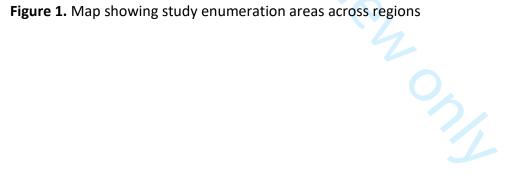
## **FIGURE TITLES**

Figure 1. Map showing study enumeration areas across regions

Figure 2. Sampling frame for the Ethiopia National Food and Nutrition Survey to inform the Ethiopian National Food and Nutrition Strategy

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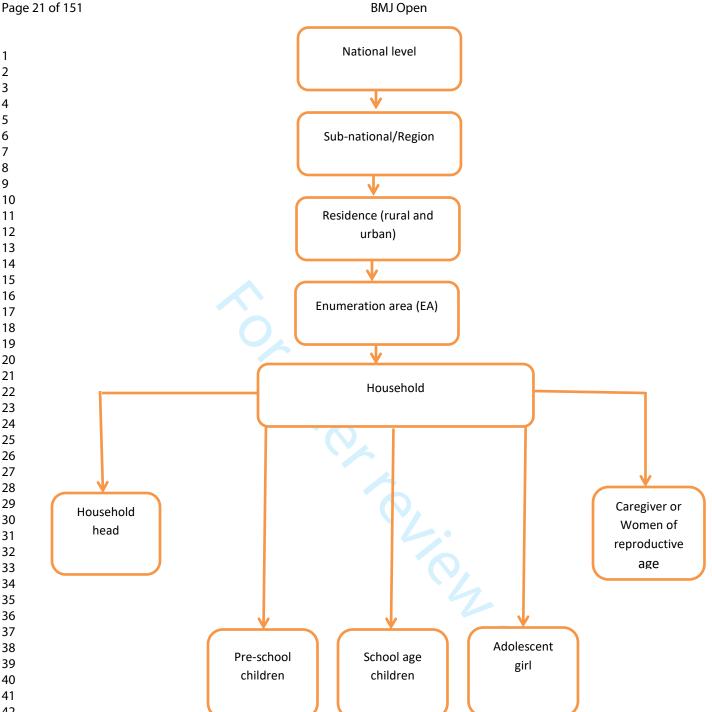


Figure 2. Sampling frame for Ethiopia National Food and Nutrition Survey to Inform the Ethiopian Food and Nutrition Strategy

Key indicators used to estimate sample size	
Vitamin A deficiency	
-	
•	
Total goiter prevalence	
Any anemia	
Zinc deficiency	
-	
-	
lodine deficiency	
Zinc deficiency	
Any anemia	
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	Vitamin A deficiency Total goiter prevalence Stunting Any anemia Zinc deficiency Prevalence of inadequate intake of zinc Vitamin A deficiency Total goiter prevalence Any anemia Zinc deficiency RBC folate deficiency Serum folate deficiency Vitamin B ₁₂ deficiency Iodized salt coverage Prevalence of inadequate intake of iron Prevalence of inadequate intake of zinc Prevalence of inadequate intake vitamin A Vitamin A deficiency Total goiter prevalence Any anemia Iodine deficiency Zinc deficiency

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Table 2S. Sample size determination and allocation

Region	Indicator used to estimate sample size	Number of EA	Number of HH	Expected number pre- school children (0-59 months)	Expected number of WRA (15- 49 years)	Expected number school age children (6-12 years)	Expected number of adolescent girls (10-19 years)	Total populatio (2019 yea projectior
Tigray	Any anemia	55	1,432	590	1,236	753	516	5,443,095
Afar	Stunting	51	1,328	539	1,096	695	406	1,901,863
Amhara	Stunting	61	1,585	619	1,253	843	531	21,842,54
Oromia	IDD	62	1,622	891	1,539	1,111	739	37,267,22
Somali	IDD	55	1,424	855	1,268	1,000	492	6,050,85
Benishangul-Gumuz	Stunting	49	1,282	555	1,127	732	475	1,126,65
SNNPR	Any anemia	59	1,528	818	1,492	1,000	692	15,763,48
Gambela	Any anemia	47	1,211	428	1,018	568	373	463,203
Harari	Any anemia	45	1,164	375	978	499	348	257,362
Addis Ababa	IDD/ TGR	54	1,411	413	1,274	405	262	3,685,68
Dire Dawa	Stunting	47	1,215	382	1,128	480	360	492,819
Sidama	Any anemia	54	1,395	747	1,363	914	632	4,322,68
Total sample size		639	16,596	7,213	14,772	9,001	5,824	98,617,47

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Document Title: DET	<b>FERMIANTION OF IODINE FROM SALT SAMPLE</b>	Effective date: November 2	0, 2020

# 1. Purpose

This procedure used to determine the amount of iodine content in the form of potassium

iodate (KIO₃) by iodometric titration from iodized salt.

# 2. Abbreviations:

g	gram	KI	Potassium Iodide
ppm	Parts Per million	$H_2SO_4$	sulfuric acid
Μ	Molarity	KIO ₃	potassium iodate
ml	mili liter	IDD	Iodine deficiency disorder
Ν	Normality	Na ₂ S ₂ O	<b>3.5H₂O</b> sodium thiosulfate penta hydrate

# 3. Principle:

Iodine released from potassium iodate by the action of sulphuric acid and the released iodine trapped with potassium iodide and titrate with sodium thiosulphate.

# 4. Material and methods

# 4.1 Reagents

- ➢ 0.005M Na₂S₂O₃.5H₂O
- $\succ$  2N H₂SO₄
- ≻ 10% KI
- > 1% Starch

# 4.2 Reagents preparation:

- <u>1%starch</u>: Dissolve 1 g of soluble starch in 100ml boiled distilled water heat the solution till starch dissolve completely.
- > <u>10% KI</u>; Dissolve 10gm of KI in 100ml deionized water.

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9	> $2N H_2 SO_4$ ; Slowly add 6 ml of concentrated H ₂ SO ₄ to 9	0 ml of deionized water make	the
10 11	final volume 100 ml.	o nii or deronized water make	
2 3	<ul> <li><u>0.005M Na₂S₂O₃;</u> Dissolve 1.24gm of Na₂S₂O₃.5H₂O in</li> </ul>	1000 ml of doionized water	
4	$\sim 0.0051 \text{ Ma}_{252} \text{ O}_{3}$ ; Dissolve 1.24gill of Na252O3.5H2O II	1 1000 mil of defomized water	
	3 Reagents stability and storage:		
7 8	➢ Na₂S₂O3.5H₂O & 10% KI reagents store in a cool & dar	k place for six months.	
19 20	➢ H₂SO₄ store at room temperature it stable indefinitely.		
21 22	Starch should be prepared daily.		
²³ 5.	Supplies and Equipment		
24 25	▶ Balance (Four-beam pan): Sensitivity = 0.01g, Capacity	v = 410g	
6 7	<ul> <li>Flask, volumetric, 1000mL, 100mL</li> </ul>	U	
.8	Measuring cylinder, 10mL, 100mL		
29 30	<ul> <li>Beakers (Pyrex)</li> </ul>		
1 2	<ul> <li>Flasks, Erlenmeyer (conical) with stopper, 250mL</li> </ul>		
3 4	<ul> <li>Pipette, volumetric, 1mL, 5mL</li> </ul>		
5	<ul> <li>Burette w/straight stopcock 10mL</li> </ul>		
6 7	➢ Burette stand		
8 9	Laboratory safety glasses		
0	<ul> <li>Parafilm, for covering beakers</li> </ul>		
2	<ul> <li>Glass bottles with stoppers, for reagents, 250mL</li> </ul>		
3 4	<ul> <li>Spatula Lab single blade 150mm SS length</li> </ul>		
-5 -6	<ul> <li>Dropper bottle, glass 25-60mL</li> </ul>		
7	<ul> <li>Hot plate</li> </ul>		
8 9 6	Sample		
50 51	<ul><li>Sample type: salt</li></ul>		
2	<ul> <li>Amount required: 50-100g</li> </ul>		
3 4		ire to direct suplight	
5 6 Prepa	Transport and storage: At room temperature avoid exposited by: Addisu Legesse and Teshome Assefa Authority: EP	[°]	
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# 7. Special Safety and Precautions

- ➢ While titrate the sample wear eye google.
- The reaction mixture should be kept in the dark before titration because a side reaction can occur when the solution is exposed to light that causes iodide ions to be oxidized to iodine.
- > Inaccurate results may occur if starch solution is used while still warm.
- If starch indicator is added too early, a strong iodine-starch complex is formed, which reacts slowly, and gives falsely elevated results.
- The reaction should be performed at mild room temperature (<30 °C), since the iodine is volatile, and the indicator solution loses sensitivity when exposed to high temperatures.</p>

# 8. Procedure

- 8.1 Weigh 10g of the salt sample into a 250mL Erlenmeyer flask with a stopper.
- 8.2 Add approximately 50 mL water, swirl to dissolve salt sample.
- 8.3 Add 1 ml 2N H₂SO₄
- 8.4 Add 5mL 10% KI. The solution should turn yellow if iodine is present.
- 8.5 Close the flask with stopper & put it in the dark place for 10 minutes in closed box (cupboard or drawer).
- 8.6 Rinse and fill burette with 0.005M Na₂S₂O₃, and adjust level to zero.
- 8.7 After 10 minutes take the flask out from drawer, and add some Na₂S₂O₃ from the titration burette Until the solution turns pale yellow.
- 8.8 Add approximately 2mL of starch indicator solution (the solution should turn dark purple) and continue titrating with 0.005M Na₂S₂O₃ until the solution becomes pink, and finally colorless.
- 8.9 Record the level of thiosulfate in the burette and convert to parts per million (ppm)

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2 3 4 5 6			Page 4 of 7
7	TERMIANTION OF IODINE FROM SALT SAMPLE	Effective date: November 2	20, 2020
9			
10	lity control:		
11 12	Control material: KIO ₃		
13	Level of iodine: 59.3-60.3ppm		
14 15	Stability: stable at room temperature for long period of	time.	
16 17	Frequency: per batch		
18 <b>10. Qua</b>	ality control preparation: Prepare 0.0047M KIO3 in 100m	l. By weighing 0.10058g i	n
20 100	ml deionized water. From this solution Pipette out 1 ml into	conical flask and follow t	he
21 22 proc	redure proceed from step 2 as described above.		
²³ 24 <b>11. Cal</b>	culation		
25 26	<b>Iodine</b> (ppm) = <b>10.6* V Na₂S₂O₃(ml</b> )		
27 28	Where: VNa ₂ S ₂ O ₃ : Volume of sodium thiosulphate ta	kes to titrate iodine in salt	
29	sult Interpretation		
31 32	<b>5ppm</b> to indicate salt with no added iodine		
22	-14.9 ppm to indicate inadequately iodized salt		

- > 15-39.9 ppm to indicate salt is adequately iodized
- > >40 ppm of iodine is not recommended.

# 13. Other Records

- ➢ Data Log sheet
- ➢ QC chart

# 14. References

- De Maeyer, Lowenstein & Thilly, 1979; World Health Organization (WHO), United Nations Children Fund (UNICEF) & ICCIDD, 2007.
- ➢ AOAC 925.56 2016

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# 15. Annex

Conversion chart for iodine in fortified salt in the form of KIO₃ (PPM)

Volume Thiosulphate	lodine	Volume	lodine								
		Thiosulphate									
(mL)	(ppm)	(mL)	(ppm)	(mL)	(ppm)	(mL)	(ppm)	(mL)	(ppm)	(mL)	(ppr
0.1	1.1	2.0	21.2	3.9	41.3	5.8	61.5	7.7	81.6	9.6	101.
0.2	2.1	2.1	22.2	4.0	42.4	5.9	62.5	7.8	82.7	9.7	102.
0.3	3.2	2.2	23.3	4.1	43.5	6.0	63.6	7.9	83.4	9.8	103.
0.4	4.2	2.3	24.4	4.2	44.5	6.1	64.7	8.0	84.8	9.9	104.
0.5	5.3	2.4	25.4	4.3	45.6	6.2	65.7	8.1	85.9	10.0	106.
0.6	6.4	2.5	26.5	4.4	46.4	6.3	66.8	8.2	86.9	10.1	107.
0.7	7.4	2.6	27.6	4.5	47.7	6.4	67.8	8.3	88.0	10.2	108.
0.8	8.5	2.7	28.6	4.6	48.8	6.5	68.9	8.4	89.0	10.3	109.
0.9	9.4	2.8	29.7	4.7	49.8	6.6	70.0	8.5	90.1	10.4	110.
1.0	10.6	2.9	30.7	4.8	50.9	6.7	71.0	8.6	91.2	10.5	111.
1.1	11.7	3.0	31.8	4.9	51.9	6.8	72.1	8.7	92.2	10.6	112.
1.2	12.2	3.1	32.9	5.0	53.0	6.9	73.1	8.8	93.3	10.7	113.
1.3	13.8	3.2	33.9	5.1	54.1	7.0	74.2	8.9	94.3	10.8	114.
1.4	14.8	3.3	35.0	5.2	55.1	7.1	75.3	9.0	95.4	10.9	115.
1.5	15.9	3.4	36.0	5.3	56.2	7.2	76.3	9.1	96.5	11.0	116.
1.6	17.0	3.5	37.1	5.4	57.2	7.3	77.4	9.2	97.5	11.1	117.
1.7	18.0	3.6	38.2	5.5	58.3	7.4	78.4	9.3	98.6	11.2	118.
1.8	19.1	3.7	39.2	5.6	59.4	7.5	79.5	9.4	99.7	11.3	119.
1.9	20.1	3.8	40.3	5.7	60.4	7.6	80.6	9.5	100.7	11.4	120.

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# **Document Change History**

Revision No.	Date approved	Nature of revision
1	February, 2019	Initial release
2	November, 2020	Typographical adjustment
		Document revision table is added
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# Determination of serum retinol using hexane as the serum sample extraction procedure

# Contents

1. Reagents and materials	2
2. Instrument (HPLC) parameters – we have Shimadzu prominence HPLC.	2
3. Sample extraction procedure	2
4. Calculation	3
5. Reference	3

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1	
2 3	1 Descents and materials
4	1. Reagents and materials
5 6	• Methanol (HPLC grade)
7	• Hexane (HPLC grade)
8 9	• Ethanol (HPLC grade/AR grade)
10 11	• Normal saline solution (0.9% NaCl)
12	• Retinol acetate (as internal standard)
13 14	• Retinol (as external standard)
15 16	CRM (Certified Reference Material) for serum retinol
17 18	• 15 ml centrifuge tube
19	• Vortex mixer
20 21	• Centrifuge (up to 4000 rpm capacity)
22 23	<ul> <li>Centrifuge tube rack</li> </ul>
24	
25	<ul> <li>Micropipettes (10-100µl and 100-1000µl)</li> </ul>
26 27	Micropipettes tips for both types
28 29	• Stopwatch
30	• HPLC Vials (1ml)
31	
32 33	2. Instrument (HPLC) parameters – we have Shimadzu prominence HPLC.
34 35	<ul> <li>Detector - SPD-10A UV/VIS at 325 nm</li> </ul>
36	<ul> <li>Column – SUPELCOSILTMLC-NH₂-NP, 25cm × 4.6mm, 5µm</li> </ul>
37 38	
39	• Mobile phase – HPLC grade methanol
40 41	Elution system – isocratic
42	
43 44	<ul> <li>3. Sample extraction procedure</li> </ul>
45	• Add 200 µl serum sample to 15ml plastic test tube.
46 47	• Add an equal volume of 50 $\mu$ g/dl retinol acetate and ethanol.
48 49	• Take 200 $\mu$ l of a serious of standards (10, 20, 40, 60, 75 $\mu$ g/dl) to the 15 ml centrifuge
50	tube and add the same volume of retinol acetate and normal saline solution.
51 52	• Mix the standard and sample solutions with a vortex mixer
53 54	• Add 1ml of HPLC grade hexane and mix for 45 seconds
55	• Centrifuge the solution at 4000rpm for 7 minutes and transfer the supernatant to other
56 57	test tubes
58 59	• The solution has to be extract twice by adding 1 ml hexane.
60	The solution has to be ordined to lee by adding 1 hit normale.

- Dry the hexane using nitrogen gas and reconstitute by 500µl HPLC grade methanol
- It has to be mix for 30 minutes using a vortex mixer and transfer to 1 ml HPLC vials
- Analyze the extracted solutions using reversed-phase HPLC by isocratic elution system, at the flow rate of 1.5 ml/min, and injection volume 30µl

N: B: - Extract and analyse the CRM using the sample extraction procedure.

# 4. Calculation

- Plot the calibration curve using area ratio of retinol (standard) and retinol acetate (internal standard) vs concentration ratio (retinol: retinol acetate).
- From the linear equation formula, Y = mx + b, the concentration of serum retinol can be calculated.

Where Y - is area ratio (retinol: retinol acetate) of sample

- M is slope and b is Y intercept
- X is the concentration of serum retinol in  $\mu g/dl$

# 5. Reference

- Quadro, Y.-K. K. and L. (2013). Reverse-Phase High-Performance Liquid Chromatography (HPLC) Analysis of Retinol and Retinyl Esters in Mouse Serum and Tissues. *Methods Mol Biol. 2010*; 652: 263–275. Doi:10.1007/978-1-60327-325-1_15., (12), 1–10. https://doi.org/10.1007/978-1-60327-325-1
- von Lucke, A., Russell, R. M., Stephensen, C. B., Gannon, B. M., Craft, N. E., Haskell, M. J., ... Raiten, D. J. (2016). Biomarkers of Nutrition for Development (BOND)—Vitamin A Review. *The Journal of Nutrition*, 1816s-1848s. https://doi.org/10.3945/jn.115.229708.FIGURE

Prepared by: Nahom Tefera (September 2020)

Reviewed by: Meseret W/yohannis

Approved by: Dr. Masresha Tessema

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Document Title: DETER	MINATION OF IODINE IN URINE	Effective date: Decem	ber, 2020

### 1 Scope

This test method is applicable for the determination of iodine from a urine sample.

### 2 Purpose

This procedure provides methods to control the amount of iodine and it's toxicity by ammonium persulfate digestion with spectrophotometric detection of the Sandell-Kolthoff Reaction method from urine samples.

### Principle

Urine is digested with ammonium persulfate. Iodide is the catalyst in the reduction of ceric ammonium sulfate (yellow) to cerous form (colorless) and is detected by the rate of color disappearance (Sandell-Kolthoff reaction).

### 4 Chemicals and Apparatus

a) Chemicals

- Ammonium persulphate
- Arsenic trioxide
- Ceric ammonium sulphate
- Potassium iodate
- Deionized water
- Sodium chloride

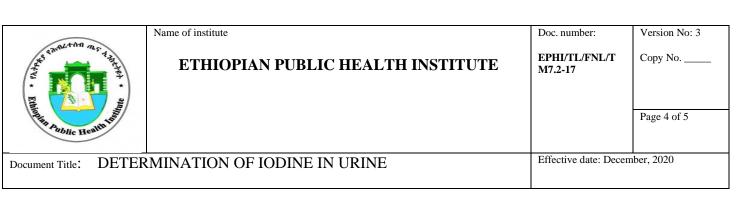
b) Apparatus

- Hot plate
- Oven
- UV-Vis spectrophotometer
- Volumetric flasks (100 2000 ml)
- Beakers (100- 1000 ml)



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	RMINATION OF IODINE IN URINE	Effective date: Dece	mber, 2020
Micropip	ette (100 – 1000 μl)		
• Pipette (S	5 – 10 ml)		
• Vortex m	ixer		
• Measurir	g cylinder (100 – 1000 ml)		
• Glass tes	t tubes (13*100 mm)		
Analytica	al balance (nearest to the 0.0001 g)		
• Erlenme	ver flask (2000 ml)		
5 Reagents p	reparation		
a) 1 M Ammoni	um persulphate		
Dissolve	114.1 g $H_2 N_2 O_8 S_2$ in deionized water and makeup to 5	00 ml with $H_2O$ .	Store away
from ligh	t. Stable for at least one month.		
b) 5 N H ₂ SO ₄			
• Slowly a	dd 139 ml concentrated (36 N) $H_2SO_4$ to about 700 ml de	eionized water (ca	areful - this
e	heat!). When cool, adjust with deionized water to a final	volume of 1 liter.	
c) 3.5 N H ₂ SO ₄			
-	dd 97 ml concentrated (36 N) $H_2SO_4$ to about 800 ml de		
-	heat!), and when cool, adjusting with deionized water to	a final volume of	1 liter
	um sulphate solution		
	48 g ceric ammonium sulphate in 1 liter $3.5 \text{ N H}_2\text{SO}_4$ . Sto	re in a dark bottle	away from
-	bom temperature. The solution is stable for months.		
e) Arsenious aci			
	) ml Erlenmeyer flask, place 20 g $As_2O_3$ and 50 g NaCl,	•	
	Add water to about 1 liter, heat gently to dissolve, cool to re	-	
is stable	2 liters, filter, store in a dark bottle away from light at roo	om temperature. T	he solution

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6 Standards preparation		
a) Stock standard solution (1 mg/ml)		
• Dissolve 0.1685 g KIO ₃ in deionized water to a final volume of 1	00 ml (1.68 g KI	O ₃ contains
1.0 g iodine). $KIO_3$ is preferred over KI because it is more stable.	It may be more co	nvenient to
make a more concentrated solution, e.g., 10 or 100 mg iodine/ml.		
b) Intermediate standard solution $(1 \mu g/ml)$		
• Dilute 100 µl stock iodine standard (1mg/ml) to 100 ml deionized	water. Store in a	dark bottle.
The solution is stable for months. Useful standards are 20, 50, 100,	150, 200, and 30	0 μg/l.
c) Serial standard dilutions		
• From intermediate standards, prepare 50, 100, 150, 200, and 25	0 μg/L useful st	andards for
calibration curve purposes.		
Note: All standard solutions should store in a dark place. The solutions are	e stable for month	s.
7 Procedures		
• Mix urine to suspend sediment using a vortex mixer.		
• Pipette 250 $\mu$ l of each urine sample into a 13 x 100 mm test tube.		
• Pipette 250 µl each iodine serial standards also into a test tube.		
• Add 1 ml 1.0 M ammonium persulfate solution to each tube.		
• Vortex all tubes using a vortex mixer		
• Heat all tubes for 60 minutes at 100° C in the oven.		
<ul> <li>Cool tubes to room temperature in a fume hood.</li> <li>Add 2.5 ml emerican orid solution. Min hus suprtary Let stand for</li> </ul>	15	
<ul> <li>Add 2.5 ml arsenious acid solution. Mix by a vortex. Let stand for</li> <li>Add 300 µl of ceric ammonium sulfate solution to each tube (quice)</li> </ul>		-30 second
intervals between successive tubes. A stopwatch should be used	•	
second interval is convenient.		
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• Allow sitting at room temperature. Exactly 30 minutes after the addition of ceric ammonium sulfate to the first tube, read its absorbance by using UV-Vis spectrophotometer at 420 nm. Read successive tubes at the same interval as when adding the ceric ammonium sulfate.

### 8 Calculation

- Construct a standard curve on graph paper by plotting log absorbance of each concentration versus iodine concentration of each standard.
- Urinary Iodine in µg/l = ((log A-b)/(m)) * 10
   Where log A is log absorbance of sample, b is Y-intercept of the calibration graph, m is the slope of the graph, and 10 is used as a conversion factor when we prepare serials of standards in µg/dl (in this case we multiply the final concentration (µg/ dl) by 10 to get the concentration in µg /l).

### 9 Quality control and safety precautions

- In each batch, the urine quality control sample (CRM or in-house prepared) should run together with sample and standards.
- While working the urine analysis and reagent preparation wear gloves, lab coat, eye goggle, lab shoe, and mouth cover.

**Remember**: During the analysis of iodine from urine, you should ensure that the laboratory working environment should free from salt samples (especially iodized salt samples) to avoid contamination. **Limitations:** If the urine sample is analyzed at a high temperature the loss of iodine occurred and the method has to detect very low iodine concentration in urine.

### 10 References

• ICCIDD, UNICEF, WHO. Dunn JT et al. Methods for measuring iodine in urine. The Netherlands, ICCIDD, 1993.

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11355279 216	Calibrator f.a.s. Proteins (5 x 1 mL)	Code 656	
<b>11355279</b> 160	Calibrator f.a.s. Proteins (5 x 1 mL, for USA)	Code 656	
<b>10557897</b> 122	Precinorm Protein (3 x 1 mL)	Code 302	
<b>10557897</b> 160	Precinorm Protein (3 x 1 mL, for USA)	Code 302	
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<b>11333127</b> 160	Precipath Protein (3 x 1 mL, for USA)	Code 303	
<b>05117003</b> 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
<b>05947626</b> 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
<b>05947626</b> 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391	
<b>05117216</b> 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
<b>05947774</b> 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
<b>05947774</b> 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392	
<b>04489357</b> 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

### English

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System information

For **cobas c** 311/501 analyzers:

### AAGP2: ACN 229

For **cobas c** 502 analyzer:

AAGP2: ACN 8229

### Intended use

In vitro test for the quantitative determination of  $\alpha_1$ -acid glycoprotein in human serum and plasma on Roche/Hitachi **cobas c** systems.

### Summary^{1,2,3,4,5}

 $\begin{array}{rcl} 33 & \alpha_1 - \text{Acid glycoprotein is synthesized in hepatocytes and consists of a} \\ 34 & \text{polypeptide chain having 5 carbohydrate chains N-glycosidically bonded to} \\ 35 & \text{it (molar mass 41000 daltons). Structurally, it belongs to the lipocalin} \\ 36 & \text{retinol-binding protein}. \\ \alpha_1 - \text{Acid glycoprotein promotes fibroblast growth and} \\ 37 & \text{interacts with collagen.} \end{array}$ 

It is a sensitive acute phase reactant whose concentration can increase by 38 a factor of 3 within 24-48 hours when inflammation occurs.  $\alpha_1$ -Acid 39 glycoprotein can also be used to differentiate between acute phase 40 reactions (elevated serum level) and estrogen effects (normal or decreased serum level) whereas the serum level of other positive reactants such as 41 ceruloplasmin and haptoglobin increases during such reactions. Along with haptoglobin it is perhaps the best protein for identifying slight in vivo hemolysis. An increased  $\alpha_1$ -acid glycoprotein level and normal haptoglobin 42 43 values indicate an acute phase reaction with concomitant slight in vivo 44 hemolysis. Moderate and isolated increases occur when glomerular 45 filtration is inhibited in the early stages of uremia. The determination is used

- 46 in the assessment of the activity of acute and recurring inflammations as
  47 well as of tumors with cell necrosis.
- 48 Various assay methods for  $\alpha_1$ -acid glycoprotein determination are available 49 such as kinetic nephelometry, radial immunodiffusion (RID) and 49 turbidimetry. The Roche  $\alpha_1$ -acid glycoprotein assay is based on the 50 principle of immunological agglutination.

### 51 **Test principle**² 52 Immunoturbidim

Immunoturbidimetric assay.

 $\begin{array}{ll} \text{53} \\ \text{54} \\ \text{55} \end{array} \text{Anti-}\alpha_1\text{-}acid glycoprotein antibodies react with antigen in the sample to form an antigen/antibody complex. Following agglutination, this is measured turbidimetrically. } \end{array}$ 

### 56 Reagents - working solutions

- 57 R1 TRIS buffer: 50 mmol/L, pH 8.0; NaCI: 300 mmol/L; PEG: 7 %;
   58 preservative; stabilizer
- 59 R2 Polyclonal anti-human α₁-acid glycoprotein antibody (goat):
   60 dependent on titer; TRIS buffer: 13 mmol/L, pH 7.5; NaCl: 198 mmol/L; preservative

R1 is in position B and R2 is in position C.

### Precautions and warnings

For in vitro diagnostic use. Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

### Reagent handling

Ready for use

### Storage and stability

AAGP2

Shelf life at 2-8 °C:

See expiration date on **cobas c** pack label.

12 weeks

On-board in use and refrigerated on the analyzer: *Diluent NaCl 9 %* Shelf life at 2-8 °C:

See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the 12 weeks analyzer:

### Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable. Serum

Plasma: Li-heparin and K₂-EDTA plasma.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:⁶ < 72 hours at 4 °C

6 months at -20 °C

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### 0003333795190c501V10.0 AAGP2 Tina-quant α1-Acid Glycoprotein Gen.2

5	Matariala provided						Comple	Diluant (NaCI)
6	Materials provided See "Reagents – working sol	utions" section t	for reagents.		Normal	10	Sample	Diluent (NaCl)
7	Materials required (but not		ion rougonio.			12 µL	9 μL	180 µL
8	<ul> <li>See "Order information" s</li> </ul>				Decreased	12 µL	4 μL	122 µL
9	<ul> <li>General laboratory equiprint</li> </ul>				Increased	12 µL	18 µL	180 µL
10	Assay				Calibration			
11 12	For optimum performance of	the assay follow	v the directions	given in this	Calibrators	S1: H ₂ O		
12	document for the analyzer co manual for analyzer-specific	ncerned. Refer assav instructio	to the appropr	iate operator's		S2-S6: C.f.a.s. Prote	eins	
14	The performance of applicati	,		not warranted		Multiply the lot-spec	fic C.f.a.s.	Proteins
15	and must be defined by the u					calibrator value by the		
16	Application for serum and	plasma				determine the stand 6-point calibration cu		trations for the
17	cobas c 311 test definition					S2: 0.140	S5: 1	40
18 19	Assay type	2-Point End				S3: 0.280	S6: 2	
20	Reaction time/Assay points	10/6-32				S4: 0.700	00.2	
20	Wavelength (sub/main)	660/340 nm			Calibration mode	RCM2		
22	Reaction direction	Increase			Calibration frequency	Full calibration		
23	Units	g/L (µmol/L, m	ng/dL)		Calibration nequency	- after reagent lot ch	ange	
24	Reagent pipetting		Diluent (H ₂ O)			- as required following		ontrol
25	R1	120 µL	-			procedures		
26 27	R2	40 µL	-		Calibratian interval mars	he automated based on	to bla	werification of
27	Sample volumes	Sample	Sampl	e dilution	Calibration interval may calibration by the labora	tory.	acceptable	venilication of
29			Sample	Diluent (NaCl)	Traceability: This metho	d has been standardize	ed against t	he reference
30	Normal	12 µL	9 μL	180 µL	preparation of the IRMM Measurements) BCR470	l (Institute for Referenc )/CRM470 (RPPHS - F	e Materials leference P	and reparation for
31	Decreased	12 µL	4 μL	122 µL	Proteins in Human Seru	m). ⁷		- oparallor rol
32	Increased	12 µL	9 μL	180 µL	Quality control			
33 34	cobas c 501 test definition				<ul> <li>For quality control, use of section.</li> </ul>	control materials as list	ed in the "C	order information"
35	Assay type	2-Point End			In addition, other suitabl	e control material can l	be used.	
36	Reaction time/Assay points	10/10-48			The control intervals and	l limits should be adap	ted to each	laboratory's
37	Wavelength (sub/main)	660/340 nm			individual requirements. limits. Each laboratory s	values obtained shoul hould establish correct	d fall within ve measur	the defined es to be taken if
38	Reaction direction	Increase			values fall outside the de	efined limits.		
39 40	Units	g/L (µmol/L, m	ng/dL)		Follow the applicable go quality control.	vernment regulations a	ind local gu	idelines for
40 41	Reagent pipetting	<b>U</b> (1 )	Diluent (H ₂ O)	1	Calculation			
42	R1	120 µL	_		Roche/Hitachi cobas c	systems automatically	calculate th	e analyte
43	R2	40 μL	_		concentration of each sa	ample.		
44	Sample volumes	Sample	Sampl	e dilution	Conversion factors:	g/L x 25 = µmol/L	mg/dL x	: 0.01 = g/L
45 46			Sample	Diluent (NaCl)		mg/dL x 0.25 = µmol/l	g/L x 10	0 = mg/dL
40 47	Normal	12 µL	9 μL	180 µL	Limitations - interferen	ice		
48	Decreased	12 µL	4 μL	122 µL	Criterion: Recovery with			cid glycoprotein
49	Increased	12 µL	9 μL	180 µL	concentration of 0.5 g/L Icterus: ⁸ No significant ir		,	or conjugated
50	aches a 500 test definition				and unconjugated biliruk	pin (approximate conjug	gated and u	inconjugated
51	cobas c 502 test definition	0 Doint End			bilirubin concentration: 1		,	4000
52	Assay type	2-Point End 10/10-48			Hemolysis: ⁸ No significa (approximate hemoglobi	int interference up to ar in concentration: 621 u	n H index of mol/L or 10	1000 00 ma/dL).
53 54	Reaction time/Assay points				Lipemia (Intralipid):8 No	significant interference	up to an L	index of 650.
55	Wavelength (sub/main)	660/340 nm			There is poor correlation triglycerides concentration	n between the L index (	correspond	s to turbidity) and
56	Reaction direction	Increase	a/dL)		Rheumatoid factors up t		terfere.	
57	Units Reagant pinotting	g/L (µmol/L, m			High dose hook-effect: N	No false result occurs u	p to an α₁-a	acid glycoprotein
58	Reagent pipetting	1001	Diluent (H ₂ O)		concentration of 11 g/L (		,	
59 60	R1 R2	120 μL	-		Drugs: No interference v common drug panels. ^{9, 1}	was found at therapeuti	c concentra	ations using
60		40 µL Samplo	- Comol	a dilution	In very rare cases, gami	mopathy, in particular t	/pe lgM (W	aldenström's
	Sample volumes	Sample	Sampi	e dilution	macroglobulinemia), ma	y cause unreliable resu	Ilts.11	

### 0003333795190c501V10 0 Tina-quant α1-Acid Glycoprotein Gen.2

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conjunction with the patient's medical history, clinical examination and other T = 0.973findings. **ACTION REQUIRED** Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi cobas c systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further 1 instructions refer to the operator's manual. cobas c 502 analyzer: All special wash programming necessary for avoiding carry-over is available 2 via the cobas link, manual input is not required. Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test. 3 Limits and ranges 4 Measuring range 0.1-4.0 g/L (2.5-100 µmol/L, 10-400 mg/dL) 5 Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:1.5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 1.5. 6 Lower limits of measurement 7 Lower detection limit of the test 0.1 g/L (2.5 µmol/L, 10 mg/dL) The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 8 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

For diagnostic purposes, the results should always be assessed in

#### 28 Expected values¹² Т 29

0.5-1.2 g/L (12.5-30 µmol/L, 50-120 mg/dL)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges

### Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

### Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

20			•	
39 40	Repeatability	Mean	SD	CV
40 41 42		g/L (µmol/L, mg/dL)	g/L (µmol/L, mg/dL)	%
43	Precinorm Protein	0.724 (18.1, 72.4)	0.00 (0.0, 0.0)	0.6
44	Precipath Protein	1.21 (30.3, 121)	0.01 (0.3, 1)	0.5
45	Human serum 1	0.642 (16.1, 64.2)	0.00 (0.0, 0.0)	0.7
46 47	Human serum 2	1.07 (26.8, 107)	0.01 (0.3, 1)	0.7
40	Intermediate presision	Mean	SD	CV
48	Intermediate precision	Mean	00	01
48 49		g/L (µmol/L,	g/L (µmol/L,	%
	memediale precision		-	
49 50 51	Precinorm Protein	g/L (µmol/L,	g/L (µmol/L, mg/dL)	
49 50 51 52		g/L (μmol/L, mg/dL)	g/L (µmol/L, mg/dL) 0.007 (0.2, 1.0)	%
49 50 51 52 53	Precinorm Protein	g/L (μmol/L, mg/dL) 0.710 (17.8, 71.0)	g/L (μmol/L, mg/dL) 0.007 (0.2, 1.0) 0.01 (0.3, 1)	% 0.9
49 50 51 52	Precinorm Protein Precipath Protein	<i>g/L (µmol/L, mg/dL)</i> 0.710 (17.8, 71.0) 1.19 (30.0, 119) 0.660 (16.5, 66.0)	g/L (μmol/L, mg/dL) 0.007 (0.2, 1.0) 0.01 (0.3, 1)	% 0.9 0.9

### Method comparison

 $\alpha_1$ -Acid glycoprotein values for human serum and plasma samples obtained on a Roche/Hitachi cobas c 501 analyzer (y) were compared with those determined using the corresponding reagent on a Т Roche/Hitachi 917 analyzer (x).

Sample size (n) = 119

### Passing/Bablok¹³

### Linear regression

2018-08, V 10.0 English



r = 0.999

The sample concentrations were between 0.489 and 3.25 g/L (12.2 and 81.3 µmol/L, 48.9 and 325 mg/dL).

### References

y = 1.012x - 0.070 g/L

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- Greiling H, Gressner AM, eds. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3rd ed. Stuttgart/New York: Schattauer Verlag 1995:236.
- Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders Company 1995;66-67.
- Ganrot K. Plasma protein pattern in acute infectious disease. Scand J Clin Lab Invest 1974:34:75-81.
- Lievens M, Bienvenu J, Buitrago JMG, et al. Evaluation of four new Tina-quant assays for determination of α1-acid glycoprotein, α1-antitrypsin, haptoglobin and prealbumin. Clin Lab 1996;42:515-520.
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- Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986:32:470-475.
- 9 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.
- Sonntag O, Scholer A. Drug interference in clinical chemistry: 10 recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.
- 11 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.
- 12 Dati F, Schumann G, Thomas L, et al. Consensus of a group of professional societies and diagnostic companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP reference material (CRM 470). Eur J Clin Chem Clin Biochem 1996;34:517-520.
- 13 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

### Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

Contents of kit



Volume after reconstitution or mixing

GTIN

Global Trade Item Number

### 0003333795190c501V10.0 **AAGP2**

Tina-quant α1-Acid Glycoprotein Gen.2

### FOR US CUSTOMERS ONLY: LIMITED WARRANTY

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### CE

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Roche Diagnostics, Indianapolis, IN US Customer Technical Support 1-800-428-2336

Roche



### Vitamin D total 25-Hvdroxvvitamin D

1 2 3

## cobas®

2011	yaroxy manini D					
REF	]					SYSTEM
						Elecsys 2010
						MODULAR ANALYTICS E170
058	<b>94913</b> 190	100				cobas e 411
						<b>cobas e</b> 601
						<b>cobas e</b> 602
Engl	ish					tion: After addition of streptavidin-coated microparticles and
Inter	ided use					(25-OH) labeled with biotin, unbound ruthenium labeled binding proteins become occupied. A complex consisting of
		or the quantitative determination		tl	ne ruthen	ylated vitamin D binding protein and the biotinylated
		human serum and plasma. This ment of vitamin D sufficiency.	s assay is to be used			(25-OH) is formed and becomes bound to the solid phase via of biotin and streptavidin.
The e	electrochemilumine	escence binding assay is intend	ed for use on			on mixture is aspirated into the measuring cell where the
Elecs	sys and <b>cobas e</b> in	nmunoassay analyzers.		n	nicropartio	cles are magnetically captured onto the surface of the
Sum						Unbound substances are then removed with oCell M. Application of a voltage to the electrode then induces
Vitan	nin D is a fat-solub	e steroid hormone precursor th exposure to sunlight. Vitamin E	at is mainly	c	hemilumi	nescent emission which is measured by a photomultiplier.
and r	nust undergo two s	successive hydroxylations in the	e liver and kidney to	• F	lesults ar	e determined via a calibration curve which is instrument-
beco	me the biologically	active 1,25-dihydroxyvitamin E	0.1			y generated by 2-point calibration and a master curve provided
		forms of vitamin D are vitamin ciferol). In contrast to vitamin D				agent barcode.
cann	ot produce vitamin	D2 which is taken up with fortifi	ed food or given by		-	rorking solutions ackpack (M, R1, R2) and the pretreatment reagents (PT1,
		plasma vitamin D ₃ and D ₂ are n and transported to the liver w				led as VITD-T.
hydro	oxylated to form vit	amin D (25-OH), i.e. 25-hydrox	yvitamin D. It is	PT1	Pretreat	ment reagent 1 (white cap), 1 bottle, 4 mL:
		vitamin D (25-OH) is the metabolas it is the major storage form				eitol 1 g/L, pH 5.5.
huma	an body. This prima	ary circulating form of vitamin D	is biologically	PT2		ment reagent 2 (gray cap), 1 bottle, 4 mL:
inacti	ive with levels applied by droxyvitamin [	oximately 1000-fold greater that D. The half-life of circulating vita	an the circulating			hydroxide 55 g/L.
	eeks.	. The nan-life of circulating vite		M		
Most	of the vitamin D (2	5-OH), measurable in serum, i	s vitamin D3 (25-OH)	М		idin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
wher takin	eas vitamin D ₂ (25 o vitamin D ₂ supple	-OH) reaches measurable level ements. ^{2,3,4} Vitamin D ₂ is consid	s only in patients lered to be less			idin-coated microparticles 0.72 mg/mL; preservative.
effec				R1		D binding protein-BPRu (gray cap), 1 bottle, 9 mL:
		or bone health. In children, seve				um labeled vitamin D binding protein 150 μg/L; bis-tris
		nown as rickets. Milder degree ed efficiency in the utilization o			propane	buffer 200 mmol/L; albumin (human) 25 g/L; pH 7.5;
Vitan	nin D deficiency ca	uses muscle weakness; in elde	rly, the risk of falling	<b>D</b> 0		oxyvitamin D~biotin (black cap), 1 bottle, 8.5 mL:
		ne effect of vitamin D on muscle cause of secondary hyperpara		ΠŹ		
Eleva	ations of PTH level	s, especially in elderly vitamin [	D deficient adults can			ted vitamin D (25-OH) 14 $\mu$ g/L; bis-tris propane buffer ol/L; pH 8.6; preservative.
resul risk c	t in osteomalacia, i of bone fractures ¹⁰	ncreased bone turnover, reduc Low vitamin D (25-OH) concer	ed bone mass and Itrations are also	<b>D</b>		
asso	ciated with lower b	one mineral density.11 In conjur	nction with other			and warnings agnostic use.
	al data, the results bolism.	may be used as an aid in the a	issessment of bone	Exer	cise the r	normal precautions required for handling all laboratory
		een shown to affect expression	of over 200 different		ents. osal of all	I waste material should be in accordance with local guidelines.
gene	s. Insufficiency has	s been linked to diabetes, differ	ent forms of cancer,			neet available for professional user on request.
		autoimmune diseases and inn	,	This	kit contai	ins components classified as follows in accordance with the
		total assay employs a vitamin [ ein to bind vitamin D ₃ (25-OH) a		Reg	ulation (E	C) No. 1272/2008:
	iin D₂ (25-OH).	- ( )			$\land$	
	principle					
		otal duration of assay: 27 minu				
re	st incubation: By in eagent 1 and 2, bou tamin D binding pr	cubating the sample (15 μL) wi und vitamin D (25-OH) is releas	th pretreatment ed from the	Dan		
	• ·	ncubating the pretreated sampl	e with the ruthenium	H290	)	May be corrosive to metals.
la	beled vitamin D bi	nding protein, a complex betwe	en the	H314	1	Causes severe skin burns and eye damage.
	tamin D (25-OH) a prmed.	nd the ruthenylated vitamin D b	inding protein is		ention:	
iC				P280		Wear protective gloves/ protective clothing/ eye protection/ face protection.
				Roe	oonse:	

### ms 05894913190V7.0 Vitamin D total

2	Vita	min D i	lotal
2			
4	25-Hydroxyvi	itamin D	
5	P303 + P361	IE ON SKIN (or bair): E	Remove/Take off immediately all
6 7	+ P353		Rinse skin with water/shower.
8	P304 + P340 + P310	IF INHALED: Remove comfortable for breathing	person to fresh air and keep ng.
9 10			SON CENTER or doctor/physician.
11	P305 + P351	IF IN EYES: Rinse cau	tiously with water for several
12	+ P338 +		act lenses, if present and easy to do.
13	P310	0	ediately call a POISON CENTER or
14		doctor/ physician.	
15		/ labeling primarily follow	e e e e e e e e e e e e e e e e e e e
16	•	e: all countries: +49-621	
17			red potentially infectious. All products
18	derived from r	individually and shown	ed exclusively from the blood of to be free from HBsAg and antibodies
19	to HCV and H	IV. The testing methods	applied were FDA-approved or
20		npliance with the Europe	an Directive 98/79/EC, Annex II,
21	List A.	a tasting mathed can m	le out the potential risk of infection
22	with absolute	certainty, the material st	hould be handled with the same level
23	of care as a pa	atient specimen. In the e	event of exposure, the directives of the
24	•	ealth authorities should l	
25	Avoid foam fo calibrators and		nd sample types (specimens,
26		,	
27	Reagent hand	-	ampled into a ready for use unit that
28	cannot be sep		embled into a ready-for-use unit that
29	All information	required for correct ope	eration is read in from the respective
30	reagent barco		
31	Storage and	stability	
32	Store at 2-8 °C	C.	
33 34	Do not freeze.		
35			in order to ensure complete automatic mixing prior to use.
36	Stability:		
37	unopened at	2-8 °C	up to the stated expiration date
38	after opening		56 days (8 weeks)
39 40			
40 41	-	010 and <b>cobas e</b> 411	21 days (3 weeks)
41		R ANALYTICS E170,	28 days (4 weeks)
43	CODAS E 001	and <b>cobas e</b> 602	
44		llection and preparation	
45			tested and found acceptable.
46			ling tubes or tubes containing
47	separating gel		a well as Liberarin plasma tubas
48	containing sep		as well as Li-heparin plasma tubes
49	Criterion: Met	hod comparison serum v	versus plasma, slope 0.9-1.1 cient of correlation > 0.9.
50 51	Serum, Li-hep	arin, K ₂ - and K ₃ -EDTA p	olasma: Vitamin D (25-OH) is stable °C, 24 weeks at -20 °C.
52			of with the Elecsus Vitamin D total

52 The stability of vitamin D (25-OH) found with the Elecsys Vitamin D total 53 assay is in line with earlier studies using a vitamin D binding protein assay and mass spectrometry.14 54

The sample types listed were tested with a selection of sample collection 55 tubes that were commercially available at the time of testing, i.e. not all 56 available tubes of all manufacturers were tested. Sample collection systems 57 from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary 58 tubes (sample collection systems), follow the instructions of the tube

59 manufacturer. 60 Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

### Materials provided

See "Reagents - working solutions" section for reagents.

### Materials required (but not provided)

- REF 05894921190, Vitamin D total CalSet, for 4 x 1 mL
- REF 05618860190, PreciControl Varia, for 2 x 3 mL each of PreciControl Varia 1 and 2
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or cobas e analyzer • Accessories for Elecsys 2010 and cobas e 411 analyzers:
- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels •

[REF] 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips Accessories for MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- . REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- . REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M
- Accessories for all analyzers:
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

### Assav

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

### Calibration

Traceability: This method has been standardized against LC-MS/MS¹⁵ which in turn has been standardized to the NIST standard.¹⁶

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

# Vitamin D total

### 25-Hydroxyvitamin D

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

### Quality control

For quality control, use PreciControl Varia.

- In addition, other suitable control material can be used.
- Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.
- The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if unknown fall establish of a defined limits.
- values fall outside the defined limits. Follow the applicable government regulations and local guidelines for
- quality control.

### Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in ng/mL or nmol/L).

Conversion	factors:
0011001010101	iuoloio.

nmol/L x 0.40 = ng/mL ng/mL x 2.50 = nmol/L

### Limitations - interference

Samples showing visible signs of hemolysis may cause interference.
 Hemoglobin concentrations > 2 g/L (> 0.124 mmol/L) may lead to elevated results.

The assay is unaffected by icterus (bilirubin < 1129 μmol/L or < 66 mg/dL),</li>
 lipemia (Intralipid < 400 mg/dL) and biotin (< 287 nmol/L or < 70 ng/mL).</li>

Criterion: For concentrations from LoQ up to 15 ng/mL, deviation is

 $\leq 1.5$  ng/mL; for concentrations > 15 ng/mL, deviation is  $\leq 10$  %.

Samples should not be taken from patients receiving therapy with high
 biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin
 administration.

- In vitro tests were performed on 17 commonly used pharmaceuticals and 5 special therapeutic drugs (Bonviva (Ibandronate), EinsAlpha (Alfacalcidol),
- Fosamax (Alendronate), Pamidron HEXAL (Pamidronate) and Zometa
   (Zoledronate)). No interference with the assay was found.
- In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These

effects are minimized by suitable test design.

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other
- 45 findings. 46 Limits a

### Limits and ranges

### 47 Measuring range

3.00-70.0 ng/mL or 7.50-175 nmol/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 3.00 ng/mL (< 7.50 nmol/L). Values above the measuring range are reported as > 70.0 ng/mL (> 175 nmol/L).

### 51 Lower limits of measurement

- 52 Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation 53 (LoQ)
- 54 Limit of Blank = 2.00 ng/mL (5.00 nmol/L)
- 55 Limit of Detection = 3.00 ng/mL (7.50 nmol/L)
- 56 Limit of Quantitation = 5.00 ng/mL (12.5 nmol/L) with a total allowable 57 relative error of  $\leq$  30 %
- The Limit of Blank, Limit of Detection and Limit of Quantitation were
- 58 determined in accordance with the CLSI (Clinical and Laboratory Standards
   59 Institute) EP17-A requirements.

## cobas®

corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative error of  $\leq$  30 %.

The total error concept describes the maximum possible error of a test result taking into account the imprecision (SD) and inaccuracy (bias) of the test system. The Total Error (TE) was determined using the RMS (Root Mean Square) model (CLSI EP17-A2). The relative allowable total error refers to the respective concentration of the sample.

### Dilution

Samples with vitamin D (25-OH) concentrations above the measuring range can be manually diluted with Diluent Universal or a suitable human serum with a low analyte concentration. The recommended dilution is 1:2. The concentration of the diluted sample must be > 30.0 ng/mL (> 75.0 nmol/L). After manual dilution, multiply the results by the dilution factor 2. The endogenous analyte concentration of the human serum used for dilution has to be taken into account.

### Expected values

Due to different standardizations between methods, result variation may arise. Clinical assessment should be taken into consideration when interpreting results.

### Health based reference values (recommended for use):

Currently there is no standard definition of the optimal vitamin D status. Many specialists consider the commonly used population based reference values too low. Health based reference values are recommended to replace population based reference values.¹⁷

Most experts agree that vitamin D deficiency should be defined as vitamin D (25-OH) of  $\leq$  20 ng/mL ( $\leq$  50 nmol/L).¹⁸ Vitamin D insufficiency is recognized as 21-29 ng/mL.¹⁸ Similarly, the US National Kidney Foundation considers levels < 30 ng/mL to be insufficient or deficient.¹⁹

The preferred level for vitamin D (25-OH) by many experts is now recommended to be  $\ge 30$  ng/mL ( $\ge 75$  nmol/L).^{18,20,21,22}

### Reference values measured in an apparently healthy population:

It should be taken into consideration that differences in vitamin D (25-OH) levels may exist with respect to gender, age, season, geographical latitude and ethnic groups.  18,20 

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Population based reference ranges should not be taken as clinical cutoff to recommend or dissuade from vitamin D supplementation. Guidance for supplementation should be taken from recent literature.^{18,19}

A reference range study was conducted with samples from apparently healthy individuals of Caucasian heritage. The age range was 20-77 years. Samples were collected between November and July in northern Germany. The values given are for information only and may vary from other published data.

			Gender			
	All (n = 453)		Female (n = 252)		Male (n = 201)	
Unit	ng/mL	nmol/L	ng/mL	nmol/L	ng/mL	nmol/L
Mean	20.6	51.5	21.6	54.0	19.4	48.5
2.5 th percentile	5.26	13.2	6.23	15.6	4.92	12.3
97.5 th percentile	47.0	118	49.9	125	42.7	107

A lower recovery may be found in particular clinical cohorts, for example dialysis patients.  $^{\rm 23}$ 

### Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

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25-Hydroxyvitamin D

### Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

			Repeatability				
Sample	Me	ean	S	D	CV		
	ng/mL	nmol/L	ng/mL	nmol/L	%		
HS ^{a)} 1	6.76	16.9	0.525	1.31	7.8		
HS 2	15.0	37.5	0.770	1.93	5.1		
HS 3	28.0	70.0	0.860	2.15	3.1		
HS 4	67.0	168	1.15	2.88	1.7		
PC ^{b)} Varia 1	19.9	49.8	0.948	2.37	4.8		
PC Varia 2	38.3	95.8	1.05	2.63	2.7		

b) PC = PreciControl

Elecsys 2010 and cobas e 411 analyzers Intermediate precision Mean SD CV Sample % nmol/L nmol/L ng/mL ng/mL HS₁ 6.76 16.9 0.724 1.81 10.7 HS₂ 15.0 37.5 1.28 3.20 8.5 HS 3 28.0 70.0 1.46 3.65 5.2 HS₄ 67.0 168 1.46 3.65 2.2 PC Varia 1 19.9 49.8 1.23 3.08 6.2 PC Varia 2 38.3 95.8 1.41 3.53 37

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers

			Re	epeatability	/
Sample	Ме	Mean SD		CV	
	ng/mL	nmol/L	ng/mL	nmol/L	%
HS 1	8.35	20.9	0.567	1.42	6.8
HS 2	15.8	39.5	0.824	2.06	5.2
HS 3	28.3	70.8	1.11	2.78	3.9
HS 4	69.6	174	1.50	3.75	2.2
PC Varia 1	20.2	50.5	0.924	2.31	4.6
PC Varia 2	39.6	99.0	1.06	2.65	2.7

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers

		Intermediate precisio				
Sample	Me	Mean		SD		
	ng/mL	nmol/L	ng/mL	nmol/L	%	
HS 1	8.35	20.9	1.10	2.75	13.1	
HS 2	15.8	39.5	1.18	2.95	7.5	
HS 3	28.3	70.8	1.83	4.58	6.5	
HS 4	69.6	174	2.37	5.93	3.4	
PC Varia 1	20.2	50.5	0.954	2.39	4.7	
PC Varia 2	39.6	99.0	1.38	3.45	3.5	

### Method comparison

1) A comparison of the Elecsys Vitamin D total assay (y) using samples measured with LC-MS/MS (x) gave the following correlations (ng/mL):

BMJ Open



0.510

Number of samples measured: 903

Passing/Bablok ²⁴	y = 1.09x -
Pearson	r – 0 894

The sample concentrations were between approximately 3 ng/mL (7.5 nmol/L) and 81 ng/mL (203 nmol/L).

2) A comparison of the Elecsys Vitamin D total assay (y) using samples measured with a commercially available vitamin D (25-OH) immunoassay (x) gave the following correlations (ng/mL):

Number of samples measured: 451

Passing/Bablok ²⁴	y = 1.29x + 1.71
Pearson	r = 0.803

The sample concentrations were between approximately 5 ng/mL (12.5 nmol/L) and 81 ng/mL (203 nmol/L).

### Analytical specificity

The specificity was assessed at 50 %  $B_0$  and the results are summarized in the following table:

Cross-reactant	Cross-reactivity (%)
25-hydroxyvitamin $D_3$	100
25-hydroxyvitamin D ₂	92
24,25-dihydroxyvitamin D ₃	149
C3-epimer of 25-hydroxyvitamin D ₃	91
1,25-dihydroxyvitamin D ₃	non detectable
1,25-dihydroxyvitamin D ₂	non detectable
Vitamin D ₃	non detectable
Vitamin D ₂	non detectable

### Functional sensitivity

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of  $\le 20$  %. 8 samples with concentrations between 0.722 ng/mL and 10.1 ng/mL were measured on several days. The functional sensitivity was determined to be 4.01 ng/mL (CV 18.5 %).

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# itamin D total

25-Hydroxyvitamin D

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

### Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
$\rightarrow$	Volume after reconstitution or mixing
GTIN	Global Trade Item Number
COBAS, COBAS E, ELE	CSYS and PRECICONTROL are trademarks of Roche. INTRALIPID is a trademark of

Fresenius Kabi AB

All other product names and trademarks are the property of their respective owners Additions, deletions or changes are indicated by a change bar in the margin

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Vitamin	B12	
Vitamin B12		

4				1	
5	REF	Σ		SYSTEM	
6		•		Elecsys 2010	
7				MODULAR ANALYTICS E170	
8	07010771 100	100		cobas e 411	
9	07212771 190	100			
10				<b>cobas e</b> 601	
11				<b>cobas e</b> 602	
12	En alla h		т.		
13	English			<b>st principle</b> mpetition principle. Total duration of assay: 27 minutes	
14	Intended use	in vitre succetitative datamainstian of vitamin D10 in			
15 16	human serum and pla		•	1st incubation: By incubating the sample (15 $\mu L)$ with t pretreatment 1 and pretreatment 2, bound vitamin B12	
17		nescence immunoassay "ECLIA" is intended for use	•	2nd incubation: By incubating the pretreated sample w	ith the ruthenium
18	,	s e immunoassay analyzers.		labeled intrinsic factor, a vitamin B12-binding protein c the amount of which is dependent upon the analyte co	
19	Summary	wed to as ashalamin in a semalar annonanatallia		sample.	
20		erred to as cobalamin, is a complex organometallic cobalt atom is situated within a corrin ring. It is a	•	3rd incubation: After addition of streptavidin-coated mi	croparticles and
21	water-soluble vitamin	which is synthesized by microorganisms. It cannot be		vitamin B12 labeled with biotin, the still-vacant sites of	the ruthenium
22		nan body and is seldom found in products of plant of vitamin B12 are meat, fish, eggs and dairy products.		labeled intrinsic factor become occupied, with formatio labeled intrinsic factor vitamin B12 biotin complex. The	
23	¹ The uptake in the ga	istrointestinal tract depends on intrinsic factor, which		becomes bound to the solid phase via interaction of bin	
24	is synthesized by the	gastric parietal cells, and on the "cubam receptor" in		streptavidin.	
25	is a lack of intrinsic fac	nost frequent cause of severe vitamin B12 deficiency ctor due to autoimmune atrophic gastritis. The disease		The reaction mixture is aspirated into the measuring of microparticles are magnetically captured onto the surfa	
26	is historically called "p	ernicious anemia", even though many patients		electrode. Unbound substances are then removed with	
27	present with mainly ne	eurologic manifestations. Examples of other causes ency are malabsorption due to gastrectomy,		ProCell/ProCell M. Application of a voltage to the elect	
28	inflammatory bowel di	sease or dietary deficiency, e.g. in strict vegetarians		chemiluminescent emission which is measured by a pl	
29 30	(vegans). ²			Results are determined via a calibration curve which is specifically generated by 2-point calibration and a mas	
31	Vitamin B12 is the cof	actor for two enzymes, methionine synthase and		via the reagent barcode.	
32		utase. ^{2,3} Methionine synthase, located in the tamin B12 in the form of methylcobalamin and	Re	agents - working solutions	
33	catalyzes the convers	ion of homocysteine to methionine, an essential	The	e reagent rackpack (M, R1, R2) and the pretreatment re	eagents (PT1,
34	amino acid. During thi	s step a methyl group is transferred from e to the amino acid. ³ This enzyme links the	PT:	2) are labeled as B12 II.	
35	methylation pathway t	hrough synthesis of the methyl donor S-Adenosyl	PT	1 Pretreatment reagent 1 (white cap), 1 bottle, 4 mL:	
36	via generation of tetra	athway in which purine and pyrimidine are synthesized hydrofolate. ³ In the form of		Dithiothreitol 1.028 g/L; stabilizer, pH 5.5.	
37	5'-deoxyadenosylcoba	alamin, vitamin B12 is also required for the	PT	2 Pretreatment reagent 2 (gray cap), 1 bottle, 4 mL:	
38 39	methylmalonyl CoA to	methylmalonyl CoA mutase, which converts succinyl CoA. This is a step in the oxidation of odd-		Sodium hydroxide 40 g/L; sodium cyanide 2.205 g/L.	
40		catabolism of ketogenic amino acids. ³ Thus, ant for DNA synthesis, regenerating methionine for	М	Streptavidin-coated microparticles (transparent cap),	1 bottle, 6.5 mL:
41	protein synthesis and	methylation, as well as for the development and initial		Streptavidin-coated microparticles 0.72 mg/mL; pres	ervative.
42	of normal CNS function	tral nervous system (CNS) and for the maintenance	R1	Intrinsic factor~ $Ru(bpy)_3^{2+}$ (gray cap), 1 bottle, 10 mL	:
43	Vitamin B12 deficienc	ies are common in wealthier countries principally		Ruthenium labeled recombinant porcine intrinsic fact	
44	among the elderly and the prevalence increase	d are most prevalent in poorer populations. In general		cobinamide dicyanide 15 µg/L; stabilizer; human ser	um albumin;
45 46	•	y impacts red blood cell synthesis, resulting in		phosphate buffer, pH 5.5; preservative.	
40 47	megaloblastic anemia	due to abnormal DNA synthesis. ³ In addition it	R2	Vitamin B12~biotin (black cap), 1 bottle, 8.5 mL:	
48	impairs neurological fu	unction, in particular demyelination of nerves in part ylation, leading to peripheral neuropathy, dementia,		Biotinylated vitamin B12 25 µg/L; biotin 3 µg/L; phos	phate buffer,
49	poor cognitive perform	nance, and depression. ³ Other effects of vitamin B12		pH 7.0; preservative.	
50	deficiency or depletion	n are increased risk of neural tube defects,	Pre	ecautions and warnings	
51		vascular and cardiovascular diseases. ³ Early because of the latent nature of this disorder and the	For	r in vitro diagnostic use.	
52	risk of permanent neu			ercise the normal precautions required for handling all ligents.	aboratory
53		test performed to confirm the diagnosis of	Dis	posal of all waste material should be in accordance wit	
54	publications suggest t	y is measurement of serum vitamin B12 level. ² Recent hat in addition the following biomarkers should be		fety data sheet available for professional user on reque	
55	measured to improve	the specificity of diagnosis: folate, methylmalonic acid		s kit contains components classified as follows in accol gulation (EC) No. 1272/2008:	uance with the
56		and holotranscobalamin. ^{2,5,6,7}	. 10	· · · · · · · · · · · · · · · · · · ·	
57	intrinsic factor specific	312 II assay employs a competitive test principle using of rvitamin B12. Vitamin B12 in the sample competes			
58	with the added vitamir	n B12 labeled with biotin for the binding sites on the	5		
59	ruthenium-labeled intr	insic factor complex ^a .		V	

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)₃²⁺)

Danger

# Vitamin B12 II

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## 

2	VILA	
3	Vitamin B12	
4 5	H290	May be corrosive to metals.
6 7	H314	Causes severe skin burns and eye damage.
8 9	H412	Harmful to aquatic life with long lasting effects.
9 10	Prevention:	
10	P234	Keep only in original container.
12		
13	P264	Wash skin thoroughly after handling.
14 15	P273	Avoid release to the environment.
16 17	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
17	Response:	
19 20	P301 + P330 + P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
21		IF ON SKIN (ar hair), Damaya/Taka aff immediataly all
22 23	P303 + P361 + P353	IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
24 25	P304 + P340	IF INHALED: Remove person to fresh air and keep comfortable for breathing.
26 27 28 29	P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
30	P310	Immediately call a POISON CENTER or doctor/physician.
31 32	P363	Wash contaminated clothing before reuse.
33	P390	Absorb spillage to prevent material damage.
34	Storage:	
35 36	P405	Store locked up.
37	P406	Store in corrosive resistant stainless steel container with a
38	1 400	resistant inner liner.
39	Disposal:	
40 41	P501	Dispose of contents/container to an approved waste
42		disposal plant.
43	-	/ labeling primarily follows EU GHS guidance.
44	1	e: all countries: +49-621-7590
45		terial should be considered potentially infectious. All products numan blood are prepared exclusively from the blood of
46 47	donors tested	individually and shown to be free from HBsAg and antibodies
47 48		IV. The testing methods applied were FDA-approved or npliance with the European Directive 98/79/EC, Annex II,
40 49	List A.	
49 50	However, as r	no testing method can rule out the potential risk of infection
50		certainty, the material should be handled with the same level atient specimen. In the event of exposure, the directives of the
52	responsible he	ealth authorities should be followed. ^{8,9}
52	Avoid foam fo	rmation in all reagents and sample types (specimens,
55	calibrators and	d controls).
55	Reagent han	dling
56		in the kit have been assembled into a ready-for-use unit that
57	cannot be sep	
58	reagent barco	n required for correct operation is read in from the respective des.
59 60	Storage and Store at 2-8 °C	

Do not freeze.

Store the Elecsys reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	84 days (12 weeks)
on the analyzers	35 days (5 weeks) onboard or 60 days when stored alternatively in the refrigerator and on the analyzer, with the total time onboard on the analyzer not exceeding 10 x 8 hours

### Specimen collection and preparation

Only the specimens listed below were tested and found acceptable. Serum collected using standard sampling tubes or tubes containing separating gel.

Na-heparin, Li-heparin, K₂-EDTA and K₃-EDTA plasma. Li-heparin plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + intercept within < ± 2x Limit of Blank (LoB) + coefficient of correlation  $\geq 0.95$ .

Stable for 2 hours at 15-25 °C, 48 hours at 2-8 °C, 56 days at (-15)-(-25) °C. Freeze once only.

Stability of serum obtained with separating tubes: 24 hours at 2-8 °C (note the data provided by the tube manufacturer).

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples.

Avoid hemolysis.

Do not use samples and controls stabilized with azide.

Vitamin B12 determinations should be performed on serum or plasma samples from fasting patients.

Note: Samples with extremely high total protein concentrations (e.g. patients suffering from Waldenström's macroglobulinemia) are not suitable for use in this assay, since they may lead to the formation of protein gel in the assay cup. Processing protein gel may cause a run abort. The critical protein concentration is dependent upon the individual sample composition. The formation of protein gel was seen in samples (spiked with human IgG or human serum albumin) having a total protein concentration > 160 g/L.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

### Materials provided

See "Reagents – working solutions" section for reagents.

### Materials required (but not provided)

- REF 07212780190, Vitamin B12 II CalSet, for 4 x 1 mL
- REF 05618860190, PreciControl Varia, for 2 x 3 mL each of PreciControl Varia 1 and 2
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or cobas e analyzer Accessories for Elecsys 2010 and cobas e 411 analyzers:
- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution

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# itamin B12 II/

- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- [REF] 11933159001, Adapter for SysClean
- REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips
- Accessories for MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers:
- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- [REF] 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M
- Accessories for all analyzers:
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

### Assav

- 26 For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's 27 28 manual for analyzer-specific assay instructions.
- 29 Resuspension of the microparticles takes place automatically prior to use. 30 Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence 31
- of numbers. 32
- MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers: 33 PreClean M solution is necessary.
- 34 Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system 35 automatically regulates the temperature of the reagents and the 36
- opening/closing of the bottles. 37

### Calibration

- 38 Traceability: This method has been standardized against the Vitamin B12 assay ([REF] 04745736). 39
- 40 Every Elecsys reagent set has a barcoded label containing specific 41 information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet. 42
- Calibration frequency: Calibration must be performed once per reagent lot 43 using fresh reagent (i.e. not more than 24 hours since the reagent kit was 44 registered on the analyzer). Renewed calibration is recommended as 45 follows:
  - after 1 month (28 days) when using the same reagent lot
  - after 7 days (when using the same reagent kit on the analyzer)
  - as required: e.g. quality control findings outside the defined limits

### Quality control

- For quality control, use PreciControl Varia.
- 51 In addition, other suitable control material can be used.
- 52 Controls for the various concentration ranges should be run individually at 53 least once every 24 hours when the test is in use, once per reagent kit, and following each calibration. 54
- The control intervals and limits should be adapted to each laboratory's 55
- individual requirements. Values obtained should fall within the defined 56 limits. Each laboratory should establish corrective measures to be taken if 57 values fall outside the defined limits.
- 58 Follow the applicable government regulations and local guidelines for quality control. 59

### Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in pmol/L or pg/mL).

pmol/L x 1.36 = pg/mL pg/mL x 0.738 = pmol/L

### Limitations - interference

The assay is unaffected by icterus (bilirubin  $\leq 1112 \,\mu$ mol/L or  $\leq 65 \,\text{mg/dL}$ ), hemolysis (Hb ≤ 0.025 mmol/L or ≤ 0.04 g/dL), lipemia (triglycerides  $\leq$  17.1 mmol/L or  $\leq$  1500 mg/dL), biotin ( $\leq$  205 nmol/L or  $\leq$  50 ng/mL), lgG  $\leq$  28 g/L, IgA  $\leq$  16 g/L and IgM  $\leq$  10 g/L.

Criterion: Recovery within ± 10 % of initial value with samples > 200 pg/mL and  $\leq \pm 20$  pg/mL with samples  $\leq 200$  pg/mL.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

Because intrinsic factor is typically used as the binding protein in serum vitamin B12 assays, anti-intrinsic factor antibodies (which are common in pernicious anemia) can lead to elevated vitamin B12 measurement values.^{2,} ^{10,11} The Elecsys Vitamin B12 II assay is designed to avoid interference due to anti-intrinsic factor antibodies.12

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findinas.

Note: The presence of immunoglobulin-vitamin B12 complexes may cause unexpectedly high values of vitamin B12.13,14

### Limits and ranges

### Measuring range

50.0-2000 pg/mL or 36.9-1476 pmol/L (defined by the Limit of Blank and the maximum of the master curve). Values below the Limit of Blank are reported as < 50.0 pg/mL or < 36.9 pmol/L. Values above the measuring range are reported as > 2000 pg/mL or > 1476 pmol/L.

### Lower limits of measurement

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

Limit of Blank = 50 pg/mL (36.9 pmol/L)

Limit of Detection = 100 pg/mL (73.8 pmol/L)

Limit of Quantitation = 150 pg/mL (111 pmol/L) with a allowable imprecision of ≤ 20 %

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from  $n \ge 60$  measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a allowable imprecision of ≤ 20 %.

It has been determined using low concentration vitamin B12 samples.

### Dilution

Samples with vitamin B12 concentrations above the measuring range can be manually diluted 1:2 with Diluent Universal. The concentration of the diluted sample must be > 738 pmol/L or > 1000 pg/mL. After manual dilution, multiply the results by the dilution factor 2.

Note: Sample-dependent non-linearity upon dilution is seen with samples having analyte levels beyond the measuring range. As Diluent Universal may contain low levels of endogenous vitamin B12, it is recommended that linearity studies be performed using a known low analyte-containing serum



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pool. Samples outside the measuring range can be diluted 1:2 with Diluent Universal; the effect of endogenous vitamin B12 concentration is insignificant at these levels.

### Expected values

Because differences may exist with respect to population and dietary status, it is recommended that normal ranges be determined by each laboratory over a suitable period of time and in a statistically significant number of assays before clinical significance is attached to the results of these tests.

The values shown below were performed on samples from an apparently healthy population, using the Elecsys Vitamin B12 II assay. The calculation is based on 135 sera (68 men, 67 women). The age range was between 20 and 78 years. Pregnant women were excluded. The reference population was selected according to normal homocysteine values.

Ν	Median		Range (2.5th-97.5th percentile)	
	pg/mL	pmol/L	pg/mL	pmol/L
135	425	314	197 <b>-</b> 771	145-569

These values should only be used as guidelines.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

### Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

### Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

		Repeatability		Intermediate precision		
Sample	Mean	SD	CV	SD	CV	
	pg/mL	pg/mL	%	pg/mL	%	
Human serum 1	176	8.86	5.0	12.7	7.2	
Human serum 2	405	13.0	3.2	17.5	4.3	
Human serum 3	960	19.7	2.1	31.0	3.2	
Human serum 4	1230	27.4	2.2	46.4	3.8	
Human serum 5	1940	40.9	2.1	72.6	3.7	
PreciControl Varia1	447	12.2	2.7	18.6	4.2	
PreciControl Varia2	934	20.2	2.2	38.4	4.1	

Elecsys 2010 and <b>cobas e</b> 411 analyzers						
	Repeatal	bility	Intermediate precision			
Sample	Mean	SD	CV	SD	CV	
	pmol/L	pmol/L	%	pmol/L	%	
Human serum 1	130	6.54	5.0	9.37	7.2	
Human serum 2	299	9.59	3.2	12.9	4.3	
Human serum 3	708	14.5	2.1	22.9	3.2	
Human serum 4	908	20.2	2.2	34.2	3.8	
Human serum 5	1432	30.2	2.1	53.6	3.7	
PreciControl Varia1	330	9.00	2.7	13.7	4.2	
PreciControl Varia2	689	14.9	2.2	28.3	4.1	

MODULAR ANALYTICS E170	, <b>cobas e</b> 601	1 and <b>cobas e</b> 602 analyzers
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	Repeata	bility	Intermediate precision		
Sample	Mean	SD	CV	SD	CV
	pg/mL	pg/mL	%	pg/mL	%
Human serum 1	176	5.84	3.3	9.14	5.2
Human serum 2	407	8.24	2.0	12.7	3.1
Human serum 3	1010	13.2	1.3	21.1	2.1
Human serum 4	1230	19.8	1.6	28.8	2.3
Human serum 5	1890	29.8	1.6	41.5	2.2
PreciControl Varia1	448	7.16	1.6	15.3	3.4
PreciControl Varia2	917	12.0	1.3	27.8	3.0

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers

	Repeata	bility	Intermediate precision		
Sample	Mean	SD	CV	SD	CV
	pmol/L	pmol/L	%	pmol/L	%
Human serum 1	130	4.31	3.3	6.75	5.2
Human serum 2	300	6.08	2.0	9.37	3.1
Human serum 3	745	9.74	1.3	15.6	2.1
Human serum 4	908	14.6	1.6	21.3	2.3
Human serum 5	1395	22.0	1.6	30.6	2.2
PreciControl Varia1	331	5.28	1.6	11.3	3.4
PreciControl Varia2	677	8.86	1.3	20.5	3.0

### Method comparison

a) A comparison of the Elecsys Vitamin B12 assay (calibrated with Vitamin B12 CalSet II; x) and the Elecsys Vitamin B12 II assay (calibrated with Vitamin B12 II CalSet; y) using clinical samples gave the following correlations (pg/mL):

Number of samples measured: 100

Passing/Bablok ¹⁵	Linear regression
y = 0.952x + 15.1	y = 0.957x + 11.6
т = 0.977	r = 0.999

The sample concentrations were between 69 and 1890 pg/mL (51 and 1395 pmol/L).

b) A comparison of the Elecsys Vitamin B12 II assay (y) and a commercially available method (x) using clinical samples gave the following correlations (pg/mL):

Number of samples measured: 106

Passing/Bablok ¹⁵	Linear regression
y = 0.923x + 4.90	y = 0.881x + 27.6
т = 0.952	r = 0.993

The sample concentrations were between 182 and 1797 pg/mL (134 and 1326 pmol/L).

c) A comparison of the Elecsys Vitamin B12 II assay on the cobas e 601 analyzer (y) and the Elecsys Vitamin B12 II assay on the cobas e 411 analyzer (x) using clinical samples gave the following correlations (pg/mL): Number of samples measured: 117

Passing/Bablok ¹⁵	Linear regression
y = 1.01x - 2.77	y = 1.01x + 3.22
т = 0.933	r = 0.995

The sample concentrations were between 56 and 1887 pg/mL (41 and 1393 pmol/L).

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# Vitamin B12 II

Vitamin B12

### Analytical specificity

The following cross-reactivities were found, tested with vitamin B12 concentrations of 129 pg/mL and 550 pg/mL.

8 9 10	Cross-reactant	Maximum concentration tested ng/mL	Cross-reactivity %
11	Cobinamide dicyanide	210	0.003
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12	Referer	2020	CE	
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42 43	con	ven RA, Drake SK, Vanjani R, et al. Markedly increased vitamin B12 centrations attributable to IgG-IgM-vitamin B12 immune complexes. 1 Chem 2006;52(11):2107-2114.		
44 45 46 47	for for	blok W, Passing H, Bender R, et al. A general regression procedure method transformation. Application of linear regression procedures method comparison studies in clinical chemistry, Part III. lin Chem Clin Biochem 1988 Nov;26(11):783-790.		
48 49 50	the ana informat	ner information, please refer to the appropriate operator's manual for lyzer concerned, the respective application sheets, the product tion and the Method Sheets of all necessary components (if e in your country).		
51 52 53	separat	(period/stop) is always used in this Method Sheet as the decimal or to mark the border between the integral and the fractional parts of al numeral. Separators for thousands are not used.		
54 55 56		Is Diagnostics uses the following symbols and signs in addition to sted in the ISO 15223-1 standard.		
50 57	CONTE	VT Contents of kit		
58 50	SYSTEM			
59 60	CALIBR			
		Jubraton		



Volume after reconstitution or mixing

Global Trade Item Number

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### 0107227841190c501V1.0 Tina-quant Soluble Transferrin Receptor II



REF	CONTENT								Analyzer(s) on which cobas c p	back(s) can be used		
07227841190	Tina-quant Solubl	e Trans	ferrin Re	ecept	or II(100	) tests)	Syst	em-ID 07 7473 1	cobas c 311, cobas c 501/502			
08753776190	Calibrator sTfR II	(3 x 1 m	ıL)				Code	es 697				
08278202190	ControlSet sTfR I											
	Level I (3 x 1 mL)						Leve	el I Code 153				
	Level II (3 x 1 mL	)					Leve	el II Code 154				
04489357190	Diluent NaCl 9 %	(50 mL)					Syst	em-ID 07 6869 3				
English									ticles coated with monoclonal anti n glycine buffer; preservative	i-human sTfR antibodie		
System information For cobas c 311/501 analyzers:								. ,	B and R2 is in position C.			
STFR2: ACN 43								Precautions and	·			
For cobas c 502								For in vitro diagno	ostic use.			
STFR2: ACN 84	39							Exercise the norm	nal precautions required for hand	ling all laboratory		
Intended use								reagents. Disposal of all wa	aste material should be in accorda	nce with local guideline		
In vitro test for th	e quantitative deter	rminatior	n of solu	ible tr	ransferri	in recept	or	Safety data sheet	t available for professional user of	n request.		
(sTfR) in human Summary ^{1,2,3,4,5}	serum and plasma	on coba	IS C SYS	iems.	5			This kit contains of Regulation (EC) N	components classified as follows	in accordance with the		
	eceptor is an integra	al memb	orane olv	copr	otein ha	avino a		•	/l-2H-isothiazol-3-one hydrochlori	de		
molecular weigh	t of 190 kilodalton (	kDa). It o	consists	of tw	/o identi	ical			5	~~		
subunits linked b C-terminal comm	by disulfide bridges.	⊨ach of nd an irc	tne mo n-lader	nome trans	ers has a sferrin n	an 85 kD nolecule	a		ay produce an allergic reaction.			
Proteolysis lead	s to the soluble forn	n of the t	transferr	rin rec	ceptor (s	sTfR). In		-	beling follows EU GHS guidance.			
plasma, the solu	ble transferrin rece aving a molecular v	otor is pr veight o	resent ir f approv	the f	iorm of a	a comple kD. The	ЭХ	Reagent handlin	ıg			
serum concentra	ation of sTfR is direct	ctly prop	ortional	to the	e conce	ntration	of	Ready for use Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.				
the receptor on t		lla ¦a	ال المسل			f. ji						
transferrin recep	on by the body's cel tor (TfR). If the intra	acellular	iron sto	res ai	re exhai	usted -		Storage and stat	bility			
corresponding to	a ferritin concentra	ation of le	ess thar	n 12 µ	ug/L - th	en more		STFR2				
depends on the	<ol> <li>The affinity of the latter's loading state</li> </ol>	transter a. As 80-	rin rece -95 % ດ	ptor to the t	u transfer	errin rin recen	tor	Shelf life at 2-8 °C	·.	See expiration date		
molecules are lo	calized on erythrop	oietic ce	ells, the ⁻	TfR co	oncentra	ation (an	ld		2.	on <b>cobas c</b> pack		
nence also the s cells. When iron	TfR concentration) deficiency exists, th	reflects t ne sTfR	the iron	requi ratior	rement	ot these				label.		
even before the	hemoglobin concer	ntration is	s signific	cantly	depres	sed. The	<del>)</del>	On-board in use a	and refrigerated on the analyzer:	26 weeks		
s I tH concentrati ferritin reflects th	on can therefore de le iron storage statu	scribe thus, A nre	ne tunct	ional sessn	Iron sta nent of t	tus while the iron	ł	Diluent NaCl 9 %	-			
status can be ob	tained by determini	ing the s	sTfR inde	ex execution and the second se				Shelf life at 2-8 °C	D:	See expiration date		
	ration/log ferritin co									on <b>cobas c</b> pack		
	o ferritin - the conce ctions, acute liver fu						. it			label.		
is possible to dif	ferentiate between a	anemia d	of chron	ic dis	ease (A	ACD) and		On-board in use a	and refrigerated on the analyzer:	12 weeks		
	nemia (IDA). Elevat emolytic anemia, tha							Specimen collec	ction and preparation			
sickle cell anem	a, megaloblastic ar	nemia, m	nyelodys	plasti	ic syndr	rome and	I		lection and preparation only use s	suitable tubes or		
	iency. Elevated sTf there is a deficience				ur durin	g		collection containers. Only the specimens listed below were tested and found acceptable.				
Parameter	Change		ACD	2	IDA +			Serum.				
Ferritin	iron stores	ida ↓	ACD ↑	_	or	-		•	(Li-, Na-, NH4+-) plasma	n of comple collection		
TIBC/TRSF				*		Ť		tubes that were co	s listed were tested with a selectic ommercially available at the time	of testing, i.e. not all		
	iron status	↑ ↓	↓ ↓	1	or	_		available tubes of	f all manufacturers were tested. S	ample collection system		
Serum iron	iron status	↓ ◆	¥		↓ ↑			affect the test res	nufacturers may contain differing r sults in some cases. When proces	sing samples in primary		
sTfR	functional iron deficiency	Ť	_		1			tubes (sample col manufacturer.	llection systems), follow the instru	uctions of the tube		
↓ decreased. ↑	increased, - unch	nanged							es containing precipitates before	performing the assav.		
Test principle ⁸		922							is and interferences section for de			
• •	ed immunoturbidime		•					sample interferen				
Human soluble transferrin receptor agglutinates with latex particles coated							d	Stability:	6 days at 15	-25 °C		
	with anti-soluble transferrin receptor antibodies. The precipitate is determined photometrically.											
with anti-soluble		r antiboo	lies. The	e prec	sipitate i	IS			15 days at 2			
with anti-soluble	ometrically.	r antiboc	lies. The	e prec	cipitate i	IS		·	15 days at 2			

Tina-quant Soluble Transferrin Receptor II

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Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory. 10 Materials provided 11 See "Reagents - working solutions" section for reagents. 12 Materials required (but not provided) 13 See "Order information" section 14 General laboratory equipment 15 Assav 16 For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's 17 manual for analyzer-specific assay instructions. 18 19 The performance of applications not validated by Roche is not warranted and must be defined by the user. 20 Application for serum and plasma 21 22 cobas c 311 test definition 23 Assay type 2-Point End 24 Reaction time/Assay points 10/8-21 25 Wavelength (sub/main) 800/570 nm 26 Reaction direction Increase 27 28 Unit mg/L (mg/dL, nmol/L) 29 Reagent pipetting Diluent (H₂O) 30 100 µL R1 31 R2 40 µL 32 Sample volumes Sample Sample dilution 33 Diluent (NaCl Sample 34 Normal 2 µL 35 36 Decreased 2μL 25 µL 75 µL 37 Increased 2μL 38 cobas c 501 test definition 39 40 Assay type 2-Point End 41 10 / 13-30 Reaction time/Assay points 42 Wavelength (sub/main) 800/570 nm 43 Reaction direction Increase 44 Unit mg/L (mg/dL, nmol/L) 45 Reagent pipetting Diluent (H₂O) 46 47 R1 100 µL 48 R2 40 µL 49 Sample volumes Sample Sample dilution 50 Diluent (NaCl) Sample 51 Normal 2 µL 52 Decreased 2μL 25 µL 75 µL 53 Increased 2μL 54 55 cobas c 502 test definition 56 2-Point End Assay type 57 Reaction time/Assay points 10 / 13-30 58 59 Wavelength (sub/main) 800/570 nm 60 Reaction direction Increase Unit mg/L (mg/dL, nmol/L)

Reagent pipetting		Diluent (H ₂ O	))
R1	100 µL	-	
R2	40 µL	-	
Sample volumes	Sample	Samp	le dilution
		Sample	Diluent (NaCl)
Normal	2 µL	-	-
Decreased	2 µL	25 µL	75 µL
Increased	2 µL		
Calibration			
Calibrators	S1: H ₂ O		
	S2-S6: Calibra	ator sTfR II	
Calibration mode	Non-linear		
Calibration frequency	Full calibration • after reagent • after 12 weel • after 6 month lot	lot change ks on-board th	ne analyzer a single reagent
	<ul> <li>as required for procedures</li> </ul>	ollowing quali	ty control
Calibration interval may be ex calibration by the laboratory.	tended based of	on acceptable	verification of
Traceability: This method has reference preparation.	been standard	ized against a	an in-house
<b>Quality control</b> For quality control, use control section.	l materials as li	sted in the "C	order information"
In addition, other suitable cont	trol material ca	n be used.	
The control intervals and limits individual requirements. Value limits. Each laboratory should values fall outside the defined	es obtained sho establish corre	ould fall within	the defined
Follow the applicable governm quality control.	nent regulations	s and local gu	idelines for
Calculation			
cobas c systems automaticall sample in the unit mg/L (mg/d	ly calculate the L, nmol/L)	analyte conc	entration of each
Conversion factors:	mg/L x	11.8 = nmol/	<b>L</b> 9,a)

 $mg/L \ge 0.1 = mg/dL$ 

a) Based on a molecular mass of 85 kDa for circulating transferrin receptor.

### Limitations - interference

Criterion: Recovery within  $\pm$  0.2 mg/L (2.36 nmol/L) of initial values of samples  $\leq 2 \text{ mg/L}$  (23.6 nmol/L) and within  $\pm 10 \%$  for samples > 2 mg/L. Icterus:¹⁰ No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:¹⁰ No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 622 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):¹⁰ No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference from rheumatoid factors up to a concentration of 1200 IU/mL.

High dose hook-effect: No false result occurs up to an sTfR concentration of 80 mg/L (944 nmol/L).

The antibodies are specific for sTfR. There is no cross-reactivity with diferrotransferrin, apotransferrin or ferritin under the assay conditions. Drugs: No interference was found at therapeutic concentrations using common drug panels.11,12

Tina-quant Soluble Transferrin Receptor II

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.¹

In very rare cases, patient samples may contain particle agglutinating proteins (e.g. heterophilic antibodies or antibodies due to abnormal immunoglobulin synthesis, such as gammopathies like MGUS* or Waldenström's macroglobulinemia) which may lead to incorrect low or high 10 results with this assay. Correct results cannot be obtained by sample dilution and these samples should be analyzed by an alternative method. 11 noclonal Gammopathy of unknown significance

12 For diagnostic purposes, the results should always be assessed in 13 conjunction with the patient's medical history, clinical examination and other 14 findings

#### **ACTION REQUIRED** 15

Special Wash Programming: The use of special wash steps is mandatory 16 when certain test combinations are run together on cobas c systems. The 17 latest version of the carry-over evasion list can be found with the NaOHD-18 SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. cobas c 502 analyzer: All special wash programming 19 necessary for avoiding carry-over is available via the cobas link, manual 20 input is required in certain cases.

#### 21 Where required, special wash/carry-over evasion programming must 22 be implemented prior to reporting results with this test.

#### 23 Limits and ranges

#### 24 Measuring range

25 0.50-20.0 mg/L (5.9-236 nmol/L)

26 Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:4 dilution. Results from 27 samples diluted using the rerun function are automatically multiplied by a 28 factor of 4 29

### Lower limits of measurement

20	
30	
	Limit of Blank. Limit of Detection and Limit of Quantitation
24	

31	LIIIII OI DIAIIK, LIIIII	Of Delection and Limit of Quantiti
32	Limit of Blank	= 0.25 mg/L (2.95 nmol/L)

33 Limit of Detection	= 0.40 mg/L (4.72 nmol/L)
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34 Limit of Quantitation = 0.50 mg/L (5.90 nmol/L)

35 The Limit of Blank and Limit of Detection were determined in accordance 36 with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements. 37

The Limit of Blank is the 95th percentile value from  $n \ge 60$  measurements of 38 analyte-free samples over several independent series. The Limit of Blank 39 corresponds to the concentration below which analyte-free samples are found with a probability of 95 %. 40

41 The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. 42

The Limit of Detection corresponds to the lowest analyte concentration 43 which can be detected (value above the Limit of Blank with a probability of 44 95 %).

45 The Limit of Quantitation is the lowest analyte concentration that can be 46 reproducibly measured with a total error of 20 %. It has been determined using low concentration sTfR samples. 47

### Expected values

48 The values shown below were performed on samples from an apparently 49 healthy population, using the Tina-guant Soluble Transferrin Receptor II 50 assay (STFR2). The calculation is based on 165 sera (101 men, 64 women). The age range was between 22 and 83 years. The analysis of the data with the 2.5 % and the 97.5 % percentile gave a soluble transferrin receptor (sTfR) range from 1.71 mg/L (20.2 nmol/L) to 4.13 mg/L 51 52 53 (48.7 nmol/L). 54

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference 55 ranges. 56

### Specific performance data

57 Representative performance data on the analyzers are given below. 58 Results obtained in individual laboratories may differ. 59

### Precision

60

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute)

## 

EP5-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days) The following results were obtained:

Repeatability SD CV Mean mg/L mg/L % Control Set sTfR II L 1 2.56 0.0389 1.5 Control Set sTfR II L 2 6.91 0.0626 0.9 3.1 Human serum 1 1.21 0.0375 2.00 2.2 Human serum 2 0.0438 Human serum 3 5.27 0.0526 1.0 Human serum 4 9.23 0.108 1.2 Human serum 5 17.7 0.9 0.157 Intermediate precision SD CV Mean mg/L mg/L % Control Set sTfR II L 1 2.56 0.0444 1.7 Control Set sTfR II L 2 6.91 0.0732 1.1 Human serum 1 1.21 0.0388 3.2 Human serum 2 2.00 0.0475 2.4 Human serum 3 5.27 0.0675 1.3 Human serum 4 9.31 0.118 1.3 Human serum 5 17.70.1921.1

### Method comparison

sTfR values for human serum and plasma samples obtained on a cobas c 501 analyzer (y) were compared with those determined using the Soluble Transferrin Receptor assay (STFR) on a cobas c 501 analyzer(x). Sample size (n) = 87

Passing/Bablok ¹⁴	Linear regression
y = 0.987x + 0.0347 mg/L	y = 0.989x + 0.0264 mg/L
т = 0.939	r = 0.996

The sample concentrations were between 0.660 and 19.1 mg/L.

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Tha-quant Soluble Transferrin Receptor II  11. Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.  12. Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.  13. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. clin Clin Chem Lab Med 2007;45(9):1240-1243.  14. Bablok W, Passing H, Bender R, et al. A general regression procedures for method transformation. Application of linear regression procedures for method transformation. Application of linear regression procedures for method transformation. Application of linear tegression procedures detection Biochem 1988 Nov;26(11):783-790.  Apoint (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used. <b>Symbols</b> McChe Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for glinition of symbols used):  COMTENT Contents of kit Content Contents of kit Content of the responstitution Content and tradomarkas en tenzens.  COMTENT Contents and tradomarkas en tenzens.  COMTENT Contents of kit Autor generation changes are indicated by a changes bar in the margin.  COMTENT Contents and tradomarkas en tenzens.  Contents of the responstitution Contents and tradomarkas en tenzens.  Part Contents of the response to more.  Contents of the response to the margin.  Contents of the response to the property of their response to more.  Contents of the segnetic structure of the response to more.  Contents of the demarkas are indicated by a change bar in the margin.  Contents of the segnetic structure of the response to more.  Contents of the demarkas are indicated by a change bar in the margin.  Cont	<ul> <li>Tina-quant Soluble Transferrin Receptor II</li> <li>11 Breuer J. Report on the Symposium "Drug effects Methods". Eur J Clin Chem Clin Biochem 1996;34</li> <li>12 Sonntag O, Scholer A. Drug interference in clinicar recommendation of drugs and their concentration interference studies. Ann Clin Biochem 2001;38:3</li> <li>13 Bakker AJ, Mücke M. Gammopathy interference in assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.</li> <li>14 Bablok W, Passing H, Bender R, et al. A general for method transformation. Application of linear refor method comparison studies in clinical chemistr. Chem Clin Biochem 1988 Nov;26(11):783-790.</li> <li>A point (period/stop) is always used in this Method Sh separator to mark the border between the integral and a decimal numeral. Separators for thousands are not of Symbols</li> <li>Roche Diagnostics uses the following symbols and sig those listed in the ISO 15223-1 standard (for USA: set definition of symbols used):</li> <li>CONTENT Contents of kit Volume for reconstitution Global Trade Item Num</li> <li>COBAS, COBAS C and TINA-QUANT are trademarks of Roche.</li> <li>All other product names and trademarks are the property of their respective owner Additions, deletions or changes are indicated by a change bar in the margin. © 2020, Roche Diagnostics</li> </ul>	35-386. hemistry: be used in drug -385. linical chemistry ression procedure Part III. J Clin t as the decimal e fractional parts of ad.	
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Image: Construction Constr	GTIN       Volume for reconstitution         GIObal Trade Item Num         COBAS, COBAS C and TINA-QUANT are trademarks of Roche.         All other product names and trademarks are the property of their respective owne         Additions, deletions or changes are indicated by a change bar in the margin.         © 2020, Roche Diagnostics         CEE         Main         Boche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannhe		
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All other product names and trademarks are the property of their respective owners. Additions, deletions or changes are indicated by a change bar in the margin. © 2020, Roche Diagnostics C C C M Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim www.roche.com	All other product names and trademarks are the property of their respective owne Additions, deletions or changes are indicated by a change bar in the margin. © 2020, Roche Diagnostics Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannhe	Roche	

## cobas®

# Ferritin

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## cobas®

4	remun					
5	REF	Σ		SYSTEM		
6		•		Elecsys 2010		
7				MODULAR ANA		
8					ALTICS ET/0	
9	<b>03737551</b> 190	100		cobas e 411		
10				cobas e 601		
11 J				cobas e 602		
12			·			
13	English				nto the measuring cell where the	
14	Intended use				ptured onto the surface of the	
15		vitro quantitative determination of ferritin in human			a voltage to the electrode then induces	
16	serum and plasma.				is measured by a photomultiplier.	
17 18		escence immuno <b>a</b> ssay "ECLIA" is intended for use e immunoassay analyzers.	<ul> <li>Results are deterr specifically general</li> </ul>	nined via a calib ated by 2-point c	ration curve which is instrument- alibration and a master curve provided	
19	Summary		via the reagent ba		·	
20		cule with a molecular weight of at least 440 kDa	a) Tris(2,2'-bipyridyl)rutheni	um(II)-complex (Ru(b	py) ₃ ²⁺ )	
21		content) and consists of a protein shell (apoferritin)	Reagents - working			
22	2500 Fe ³⁺ ions (in liver	on core containing an average of approx.	The reagent rackpack	is labeled as FE	ERR.	
23		gomers, and when it is present in excess in the cells	M Streptavidin-coa	ted microparticle	es (transparent cap), 1 bottle, 6.5 mL:	
24	of the storage organs th	ere is a tendency for condensation to lerin to occur in the lysosomes.	Streptavidin-coated microparticles 0.72 mg/mL; preservative.			
25		an be distinguished with the aid of isoelectric	R1 Anti-Ferritin-Ab~biotin (gray cap), 1 bottle, 10 mL:			
26	focusing. ² This microhe	terogeneity is due to differences in the contents of	Biotinylated monoclonal anti-ferritin antibody (mouse) 3.0 mg/L;			
27	the acidic H and weakly	basic L subunits. The basic isoferritins are			H 7.2; preservative.	
28	the liver, spleen, and bo	one marrow. ^{1,3}			cap), 1 bottle, 10 mL:	
29		und mainly in the myocardium, placenta, and tumor				
30	tissue. They have a low	er iron content and presumably function as			(mouse) labeled with ruthenium iffer 100 mmol/L, pH 7.2;	
31		ansfer of iron in various syntheses.4,5,6	preservative.	L, priospriate bu	iner 100 mmor∠, μ⊓ 7.2,	
32	The determination of te	rritin is a suitable method for ascertaining the iron etermination of ferritin at the beginning of therapy				
33	provides a representativ	e measure of the body's iron reserves. A storage	Precautions and wa	•		
34	deficiency in the reticulo	o-endothelial system (RES) can be detected at a	For in vitro diagnostic		red for handling all laboratory	
35	very early stage. ⁷		reagents.	necaulions requi		
36	detection of prelatent in	alue of 20 µg/L (ng/mL) has proved useful in the on deficiency. This value provides a reliable	Disposal of all waste	material should b	be in accordance with local guidelines.	
37	indication of exhaustion	of the iron reserves that can be mobilized for			sional user on request.	
38	hemoglobin synthesis. I	_atent iron deficiency is defined as a fall below the threshold. These two values necessitate no further	calibrators and contro		nd sample types (specimens,	
39		even when the blood picture is still morphologically	Reagent handling			
40	normal. If the depresse	d ferritin level is accompanied by hypochromic,		it have been ass	embled into a ready-for-use unit that	
41	•	manifest iron deficiency is present. ¹	cannot be separated.			
42 43	When the ferritin level is disorder can be ruled or	s elevated and the possibility of a distribution ut, this is a manifestation of iron overloading in the		ed for correct ope	eration is read in from the respective	
44	body. 400 µg/L (ng/mL)	ferritin is used as the threshold value. Elevated	reagent barcodes.			
45		encountered with the following tumors: acute	Storage and stability			
46		ease and carcinoma of the lung, colon, liver and ation of ferritin has proved to be of value in liver	Store at 2-8 °C.			
47	metastasis. Studies ind	cate that 76 % of all patients with liver metastasis	Do not freeze.			
48	have ferritin values abo	ve 400 µg/L (ng/mL). Reasons for the elevated crosis, blocked erythropoiesis or increased synthesis			in order to ensure complete a automatic mixing prior to use.	
49	in tumor tissue.	iosis, bioched erythiopolesis of increased synthesis				
50		antibodies - M-4.184 and M-3.170 - are used to	Stability:			
51	form the sandwich com	plex in the assay.	unopened at 2-8 °C		up to the stated expiration date	
52	Test principle		after opening at 2-8	°C	12 weeks	
53		al duration of assay: 18 minutes.	on the analyzers		6 weeks	
54		of sample, a biotinylated monoclonal	Specimen collection	and properatio	n	
55		ody, and a monoclonal ferritin-specific antibody nium complex.	•		tested and found acceptable.	
56		r addition of streptavidin-coated microparticles, the	Serum collected using		•	
57	complex becomes b	ound to the solid phase via interaction of biotin and	Li-, Na-heparin, K ₃ -EI		•	
58	streptavidin.		· · · · · · · · · · · · · · · · · · ·		Its must be corrected by + 10 %.	
59					f serum value or slope	
60					tical sensitivity (LDL) + coefficient of	
			Stable for 7 days at 2	-8 °C, 12 months	s at -20 °C. ⁸	

1/4 For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

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# **Ferritin**

### Ferritin

manufacturer.

## cobas®

### Calibration

Traceability: The Ferritin assay (REF) 03737551) has been standardized against the Ferritin assay (REF) 11820982). The Ferritin assay (REF) 11820982) has been standardized against the Enzymun-Test Ferritin method. This in turn has been standardized against the 1st International Standard (IS) NIBSC (National Institute for Biological Standards and Control) "Reagent for Ferritin (human liver)" 80/602.

Recovery studies, including a published study,⁹ to assess traceability of the Elecsys Ferritin assay to more recent international standards (2nd IS 80/578 and 3rd IS 94/572) have been conducted, with results showing very good agreement.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

*Calibration frequency:* Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

### **Quality control**

For quality control, use PreciControl Tumor Marker or PreciControl Varia. In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

### Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in  $\mu$ g/L or ng/mL).

### Limitations - interference

The assay is unaffected by icterus (bilirubin < 1112  $\mu$ mol/L or < 65 mg/dL), hemolysis (Hb < 0.31 mmol/L or < 0.5 g/dL), lipemia (Intralipid < 3300 mg/dL) and biotin (< 205 nmol/L or < 50 ng/mL).

Criterion: Recovery within ± 10 % of initial value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 2500 IU/mL.

There is no high-dose hook effect at ferritin concentrations up to 100000  $\mu$ g/L (ng/mL).

In vitro tests were performed on 19 commonly used pharmaceuticals. No interference with the assay was found.

Iron²⁺- and iron³⁺-ions at therapeutic concentrations do not interfere with the Elecsys Ferritin assay.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

### Limits and ranges

### Measuring range

0.500-2000 µg/L (ng/mL) (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.500 µg/L (ng/mL). Values above the measuring range are reported as > 2000 µg/L (ng/mL) (or up to 100000 µg/L (ng/mL) for 50-fold diluted samples).

	inutacturer.	(
	ntrifuge samples containing precipitates before performing the assay.	F
	not use heat-inactivated samples.	E
	not use samples and controls stabilized with azide.	() S
me	sure the samples, calibrators and controls are at 20-25 °C prior to asurement.	E it
the	e to possible evaporation effects, samples, calibrators and controls on analyzers should be analyzed/measured within 2 hours.	n (
	terials provided e "Reagents – working solutions" section for reagents.	l r
Ма	terials required (but not provided)	t
•	REF] 03737586190, Ferritin CalSet, 4 x 1 mL	
•	REF 11776452122, PreciControl Tumor Marker, for 2 x 3 mL each of PreciControl Tumor Marker 1 and 2 or REF 05618860190, PreciControl Varia, for 2 x 3 mL each of PreciControl Varia 1 and 2	•
		F
-	REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or	l
_	REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent	0
-	General laboratory equipment	f
Ac	Elecsys 2010, MODULAR ANALYTICS E170 or <b>cobas e</b> analyzer cessories for Elecsys 2010 and <b>cobas e</b> 411 analyzers:	۲ ir
•	REF 11662988122, ProCell, 6 x 380 mL system buffer	li
•	REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution	V F
•	REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive	C
•	REF 11933159001, Adapter for SysClean	(
•	REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels	ا ج
	REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips cessories for MODULAR ANALYTICS E170, <b>cobas e</b> 601 and <b>bas e</b> 602 analyzers:	ן ו ו
•	REF 04880340190, ProCell M, 2 x 2 L system buffer	י <
•	REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution	(
•	REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use	k a
•	[REF] 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change	N 0
•	REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags	1
•	REF 03023150001, WasteLiner, waste bags	i
•	REF 03027651001, SysClean Adapter M	ŀ
Ac	cessories for all analyzers:	E
•	REF 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution	 8
As	say	e
do	r optimum performance of the assay follow the directions given in this cument for the analyzer concerned. Refer to the appropriate operator's inual for analyzer-specific assay instructions.	f
Re Re exe	suspension of the microparticles takes place automatically prior to use. ad in the test-specific parameters via the reagent barcode. If in ceptional cases the barcode cannot be read, enter the 15-digit sequence numbers.	L N C
(20 reg	ng the cooled reagents to approx. 20 °C and place on the reagent disk 0 °C) of the analyzer. Avoid foam formation. The system automatically gulates the temperature of the reagents and the opening/closing of the ttles.	r r C

The sample types listed were tested with a selection of sample collection

available tubes of all manufacturers were tested. Sample collection systems

tubes that were commercially available at the time of testing, i.e. not all

from various manufacturers may contain differing materials which could

tubes (sample collection systems), follow the instructions of the tube

affect the test results in some cases. When processing samples in primary

# Ferriti

### Lower limits of measurement

Lower detection limit of the test

Lower detection limit: 0.50 µg/L (ng/mL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

### Dilution

Samples with ferritin concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:50 (either automatically by the MODULAR ANALYTICS E170, Elecsys 2010 or cobas e analyzers or manually). The concentration of the diluted sample must be > 40  $\mu$ g/L (ng/mL).

16 After manual dilution, multiply the result by the dilution factor.

17 After dilution by the analyzers, the MODULAR ANALYTICS E170, 18 Elecsys 2010 and cobas e software automatically takes the dilution into 19 account when calculating the sample concentration.

#### 20 Expected values

21 Results of a study with the Enzymun-Test Ferritin method on samples from 224 healthy test subjects (104 women - mainly premenopausal - and 22 120 men) are given below. The values correspond to the 5th and 95th percentiles.¹⁰ 23

24 Men, 20-60 years: 30-400 µg/L (ng/mL)

25 Women, 17-60 years: 13-150 µg/L (ng/mL)

26 Each laboratory should investigate the transferability of the expected values 27 to its own patient population and if necessary determine its own reference ranges. 28

### Specific performance data

Representative performance data on the analyzers are given below. 30 Results obtained in individual laboratories may differ.

### Precision

32 Precision was determined using Elecsys reagents, pooled human sera and 33 controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory 34 Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). 35 The following results were obtained:

		Intermedia sioi			
Sample	Mean µg/L (ng/mL)	SD µg/L (ng/mL)	CV %	SD µg/L (ng/mL)	CV %
Human serum 1	1.45	0.101	7.0	0.168	11.6
Human serum 2	11.9	0.411	3.5	0.798	6.7
Human serum 3	19.2	0.780	4.1	1.47	7.7
Human serum 4	376	10.8	2.9	17.2	4.6
Human serum 5	1361	26.5	1.9	84.4	6.2
PreciControl Varia 1	134	1.96	1.5	2.75	2.1
PreciControl Varia 2	858	15.1	1.8	21.7	2.5

### MODULAR ANALYTICS E170. cobas e 601 and cobas e 602 analyzers

	R	Repeatability			Intermediate precision	
Sample	Mean µg/L (ng/mL)	SD µg/L (ng/mL)	CV %	SD μg/L (ng/mL)	CV %	
Human serum 1	1.12	0.139	12.4	0.263	23.4	
Human serum 2	12.3	0.467	3.8	0.789	6.4	
Human serum 3	20.5	0.837	4.1	1.67	8.1	
Human serum 4	392	8.14	2.1	16.9	4.3	

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### MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers

	R	epeatabili	ty	Intermediat sior	-
Sample	Mean µg/L (ng/mL)	SD µg/L (ng/mL)	CV %	SD μg/L (ng/mL)	CV %
Human serum 5	1449	35.6	2.5	92.8	6.4
PreciControl Varia 1	140	2.31	1.7	3.53	2.5
PreciControl Varia 2	900	14.4	1.6	25.0	2.8

### Method comparison

A comparison of the Ferritin assay, REF 03737551 (y) with the Ferritin assay, REF 11820982 (x) using clinical samples gave the following correlations:

Number of samples measured: 134

Passing/Bablok ¹¹	Linear regression
y = 1.00x + 0.72	y = 0.99x + 4.11
т = 0.984	r = 0.999

The sample concentrations were between approximately 2.68 and 1891 µg/L (ng/mL).

### Analytical specificity

Human liver ferritin: 100 % recovery

Human spleen ferritin: 85 % recovery

Human heart ferritin: 1 % recovery

### References

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

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### Symbols Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
$\rightarrow$	Volume after reconstitution or mixing

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Roche

# Elecsys Folate RBC

cobas
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### English

### System information

For cobas e 411 analyzer: test number 1210

For cobas e 601 and cobas e 602 analyzers: Application Code

### Number 272

Intended use This assay is used for the in vitro quantitative determination of folate in erythrocytes (red blood cells, RBC). The electrochemiluminescence binding assay is intended for use on the Elecsys and **cobas e** immunoassay

### analyzers. Summary

Summary
 Folate belongs to the family of B-group vitamins composed of an aromatic pteridine ring linked through a methylene group to p-aminobenzoic acid and a glutamate residue. Folate (folic acid) is vital for normal cellular functions and plays an essential role in nucleic acid synthesis, methionine regeneration, shuttling and redox reactions of one-carbon units required for normal metabolism and regulation.^{1,2}
 The folate metabolism can be exemplified as a cycle, where folate facilitates the transfer of one-carbon units from one molecule to another

required in various biochemical reactions: for example, tetrahydrofolate
(THF) accepts a single carbon unit from serine, which is reduced in a
number of steps to 5-methyltetrahydrofolate (5-MTHF). 5-MTHF gives its
methyl group to homocysteine, which is - with involvement of methionine
synthase and vitamin B12 - enzymatically converted to methionine. The
resulting THF starts again the cycle of methyl group synthesis. From
(SAM).³ SAM serves as a methyl group donor in several methylation
reactions, like DNA, RNA and protein methylation.¹

The methionine cycle is highly sensitive to folate deficiency: with a low folate status, the ability of the cell to re-methylate homocysteine is impaired and this results in increased homocysteine concentrations in plasma.²

Folate also plays an essential role in the synthesis of purine and pyrimidine
precursors of nucleic acids. Altered distribution of methyl groups and
impaired DNA synthesis play an essential role in the development of
cancers. Abnormal folate status has also been linked with the development
of diseases like cardiovascular diseases, neural tube defects, cleft lip and
palate, late pregnancy complications, neurodegenerative and psychiatric
disorders.^{1,2}

Folate belongs to the group of essential vitamins, i.e. it cannot be
synthesized by the human organism and therefore must be absorbed from
diet. Primary sources of folates are green and leafy vegetables, sprouts,
fruits, brewer's yeast and liver.^{1,2}

- Folate deficiency can be caused by decreased nutritional intake, poor absorption of ingested folate in the intestine or increased demand of folate, for example during physical activity or pregnancy. Deficiency of folate can also be a result of liver diseases or impaired folate metabolism due to genetic defects or drug interactions.²
- A clinical manifestation of both folate and vitamin B12 deficiency is the so called megaloblastic (macrocytic) anemia: due to the affected DNA synthesis and cell maturation, especially involving the cells of
- synthesis and cen maturation, especially involving the cens of erythropoiesis, the total count of erythrocytes is significantly reduced. The hemoglobin synthesis capacity however is normal, which leads to abnormally large erythrocyte precursors ("macrocytes" or "megaloblasts"),
- 54 Serum folate concentrations may be affected by recent folate intakes,
- Setum totate concentrations may be anected by recent totate intakes, whereas red blood cell (RBC) folate is a measure of the folate intake across the 90-120 days lifespan of erythrocytes. Thus, folate concentrations in RBC give a more accurate picture of a patient's underlying folate status than serum folate concentrations, and are considered by experts as the better measure for folate status.⁵
- Because vitamin B12 and folate are closely interrelated in the cellular
   one-carbon unit metabolism, and also hematologic and clinical consequences of the two vitamin deficiency states might be similar, it is

advisable to determine both parameters simultaneously in patients with the relevant symptoms of vitamin deficiency.  $^{\!\!\!3,4}$ 

### Test principle

Competition principle. Total duration of assay: 27 minutes.

Whole blood treated with anticoagulants (heparin or EDTA) is mixed with ascorbic acid solution and incubated for approximately 90 minutes at 20-25 °C. Lysis of the erythrocytes takes place, with liberation and stabilization of the intracellular folate. The resulting hemolysate sample is then used for subsequent measurement.

- 1st incubation: By incubating 25 µL of hemolysate sample with the folate pretreatment reagents 1 and 2, bound folate is released from endogenous folate binding proteins.
- 2nd incubation: By incubating the pretreated sample with the ruthenium labeled folate binding protein, a folate complex is formed, the amount of which is dependent upon the analyte concentration in the sample.
- 3rd incubation: After addition of streptavidin-coated microparticles and folate labeled with biotin, the unbound sites of the ruthenium labeled folate binding protein become occupied, with formation of a ruthenium labeled folate binding protein-folate biotin complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrumentspecifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

### Reagents - working solutions

The reagent rackpack (M, R1, R2) is labeled as RBC-FOL.

- PT1 Pretreatment reagent 1 (white cap), 1 bottle, 4 mL: Sodium 2-mercaptoethanesulfonate (MESNA) 40 g/L, pH 5.5.
- PT2 Pretreatment reagent 2 (gray cap), 1 bottle, 5 mL: Sodium hydroxide 25 g/L.
- M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Folate binding protein~Ru(bpy)₃²⁺ (gray cap), 1 bottle, 9 mL: Ruthenium labeled folate binding protein 75 μg/L; human serum albumin (stabilizer); borate/phosphate/citrate buffer 70 mmol/L, pH 5.5; preservative.
- R2 Folate~biotin (black cap), 1 bottle, 8 mL:

Biotinylated folate 17  $\mu$ g/L; biotin 120  $\mu$ g/L; human serum albumin (stabilizer); borate buffer 100 mmol/L, pH 9.0; preservative.

### Precautions and warnings

### For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

2-methyl-2H-isothiazol-3-one hydrochloride

EUH 208 May produce an allergic reaction.

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# Elecsys Folate RBC



- For the determination of folate in RBC: Determine hematocrit in whole blood samples and record the value.
- Preparation of the hemolysate sample

Mix 3.0 mL of Folate RBC Hemolyzing Reagent (ascorbic acid solution, 0.2 %) and 100  $\mu L$  of well-mixed whole blood, avoiding foam formation. Incubate with closed caps for 90  $\pm$  15 minutes at 20-25 °C.

### Stability:

Whole blood: 2 hours at 20-25 °C8, 24 hours at 2-8 °C, 1 month at -20 °C (± 5 °C) (only EDTA-blood).

Hemolysate sample: 1 month at -20 °C (± 5 °C), freeze only once. The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all

available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Samples should not subsequently be altered with additives (biocides, anti-oxidants or substances possibly changing the pH of the sample) in order to avoid erroneous folate recovery.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

If measurements cannot be carried out within 2 hours please store the hemolysate sample at -20 °C (± 5 °C).

### Materials provided

See "Reagents - working solutions" section for reagents.

### Materials required (but not provided)

- REF 05944309190, Folate RBC CalSet, for 4 x 1.0 mL
- REF 05944317190, Folate RBC Hemolyzing Reagent kit for 4 x 200 mL, contains ascorbic acid
- General laboratory equipment

cobas e analvzer

Additional materials for the **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- [REF] 11706799001, AssayTip, 30 x 120 pipette tips
- [REF] 11800507001, Clean-Liner

Additional materials for cobas e 601 and cobas e 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REFI 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M
- Additional materials for all analyzers:
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution
- H290 May be corrosive to metals. H314 Causes severe skin burns and eye damage. Prevention. P280 Wear protective gloves/ protective clothing/ eye protection/ face protection. **Response:** P301 + P330 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. + P331 P303 + P361 IF ON SKIN (or hair): Take off immediately all contaminated + P353 clothing. Rinse skin with water. P304 + P340 IF INHALED: Remove person to fresh air and keep + P310 comfortable for breathing. Immediately call a POISON CENTER/ doctor. P305 + P351 IF IN EYES: Rinse cautiously with water for several + P338 minutes. Remove contact lenses, if present and easy to do. + P310 Continue rinsing. Immediately call a POISON CENTER/ doctor. P390 Absorb spillage to prevent material damage. Product safety labeling follows EU GHS guidance. Contact phone: all countries: +49-621-7590 All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.^{6,7} Avoid foam formation in all reagents and sample types (specimens, calibrators and controls). **Reagent handling** The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated. All information required for correct operation is read in from the respective 47 reagent barcodes. 48 Storage and stability 49 Store at 2-8 °C. 50 Do not freeze. 51 Store the Elecsys reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use. 52 53 Stability: 54 unopened at 2-8 °C up to the stated expiration date 55 after opening at 2-8 °C 8 weeks 56 on the analyzers 2 weeks 57 58 Specimen collection and preparation Only the specimens listed below were tested and found acceptable.
- 59 Hemolysate prepared from whole blood treated with anticoagulants 60 (Na-heparin or K₃-EDTA).

# Elecsys Folate RBC

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### Assay

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The well-mixed hemolysate sample is placed in the sample zone of the analyzer and recorded by entering the sample identification data. Complete determinations on the analyzer within 2 hours after finalizing the preparation of the hemolysate sample.

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in

exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

cobas e 601 and cobas e 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

### Calibration

Traceability: This method has been standardized against the Elecsys Folate III assay (REF 04476433190)/RBC application.

23 The standardization of the Elecsys Folate RBC assay includes the volume correction to account for the preparation of hemolysate sample (1:31 vol/vol).

25 Every Elecsys reagent set has a barcoded label containing specific 26 information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot 28 using fresh reagent (i.e. not more than 24 hours since the reagent kit was 29 registered on the analyzer). 30

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

### Quality control

For quality control, use commercially available whole blood control material.

38 Controls for the various concentration ranges should be run individually at 39 least once every 24 hours when the test is in use, once per reagent kit, and following each calibration. 40

41 The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined 42 limits. Each laboratory should establish corrective measures to be taken if 43 values fall outside the defined limits.

44 If necessary, repeat the measurement of the samples concerned. 45

Follow the applicable government regulations and local guidelines for quality control.

### Calculation

### 1. Whole blood folate (from hemolysate sample)

The standardization of the Elecsys Folate RBC assay includes the volume correction to account for the preparation of hemolysate sample (1:31 vol/vol).

The analyzer automatically calculates the analyte concentration of each sample in nmol/L or ng/mL.

53	·····	
54	Conversion factors:	nmol/L x 0.44 = ng/mL
55		ng/mL x 2.27 = nmol/L
56	2. RBC folate	
57	To calculate the folate concentration	
58	sample (RBC folate), the predeter	mined sample specific hematocrit value
59	must be taken into account using t	ne following equation:
60	BBC foloto - ana	lyzer result v 100

	analyzor robuit	100	
RBC folate =	% hematocrit	× 100	

#### Limitations - interference

The assay is unaffected by icterus (bilirubin < 564 µmol/L or < 33 mg/dL) lipemia (Intralipid < 1500 mg/dL), biotin (< 86.1 nmol/L or < 21 ng/mL), IgG < 16 g/L and IgA < 4.0 g/L.

Criterion: Recovery within ± 10 % of initial value with samples > 155 ng/mL and  $\leq \pm 15.5$  ng/mL with samples  $\leq 155$  ng/mL.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1000 IU/mL.

In vitro tests were performed on 16 commonly used pharmaceuticals and in addition on human erythropoietin. No interference with the assay was found.

It is contraindicated to measure samples of patients receiving therapy with certain pharmaceuticals, e.g. methotrexate or leucovorin, because of the cross-reactivity of folate binding protein with these compounds.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

In rare cases, samples with low erythrocyte folate concentration, but high serum folate concentration can occur. In these cases, a correction of the folate concentration in erythrocytes by the serum folate concentration with the following equation is recommended*:

* expected values can be used as an indicator for high serum folate concentration

Corrected RBC folate concentration =

	, serum folate		100 - % hematocrit	
-	( concentration	Х	% hematocrit	)

concentration Example

**BBC** folate

RBC folate concentration: 241 (ng/mL RBC);

serum folate concentration: 10.5 (ng/mL S);

hematocrit measured (%) = 45

Corrected RBC folate concentration =

241 ng/mL RBC	- (10.5 ng/r	mLS x	<u>100 - 45</u> 45	) = 228 ng/mL RBC
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### Limits and ranges

### Measuring range

120-620 ng/mL or 272-1407 nmol/L (defined by the Limit of Quantitation and the maximum of the master curve). Values below the Limit of Quantitation are reported as < 120 ng/mL (< 272 nmol/L). Values above the measuring range are reported as > 620 ng/mL (> 1407 nmol/L). Values are not corrected for the sample hematocrit.

### Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation:

Limit of Blank = 20.0 ng/mL

Limit of Detection = 46.5 ng/mL

Limit of Quantitation = 120 ng/mL

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative

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# Elecsys Folate RBC

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error of  $\leq 30$  %. It has been determined using low concentration folate samples.

### Dilution

Hemolysate samples with folate concentrations above the measuring range can be diluted manually with Elecsys Folate RBC Hemolyzing Reagent (ascorbic acid solution, 0.2 %). The recommended dilution is 1:2. The concentration of the diluted sample must be > 265 ng/mL or > 602 nmol/L. After manual dilution, multiply the results by the dilution factor 2.

### Expected values

The values shown below were measured on samples from an apparently healthy population, using the Elecsys Folate III/RBC application. The values can be applied for the Elecsys Folate RBC assay on all Elecsys and **cobas e** analyzers. The calculation is based on 290 sera (96 men,

194 women) from an European population. The age range was between 18 and 65 years. Pregnant or lactating women were excluded. The reference population was selected according to normal homocysteine values. The following values were obtained:

Whole blood folate (from hemolysate samples)							
	Ν	Mee	dian	2.5 th -97.5 th percentile			
		nmol/L	ng/mL	nmol/L	ng/mL		
Europe	290	673	296	481-1212	212-534		

The measured hematocrit value in this study showed a range from 37.1-46.1 %.

RBC folate (folate in erythrocyte fraction)								
	N	Med	dian	2.5 th -97.5 th	percentile			
		nmol/L ng/mL		nmol/L	ng/mL			
Europe	290	1657	730	1187-2854	523-1257			

If pathologically low hematocrit values are considered for calculation of RBC folate in the erythrocyte fraction, elevated RBC folate concentrations may be observed. No medical conclusion should be based on the calculation considering hematocrit values in such cases. Instead, whole blood folate results (from hemolysate samples) and suitable expected values may be used.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

### Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

### Precision

Precision was determined using Elecsys reagents and hemolysate samples in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). Results are given as whole blood folate (from hemolysate sample). The following results were obtained:

cobas e 411 analyzer								
		Repeatability				ermediat recision	е	
Sample	Mean		SD		CV	S	D	CV
	nmol/L	ng/mL	nmol/L	ng/mL	%	nmol/L	ng/mL	%
HL ^{a)} 1	154	68.0	11.7	5.17	7.6	21.9	9.65	14.2
HL 2	352	155	17.5	7.73	5.0	27.7	12.2	7.9
HL 3	618	272	25.4	11.2	4.1	38.4	16.9	6.2
HL 4	1195	527	38.8	17.1	3.3	56.3	24.8	4.7

a) HL = Hemolysate

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cobas e 601 and cobas e 602 analyzers								
			Repeatability				ermediat recision	e
Sample	Mean		SD CV		CV	S	D	CV
	nmol/L	ng/mL	nmol/L	ng/mL	%	nmol/L	ng/mL	%
HL 1	138	61.0	12.1	5.31	8.8	14.3	6.32	10.4
HL 2	434	191	26.1	11.5	6.0	28.4	12.5	6.5
HL 3	586	258	32.0	14.1	5.5	34.3	15.1	5.9
HL 4	1317	580	29.1	12.8	2.2	44.7	19.7	3.4

### Method comparison

a) A comparison of the Elecsys Folate RBC assay (calibrated with Folate RBC CalSet; y) and the Elecsys Folate III/RBC application (calibrated with Folate III CalSet; x) using hemolyzed clinical samples gave the following correlations (ng/mL). Results are given as whole blood folate (from hemolysate sample).

Number of samples measured: 187

Passing/Bablok9	Linear regression
y = 1.02x - 14.1	y = 1.00x - 12.0
т = 0.869	r = 0.985

The sample concentrations were between 151 and 551 ng/mL (343 and 1251 nmol/L).

b) A comparison of the Elecsys Folate RBC assay on the MODULAR ANALYTICS E170 analyzer (y) with the Elecsys Folate RBC assay on the Elecsys 2010 analyzer (x) (both tests have been calibrated with Folate RBC CalSet) using hemolyzed clinical samples gave the following correlations (ng/mL). Results are given as whole blood folate (from hemolysate sample).

Number of samples measured: 187

Passing/Bablok	Linear regression
y = 1.04x + 1.94	y = 1.02x + 8.07
т = 0.814	r = 0.970

The sample concentrations were between 137 and 557 ng/mL (311 and 1264 nmol/L).

### References

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

### Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
$\rightarrow$	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

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### CE

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### 0004628918190c501V12.0 CRPł

Cardiac C-Reactive Protein (Latex) High Sensitive

Order information

REF	CONTENT		Analyzer(s) on which <b>cobas c</b> pack(s) can be used
<b>04628918</b> 190	Cardiac C-Reactive Protein (Latex) High Sensitive (300 tests)	System-ID 07 6866 9	Roche/Hitachi cobas c 311, cobas c 501/502
11355279 216	Calibrator f.a.s. Proteins (5 x 1 mL)	Code 656	
11355279 160	Calibrator f.a.s. Proteins (5 x 1 mL, for USA)	Code 656	
<b>20766321</b> 322	CRP T Control N (5 x 0.5 mL)	Code 235	
10557897 122	Precinorm Protein (3 x 1 mL)	Code 302	
<b>10557897</b> 160	Precinorm Protein (3 x 1 mL, for USA)	Code 302	
<b>05117003</b> 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
<b>05947626</b> 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
<b>05947626</b> 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391	
<b>04489357</b> 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

### English

#### 20 System information 21

For cobas c 311/501 analyzers: 22

23 CRPHS: ACN 217

For cobas c 502 analyzer: 24

**CRPHS: ACN 8217** 25

### Intended use

In vitro test for the quantitative determination of C-reactive protein (CRP) in 27 human serum and plasma on Roche/Hitachi cobas c systems. Measurement of CRP is of use for the detection and evaluation of 28 29 inflammatory disorders and associated diseases, infection and tissue injury. Highly sensitive measurement of CRP may also be used as an aid in the 30 assessment of the risk of future coronary heart disease. When used as an 31 adjunct to other laboratory evaluation methods of acute coronary 32 syndromes, it may also be an additional independent indicator of recurrent 33 event prognosis in patients with stable coronary disease or acute coronary syndrome. 34

### Summary1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21

35 C-reactive protein is the classic acute phase protein in inflammatory 36 reactions. It is synthesized by the liver and consists of five identical 37 polypeptide chains that form a five-member ring having a molecular weight of 105000 daltons. CRP is the most sensitive of the acute phase reactants 38 and its concentration increases rapidly during inflammatory processes. 39 Complexed CRP activates the complement system beginning with C1q. CRP then initiates opsonization and phagocytosis of invading cells, but its 40 main function is to bind and detoxify endogenous toxic substances 41 produced as a result of tissue damage. 42

CRP assays are used to detect systemic inflammatory processes (apart 43 from certain types of inflammation such as SLE and Colitis ulcerosa); to assess treatment of bacterial infections with antibiotics; to detect 44 intrauterine infections with concomitant premature amniorrhexis; to 45 differentiate between active and inactive forms of disease with concurrent 46 infection, e.g. in patients suffering from SLE or Colitis ulcerosa; to therapeutically monitor rheumatic disease and assess anti-inflammatory therapy; to determine the presence of post-operative complications at an early stage, such as infected wounds, thrombosis and pneumonia, and to 47 48 49 distinguish between infection and bone marrow transplant rejection.

50 Sensitive CRP measurements have been used and discussed for early 51 detection of infection in pediatrics and risk assessment of coronary heart disease. Several studies came to the conclusion that the highly sensitive 52 measurement of CRP could be used as a marker to predict the risk of 53 coronary heart disease in apparently healthy persons and as an indicator of recurrent event prognosis. Increases in CRP values are non-specific and should not be interpreted without a complete clinical history. The American Heart Association and the Centers for Disease Control and Prevention have 54 55 56 made several recommendations concerning the use of high sensitivity C-Reactive Protein (hsCRP) in cardiovascular risk assessment.²¹ Testing for any risk assessment should not be performed while there is an indication of infection, systemic inflammation or trauma. Patients with persistently unexplained hsCRP levels above 10 mg/L (95.2 nmol/L) should 57 58 59 60 be evaluated for non-cardiovascular etiologies. When using hsCRP to assess the risk of coronary heart disease, measurements should be made on metabolically stable patients and compared to previous values.

Optimally, the average of hsCRP results repeated two weeks apart should be used for risk assessment. Screening the entire adult population for hsCRP is not recommended, and hsCRP is not a substitute for traditional cardiovascular risk factors. Acute coronary syndrome management should not depend solely on hsCRP measurements. Similarly, application of secondary prevention measures should be based on global risk assessment and not solely on hsCRP measurements. Serial measurements of hsCRP should not be used to monitor treatment.

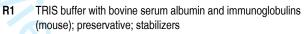
Various assay methods are available for CRP determination, such as nephelometry and turbidimetry. The Roche CRP assay is based on the principle of particle-enhanced immunological agglutination.

### Test principle^{22,23}

Particle enhanced immunoturbidimetric assay.

Human CRP applutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically.

### **Reagents - working solutions**



- R2 Latex particles coated with anti-CRP (mouse) in glycine buffer; preservative; stabilizers
- R1 is in position B and R2 is in position C.

### **Precautions and warnings**

### For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

### Reagent handling

Ready for use

Mix **cobas c** pack well before placing on the analyzer.

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

### Storage and stability

CRPHS	
Shelf life at 2-8 °C:	See expiration date on <b>cobas c</b> pack label.
On-board in use and refrigerated on the analyzer:	12 weeks
Diluent NaCl 9 %	
Shelf life at 2-8 °C:	See expiration date on <b>cobas c</b> pack label.

On-board in use and refrigerated on the analyzer: 12 weeks 0004628918190c501V12.0

## cobas®

3	Cardiac C-Reactive Protein	(Latex) High	Sensitive					
4								
5 6	Specimen collection and pr				Reaction direction	Increase		
7	For specimen collection and p collection containers.	preparation on	ly use suitable t	ubes or	Units	mg/L (nmol/	'L, mg/dL)	
8	Only the specimens listed bel	low were teste	d and found acc	ceptable.	Reagent pipetting		Diluent (H ₂ C	D)
9	Serum.				R1	82 µL	42 µL	
10	Plasma: Li-heparin and K ₂ -EI The sample types listed were	•	coloction of com	anle collection	R2	28 µL	20 µL	
11	tubes that were commercially	available at th	time of testing	g, i.e. not all				
12	available tubes of all manufac	cturers were te	sted. Sample co	ollection systems	Sample volumes	Sample	Sam	ple dilution
13	from various manufacturers n affect the test results in some	cases. When	processing sam	nples in primary			Sample	Diluent (NaCl)
14	tubes (sample collection syste	ems), follow th	e instructions of	f the tube	Normal	6 µL	-	_
15	manufacturer. Centrifuge samples containin	a proginitatos l	boforo porformi	ng the accev	Decreased	6 µL	10 µL	140 µL
16 17	See the limitations and interfe	• · ·	-		Increased	ομL		-
18	sample interferences.				morodood	ο μ <b>=</b>		
19	Sample stability claims were	established by	experimental d	ata by the	cobas c 502 test defini	tion		
20	manufacturer or based on ref temperatures/time frames as	stated in the n	re and only for t nethod sheet. It	the is the	Assay type	Rate A		
21	responsibility of the individual	l laboratory to	use all available	e references	Reaction time / Assay po	pints 10/12-70		
22	and/or its own studies to dete laboratory.	ermine specific	stability criteria	tor its	Wavelength (sub/main)	– /546 nm		
23					Reaction direction	Increase		
24	Stability: ²⁴		ys at 15-25 °C		Units	mg/L (nmol/	'L, mg/dL)	
25			nths at 2-8 °C		Reagent pipetting		Diluent (H ₂ C	D)
26 27		3 yea	rs at (-15)-(-25)	°C	R1	82 µL	42 µL	
27 28	Materials provided				R2	28 µL	20 μL	
28 29	See "Reagents - working solu	utions" section	for reagents.			·		
30	Materials required (but not	provided)			Sample volumes	Sample	Sam	ple dilution
31	<ul> <li>See "Order information" set</li> </ul>	ection					Sample	Diluent (NaCl)
32	<ul> <li>General laboratory equips</li> </ul>	nent			Normal	6 µL	–	_
33	Assay				Decreased	6 μL	10 µL	140 µL
34	For optimum performance of document for the analyzer co	ncerned. Refe	r to the appropri	ate operator's	Increased	0 μ∟ 12 μL	- το μ <b>ε</b>	
35	manual for analyzer-specific a	assay instruction	ons.			12 με		
36 37	The performance of application and must be defined by the u		ed by Roche is	not warranted	Calibration			
38	Application for serum and p				Calibrators	S1: H ₂ O		
39		Jiasilia				S2: C.f.a.s. Prote	ins	
40	cobas c 311 test definition	<b>D</b>				Multiply the lot-sp		
41	Assay type	Rate A				value by the factor standard concent		
42	Reaction time / Assay points	10/7-57				curve:		
43	Wavelength (sub/main)	– /546 nm				S2: 0.0125	S5: 0.1	00
44 45	Reaction direction	Increase				S3: 0.0250	S6: 0.2	
45 46	Units	mg/L (nmol/L	., mg/dL)			S4: 0.0500	00.0.2	
47	Reagent pipetting		Diluent (H ₂ O)		Calibration mode	Line Graph		
48	R1	82 µL	42 µL		Calibration frequency	Full calibration		
49	R2	28 µL	20 µL		Calibration nequency	after reagent lot	change	
50						• as required follo	-	ntrol procedures
51	Sample volumes	Sample	Sampl	e dilution	Calibration interval may	be extended base	d on acceptable	e verification of
52			Sample	Diluent (NaCl)	calibration by the labora	•		
53	Normal	6 µL	-	_	Traceability: This methor preparation of the IRMM	d has been standa (Institute for Refe	rdized against rence Materials	the reference
54 55	Decreased	6 μL	10 µL	140 μL	Measurements) BCR470	)/CRM470 (RPPH	S - Reference I	Preparation for
55 56	Increased	6 μL	-	_	Proteins in Human Seru	m). ²⁵		
57					Quality control	option material-	- liotod != 11 - "4	
58	cobas c 501 test definition				For quality control, use on section.	control materials as	s listed in the "(	ruer information"
59	Assay type	Rate A			In addition, other suitable	e control material	can be used.	
60	Reaction time / Assay points	10/12-70			The control intervals and			
	Wavelength (sub/main)	– /546 nm			individual requirements.	values obtained s	nould fall withir	n the defined

1		CRPHS	
2 3			
4		Cardiac C-Reactive Protein (Latex) High Sensitive	
5 6		limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.	Expected values Consensus referenc
7 8		Follow the applicable government regulations and local guidelines for quality control.	IFCC/CRM 470
9		Calculation	mg/dL
10		Roche/Hitachi <b>cobas c</b> systems automatically calculate the analyte concentration of each sample.	< 0.5
11			The CDC/AHA recor for CVD risk assess
12 13		Conversion factors: $mg/L \ge 9.52 = nmol/L$	
14		$mg/L \ge 0.1 = mg/dL$	hsCRP level (mg/L)
15		Limitations - interference	< 1.0
16	I	Criterion: Recovery within $\pm$ 10 % of initial values at CRP levels of 1.0 mg/L. Icterus: ²⁶ No significant interference up to an I index of 60 for conjugated	1.0-3.0
17		bilirubin and unconjugated bilirubin (approximate conjugated and	> 3.0
18		unconjugated bilirubin concentration: 60 mg/dL or 1026 µmol/L).	Patients with higher myocardial infarctior
19 20		Hemolysis: ²⁶ No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 622 µmol/L or 1000 mg/dL).	5-95 % reference int
20		Lipemia (Intralipid): ²⁶ No significant interference up to an L index of 600.	Neonates (0-3 week
22		There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.	Children (2 months-
23		Rheumatoid factors: No significant interference from rheumatoid factors up	It is important to m phase of the illness
24		to a concentration of 1200 IU/mL.	Roche has not evalu
25		Drugs: No interference was found at therapeutic concentrations using common drug panels. ^{27,28}	Each laboratory sho
26 27		Therapeutic drugs: Significantly decreased CRP values may be obtained	to its own patient po ranges.
28		from samples taken from patients who have been treated with carboxypenicillins.	Increases in CRP va
29		High dose hook-effect: No false result occurs up to a CRP concentration of	without a complete of
30		1000 mg/L.	When using hsCRP measurements shou
31		In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results. ²⁹	compared to previou repeated two weeks
32 33		Although measures were taken to minimize interference caused by human	Measurements shou
34		anti-mouse antibodies, erroneous findings may be obtained from samples taken from patients who have been treated with monoclonal mouse	<ul> <li>are being used for rish</li> <li>hsCRP levels of above</li> </ul>
35		antibodies or have received them for diagnostic purposes.	non-cardiovascular of
36		For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other	performed while thei trauma. ²¹
37		findings.	Specific performan
38 39			Representative perfe
40		Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi	Results obtained in i
41		cobas c systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further	Precision was deterr
42		instructions refer to the operator's manual. cobas c 502 analyzer: All	protocol with repeate
43		special wash programming necessary for avoiding carry-over is available via the <b>cobas</b> link, manual input is required in certain cases.	per run, 1 run per da
44 45		Where required, special wash/carry-over evasion programming must	Repeatability
46		be implemented prior to reporting results with this test.	
47		Limits and ranges	Precinorm Protein
48		Measuring range 0.15-20.0 mg/L (1.43-190 nmol/L, 0.015-2.0 mg/dL)	CRP T Control N
49		Determine samples having higher concentrations via the rerun function.	Human serum 1
50 51		Dilution of samples via the rerun function is a 1:15 dilution. Results from	Human serum 2
52		samples diluted using the rerun function are automatically multiplied by a factor of 15.	Intermediate
53		Lower limits of measurement	precision
54		Lower detection limit of the test	Precinorm Protein
55		0.15 mg/L (1.43 nmol/L, 0.015 mg/dL)	CRP T Control N
56		The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying	Human serum 3
57 58		3 standard deviations above that of the lowest standard (standard $1 + 3$ SD, repeatability, n = 21).	Human serum 4
58 59		Functional sensitivity	Method compariso
60		0.3 mg/L (2.96 nmol/L, 0.03 mg/dL)	CRP values for hum
		The functional sensitivity is the lowest CRP concentration that can be	Roche/Hitachi coba
		reproducibly measured with an inter-assay coefficient of variation of < 10 $\%$ .	

## Cohas®

Consensus reference interval for adults: ³⁰	Consensus	reference	interval	for	adults:30
--------------------------------------------------------	-----------	-----------	----------	-----	-----------

mg/dL	mg/L	nmol/L
< 0.5	< 5.0	< 47.6

ommended the following hsCRP cut-off points (tertiles) sment:21,31

hsCRP level (mg/L)	hsCRP level (nmol/L)	Relative risk
< 1.0	< 9.52	low
1.0-3.0	9.52-28.6	average
> 3.0	> 28.6	high

r hsCRP concentrations are more likely to develop on and severe peripheral vascular disease. tervals of neonates and children:32

ks): 0.1-4.1 mg/L (0.95-39.0 nmol/L)

-15 years): 0.1-2.8 mg/L (0.95-26.7 nmol/L)

### nonitor the CRP concentration during the acute SS.

luated reference ranges in a pediatric population. ould investigate the transferability of the expected values opulation and if necessary determine its own reference

alues are non-specific and should not be interpreted clinical history.

to assess the risk of coronary heart disease, uld be made on metabolically stable patients and build be made on metabolically stable patients and bus values. Optimally, the average of hsCRP results is apart should be used for risk assessment. build be compared to previous values. When the results risk assessment, patients with persistently unexplained bove 10 mg/L (95.2 nmol/L) should be evaluated for r origins. Testing for any risk assessment should not be ere is indication of infection, systemic inflammation or

### nce data

formance data on the analyzers are given below. individual laboratories may differ.

rmined using human samples and controls in an internal tability (n = 21) and intermediate precision (3 aliquots ay, 21 days). The following results were obtained:

Repeatability	Mean	SD	CV
	mg/L (nmol/L, mg/dL)	mg/L (nmol/L, mg/dL)	%
Precinorm Protein	9.00 (85.7, 0.900)	0.10 (1.0, 0.010)	1.2
CRP T Control N	4.34 (41.3, 0.434)	0.04 (0.4, 0.004)	1.0
Human serum 1	15.9 (151, 1.59)	0.1 (1, 0.01)	0.4
Human serum 2	0.54 (5.14, 0.054)	0.01 (0.10, 0.001)	1.6
Intermediate	Mean	SD	CV
Intermediate precision	Mean mg/L (nmol/L, mg/dL)	SD mg/L (nmol/L, mg/dL)	CV %
precision	mg/L (nmol/L, mg/dL)	mg/L (nmol/L, mg/dL)	%
precision Precinorm Protein	mg/L (nmol/L, mg/dL) 9.06 (86.3, 0.906)	mg/L (nmol/L, mg/dL) 0.11 (1.1, 0.011)	% 1.3

### on

nan serum and plasma samples obtained on a as c 501 analyzer (y) were compared with those

### 0004628918190c501V12 0 Cardiac C-Reactive Protein (Latex) High Sensitive

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53 54

	lyzer (x). nple size (n) = 192	
Pas	sing/Bablok ³³	Linear regression
y =	0.992x + 0.254 mg/L	y = 0.946x + 0.514 mg/L
т =	0.944	r = 0.996
	e sample concentrations were 7 mg/L (4.76 and 188 nmol/L,	
	erences	
1	Methods. Vol II. Philadelphia	
2	Pathobiochemie, 3rd ed. Stu 1995:234-236.	s. Lehrbuch der Klinischen Chemie un ttgart/New York: Schattauer Verlag
3	Thomas L, Messenger M. Pa Entzündung. Lab med 1993;	thobiochemie und Labordiagnostik de 17:179–194.
4	Pathology 1991;23:118-124.	AW. C-reactive protein: A critical revie
5	WB Saunders Co; 1995.	aboratory Tests. 3rd ed. Philadelphia,
6	Wasunna A, Whitelaw A, Ga bacterial infection in preterm Mar;149(6):424-427.	llimore R, et al. C-reactive protein and infants. Eur J Pediatr 1990
7		nore JR, et al. The prognostic value o myloid A protein in severe unstable 331:417-424.
8	Kuller LH, Tracy RP, Shaten coronary heart disease in the Epidem 1996;144:537-547.	J, et al. Relation of c-reactive protein MRFIT nested case control study. Ar
9	to the Predictive Value of To	ekens CH, et al. C-Reactive Protein Ac tal and HDL Cholesterol in Determinin ction. Circulation 1998;97:2007-2011.
10		mpfer MJ, et al. Plasma Concentratior of Developing Peripheral Vascular :425-428.
11		mpfer MJ, et al. Inflammation, Aspirin, isease in Apparently Healthy Men. N I
12	Danesh J, Wheeler JG, Hirso	chfield GM, et al. C-Reactive Protein a Inflammation in the Prediction of Corc 2004;350(14):1387-1397.
13	Other Markers of Inflammatic	Buring JE, et al. C-Reactive Protein ar on in the Prediction of Cardiovascular Med 2000;342(12):836-843.
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15	Reactive Protein Levels and	Hakanen M, et al. Elevated Serum C- Early Arterial Changes in Healthy o Vasc Biol, (August) 2002;1323-1328
16		S. Increased C-Reactive Protein Level in Patients with Stable Coronary Arter urnal 2003;145 (2):248-253.
17		zitzoglou E, et al. C-Reactive Protein aphic Characteristics of Coronary Lesi 86.
18		H, et al. C-Reactive Protein and Ische Blockers and Statins. Circulation
19	Reactive Protein Within 14 D	/eil KM, et al. Simvastatin Lowers C- ays. An Effect Independent of Low-De uction. Circulation 2002;106:1447-145.

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

### Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

CONTENT

Contents of kit

Volume after reconstitution or mixing

GTIN

Global Trade Item Number

### 0004628918190c501V12.0

Cardiac C-Reactive Protein (Latex) High Sensitive

#### FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL

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### CE

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Roche Diagnostics, Indianapolis, IN US Customer Technical Support 1-800-428-2336 Roche

### cobas®

# Elecsys Folate III

cobas®
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REF		$\Sigma$	SYSTEM
		V	
07559992190	07559992500	100	<b>cobas e</b> 601
01000002100		100	<b>cobas e</b> 602
English		<ul> <li>1st incubation: By i</li> </ul>	ncubating 25 $\mu$ L of sample with the folate
System information		pretreatment reage	nts 1 and 2, bound folate is released from
For cobas e 411 analyzer: test		endogenous folate	
Number 721	602 analyzers: Application Code	labeled folate bindi	incubating the pretreated sample with the ruth ng protein, a folate complex is formed, the arr t upon the analyte concentration in the sample
Intended use Binding assay for the in vitro g	uantitative determination of folate in human		r addition of streptavidin-coated microparticle
serum and plasma.			biotin, the unbound sites of the ruthenium labe in become occupied, with formation of a ruthe
	for use on Elecsys and <b>cobas e</b>	labeled folate bindi	ng protein-folate biotin complex. The entire co
immunoassay analyzers.		becomes bound to streptavidin.	the solid phase via interaction of biotin and
Summary	P group vitaming compared of an aromatic	•	re is aspirated into the measuring cell where t
pteridine ring linked through a r	B-group vitamins composed of an aromatic methylene group to p-aminobenzoic acid and	microparticles are r	magnetically captured onto the surface of the
a glutamate residue. Folate (fo	lic acid) is vital for normal cellular functions		d substances are then removed with Application of a voltage to the electrode then
	ucleic acid synthesis, methionine ox reactions of one-carbon units required for	chemiluminescent	emission which is measured by a photomultip
normal metabolism and regulat	tion. ^{1,2}		ined via a calibration curve which is instrume
	exemplified as a cycle, where folate arbon units from one molecule to another	via the reagent bar	ted by 2-point calibration and a master curve code or e-barcode.
required in various biochemical	I reactions: for example, tetrahydrofolate	Reagents - working s	
(THF) accepts a single carbon number of steps to 5-methylteti	unit from serine, which is reduced in a rahydrofolate (5-MTHF). 5-MTHF gives its	The reagent rackpack	(M, R1, R2) and the pretreatment reagents (F
methyl group to homocysteine,	which is - with involvement of methionine	PT2) are labeled as Fo	bi III.
	zymatically converted to methionine. The cycle of methyl group synthesis. From	PT1 Pretreatment rea	agent 1 (white cap), 1 bottle, 4 mL:
methionine, the methyl groups	are transferred to S-adenosylmethionine	Sodium 2-merca	ptoethanesulfonate (MESNA) 40 g/L, pH 5.5.
(SAM). ³ SAM serves as a meth reactions, like DNA, RNA and p	nyl group donor in several methylation	PT2 Pretreatment rea	agent 2 (gray cap), 1 bottle, 5 mL:
· · · ·	sensitive to folate deficiency: with a low	Sodium hydroxic	le 25 g/L.
	ell to re-methylate homocysteine is impaired mocysteine concentrations in plasma. ²	M Streptavidin-coa	ted microparticles (transparent cap), 1 bottle,
	role in the synthesis of purine and pyrimidine	6.5 mL:	
precursors of nucleic acids. Alt	ered distribution of methyl groups and	Streptavidin-coa	ted microparticles 0.72 mg/mL; preservative.
cancers. Abnormal folate status	n essential role in the development of s has also been linked with the development	R1 Folate binding p	rotein~Ru(bpy) $_{3}^{2+}$ (gray cap), 1 bottle, 9 mL:
	diseases, neural tube defects, cleft lip and ations, neurodegenerative and psychiatric		ed folate binding protein 75 $\mu$ g/L; human seru
disorders. ^{1,2}	allons, neurodegenerative and psychiatric	albumin (stabiliz pH 5.5; preserva	er); borate/phosphate/citrate buffer 70 mmol/l
	essential vitamins, i.e. it cannot be		
diet. Primary sources of folates	anism and therefore must be absorbed from are green and leafy vegetables, sprouts,		ack cap), 1 bottle, 8 mL:
fruits, brewer's yeast and liver.	1,2		te 17 μg/L; biotin 120 μg/L; human serum albu te buffer 100 mmol/L, pH 9.0; preservative.
	d by decreased nutritional intake, poor the intestine or increased demand of folate,	Precautions and war	
for example during physical act	tivity or pregnancy. Deficiency of folate can	For in vitro diagnostic	
also be a result of liver disease genetic defects or drug interact	s or impaired folate metabolism due to	Exercise the normal pr	ecautions required for handling all laboratory
• •	folate and vitamin B12 deficiency is the so	reagents. Disposal of all waste m	naterial should be in accordance with local gu
	ic) anemia: due to the affected DNA		lable for professional user on request.
synthesis and cell maturation, e erythropoiesis, the total count of	of erythrocytes is significantly reduced. The	This kit contains comp Regulation (EC) No. 12	onents classified as follows in accordance wit
hemoglobin synthesis capacity	however is normal, which leads to	2-methyl-2H-isothiazol	
which have an elevated hemog	recursors ("macrocytes" or "megaloblasts"), lobin content ("hyperchromic anemia"). ^{3,4}	-	
Because vitamin B12 and folate	e are closely interrelated in the cellular	EUH 208 May pro	oduce an allergic reaction.
	nd also hematologic and clinical in deficiency states might be similar, it is		
advisable to determine both pa	rameters simultaneously in patients with the		
relevant symptoms of vitamin d	leticiency. ^{3,4}		
Loct principlo			
Test principle Competition principle. Total due	ration of accave 27 minutos	Danger	

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## Elecsys Folate III



H314	Causes severe skin burns and eye damage.					
Prevention:						
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.					
Response:						
P301 + P330 + P331	IF SWALLOWED: Rins	WALLOWED: Rinse mouth. Do NOT induce vomiting.				
P303 + P361 + P353	IF ON SKIN (or hair): 1 clothing. Rinse skin wit	Fake off immediately all contaminate th water.				
P304 + P340 + P310	comfortable for breathi	person to fresh air and keep ng. SON CENTER/ doctor.				
P305 + P351 + P338 + P310	minutes. Remove cont Continue rinsing. Imme	ttiously with water for several act lenses, if present and easy to do ediately call a POISON CENTER/				
	doctor.					
P390	Absorb spillage to prev	vent material damage.				
Product safety	labeling follows EU GH	IS guidance.				
	: all countries: +49-621					
derived from h donors tested i to HCV and HI	uman blood are prepare individually and shown V. The testing methods	red potentially infectious. All product ed exclusively from the blood of to be free from HBsAg and antibodie used assays approved by the FDA ean Directive 98/79/EC, Annex II,				
with absolute of care as a pa	ertainty, the material sh	le out the potential risk of infection nould be handled with the same leve event of exposure, the directives of t be followed. ^{5,6}				
Avoid foam for calibrators and		nd sample types (specimens,				
cannot be sepa	n the kit have been ass arated. required for correct ope	embled into a ready-for-use unit tha eration is read in from the respective				
Storage and s						
Store at 2-8 °C						
Do not freeze. Store the Elec:		in order to ensure complete a automatic mixing prior to use.				
Do not freeze. Store the Elec:						
Do not freeze. Store the Elecs availability of th	ne microparticles during					
Do not freeze. Store the Elec: availability of the Stability:	ne microparticles during 2-8 °C	g automatic mixing prior to use.				
Do not freeze. Store the Elect availability of the Stability: unopened at 2	ne microparticles during 2-8 °C at 2-8 °C	up to the stated expiration date				

separating gel.

Li-heparin plasma. Li-heparin plasma tubes containing separating gel can be used.

Criterion: Method comparison serum versus Li-heparin plasma, slope 0.9-1.1 + intercept within <  $\pm 2x$  Limit of Blank (LoB), coefficient of correlation  $\ge 0.95$ .

Serum: Stable for 2 hours at 15-25 °C, 48 hours at 2-8 °C, 28 days at -20 °C ( $\pm$  5 °C). Freeze only once. Protect from light. Store the samples at 2-8 °C if they cannot be measured immediately.

Li-heparin plasma: Stable for 2 hours at 15-25 °C, 48 hours at 2-8 °C, 28 days at -20 °C ( $\pm$  5 °C). Freeze only once. Protect from light. Store the samples at 2-8 °C if they cannot be measured immediately.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Samples should not subsequently be altered with additives (biocides, anti-oxidants or substances possibly changing the pH of the sample) in order to avoid erroneous folate recovery.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples.

Ensure the samples, calibrators and controls are at 20-25  $^\circ\mathrm{C}$  prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

*Note:* Hemolysis may significantly increase folate values due to high concentrations of folate in red blood cells. Therefore, hemolyzed samples are not suitable for use in this assay. Samples for folate determinations should be collected from fasting persons.

#### Materials provided

See "Reagents - working solutions" section for reagents.

#### Materials required (but not provided)

- REF 07560001190, Folate III CalSet, for 4 x 1.0 mL
- REF 05618860190, PreciControl Varia, for 4 x 3.0 mL
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment

#### cobas e analyzer

- Additional materials for the cobas e 411 analyzer:
- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials for cobas e 601 and cobas e 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- [REF] 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M
- Additional materials for all analyzers:

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## Elecsys Folate III



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 REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

#### Assay

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For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions. Resuspension of the microparticles takes place automatically prior to use.

Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.
<b>cobas e</b> 601 and <b>cobas e</b> 602 analyzers: PreClean M solution is necessary.
Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.
Calibration
Traceability: This method has been standardized against the WHO International Standard NIBSC code: 03/178.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

*Calibration frequency:* Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

#### Quality control

For quality control, use PreciControl Varia.

In addition, other suitable control material can be used.

- Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.
- 3 The control intervals and limits should be adapted to each laboratory's
- individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if
- values fall outside the defined limits. If necessary, repeat the measurement of the samples concerned.
- Follow the applicable government regulations and local guidelines for quality control.

#### Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in nmol/L or ng/mL).

F/	Conversion factors:	nmol/L x 0.44 = ng/mL
8		ng/mL x 2.27 = nmol/L

#### Limitations - interference

- The assay is unaffected by icterus (bilirubin  $\leq$  496 µmol/L or  $\leq$  29 mg/dL), lipemia (Intralipid  $\leq$  1500 mg/dL), biotin ( $\leq$  86.1 nmol/L or  $\leq$  21 ng/mL), IgG  $\leq$  16 g/L, IgA  $\leq$  4.0 g/L and IgM  $\leq$  10 g/L.
- Criterion: Recovery within  $\pm$  10 % of initial value with samples > 4 ng/mL and  $\leq \pm$  0.4 ng/mL with samples  $\leq$  4 ng/mL.
- 55 Hemolysis may significantly increase folate values due to high
- 56 concentrations of folate in red blood cells. Therefore, hemolyzed samples
  57 are not suitable for use in this assay.
- Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.
- 60 No interference was observed from rheumatoid factors up to a concentration of 1000 IU/mL.

In vitro tests were performed on 16 commonly used pharmaceuticals and in addition on human erythropoietin. No interference with the assay was found.

It is contraindicated to measure samples of patients receiving therapy with certain pharmaceuticals, e.g. methotrexate or leucovorin, because of the cross-reactivity of folate binding protein with these compounds.

Samples with extremely high total protein concentrations (hyperproteinemia) are not suitable for use in this assay. Hyperproteinemia may be caused by, but not limited to, the following conditions: Lymphoma^{7,8}, bone marrow disorders such as multiple myeloma, monoclonal gammopathy of undetermined significance (MGUS), Waldenström macroglobulinemia, plasmocytoma^{7,8,9,10,11,12,13}, Amyloidosis^{13,14}. Respective samples may lead to the formation of protein gel in the assay cup, which may cause a run abort. The critical total protein concentration is dependent upon the individual sample composition.

In rare cases, interference due to extremely high titers of antibodies to streptavidin and ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with RBC folate, the patient's medical history, clinical examination, and other findings.

#### Limits and ranges

#### Measuring range

0.6-20.0 ng/mL or 1.36-45.4 nmol/L (defined by the Limit of Blank and the maximum of the master curve). Values below the Limit of Blank are reported as < 0.6 ng/mL (< 1.36 nmol/L). Values above the measuring range are reported as > 20.0 ng/mL (> 45.4 nmol/L).

#### Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.6 ng/mL (1.36 nmol/L)

Limit of Detection = 1.2 ng/mL (2.72 nmol/L)

Limit of Quantitation = 2.0 ng/mL (4.54 nmol/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from  $n \ge 60$  measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative error of  $\leq 20$  %.

It has been determined using low concentration folate samples.

#### Dilution

Samples with folate concentrations above the measuring range can be diluted manually with Diluent Universal. The recommended dilution is 1:2. The concentration of the diluted sample must be > 8.5 ng/mL or > 19.3 nmol/L. After manual dilution, multiply the results by the dilution factor 2.

#### Expected values

Referring to "The American Journal of Clinical Nutrition"¹⁵ serum folate (folic acid) values were found as follows:

Sex	Age	Ν	Median		2.5th-97.5th percentile	
	years		ng/mL	nmol/L	ng/mL	nmol/L
Both	all	23345	13.0	29.5	4.6-34.8	10.4-78.9
Male	all	11387	12.3	27.9	4.5-32.2	10.2-73.0
Female	all	11958	13.6	30.1	4.8-37.3	10.9-84.5
Both	4-11	3595	17.2	39.0	8.6-37.7	19.5-85.4
Both	12-19	6390	12.1	27.4	5.0-27.2	11.3-61.6
Both	20-59	8689	11.6	26.3	4.4-31.0	10.0-70.2

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### 07559992500V4.0 **Elecsys Folate III**

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Sex	Age	Ν	Med	lian	2.5 th -97.	5 th percentile			
	years		ng/mL	nmol/L	ng/mL	nmol/L			
Both	≥ 60	4671	16.6	37.6	5.6-45.8	12.7-103			
These values were obtained in the USA during the National Health and Nutrition Examination Survey (NHANES), 1999-2004. The values shown below were performed on samples from an apparently lealthy population, using the Elecsys Folate III assay. The calculation is based on 404 sera (177 men, 227 women). The age ange was between 20 and 65 years. Pregnant or lactating women were excluded. The reference population was selected according to normal									
homocyste N	eine values	s. Median		2.	5 th -97.5 th p	ercentile			
-	ng/mL		nmol/L		/mL	nmol/L			
404	8.94		20.3 🧹	3.89	3.89-26.8				
25 sample	<i>ficient sam</i> s consider	ed to be c	deficient ^{a)} i	n serum f	olate conce	entration wer			
assessed below the	using the I 2.5 th perce	Elecsys Fo entile as g	plate III as iven in the	say. All sa table abc	amples wer ve.	e found to be			
a) Folate defi available fola	ciency was a te assays.	ssessed by r	neasurement	of serum fol	ate by two co	mmercially			
Represent	Specific performance data Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.								
Precision Precision was determined using Elecsys reagents, pooled human sera an controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:									
		col	<b>bas e</b> 411	analyzer					

cobas e 411 analyzer							
		Repeatability			Intermediate precision		
Sample	Mean	SD	CV	SD	CV		
	ng/mL	ng/mL	%	ng/mL	%		
Human serum 1	1.88	0.150	8.0	0.205	10.9		
Human serum 2	3.92	0.200	5.1	0.318	8.1		
Human serum 3	11.9	0.346	2.9	0.571	4.8		
Human serum 4	13.4	0.301	2.2	0.574	4.3		
Human serum 5	17.8	0.440	2.5	0.666	3.7		
PreciControl Varia1	3.24	0.215	6.6	0.309	9.5		
PreciControl Varia2	11.6	0.314	2.7	0.566	4.9		

cobas e 411 analyzer							
		Repeata	bility	Interme precis			
Sample	Mean	SD	CV	SD	CV		
	nmol/L	nmol/L	%	nmol/L	%		
Human serum 1	4.27	0.341	8.0	0.465	10.9		
Human serum 2	8.90	0.454	5.1	0.722	8.1		
Human serum 3	27.0	0.785	2.9	1.30	4.8		
Human serum 4	30.4	0.683	2.2	1.30	4.3		

cobas e 411 analyzer							
		Repeata	bility	Interme precis			
Sample	Mean	SD	CV	SD	CV		
	nmol/L	nmol/L	%	nmol/L	%		
Human serum 5	40.4	0.999	2.5	1.51	3.7		
PreciControl Varia1	7.35	0.488	6.6	0.701	9.5		
PreciControl Varia2	26.3	0.713	2.7	1.28	4.9		

cobas e 601 and cobas e 602 analyzers								
			Repeatability		ediate sion			
Sample	Mean	SD	CV	SD	CV			
	ng/mL	ng/mL	%	ng/mL	%			
Human serum 1	1.66	0.255	15.4	0.268	16.1			
Human serum 2	4.10	0.219	5.4	0.303	7.4			
Human serum 3	11.1	0.449	4.1	0.503	4.6			
Human serum 4	12.2	0.454	3.7	0.467	3.8			
Human serum 5	16.4	0.502	3.1	0.625	3.8			
PreciControl Varia1	2.34	0.189	8.1	0.228	9.8			
PreciControl Varia2	10.1	0.443	4.4	0.489	4.9			

cobas e 601 and cobas e 602 analyzers

	Repeata	bility	Intermediate precision		
Sample	Mean	SD	CV	SD	CV
	nmol/L	nmol/L	%	nmol/L	%
Human serum 1	3.77	0.579	15.4	0.608	16.1
Human serum 2	9.31	0.497	5.4	0.688	7.4
Human serum 3	25.2	1.02	4.1	1.14	4.6
Human serum 4	27.7	1.03	3.7	1.06	3.8
Human serum 5	37.2	1.14	3.1	1.42	3.8
PreciControl Varia1	5.31	0.429	8.1	0.518	9.8
PreciControl Varia2	22.9	1.01	4.4	1.11	4.9

#### Method comparison

a) A comparison of the Elecsys Folate III assay (traceable to WHO IS 03/178; y) and the Elecsys Folate III assay prior to standardization against WHO IS 03/178 (x) using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 113

Passing/Bablok ¹⁶	Linear regression
y = 1.14x - 1.97	y = 1.11x - 1.77
т = 0.939	r = 0.994

The sample concentrations were between 2.1 and 18 ng/mL (4.8 and 41 nmol/Ĺ).

b) A comparison of the Elecsys Folate III assay (y) with a commercially available method (x) using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 106

Passing/Bablok ¹⁶	Linear regression
y = 0.980x - 0.095	y = 1.09x - 0.659
т = 0.924	r = 0.984
The sample concentrations were bet	veen 1 9 and 17 ng/ml (4 3

concentrations were between 1.9 and 17 ng/mL (4.3 and 39 nmol/Ĺ).

## Elecsys Folate III



c) A comparison of the Elecsys Folate III assay on the cobas e 601 analyzer (y) with the Elecsys Folate III assay on the cobas e 411 analyzer (x) using clinical samples gave the following correlations (ng/mL): Number of samples measured: 105

Passing/Bablok ¹⁶	Linear regression
y = 1.05x - 0.303	y = 0.981x + 0.143
т = 0.868	r = 0.982

The sample concentrations were between 1.6 and 19 ng/mL (3.6 and 43 nmol/L).

#### Analytical specificity

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The following cross-reactivities were found, tested with folate concentrations of approximately 3.5 ng/mL, 10 ng/mL and 19 ng/mL.

Cross-reactant	Concentration tested ng/mL	Cross-reactivity %
Amethopterin	750	2.5
Aminopterin	750	4.4
Folinic acid	750	0.7

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- For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

#### Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
$\longrightarrow$	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

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FNS/1	Sentila Listing Fo						
FNS EA no							
Serial no of	Address	Residence	Serial no	Name of HH	Occupie	Serial no	Remark(mark "X" fo
structure	description of		House/HH	head	d	occupied	selected HH)
	structure	(Y/N)	in the		(Y/N)	EA	
			structure				
					-		
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#### Annex 2: Nutrition direct and indirect interventions/indicators assessment questionnaire <u>Nutrition direct and indirect Interventions Questionnaire</u>

Module 1: Household identifier, characteristics and socio-demographic status

Househo	old identifier and character	istics				
101	Region Code					
102	Woreda Code					
103	Kebele Code					
104	Gote Code					
105	Household Code					GPS Coordinates
		_	<u>_      </u>	I		
106	Unique Household Code	 				
		Region	Woreda	Kebele	EA	Household Code
		Code	Code	Code	Code	
107	Residence	1 = Urban				
		2 = Rural				
108	Do you own this house?	1 = Yes				
		0 = No				
109		1 = No walls				
	material of the walls?		•	ne, wood, mud	, straw)	
		3 = Stone wi				
	Observe		icks with cerr	ient		
110	Mart is the main flags	99 = Other				
110			loor (earth/s			
	material?			od/palm/ban	-	
	Observe	tiles/cement		l wood/ vinyl/		
	Observe	99 = Other	(carpet)			
111	What is the main		rass or leave			
	material of the roof?	2 = Iron shee		3		
	Observe	99 = Other				
112	What type of fuel does	1 = Dung				
	your household mostly	2 = Firewood	d/straw			
	use for cooking?	3 = Charcoal				
	C C	4 = Kerosene	2			
	Do not read list	5 = Gas (met	hane/biogas			
		6 = Electricit	У			
		99 = Other				
113	Is the house connected	1 = Yes				
	to electricity?	0 = No				
	In total, how many of the		amp/pressur	e lamp		
	following items are	Mobile phor	ne			
116		Cart				
117	this household?	Bicycle				
118		Motorcycle				
119	Add the household total	Radio				
120	for each item	Television				

		Car/tractor/Bajaj	
122	Does this household own	1 = Yes	
	any livestock, herds,	0 = No (Go to→131)	
	other farm animals, or		
	poultry?		
123	In total, how many of the	Milk cows, oxen or bulls?	
124	following animals are	Other cattle?	
125	owned by residents of	Horses, donkeys, or mules?	
126	this household?	Camels	
127	Add the household total	Goats?	
128	for each item	Sheep?	
129		Chickens or other poultry?	
130		Beehives?	
131	Does any member of this	1 = Yes	
	household own any	0 = No	
	agricultural land?		
132	How often does anyone	1 = Daily	
	smoke inside your	2 = Weekly	
	house?	3 = Monthly	
		4 = Less once monthly	
	Would you say daily,	5 = Never	
	weekly, monthly, less		
	often than once a month,	6	
	or never?		
Househ	old head socio-demographi	c status	
133	Age in years		
134	Marital status	1. Single	
134	Marital status	2. Married	
134	Marital status	<ol> <li>Married</li> <li>Divorced</li> </ol>	I1
134	Marital status	<ol> <li>Married</li> <li>Divorced</li> <li>Separated</li> </ol>	
		<ol> <li>Married</li> <li>Divorced</li> <li>Separated</li> <li>Widowed</li> </ol>	
	What is the highest level	<ul> <li>2. Married</li> <li>3. Divorced</li> <li>4. Separated</li> <li>5. Widowed</li> <li>1. None</li> </ul>	
	What is the highest level of school the head of the	<ul> <li>2. Married</li> <li>3. Divorced</li> <li>4. Separated</li> <li>5. Widowed</li> <li>1. None</li> <li>2. Primary</li> </ul>	
	What is the highest level	<ul> <li>2. Married</li> <li>3. Divorced</li> <li>4. Separated</li> <li>5. Widowed</li> <li>1. None</li> <li>2. Primary</li> <li>3. Secondary</li> </ul>	
	What is the highest level of school the head of the	<ol> <li>Married</li> <li>Divorced</li> <li>Separated</li> <li>Widowed</li> <li>None</li> <li>Primary</li> <li>Secondary</li> <li>Technical/vocational certificate</li> </ol>	
	What is the highest level of school the head of the	<ol> <li>Married</li> <li>Divorced</li> <li>Separated</li> <li>Widowed</li> <li>None</li> <li>Primary</li> <li>Secondary</li> <li>Technical/vocational certificate</li> <li>Higher / university/ college</li> </ol>	
	What is the highest level of school the head of the	<ol> <li>Married</li> <li>Divorced</li> <li>Separated</li> <li>Widowed</li> <li>None</li> <li>Primary</li> <li>Secondary</li> <li>Technical/vocational certificate</li> <li>Higher / university/ college</li> <li>98.Don't know</li> </ol>	
135	What is the highest level of school the head of the household completed?	<ol> <li>Married</li> <li>Divorced</li> <li>Separated</li> <li>Widowed</li> <li>None</li> <li>Primary</li> <li>Secondary</li> <li>Technical/vocational certificate</li> <li>Higher / university/ college</li> <li>98.Don't know</li> <li>99.Other (specify)</li> </ol>	
135	What is the highest level of school the head of the household completed? What is the religion of	<ol> <li>Married</li> <li>Divorced</li> <li>Separated</li> <li>Widowed</li> <li>None</li> <li>Primary</li> <li>Secondary</li> <li>Technical/vocational certificate</li> <li>Higher / university/ college</li> <li>98.Don't know</li> <li>99.Other (specify)</li> <li>Orthodox</li> </ol>	
135	What is the highest level of school the head of the household completed?	<ol> <li>Married</li> <li>Divorced</li> <li>Separated</li> <li>Widowed</li> <li>None</li> <li>Primary</li> <li>Secondary</li> <li>Technical/vocational certificate</li> <li>Higher / university/ college</li> <li>98.Don't know</li> <li>99.Other (specify)</li> <li>Orthodox</li> <li>Protestant</li> </ol>	
135	What is the highest level of school the head of the household completed? What is the religion of	<ol> <li>Married</li> <li>Divorced</li> <li>Separated</li> <li>Widowed</li> <li>None</li> <li>Primary</li> <li>Secondary</li> <li>Technical/vocational certificate</li> <li>Higher / university/ college</li> <li>98.Don't know</li> <li>99.Other (specify)</li> <li>Orthodox</li> <li>Protestant</li> <li>Catholic/ other Christian</li> </ol>	
135	What is the highest level of school the head of the household completed? What is the religion of	<ol> <li>Married</li> <li>Divorced</li> <li>Separated</li> <li>Widowed</li> <li>None</li> <li>Primary</li> <li>Secondary</li> <li>Technical/vocational certificate</li> <li>Higher / university/ college</li> <li>98.Don't know</li> <li>99.Other (specify)</li> <li>Orthodox</li> <li>Protestant</li> <li>Catholic/ other Christian</li> <li>Muslim</li> </ol>	
135	What is the highest level of school the head of the household completed? What is the religion of	<ol> <li>Married</li> <li>Divorced</li> <li>Separated</li> <li>Widowed</li> <li>None</li> <li>Primary</li> <li>Secondary</li> <li>Technical/vocational certificate</li> <li>Higher / university/ college</li> <li>98.Don't know</li> <li>99.Other (specify)</li> <li>Orthodox</li> <li>Protestant</li> <li>Catholic/ other Christian</li> <li>Muslim</li> <li>No religion</li> </ol>	
135	What is the highest level of school the head of the household completed? What is the religion of	<ol> <li>Married</li> <li>Divorced</li> <li>Separated</li> <li>Widowed</li> <li>None</li> <li>Primary</li> <li>Secondary</li> <li>Technical/vocational certificate</li> <li>Higher / university/ college</li> <li>98.Don't know</li> <li>99.Other (specify)</li> <li>Orthodox</li> <li>Protestant</li> <li>Catholic/ other Christian</li> <li>Muslim</li> <li>No religion</li> <li>98.Don't know</li> </ol>	
135	What is the highest level of school the head of the household completed? What is the religion of	<ol> <li>Married</li> <li>Divorced</li> <li>Separated</li> <li>Widowed</li> <li>None</li> <li>Primary</li> <li>Secondary</li> <li>Technical/vocational certificate</li> <li>Higher / university/ college</li> <li>98.Don't know</li> <li>99.Other (specify)</li> <li>Orthodox</li> <li>Protestant</li> <li>Catholic/ other Christian</li> <li>Muslim</li> <li>No religion</li> </ol>	

#### Module 2: Child health

201	each separately, starting with the youngest. Child's code		1 11
201	Mother's name	Mothor's given name	_
		Mother's given name	
203	Mother's age	1-Nono	
204	Mother's education level	1=None	
		2=Primary	
		3=Secondary	
		4=Technical/vocational certificate	
		5=Higher / university/ college 98=Don't know	
205	And the dense of the last of	99. Other (specify)	
205	Mother's marital status	1=Single	
	O,	2 =Married	
		3=Divorced	
	<b>A</b>	4 =Separated	
200		5=Widowed	
206	Mother's religion	1=Orthodox	
		2=Protestant	
		3=Catholic/ other Christian	
		4=Muslim	
		5=No religion 98=Don't know	
207	Mother's ethnicity	99=Other religion (specify) Specify	
207	Child's name	Child's given name	
209	Child (NAME) sex	1 = Boy	
205		2 = Girl	
210	Child (NAME) age?	Age in months or age at the time of	
		the child's death	_
211	In the last six months, was (NAME) given	1 = Yes	
	any vitamin A supplement?	0 = No	
		98 = Don't know	
212	When was the child (NAME) given the vitamin A supplement?	Specify	
213	In the last 12 months, was (NAME) given any iron	1 = Yes	_
	tablet or syrup or supplement?	0 = No	
		98 = Don't know	
214	In the last 6 months, was (name) given any	1 = Yes	
	medicine for intestinal worms?	0 = No	_
		98 = Don't know	
215	In the last 3 months, has any healthcare provider	1 = Yes, 0 = No, 98 = Don't know	
	measured?	Weight	_
		Height/length	_
		MUAC	_
216	Has (name) had diarrhea in the last 2 weeks?	1 = Yes	
		0 = No (Go to 224)	

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2		98 = Don't know	
217	Now I would like to know how much was the child	1 = Much less	
	given to drink during diarrhea, including breast	2 = Somewhat less	
	milk. Was the child given less than usual to drink,	3 = About the same	
	about the same amount, or more than usual to	4 = More	
3	drink?	5 = Nothing to drink	
		98 = Don't know	
0 218	When the child had diarrhea, was he/she given	1 = Much less	
1	less than usual to eat, about the same amount,	2 = Somewhat less	
2	more than usual, or nothing to eat?	3 = About the same	
3		4 = More	
4		5 = Nothing to drink	
5 6		98 = Don't know	
7 219	Did you seek advice or treatment for the diarrhea	1 = Yes	
8	from any source?	0 = No <b>(Go to 221)</b>	''
9 220	Where did you seek advice or treatment?	1= Government hospital	
0	Anywhere else?	2= Government health center	
1		3= Government health post	
22	Probe to identify the type of source.	4 = Mobile clinic	
23	If unable to determine if public, private, or	5 = Community health worker/	
24	NGO sector, record '21' and write the name	fieldworker	
25	Of the place(s).	6 = Other public sector (specify)	
27		7 = Private hospital	
28		8 = Private clinic	
29	Ň	9 = Pharmacy	
80		10 = Private doctor	
31		11 = Mobile clinic	
32		12 = Community health	''
3		worker/fieldworker	
4		13 = Other private medical sector	
5		(specify)	
6 7		14 = NGO hospital	
8		14 = NGO (lospital) 15 = NGO clinic	
9		16 = Other NGO medical sector	
0			
1		(specify) 17 = Shop	
2		17 – Shop 18 = Traditional practitioner	
-3		19 = Market	
4		20 = Itinerant drug seller	
-5		99 = Other (specify)	
.7	Mac (name) given any of the following at any time		
8 221	Was (name) given any of the following at any time since (name) started having diarrhea:	1 = Yes, 0 = No, 98 = Don't know	
221	אוונים אווופן אנמו נפט וומעוווצ טומודוופמ.	Fluid from ORS packet	<u>    </u>
io 222	4	Zinc	<u>      </u>
223		Homemade fluid	<u>     </u>
2 224	Has (name) been ill with a fever at any time in the	1 = Yes	
53	last 2 weeks?	0 = No ( <b>Go to 226</b> )	
4 5 225		98 = Don't know	
5 <u>225</u> 6	Where did you seek advice or treatment for	1= Government hospital	
57			
58	5		
59	For poor review each that //harden as have	m/site/about/avidaliace.victural	
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	fever?	2= Government health center	
	Anywhere else?	3= Government health post	
		4 = Mobile clinic	
	Probe to identify the type of source.	5 = Community health worker/	
	If unable to determine if public, private, or	fieldworker	
	NGO sector, record '21' and write the name	6 = Other public sector (specify)	
	Of the place(s).	7 = Private hospital	
		8 = Private clinic	
		9 = Pharmacy	
		10 = Private doctor	
		11 = Mobile clinic	
		12 = Community health	
		worker/fieldworker	
		13 = Other private medical sector	
		(specify)	
		14 = NGO hospital	
		15 = NGO clinic	
		16 = Other NGO medical sector	
		(specify)	
	$\mathbf{N}$	17 = Shop	
		18 = Traditional practitioner	
		19 = Market	
		20 = Itinerant drug seller	
		99 = Other (specify)	
226	Was Child (Name) ever breastfed?	1 = Yes	
		0 = No ( <b>Go 228</b> )	
227	How many months the child (NAME) was breastfe	d?	
	opometric and clinical nutrition assessment		
228	Weight	4	
229	Height/length		
230	MUAC		_
231	Presence of bilateral oedema for children 6-59	1 = Yes	
	months	0 = No	
232	Bitot spot	1 = Yes	
		0 = No	

	nodule is to be administered to the mother/care	÷	
living	with respondents. Verify that the respondent yo	u are speaking to is the mother/care	giver of the chi
301	Was Child (Name) ever breastfed?	1 = Yes 0 = No ( <b>Go to 304</b> ) 98 = Don't know	_  _
302	Was Child (NAME) given the first milk (colostrum) after birth?	1 = Yes 0 = No 98 = Don't know	
303	How long after birth did you first put (NAME) to the breast, even if your breast milk did not arrive?	<ol> <li>1 = Immediately after birth, or within 1 hour</li> <li>2 = Between 1 and 24 hours</li> <li>3 = More than 24 hours after delivery</li> <li>98 = Don't know</li> </ol>	
304	Child (NAME) alive now?	1 = Yes 0 = No ( <b>Go to 401</b> )	_  _
305	Was (NAME) breastfed yesterday from sunrise until today sunrise? NB: Breastfeeding could be by the mother herself or by wet mother.	1 = Yes 0 = No ( <b>Go to 307</b> ) 98 = Don't know	
306	Sometimes babies are fed breast milk in different ways, for example by spoon, cup or bottle. This can happen when the mother cannot always be with her baby. Sometimes babies are breastfed by another woman, or given breast milk from another woman by spoon, cup or bottle or some other way. This can happen if a mother cannot breastfeed her own baby. Did (NAME) consume breast milk in any of these ways yesterday from sunrise until today sunrise?	1 = Yes 0 = No 98 = Don't know	
307	Now I would like to ask you about some medicines and vitamins that are sometimes given to infants. Was (NAME) given any vitamin drops or other medicines as drops yesterday from sunrise until today sunrise?	1 = Yes 0 = No 98 = Don't know	
308	Was (NAME) given Lemlem or ORS in the last two weeks?	1 = Yes 0 = No 98 = Don't know	_  _

309	In the last 6 months, did any healthcare	1 = Yes	
	provider or community health worker talk	0 = No	
	with you about how and what to feed your child?	98 = Don't know	11
310	Now, I would like to ask you about some	Did (NAME) have any (item from	
	liquids that (NAME) may have had yesterday	list)?	
	from sunrise until today sunrise?	1 = Yes	
		0 = No (Go to 321)	
	If yes to Q310, read the list of liquids starting with 'plain water'.	98 = Don't know	
311	Plain water	1 = Yes	
		0 = No	
		98 = Don't know	
312	Infant formula such as S-26?	1 = Yes	
		0 = No <b>(Go to 314)</b>	
		98 = Don't know	
313	How many times infant		
	formula such as S-26?		
314	Milk such as tinned,	1 = Yes	
	powdered, or fresh animal	0 = No <b>(Go to 316)</b>	
	milk?	98 = Don't know	
315	How many times milk drink?		
316	Yogurt drink?	1 = Yes	
		0 = No	
		98 = Don't know	
317	Chocolate flavored drink?	1 = Yes	
		0 = No	
		98 = Don't know	
318	Sodas malt drinks or operation	1 = Yes	
	Sodas, malt drinks or energy drinks?	0 = No	
	UTITIKS!	98 = Don't know	
319	Clear broth or clear soup?	1 = Yes	
		0 = No	
		98 = Don't know	
320	Any other liquids?	1 = Yes	
		0 = No	
		98 = Don't know	
Now foods would	I would like to ask you about foods that (NAME) s your child ate whether at home or somewhere d like to know whether your child ate the food e	98 = Don't know         1 = Yes         0 = No         98 = Don't know         had yesterday during the day or nigh         else. I will ask you about different typ         ven if it was combined with other foor	pes of foods,
	er 'yes' for any food or ingredient used in a smal		aro not in the li
belov	R FOODS: Please write down other foods in this v	box that respondent mentioned but	are not in the lis
	rday during the day or at night, did (NAME) eat:		
Yeste	ady during the day of at hight, and (NAME) cat.		
Yeste 321	Did the child ate any solid or semi-solid food y		Eaten?

			0 = No (Go to 342)
322	Yogurt, other than yogurt drink?		98 = Don't know
323	How many times did child (NAME) eat yogurt?		
323	Injera, bread, rice, noodles, pasta, macaroni, porridge	or other foods made from	II
524	grains such as tef, oats, maize, barley?	, of other loous made from	
325	Any commercially fortified baby food like Fafa, Hilina,	Cerilak. Cerifam. Mother	
	Choice?	, ,	
326	Pumpkin, carrots, squash, or sweet potatoes that are	yellow or orange inside?	
327	White potatoes, white yams, bulla, kocho, manioc, ca	ssava or any other foods	1 1
	made from roots?		II
328	Any dark green leafy vegetables (kale, dark green lett	uce, moringa)?	
329	Any other vegetable?		
330	Ripe mangoes, ripe papayas (insert other local vitami	n a-rich fruits)?	
331	Any other fruit?		
332	Liver, kidney, heart, or other organ meats?		
333 334	Any meat, such as beef, pork, lamb, goat, chicken?		<u> </u>
335	Egg? Fresh or dried fish, shellfish, or seafood?		
336	Any foods made from beans, peas, lentils, nuts, or see	2945	
337	Cheese or other food made from milk?	203:	<u> </u>
338	Any sugary foods such as chocolates, sweets, candies	. pastries, cakes, or biscuits	
339	Any savory junk foods, such as crisps/chips/salted bis	-	
340	Any other solid, semi-solid, or soft food?	·	
341	How many times did (NAME) eat solid, semi-solid,	Fill in the number of times.	
	or soft foods other than liquids yesterday during the	98 = Don't know	
	day or at night?		
342	Did (NAME) drink anything from a bottle with a	1 = Yes	
	nipple yesterday during the day or night?	0 = No	
		98 = Don't know	

#### Module 4: KAP of mothers or caregivers on children's care and feeding

-	going to read you some knowledge question questions.	ons about breastfeeding. Please tell me y	our answers on
401	How long after birth should a baby start breastfeeding?	<ul> <li>1 = Immediately, within 1 hour of delivery</li> <li>2 = Some hours later but within 24 hours</li> <li>3 = After 1 day</li> <li>4 = After 2 days</li> <li>5 = After &gt;3 days</li> <li>6 = Does not think a baby should be breastfed</li> <li>98 = Don't know</li> </ul>	IIII
402	How long should a baby receive nothing other than breast milk?	1 = From birth to six months 2 = Other 98 = Don't know	1111
403	How often should a baby younger than six months be breastfed or fed with breast milk?	1 = On-demand, whenever the baby wants 2 = Other 98 = Don't know	
404	How much should a child be fed when he/she is sick?	1 = Less frequent than usual 2 = Same as usual 3 = More than usual 98 = Don't know	_  _
405	How often should a child be fed when he/she is sick?	<ol> <li>1 = Less frequently than usual</li> <li>2 = Same as usual</li> <li>3 = More frequently than usual</li> <li>98 = Don't know</li> </ol>	
406	At what age should a baby first start to receive foods in addition to breast milk?	Months of age (Specify) 98= Don't know	
407	At what age should children begin observing fasting days if that is their culture or religion? (If <2 years, enter age in months.)	Years of age (Specify) 98=Don't know/remember	_  _
408	Have you ever heard of child stunting?	1 = Yes 0 = No 98 = Don't know	1_11_1
409	What age are children at the highest risk of becoming stunted?	Years of age (Specify) Months of age (Specify) 98 = Don't know/remember	_  _
410	What are the consequences of stunting for young children? Mark all that are mentioned by the respondent	<ol> <li>1 = Higher risk of severe infectious diseases</li> <li>2 = Poor educational performance</li> <li>3 = Weaker immune system</li> <li>4 = Low adult wages</li> <li>5 = Lost productivity</li> <li>6 = Excessive weight gain in later life</li> </ol>	IIII



		<ul> <li>7 = Increased risk of nutrition-related chronic diseases in adult life</li> <li>8 = Increased mortality rate</li> <li>98= Don't know</li> <li>99 = Other</li> </ul>	
411	Poor diet during pregnancy and the first two years of child age can cause child stunting	1 = Agree 2 = Do not agree 98 = Don't know	
-	going to read you some statements about		
	ers who live in a community like yours. Ple are no correct answers! I would like to kr		ments. Remenn
412	The colostrum (the "first yellowish	1 = Strongly disagree	1 11 1
712	milk") is not good for the baby and	2 = Disagree	11
	should be discarded	3 = Agree somewhat	
		4 = Agree	
		5 = Strongly agree	
		98 = Don't know	
413	It is good to exclusively breastfeed	1 = Strongly disagree	
	give a baby only breast milk and no	2 = Disagree	
	other foods or liquids for the first six	3 = Agree somewhat	
	months	4 = Agree	
		5 = Strongly agree	
		98 = Don't know	
414	If a child is sick (for example has 🛛 🧹	1 = Strongly disagree	
	fever/diarrhea) breastfeeding must be	2 = Disagree	
	continued	3 = Agree somewhat	
		4 = Agree	
		5 = Strongly agree	
		98 = Don't know	
415	A child should eat eggs, cow milk, or	1 = Strongly disagree	
	meat even on fasting days	2 = Disagree	
		3 = Agree somewhat	
		4 = Agree	
		5 = Strongly agree	
110	Fating a most from different food	98 = Don't know	
416	Eating a meal from different food groups is not necessary until children	1 = Strongly disagree 2 = Disagree	
	are old enough to go to school	3 = Agree somewhat	
		4 = Agree	
		5 = Strongly agree	
		98 = Don't know	
417	It is good to feed a two years child at	1 = Strongly disagree	1 11 1
	least four times each day	2 = Disagree	''''
		3 = Agree somewhat	
		4 = Agree	
		5 = Strongly agree	
		98 = Don't know	
418	A mother should eat nutritious food	1 = Strongly disagree	1 11 1

	(four) times daily from the time of	2 = Disagree	
	pregnancy	3 = Agree somewhat	
		4 = Agree	
		5 = Strongly agree	
		98 = Don't know	
419	A mother should take iron folic acid	1 = Strongly disagree	_  _
	tablets during pregnancy	2 = Disagree	
		3 = Agree somewhat	
		4 = Agree	
		5 = Strongly agree	
		98 = Don't know	
420	A mother should take iodized salt	1 = Strongly disagree	
	during pregnancy	2 = Disagree	
		3 = Agree somewhat	
		4 = Agree	
		5 = Strongly agree	
		98 = Don't know	
421	A mother should take de-worming	1 = Strongly disagree	
	medicines during pregnancy	2 = Disagree	
		3 = Agree somewhat	
		4 = Agree	
		5 = Strongly agree	
		98 = Don't know	

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	This section is administered for Adolescent girls 10-19 years old		
	ned consent and Assent Form to the respondent Read the cons	sent (for mothers of adole	scent gi
	ssent (adolescent girls) form		T
501	Girl's code		_
502	Girl's name	Given name	
503	Girl's age		
504	Girl's education level	1=None	
		1=Primary	
		2=Secondary	
		3=Technical/vocational	
		certificate	
		98=Don't know	
		99=Other (specify)	
505	Girl's marital status	1 = Single	
505	Girl's mantal status	2 = Married	
		3 = Divorced	
		4 = Separated	
		5 = Widowed	
506	Girl's religion	1=Orthodox	
300		2=Protestant	
		3=Catholic/ other Christian	
		4=Muslim	
		5=No religion	
		98=Don't know	
507		Other religion (specify)	
507	Girl's ethnicity	specify	
508	Are you currently a student	1 = Yes	
		0 = No	
509	Were you given any iron/foliate tablets at school or out of	1 = Yes	II
	school? ( <i>show the tablet)</i>	0 = No ( <b>Go to 511)</b>	
510	How many weeks per month have you taken the iron	Weeks per month	_
	tablets?	(specify)	
		98 = don't know	
511	Were you given any drug for intestinal worms at school or	1 = Yes	
	out of school in the last six months?	0 = No	
512	Have you received any nutrition counseling in the last six	1 = Yes	II
	months?	0 = No	
513	Did you receive nutritional assessment services in health	1 = Yes	
	facilities when you went for any kind of health service?	0 = No	
514	Is there any food taboo for adolescent girls in your	1 = Yes	
	community?	0 = No ( <b>Go to 516</b> )	
515	Mention types of food taboo?		
Anthro	opometry and clinical nutrition assessment		
516	Weight (in kg)		
517	Height (in CM)		
	Waist circumference (in CM)		

519 Goiter	1 = Yes, 0 = No
	14
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#### For currently pregnant women 601 Woman's code 602 Woman's name Given name 603 Woman's age 604 Woman's education level 1=None 2=Primary 3=Secondary 4=Technical/vocational certificate 5=Higher / university / college 98=Don't know 99=Other (specify) 605 Woman's marital status 1 = Single 2 = Married 3 = Divorced 4 = Separated 5 = Widowed 606 Woman's religion 1=Orthodox 2=Protestant 3=Catholic/ other Christian 4=Muslim 5=No religion 98=Don't know 99=Other religion (specify) 607 Woman's ethnicity Specify 608 What was your age at first marriage Year (specify) |__||__| 96=Not applicable 98=Don't know 609 Year (specify) What was your age at your first pregnancy 96=Not applicable 98=Don't know Were you pregnant in the last 3 years? 1= Yes 610 0=No (Go to 629) Did you see anyone for antenatal care for the 611 1 = Yespregnancy? 0 = No (Go to 629) 612 Whom did you see for antenatal care? 1=Health personnel Probe to identify each type of person and 2 = Doctor record all 3 = Nurse 4 = Midwife 5= Health officer 6 = Health extension worker 7 = Other person 8 = Traditional birth Attendant 99 = Other (specify)613 Where did you receive antenatal care for this 1= My home 1 11 pregnancy? 2 = Her homeAnywhere else? 3 = Other home 4 = Health center

#### Module 6: Reproductive age women (15-49 Years)

		5 = Government Hospital 6 = Private Clinic or Hospital 7 = Health post 8 = NGO clinic or hospital 99 = Other SPECIFY	
614	How many months pregnant were you when you first received antenatal care for this pregnancy?	Months (Specify) 98 = Don't know	
615	How many times did you receive antenatal care during this pregnancy?	Number of times (specify) 98 = Don't know	
616	As part of your antenatal care during this pregnancy, were any of the following done at least once:	1 = Yes 0 = No Was your blood pressure measured?	_
		Did you give a urine sample? Did you give a blood sample? Did a health care provider talk with	    
		you about which foods to eat while pregnant? Did a health care provider weigh	
		you? Did a health care provider talk with you about your weight?	
		Did a health care provider talk with you about breastfeeding?	
617	During this pregnancy, did you ever receive food or cash assistance from government, an NGO, religious institution or other group?	1 = Yes 0 = No ( <b>Go to 619</b> )	
618	What type of assistance did you receive?	1 = Cash only 2 = Food only 3 = Cash and food mix 99 = Other (specify)	
619	During this pregnancy, were you given or did you buy any iron tablets or iron syrup?	1 = Yes 0 = No <b>(Go to 622)</b> 98 = Don't Know <b>(Go to 622)</b>	
	show tablets/syrup/multiple micronutrient supplement		
620	Where did you get the iron tablet or syrup from?	<ol> <li>1 = Govt. Health Facility</li> <li>2 = Private Health Facility</li> <li>3 = Mobile Clinic</li> <li>4 = CHW</li> <li>5 = [Mass Distribution Campaign – Add Local Name]</li> <li>6 = Pharmacy</li> <li>7 = Shop/Market</li> <li>8 = School</li> <li>98 = Don't Know</li> </ol>	111
621	During this pregnancy, for how many days did you take the iron tablets? <i>If answer is not numeric, probe for</i>	99= Other Number of days (specify) 98 = Don't Know	

	approximate number of days.		
622	During this pregnancy, did you take any drug	1 = Yes	
	for intestinal worms?	0 = No	
		98 = Don't Know	
623	During this pregnancy, did any health care	1 = Yes	
	provider talk with you about breastfeeding?	0 = No	
		98 = Don't Know	
624	During this pregnancy, did you practice	1 = Yes	
•= ·	fasting?	0 = No	''
		98 = Don't Know	
625	At your last ANC visit, did the health provider	1 = Yes	
025	weigh you?	0 = No	''
		98 = Don't Know	
626	Duning this guardeney has used here the		
626	During this pregnancy has your health	1 = Yes	
	provider given you information about your	0 = No ( <b>Go to 629</b> )	
	weight gain?	98 = Don't Know	
627	During your pregnancy have you been thin for	1 = Yes	
	your height?	0 = No	
		98 = Don't Know	
628	Did you received treatment for malnutrition?	1 = Yes	
		0 = No	
Anthrop	ometry		
629	Weight (in kg)		
630	Height (in CM)		
631	MUAC		
632	Waist circumference (in cm)		
633	Goiter	1 = Yes	
000	Goitei	0 = No	
		0 - 100	

Yest	erday, during the day or at night did you eat or drink:	
S.N	Question	Response
		1 = Yes
		0 = No
701	Woman's code	
702	Woman's name	Given name
703	Woman's age	
704	Woman's education level	0=None
		1=Primary
		2=Secondary
		3=Technical/vocati
		certificate
		4=Higher / univers
		college
		98=Don't know
		99=Other (specify)
705	Woman's marital status	1= Single
		2= Married
		3= Divorced
		4= Separated
706	Waman'a religion	5= Widowed 1=Orthodox
/06	Woman's religion	2=Protestant
		3=Catholic/other
		Christian
		4=Muslim
		5=No religion
		98=Don't know
		99=Other religion
		(specify)
707	Woman's ethnicity	Specify
708	Any vegetables or roots that are orange-colored inside, like: pumpkin,	
	carrots, squash, or sweet potatoes that are yellow or orange inside	
709	Any white roots and tubers or plantains, such as: white potatoes, white	
	yams, manioc/cassava/yucca, cocoyam, taro or any other foods made	
	from white-fleshed roots or tubers, or plantains	
710	Any dark green leafy vegetables, such as: [list examples of any medium-to-	
	dark green leafy vegetables including wild/foraged leaves]	
711	Any fruits that are dark yellow or orange inside, like: ripe mango, ripe	
	рарауа	
712	Any other fruits	
713	Any other vegetables	
714	Any meat made from animal organs, such as: liver, kidney, heart or other	
	organ meats or blood-based foods, including from wild game	
715	Any other types of meat or poultry, like: beef, pork, lamb, goat, rabbit,	
	wild game meat, chicken, duck, other birds	

#### **Module 7: Women Dietary Diversity**

716	Any eggs	
717	Any fish or seafood, whether fresh or dried	
718	Any beans or peas, such as: mature beans or peas (fresh or dried seed),	
	lentils, or bean/pea products, including hummus, tofu and tempeh	
719	Any nuts or seeds, like tree nut, groundnut/peanut, or certain seeds or	
	nut/seed "butters" or pastes	
720	Any milk or milk products, such as: milk, cheese, yoghurt or other milk	
	products, but not including butter, ice cream, cream or sour cream	
721	Any oils and fats	
722	Any savory and fried snacks, such as: crisps and chips, fried dough, other	
	fried snacks	
723	Any sweets, such as: sugary foods, such as chocolates, candies,	
	cookies/sweet biscuits and cakes, sweet pastries or ice cream	
724	Any sugar-sweetened beverages, like: sweetened fruit juices and "juice	
	drinks", soft drinks/fizzy drinks, chocolate drinks, malt drinks, yoghurt	
	drinks, sweet tea or coffee with sugar	
725	Any condiments and seasonings, such as: ingredients used in small	
	quantities for flavour, such as chilies, spices, herbs, fish powder, tomato	
	paste, flavor cubes or seeds	
726	Any other beverages and foods like tela, tej, bordea, arkea, cheka, tselo	
727	Did you eat anything (meal or snack) OUTSIDE of the home yesterday?	
728	Did you fast yesterday during the day or night?	

No	Question	Answer	
		1 = Yes	
		0 = No	
801	Do you often have headaches?		
802	Is your appetite poor?		
803	Do you sleep badly?		
804	Are you easily frightened?		
805	Do your hands shake?		
806	Do you feel nervous, tense or worried?		
807	Is your digestion poor?		
808	Do you have trouble thinking clearly?		_
809	Do you feel unhappy?		
810	Do you cry more than usual?		
811	Do you find it difficult to enjoy your daily activities?		
812	Do you find it difficult to make decisions?		
813	Is your daily work suffering?		
814	Are you unable to play a useful part in life?		I
815	Have you lost interest in things?		
816	Do you feel that you are a worthless person?		
817	Has the thought of ending your life been on your mind?		
818	Do you feel tired all the time?		
819	Do you have uncomfortable feelings in your stomach?	4	
820	Are you easily tired?		

Module 8: SELF-REPORTING	QUESTIONNAIRE	(SRQ-20)
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	Module 9: Women empowerme	ent	
901	Identify the most senior mother of the mothers who have a selected child. She is the mother who should respond to the rest of this interview from this point.		_
902	In the past major growing season (Meher) and minor growing (Belg) season, not including the current season, did you work on the family farm?	1 = Yes 0 = No → <b>Go to 913)</b>	_
903	What sort of work you did on the family farm?	1 = Home (kitchen) gardening	
904		2 = Fieldwork	
905		3 = Cash crop farming	
906		4 = Dairy processing	
907		5 = Poultry rearing	
908		6 = Raising livestock	
909		7 = Fishpond/ aquaculture	
910		99 = Other (specify)	
911	From the work that you did on the farm did your household earn any money?	1 = Yes 0 = No	
912	Who usually decides how the money you earn will be used?	98 = Don't know 1 = Self 2 = Husband	
	READ THE LIST.	3 = Self and husband jointly 4 = Someone else	
913	Who usually makes decisions about major household purchases/sell such as cattle or livestock?	1 = Self 2 = Husband 3 = Self and husband jointly 4 = Someone else	
914	Who usually makes decisions about minor household purchases/sell such as spices/oils, soap, utensils, or daily household needs?	1 = Self 2 = Husband 3 = Self and husband jointly 4 = Someone else	
915	Who usually makes decisions about health care for your children?	1 = Self 2 = Husband 3 = Self and husband	
	READ THE LIST.	jointly 4 = Someone else	
916	Do you have husband?	1 = Yes 0 = No ( <b>Go to 918</b> )	_
917	Who usually decides how the money your husband earns will be used?	1 = Self 2 = Husband 3 = Self and husband	
	READ THE LIST.	jointly 4 = Someone else	

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918	Do you have children?	1 = Yes 0 = No ( <b>Go to 923)</b>	
919	Does your husband help you care for the children?	1 = Yes 0 = No ( <b>Go to 923)</b>	
920	Does he help care for the children almost every day, at least once a week, or rarely?	1 = Every day 2 = At least once a week 3 = Rarely	_
921	Does your husband help you with household chores like cooking, cleaning the house, fetching water, collecting firewood or other domestic work?	1 = Yes 0 = No ( <b>Go to 923)</b>	
922	Does he help almost every day, at least once a week, or rarely?	1 = Every day 2 = At least once a week 3 = Rarely	

923	At any time	Use improved seed varieties for any of your	1 = Yes	
	during the	crops?	0 = No	_  _
	previous	$\sim$	98 = Don't know	
924	major seasons	Keep improved varieties of livestock?	1 = Yes	
	(Meher) and		0 = No	
	minor season		98 = Don't know	
925	(Belg) not	Use animal manure to improve you crops	1 = Yes	
	including the	yield?	0 = No	
	current		98 = Don't know	
926	season,	Use any other source of fertilizer on your	1 = Yes	
		crops?	0 = No	
	Did you:		98 = Don't know	
927		Irrigate your crops?	1 = Yes	
			0 = No	
			98 = Don't know	
928		Rotate your crops from one field to another	1 = Yes	
		when planting?	0 = No	
			98 = Don't know	
929		Harvest water during the rains?	1 = Yes	
			0 = No	_  _
			98 = Don't know	
930		Practice intercropping?	1 = Yes	
			0 = No	
			98 = Don't know	
931	Have you ever t	aken any steps to reduce soil erosion on your	1 = Yes	
	farm?		0 = No ( <b>Go to 936)</b>	
			98 = Don't know ( <b>Go to</b>	
			936)	
			For each mentioned: 1=Yes	0=No
932	What steps did	you take to reduce soil erosion?	Plant trees or shrubs	
933			Terracing	

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934 935		Use drainage system	1
	-	Other	 
936	Have you received any inputs for your farm from a	1 = Yes	I.
550	social/government program?	0 = No ( <b>Go to 1001</b> )	I.
937	What farm inputs have you received?	For each mentioned: 1=Yes	0=Nc
938		Seeds	
939	-	Improved seeds	
940	-	Livestock or poultry	1
941	1	Improved varieties of	
		livestock/ poultry	I
942	]	Aquaculture (fish)	
943		Fertilizer	
944		Other	

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Module	10:	WASH
wiouule	<b>TO</b> .	WASH

	Module 10: WASH				
No	Question	choices			
1001	What is the main source of drinking water	1 = Piped into dwelling			
	for the household?	2 = Piped to yard/plot			
		3 = Piped to neighbour			
	Do not read list	4 = Public tap/standpipe			
		5 = Tube well or borehole			
		6 = Protected well			
		7 = Unprotected well			
		8 = Protected spring			
		9 = Unprotected spring			
		10 = Rainwater			
		11 = Tanker truck			
		12 = Cart with small tank			
		13 = Surface water (river/dam/			
		Lake/pond/stream/canal/ irrigation			
		channel)			
		14 = Bottled water			
		99 = Other			
1002	Do you do anything to your household	1 = Yes			
1002	water to make it safer to drink?	0 = No ( <b>Go to 1004</b> )	''		
		98 = Don't know ( <b>Go to 1004</b> )			
1002	What is the main thing you do to make the	1 = Boil			
1003	What is the main thing you do to make the water safer?	2 = Add bleach/chlorine	''		
	water saler:	3 = Strain through a cloth			
		4 = Use water filter (ceramic/			
		Sand/composite/etc)			
		5 = Solar disinfection			
		6 = Let it stand and settle			
		7 = Other(specify) 98 = Don't know			
1004	And an internet of the first factories and the second second for		<u> </u>		
1004	What kind of toilet facility do members of	1 = Flush to piped sewer system			
	your household usually use? If not possible	2 = Flush to septic tank			
	to determine, ask for Permission to	3 = Flush to pit latrine			
	observe the facility.	4 = Flush to somewhere else			
		5 = Flush, don't know where			
		6 = Ventilated improved pit latrine			
		7 = Pit latrine with slab			
		8 = Pit latrine without slab/open pit			
		9 = Composting toilet			
		10 = Bucket toilet			
		11 = Hanging toilet/hanging latrine			
		12 = No facility/bush/field			
		99 = Other (specify)			
1005	How does your HH primarily dispose of HH	1 = Collected by municipality			
	waste?	2 = Buried			
		3 = Collected by private establishment			
		4 = Dumped in street/open space			

		5 = Disposed in the compound 6 = Dumped in river	
		7 = Burned 98 = Other	
1006	Do you have separate cooking room?	1 = Yes	
1000	bo you have separate cooking room:	2 = No (Go to 1008)	
1007	Does the stove or cooking room have a	1 = yes	1 11
	chimney?	2 = No	·
1008	What type of fuel or energy source is used	1 = Alcohol/ethanol	
	in this cook stove?	2 = Gasoline/diesel	
		3 = Kerosene/paraffin	
		4 = Coal/lignite	
		5 = Charcoal	
		6 = Wood	
		7 = Straw/shrubs/grass	
		8 = Agricultural crop	
		9 = Animal dung/waste	
		10 = Processed biomass (pellets) or	
		woodchips	
		11 = Garbage/plastic	
		12 = Sawdust	
		96 = Other (specify)	
1009	Do you have a confined space (beret/gata)	1 = Yes	
	to keep livestock?	0 = No	
		96 = Not applicable	·
1010	Do you keep poultry in cages/confined	1 = Yes	
	systems (kote)?	0 = No	
1011	And shall a shall be a shall be shall be the shall be for a	96 = Not applicable	
	What do you think are the activities before	For each mentioned: 1=Yes 0=No	
1012		Before preparing food	<u>                                     </u>
1013	soap?	Before touching or eating food	<u>                                     </u>
1014	DO NOT PROMPT	Before feeding a child or other person	
	DO NOT PROMPT.	Praying	<u> </u>
1016		Don't know	
	What do you think are the activities after	For each mentioned: <b>1=Yes 0=No</b>	
1018		After defecation or urinating	<u>                                     </u>
1019	-	After handling animals and their waste	<u>  !!</u>
1020		After housework or fieldwork	<u> </u>
1021	DO NOT PROMPT.	After touching pets or handling animals	
1000		and their waste	<u> </u>
1022		After blowing nose or coughing	<u>                                     </u>
1023		After cleaning a child's bottom	<u>  !!</u>
1024		None	
1025	poultry and livestock in a confined space?	To keep out of house	
1026	For each mentioned: 1=Yes 0=No	To keep away from water source	
		To reduce infectious disease	

Other
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SN	Question	Response	
1101	Does your household use cooking oil to prepare foods or add to foods at home?	1 = Yes 0 = No <b>→Go to 1103</b>	
1102	The last time your household get cooking oil, where did you get it from?	<ul> <li>1=Purchased from market/shop/kiosk/wholesaler/street vendor</li> <li>2= Homemade or obtained from local farm or local small factory/processor</li> <li>3= Received from food aid/social</li> </ul>	
		protection program 4 = Other (specify):	
	-	98= Don't know/remember	
1103	Does your household prepare foods using wheat flour at home, such as bread, kita, injera?	1 = Yes 0 = No <b>→Go to 1105</b>	
1104	The last time your household get wheat flour, where did you get it from?	1=Purchased from market/shop/kiosk/wholesaler/street vendor 2= Homemade or obtained from local farm or local small factory/processor	
		<ul> <li>3= Received from food aid/social protection program</li> <li>4 = Other (specify):</li> <li>98= Don't know/remember</li> </ul>	
1105	I would like to check whether the salt used in your household is iodized. May I have a sample of the salt used to cook meals in your household? Test salt for iodine	1=lodine present 2= No iodine 3= Household uses salt but there is no salt in household 4= Household does not use salt 5= Salt not tested, specify reason	II
1106	The last time your household get salt, where did you get it from?	<ul> <li>1=Purchased from market/shop/kiosk/wholesaler/street vendor</li> <li>2= Homemade or obtained from local farm or local small factory/processor</li> <li>3= Received from food aid/social protection program</li> <li>98= Don't know/remember</li> </ul>	

1 2	
3 4	99 = Other (specify):
$\begin{array}{c} 5\\ 6\\ 7\\ 8\\ 9\\ 10\\ 11\\ 12\\ 13\\ 14\\ 15\\ 16\\ 17\\ 18\\ 19\\ 20\\ 21\\ 22\\ 23\\ 24\\ 25\\ 26\\ 27\\ 28\\ 29\\ 30\\ 31\\ 32\\ 33\\ 34\\ 35\\ 36\\ 37\\ 38\\ 39\\ 40\\ 41\\ 42\\ 43\\ 44\\ 45\\ 46\\ 47\\ 48\\ 49\\ 50\\ 51\\ 52\\ 53\\ 54\\ 55\\ 56\\ 57\\ \end{array}$	to peer eview only
58 59 60	28 For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

About	t the household		
1201	Does any member of the household own any agricultural land (purchased or own?	1 = Yes 0 = No ( <b>Go to 1212)</b>	
1202	How many hectares of agricultural land do members of this household own? Note: Convert local land measurement unit into hector after discussing with agriculture focal person/AEW.	Enter total number of hectares (If less than 1, Enter in decimals (example 0.5) Enter 9999 if hectares are not known	
1203	In the past major growing season (Meher) and minor growing (Belg) season, not including the current season, did you work on the family farm?	1 = Yes 0 = No ( <b>Go to 1211)</b>	
	What sort of work did you do on the	1 = Yes 0 =	No
	family farm?	1 = Home (kitchen) gardening	
1204	READ THE LIST	2 = Fieldwork	
1205	READ THE LIST	3 = Cash crop farming	
1206		4 = Producing dairy	
1207		5 = Rearing poultry	
1208		6 = Raising livestock	
1209		7 = Fishpond/ aquaculture	
1210		99 = Other	
1211	Does this household own any livestock, herds, other farm animals, or poultry?	1 = Yes 0 = No → Go to 1221	
	How many of the following animals do	For each: Enter number. If none, e	nter 0
1212	this household own?	Chickens	
1213		Goats	
1214		Sheep	
1215		Donkeys	
1216		Horses	
1217		Mules	
1218		Camels	
1219		Milk cows	
1220		Oxen	

In the past 2 growing seasons (Meher and Belg), not including the current season, please describe all the crops (cereals, legumes, vegetables, fruits, seeds, and other crops) grown on your household farm.

Please also describe all animal source foods (meat, eggs, milk, dairy, fish, other) that you have produced on your household farm in same period.

Write down all crops and animals mentioned by the respondent. When the respondent has finished, probe for crops and animal source foods not mentioned. Then ask about production/yields in the relevant units. Ask the respondent to estimate the amount of the total production that went to sales, food consumption, and storage/losses/other uses.

	Γ				1				
	Group	Crop	Did HH	Season	Amount			ajor seasons (Meher)	
			cultivate	1=Meher		and Min		elg) not including the	
			crop?	2=Belg			current		
			1 = yes	3=Both		Sold	Consumed	Storage, losses,	
			0 = No					animal feed or other	
								uses	
1221	Staples	Maize							
1222		Teff	_					_  _	
1223		Wheat	_						
1224		Barley	_					_  _	
1225		Sorghum	_						
1226		Millet	_			1_11_1			
1227		Rice	_						
1228		Emmer			_				
		wheat							
		(oaths)							
1229		Other	_						
		cereals							
1230	Pulses	Bean	_						
1231	(legumes)	Haricot	_		_			_  _	
		bean							
1232		Lentil	_						
		(Miser)							
1233		Grass pea	_		_				
		(guaya)							
1234		Chickpea							
1235		Field pea	_						
		(Ater)							
1236		Soya bean							

107 of 151				BMJ Op	ben			
1237		Other						
_		legumes			''		11	11
1238		Niger seed						
		(Nug)						
1239		Sunflower	<u> _ </u>					<u> </u>
1240		Sesame	<u> </u>					<u> </u>
1241		Linseed						<u> </u>
1242	Oil Crops	Rapeseed (Gomenzer)	II					1111
1243		Lupine					_  _	
		(Gibto)						
1244		Nuts						_  _
1245		Other oil crops	_					
1246	Root	Cassava	<u> _ </u>					_  _
1247	crops/	Enset						
1248	tubers/	Irish potato						
1249	vegetables	Sweet	IR					
		potato						
		Sweet						
1250		potato -						
		orange flesh						
1251		Onion	<u> </u>					
1251		Pepper	<u> </u>					<u> </u>
1252		Tomato	<u> </u>					<u> </u>
1254		Cabbage	<u> </u>					
1255		Other light	<u> </u>					
		green leafy	''		''		11	11
		vegetables						
1256		Kale						
1257		Other dark						
		green leafy						
1258		vegetables Carrot						
1258		Other roots	II		<u> </u>			<u> </u>
1255		or tubers				''	''	! <u></u> !! <u></u> !
1260		Other	1 1					
		vegetables	II					
1261	Perennial	Coffee						_  _
1262	crops/	Chat (khat)						
1263	fruits	Banana						
1264		Orange						
1265		Mango	_					<u> </u>
1266		Нор						
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1267	Avocado						_  _
1268	Lemon						
1269	Рарауа						
1270	Guava			_			
1271	Water						
	Melon	II		II			
1272	Tirngo fruit						
1273	Other						
	perennial			II			
1274	crops Other fruits						
		<u> </u>	<u> </u>	II			
	Other fruits						

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1	2

(unit) <b>1=yes</b> <b>0=no</b> ( <i>lf no, skip</i> <b>Season</b> <b>1=Meher</b> <b>2=Belg</b> <b>Sold</b> <b>Consumed</b> <b>Storage</b> ,	$ \left  \begin{array}{c c c c c c c c c c c c c c c c c c c $	e	Group	Animal source food	Does HH produce?	Amount	-	previous Majoı (Belg) not inclı		
Image:	Image: series of the			(unit)	-		Season			
eggs       I_I       I_I       I_I_I       I_I_I       I_I_I       I_I_I         1276       Chicken meat       I_I       I_II       I_III       I_IIII       I_IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	eggs       I_I       I_I       I_I_I       I_I_				<u>(If no, skip</u> <u>to the</u>		2=Belg	Sold	Consumed	losses, anima feed or
1276       Chicken meat       I_I       I_I       I_II       I_III       I_III       I_IIII       I_IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	1276       Chicken meat       II       II <td>1275 A</td> <td>All</td> <td></td> <td></td> <td>۱۱</td> <td>  </td> <td>   </td> <td>   </td> <td>   </td>	1275 A	All			۱۱				
1278       Goat meat       II	1278       Goat meat       II	1276		Chicken					_	
1275       Camel milk       II       I	1275       Camel milk       II       I	1277		Goat milk				_	_	_
1280       Sheep meat       II       II       III       III       III       III       III       III       III       IIII       IIIII       IIIII       IIIII       IIIII       IIIII       IIIIII       IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	1280       Sheep meat       II	1278		Goat meat	1			_	_	_
1281       Cow milk       II	1281       Cow milk       II       I	1279		Camel milk				_	_	_
1282     Cow other dairy     II     II     II     II     II       1283     Beef     II     II     II     II     II       1284     Other meat (e.g. camel, horse)     II     II     II     II       1285     Farmed fish     II     II     II     II	1282       Cow other dairy       II	1280		Sheep meat				_	_	_
1283     dairy     II     II     II_I     II_I     II_I       1284     Beef     II     II     III     II_I     III       1284     Other meat (e.g. camel, horse)     II     III     IIII     IIII     IIII       1285     Farmed fish     II     III     IIII     IIII     IIII     IIIII	1283     dairy     II     II     II_I     II_I     II_I     II_I     II_I     II_I     II     <	1281		Cow milk				_		_
1284     Other meat (e.g. camel, horse)     II     II     II     II       1285     Farmed fish     II     II     II     II     II	1284     Other meat (e.g. camel, horse)     II     II     II     II       1285     Farmed fish     II     II     II     II     II	1282								III
(e.g. camel, horse)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  _         _         _         _         _         _         _         _         _         _         _         _         _         _         _         _         _         _         _         _         _         _         _         _         _         _         _  <td>(e.g. camel, horse)                                                                                                                                                                                                                      </td> <td>1283</td> <td></td> <td>Beef</td> <td></td> <td></td> <td>  </td> <td>  _ </td> <td>  _ </td> <td>  _ </td>	(e.g. camel, horse)	1283		Beef				_	_	_
1285         Farmed fish         I_I         I_I_I         I_I_I <thi_i_i< th="">         I_I_I         I_I_I         &lt;</thi_i_i<>	1285         Farmed fish         I_I         I_I <thi_i< th="">         I_I         <th< td=""><td>1284</td><td></td><td>(e.g. camel,</td><td></td><td></td><td>I_I</td><td>   </td><td>   </td><td>111</td></th<></thi_i<>	1284		(e.g. camel,			I_I			111
		1205								



Module 13: Household food insecuri	ty
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	Module 13: Household food insecurity		
Now	I would like to ask you some questions about food. During the la	st 12 MONTHS, was	there a tim
wher	1:		
SN	Questions	Answers	
1301	You or others in your household worried about not having	1=Yes	
	enough food to eat because of a lack of money or other	0= No	
	resources?	98=Don't know	
		97=Refused	
1302	Still thinking about the last 12 MONTHS, was there a time when	1=Yes	
	you or others in your household were unable to eat healthy and	0= No	
	nutritious food because of a lack of money or other resources?	98=Don't know	
		97=Refused	
1303	Was there a time when you or others in your household ate only	1=Yes	
	a few kinds of foods because of a lack of money or other	0= No	
	resources?	98=Don't know	
		97=Refused	
1304	Was there a time when you or others in your household had to	1=Yes	
	skip a	0= No	
	meal because there was not enough money or other resources to	98=Don't know	
	get	97=Refused	
	food?		
1305	Still thinking about the last 12 MONTHS, was there a time when	1=Yes	
	you or others in your household ate less than you thought you	0= No	
	should because of a lack of money or other resources?	98=Don't know	
		97=Refused	
1306	Was there a time when your household ran out of food because	1=Yes	
	of a lack of money or other resources?	0= No	
		98=Don't know	
	4	97=Refused	
1307		1=Yes	
	hungry but did not eat because there was not enough money or	0= No	
	other resources for food?	98=Don't know	
		97=Refused	
1308	Was there a time when you or others in your household went	1=Yes	
	without eating for a whole day because of a lack of money or	0= No	
	other resources?	98=Don't know	
		97=Refused	

	Module 14: Employment and socia	l protection	
S.N.	Questions	Response	
1401	Since last year, what has been the main livelihood or	1 = Sale of self-produced	
	income source of the HH?	2 = horticulture crops Sale of	
		self-produced field crops	
	(DO NOT READ LIST. PROBE FOR ONE RESPONSE)	3 = Own business (including	
		commerce, livestock rearing)	
		4 = Wage employment	
		5 = Remittances	
		6 = Property income	
		7 = Government	
		transfers/NGO support	
		8 = Pension	
		99 = Other	
1402	Since last year, have there been other livelihood or	1 = Sale of self-produced	1 11
1402		-	
	income sources for the HH?	2 = horticulture crops Sale of	
		self-produced field crops	
	(DO NOT READ LIST. PROBE FOR ALL RESPONSES)	3 = Own business (including	
		commerce, livestock rearing)	
		4 = Wage employment	
		5 = Remittances	
		6 = Property income	
		7 = Government	
		transfers/NGO support	
		8 = Pension	
	· · · · · · · · · · · · · · · · · · ·	99 = Other	
1403	Since last year, did anyone in your HH receive any kind of	1 = Yes	
	food or cash assistance from the government, NGO, or	0 = No	
	other agencies? Clarify: This is not formal employment or	98 = Don't know	
	pension. However, it may or may not be conditional on		
	work.		
1404	Since last year, which members of this HH were targeted	1 = All HH members	
	to receive this support?	2 = Specific HH members	
		98 = Don't know	
1405	Which specific HH members received food or cash	Link this back to the HH roster	
	assistance?	and have interviewer select	
	Clarify: This includes children whose parents receive cash	names.	
	on their behalf.	98 = Don't know	
1406	Which of these categories apply to the persons who	1 = Pregnant women	1 11
	received food or cash assistance?	2 = Lactating women	''
		3 = Children under 5 years	
	(READ RESPONSES ALOUD. SELECT ALL THAT APPLY)	4 = Elderly	
		5 = Disabled person	
		6 = None of the above	
1407	Since last year which food as social assistance are sur-		
1407	Since last year, which food or social assistance program	1 = PSNP	'''
	did members of the HH receive support from? (DO NOT	2 = Community Care Coalition	
	READ LIST ALOUD. PROBE FOR ALL RESPONSES)	3 = Other assistance program	
		98 = Don't know	<u> </u>
1408	Since last year, what was the form of assistance that	1 = Cash only transfer	
	members of your HH received form these programs:	2 = Food only transfer	
	food, cash or both food and cash?	3 = Cash and food mix	
		3 = Cash and food mix 99 = Other	

Module 14: Employment and social protection

1409	Is this HH currently receiving food or cash?	1 = Yes	
		0 = No	
		98 = Don't know	
1410	Since last year, how has your HH used the food received?	1 = HH consumption	
	(READ RESPONSES ALOUD. SELECT ALL THAT APPLY)	2 = Sold food for cash	
		3 = Other activities	
		98 = Don't know	

1501.	How do you preserve	1 = Fertilization (Chemicals, animal manure, green manure etc)
	soil fertility?	2 = Crop rotation (Cultivation of a series of dissimilar types of crops in the same area in sequential seasons)
	[Multiple answer is allowed! Do not read the choices. Listen and	3=Intercropping (Cultivation of two or more dissimilar types of crops in the same area in the same season)
	the choices. Listen and mark the one they	4=Tillage
	mention]	99=Other (specify):
1502.	Do you apply fertilizers?	1= Yes
		0= No (Go to 1506)
1503.	Which kinds of fertilizers	1= Chemical fertilizers
	do you use more often?	2 = Organic fertilizers (non-chemicals like animal manure, green manure compost, etc.) (Go to $\rightarrow$ 1505)
1504.	Which chemical	1 = UREA
	fertilizers are used most	2 = DAP
	often, can you specify the type/name?	3= NPS
		98= Don't know
		99= Other (specify):
1505.	are used more often, can you specify the	1 = Livestock manure
		2 = Poultry manure
	type?	99=Other (specify)
1506.	What is the most dominant cereals/crop	1 = Wheat 2 = Teff
	you produce on your	2 = Tell 3 = Maize
	farm?	99= Other (Specify):
	[Only one answer is	33- Other (Specify).
	<i>allowed</i> ! Do not read the choices. Listen,	
	mark/specify the one	
	they mention]	
1507.	How far is your	1= within 500 meter radius
	agricultural land from	2= 500 -1000 meter radius
	your house? [In case they own many	3= 1000 - 3000 meter radius
	farms, Consider only the one with the dominant	4= More than 3000 meter
	crop]	

# Module 15: Soil information questionnaire

# Observational checklist for soil sampling

For peer terien ony

r a (	ae i 15 of il	51 BMJ Open
	ge 115 of 1 1	Region
1	2	Zone
2	3	Woreda
3 - 4	4	Kebele
5 6	5	Gote Code
7	6	Household Code
9		Sample code
10 11	7	Crop history
12 13	7.1	Last two-year crop (Please, specify the crop harvested in 2011, growing season)
14 15	7.2	Last crop (Please, specify the crop harvested from the previous growing season)
16	7.3	Crop to be planted for the current season
17 18	7.4	Please write the intended planting dates (Year and Month)
19 20	7.5	When was the last time the agricultural field gets tilled?
21		1 = 0 to 3 months ago
22 23		2 = 3 to 6 months ago
24 25		1 = 6 to 12 months ago
26 27		2 = before a year
28	8	Fertilizer utilization
29 30	8.1	Which fertilizer is applied
30 31	8.1	Which fertilizer is applied     1 = Chemical fertilizer
30 31 32 33	8.1	1 = Chemical fertilizer 2 = Organic fertilizer (Go to 8.4)
30 31 32 33 34 35	8.1	1 = Chemical fertilizer         2 = Organic fertilizer (Go to 8.4)         3 = Both 1&2
30 31 32 33 34 35 36 37	8.1	1 = Chemical fertilizer         2 = Organic fertilizer (Go to 8.4)         3 = Both 1&2         4 = Fertilizer not applied (Go to 9)
30 31 32 33 34 35 36 37 38	8.1	1 = Chemical fertilizer         2 = Organic fertilizer (Go to 8.4)         3 = Both 1&2
30 31 32 33 34 35 35 37 38 39 40		1 = Chemical fertilizer         2 = Organic fertilizer (Go to 8.4)         3 = Both 1&2         4 = Fertilizer not applied (Go to 9)
30 31 32 33 34 35 36 37 38 40 41 42		1 = Chemical fertilizer         2 = Organic fertilizer (Go to 8.4)         3 = Both 1&2         4 = Fertilizer not applied (Go to 9)         Which Chemical fertilizer is applied         1 = UREA         2 = DAP
30 31 32 33 34 35 36 37 38 39 40 4		1 = Chemical fertilizer         2 = Organic fertilizer (Go to 8.4)         3 = Both 1&2         4 = Fertilizer not applied (Go to 9)         Which Chemical fertilizer is applied         1 = UREA         2 = DAP         3 = NPS
30 32 33 34 35 37 38 39 40 42 43 44 43 45		1 = Chemical fertilizer   2 = Organic fertilizer (Go to 8.4)   3 = Both 1&2   4 = Fertilizer not applied (Go to 9)   Which Chemical fertilizer is applied   1 = UREA   2 = DAP   3 = NPS   4 = Other, please specify
30 32 33 35 36 37 38 40 42 44 44 45 47		1 = Chemical fertilizer         2 = Organic fertilizer (Go to 8.4)         3 = Both 1&2         4 = Fertilizer not applied (Go to 9)         Which Chemical fertilizer is applied         1 = UREA         2 = DAP         3 = NPS         4 = Other, please specify         Please write the last date (Year and Month) you applied chemical fertilizer?
301233456789001424444444444444444444444444444444444	8.2	1 = Chemical fertilizer         2 = Organic fertilizer (Go to 8.4)         3 = Both 1&2         4 = Fertilizer not applied (Go to 9)         Which Chemical fertilizer is applied         1 = UREA         2 = DAP         3 = NPS         4 = Other, please specify         Please write the last date (Year and Month) you applied chemical fertilizer?         Which Organic fertilizer is applied
3 3 3 3 3 5 6 7 8 9 0 1 2 3 3 3 3 3 3 9 0 1 2 3 4 4 4 5 6 7 8 9 0 1 2 3 3 3 3 4 4 4 5 6 7 8 9 0 1 2 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 3 3 3 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 3 3 3 3 3 3 3 3 3 4 4 5 6 7 8 9 0 1 2 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3	8.2	1 = Chemical fertilizer         2 = Organic fertilizer (Go to 8.4)         3 = Both 1&2         4 = Fertilizer not applied (Go to 9)         Which Chemical fertilizer is applied         1 = UREA         2 = DAP         3 = NPS         4 = Other, please specify         Please write the last date (Year and Month) you applied chemical fertilizer?         Which Organic fertilizer is applied         1 = Animal Manure
3 0 1 2 3 3 3 5 6 7 8 9 0 1 2 3 4 4 5 6 7 8 9 0 1 2 3 4 4 5 6 7 8 9 0 1 2 3 4 4 5 5 2 5 2 5 2 5 2 5 2 5 2 5 2 5 2 5	8.2	1 = Chemical fertilizer         2 = Organic fertilizer (Go to 8.4)         3 = Both 1&2         4 = Fertilizer not applied (Go to 9)         Which Chemical fertilizer is applied         1 = UREA         2 = DAP         3 = NPS         4 = Other, please specify         Please write the last date (Year and Month) you applied chemical fertilizer?         Which Organic fertilizer is applied         1 = Animal Manure         2 = Green Manure
3 0 1 2 3 3 3 5 6 7 8 9 0 1 2 3 4 4 5 6 7 8 9 0 1 2 3 4 4 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	8.2	1 = Chemical fertilizer         2 = Organic fertilizer (Go to 8.4)         3 = Both 1&2         4 = Fertilizer not applied (Go to 9)         Which Chemical fertilizer is applied         1 = UREA         2 = DAP         3 = NPS         4 = Other, please specify         Please write the last date (Year and Month) you applied chemical fertilizer?         Which Organic fertilizer is applied         1 = Animal Manure
3 3 3 3 3 3 3 3 3 4 4 4 4 4 4 4 4 4 4 5 5 5 5	8.2	1 = Chemical fertilizer         2 = Organic fertilizer (Go to 8.4)         3 = Both 1&2         4 = Fertilizer not applied (Go to 9)         Which Chemical fertilizer is applied         1 = UREA         2 = DAP         3 = NPS         4 = Other, please specify         Please write the last date (Year and Month) you applied chemical fertilizer?         Which Organic fertilizer is applied         1 = Animal Manure         2 = Green Manure         3 = Compost         4 = Other, please specify
3 3 3 3 3 5 6 7 8 9 0 1 2 3 4 4 5 6 7 8 9 0 1 2 3 4 5 6 7 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	8.2	1 = Chemical fertilizer         2 = Organic fertilizer (Go to 8.4)         3 = Both 1&2         4 = Fertilizer not applied (Go to 9)         Which Chemical fertilizer is applied         1 = UREA         2 = DAP         3 = NPS         4 = Other, please specify         Please write the last date (Year and Month) you applied chemical fertilizer?         Which Organic fertilizer is applied         1 = Animal Manure         2 = Green Manure         3 = Compost
3 3 3 3 3 3 3 3 3 4 4 4 4 4 4 4 4 4 4 5 5 5 5	8.2 8.2 8.3 8.4	1 = Chemical fertilizer         2 = Organic fertilizer (Go to 8.4)         3 = Both 1&2         4 = Fertilizer not applied (Go to 9)         Which Chemical fertilizer is applied         1 = UREA         2 = DAP         3 = NPS         4 = Other, please specify         Please write the last date (Year and Month) you applied chemical fertilizer?         Which Organic fertilizer is applied         1 = Animal Manure         2 = Green Manure         3 = Compost         4 = Other, please specify

1 2	9.1	Observation: What is the colour of the soil you are about to sample
3		1 = Dark brown/Black
4 5		2 = Red
6 7		3 = Grey
8 9		4 = Other, please specify
10	9.1	Observation: Field area landscape
11 12		1 = plains/level grounds
13 14		2 = Sloppy/Inclined
15	9.4	Observation: Is there a standing crop on the sampling field or to the nearby farmland.
16 17		1 = Yes
18 19		2 = No
20 21	9.3	Observation: Is sampling field tilled/ is it being tilled at the time of sampling.
22		1 = Yes
23 24		2 = No
25 26	10	Distance of the farmland to the nearby houses
27		1 = below 100meter
28- 29		2 = 100 to 500meter
30 31		3 = 500 to 1000meter
32 33		4 = More than 1000meter
34		Please take picture for the surrounding environment i.e., plot, houses, anything permanent or even moving
35 36	11	cattle
37 38	12	Please capture GPS for the sampled farmland
39	13	Name of sample collector
40- 41		LIST ANY ABNORMAL CONDITIONS OR SPECIFIC INFORMATION DESIRED:
42 43		
44 45		
46		
47 48		
49 50		
51		
52 53		
54 55		
56		
57 58		
59		
60		

	for the data collectors: Among the household men hild under-five years of age and women of the repr	•	
Part	1: Before 24-hr recall		
	24-hour c	lietary recall	
	EA code     Household code   _	_  Line Number   _  Child ID   _	
ι	Jnique ID Woman:        Uni	que ID Child:   _ _ _ _ _ _ _	_
Intervie	ew Date: Date// <b>Day -</b> 01=Mon 0	02=Tue 03=Wed 04=Thu 05=Fri 06=Sat	t 07=Sui
	Date of food intake / _ /		
	Question	Coding category	skip
1.	Enumerator Code:		
2.	For which target group is the recall being done?	0. Woman 1. Child	
3.	Recall number	0. Recall 1	
		1. Recall 2	
4.	Name of the woman interviewed	<u></u>	
5.	Age of the woman (in complete years)	Age in years ()	
6.	Name of child		
7	Data of hirth (DOD), Use Ethiopian colondar		
7.	Date of birth (DOB): Use Ethiopian calendar	_ _ / _ _ /20 _ _	
8.	Age of the child (in complete month)	months	
9.	Child's sex:	0=Male 1= Female	
10.			
20.	Food weighing scale number:		
11.	Was yesterday's food intake different from	1=Yes 0=No	No →
	your usual diet?		
12.	If yes	1=Holyday/celebration	
	If yes,	2=I was sick	
		3=Other	
13.	Was [child name] yesterday's food intake	1=Yes	
±0.	1 that forma namely yester day 5 1000 make		No →

	If yes,	1=Holyday/celebration
		2=I was sick
		3=Other
15.		1=Yes
	Did you take medicine/supplement yesterday?	0=No
		If yes, name:
16.		1=Yes
	Did [child name] take medicine/supplement	0=No
	yesterday?	If yes, name:
		· ·

# Procedures to collect the required information

# 1. Pass 1: list all foods and drinks consumed during the 24-hour period.

Now I would like to ask you about the foods and drinks that [YOU/ YOUR CHILD] consumed yesterday from the time you work up until you went to sleep, sunrise yesterday to sunrise today. Please list all foods or drinks you ate, weather you ate or drank them at home or somewhere else. Please think about snacks and small meals as well as main meals.

- 1) "WHAT WAS THE FIRST THING [you/ your child] ATE YESTERDAY AFTER SUNRISE?"
- 2) "WHEN WAS THAT"
- 3) "DID [you/your child] HAVE ANYTHING WITH THAT?
- 4) "WHAT DID [you/he/she] HAVE?"
- 5) "WHAT IS THE NEXT THING [you/ your child] ATE OR DRANK AND WHEN WAS THAT?"
- 6) REPEAT questions 3-5 until you have a full record for both DAY AND NIGHT
  - a. The reference period is from sunrise yesterday to sunrise this morning. If they wake up at a different time than sunrise, you can use the time from waking up yesterday until waking up today

# 2. Pass 2: get more detail about each food.

- 7) "NOW, PLEASE DESCRIBE EACH FOOD [you/ your child] ATE YESTERDAY"
- 8) "WHAT TYPE WAS IT?"
- 9) "WHERE DID YOU GET IT?"
- 10) "WHAT ARE THE INGREDIENTS?"
  - a. Use standard "probes" (probing questions) to get these details for each food.
- 11) "HOW MUCH DID THIS RECIPE MAKE?" or "WHAT WAS THE TOTAL AMOUNT THIS MADE?"
- 12) "HOW WAS The Recipe PREPARED?"
  - a. Identify the cooking methods used (particularly if raw, fermented, or fried in oil).

# 3. Pass 3: estimate the amount consumed of each food on the list

- 13) "HOW MUCH OF [name the first food] DID [you/ your child] CONSUME?"
  - a. Help the mother remember and **estimate the amount** of each food or recipe that her child ate and that she herself ate.

# 14) "WAS ANY LEFT OVER?"

- a. If any food is leftover from what the mother served to the child, enter that amount.
- 15) "PLEASE HELP ME ESTIMATE THE AMOUNT OF FOOD YOU ARE OR USED IN THE RECIPE"

Use following portion size estimation method to estimate the amount of food/ingredient eaten or used in a recipie 1. Direct weight (g) 2.Proxy weight (g) 3. Water (g) 4. Number 5. Other (specify).

# 4. Pass 4: verify everything consumed

a. Quickly read the information back to the respondent, "HAVE I FORGOTTEN TO ADD ANYTHING?"

Part 2. Quick list

# Pass 1

Please describe the foods the foods and drinks that [YOU/ YOUR CHILD] consumed yesterday from the time you work up until you went to sleep (sunrise yesterday to sunrise today). Please list all foods or drinks you ate, weather you ate or drank them at home or somewhere else. Please think about snacks and small meals as well as main meals.

Write down all foods and drinks mentioned. When composite dishes are mentioned, ask for the list of ingredients

When the respondent has finished, probe for meals and snacks not mentioned.

Early morning	Mid-morning	Noon	Afternoon	Evening	Late evening
		Ô.			
			D,		
			5.		
			0		
			2		
			C		
				4	

to beet terien only

	What was the first			How was	Place of	0	þ		u		Recipe	informatio	n
Foo d No.	thing [YOU/ YOUR CHILD] ate or drank after sunrise yesterday? Any else?	Time of meal	Please describe this food / beverage/ ingredient:	this prepared ?	preparat ion	How was the food / Ing.	Amount served	Amount serve Amount left over	Amount eaten	State of each	Cooking method of	Total amount of recipe	Links to food/
1													
	Ingredient:		Description										
					NA								
			10		NA								
					NA								
					NA								
					NA								
2					),								
					NA							_	
					NA							-	
					NA							-	
					NA							-	
					NA			0					
3													<b>_</b>
					NA							-	
					NA							-	
					NA							-	
					NA							-	
					NA								

Time of meal: 1. Early morning 2. Mid-Morning 3. Noon 4. Afternoon 5. Evening 6. Late evening

Place of preparation: 1. Home 2. Outside home

How was it prepared: 1=raw/ no change/ as purchased; 2=fermented; 3=fried; 04=cooked or boiled – wet heat; 5=baked/grilled/ broiled – dry heat; 6=local miller; 7=blanched (dipped in boiling water); 8=other For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

What was the first			How was		0	þ		ç		Recipe	information	1
thing [VOLL/ VOLIB	Time of meal	Please describe this food / beverage/ ingredient:			How was the food / Ing.	Amount serve	Amount left over	Amount eate	State of each	Cooking method of	Total amount of recipe	Links to food/
Ingredient:		Description										
			1	NA								
			r k	NA								
				NA	-						-	
											-	
				NA								
				NA							-	
				NA								
				NA							-	
							-				-	
				NA								
				NIA								
 											-	
							-				-	
	CHILD] ate or drank after sunrise yesterday? Any else?	thing [YOU/ YOUR CHILD] ate or drank after sunrise yesterday? Any else?	thing [YOU/ YOUR CHILD] ate or drank after sunrise yesterday? Any else?       Time of meal       Please describe this food / beverage/ ingredient:         Vesterday? Any else?       Image: Comparison of the second	What was the first thing [YOU/ YOUR CHILD] ate or drank after sunrise yesterday? Any else?Time of mealPlease describe this food / beverage/ ingredient:this prepared ?Image: What was the first thing [YOU/ YOUR CHILD] ate or drank after sunrise yesterday? Any else?Time of mealPlease describe this food / beverage/ ingredient:this prepared ?	What was the first thing [YOU/ YOUR CHILD] ate or drank after sunrise yesterday? 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Any else?Please describe this food / beverage/ ingredient:this prepared ?prepared sionge y yge y 	What was the first thing [YOU/ YOUR CHILD] ate or drank after sunrise yesterday? Any else?Please describe this food / beverage/ ingredient:prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared ?prepared 	What was the first thing [YOU/ YOUR CHILD] ate or drank after sunrise yesterday? Any else?       Please describe this food / beverage/ ingredient:       this prepared ?       prepared ion       and after sunrise yesterday? Any else?       and beverage/ ingredient:       fine prepared ?       and sunrise       and       and sunrise       and <td>What was the first thing [YOU/YONR CHILD] are of meal after sunrise yesterday? Any else?       Please describe this food / beverage/ ingredient:       this prepared ?       prepared ?</td> <td>What was the first thing  YOU/ YOUR CHILD] ate or drank after sunrise yesterday? 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What was the first	Time of	Please describe this food /	How was	Place of	тοЭ	4 E 0	<b>4</b> E 0	4 E 0	F	Recipe i	nformation	)
				NA								

Time of meal: 1. Early morning 2. Mid-Morning 3. Noon 4. Afternoon 5. Evening 6. Late evening

Place of preparation: 1. Home 2. Outside home

 How was it prepared: 1=raw/ no change/ as purchased; 2=fermented; 3=fried; 04=cooked or boiled – wet heat; 5=baked/ grilled/ broiled – dry heat; 6=local miller; 7=blanched (dipped in boiling water); 8=other

For peer review only

	IDENTIF	ICATION	
нно	00. CLUSTER (EA) NAME	HH01. CLUSTER NUMBER:	
HH0	2. HH NUMBER:	HH03. RESIDENCE (RURAL=1, URBAN=2):	
	04. RESPONDENT LINE NUMBER:	HH05 CHILD LINE NUMBER	
нно	06. INTERVIEWER NAME	HH07. TEAM LEADER, NAME:	
	CODE:	CODE:	
HH0	08. SUPERVISOR NAME: CODE		
PAR [.]	SCHOOL CHILDREN 6-59 MONTHS OLD	bout your child. Fill or Circle the correct answer	
PAR I wo	T I: CHILD HEALTH QUESTIONS uld like to ask you some health and food questions a	2.	
PAR [®] I wo S.N	T I: CHILD HEALTH QUESTIONS uld like to ask you some health and food questions a	Response	
PAR I wo S.N	T I: CHILD HEALTH QUESTIONS uld like to ask you some health and food questions a Questions What is the birth date of the child? In day/month	Response         / year (How many months       Birth Date:         (Day/Month/Year)	
<b>PAR</b> I wo <b>S.N</b> 1	T I: CHILD HEALTH QUESTIONS uld like to ask you some health and food questions a Questions What is the birth date of the child? In day/month old is this child?) NOTE FOR INTERVIEWERS	Response         / year (How many months       Birth Date:	
PAR [.]	T I: CHILD HEALTH QUESTIONS uld like to ask you some health and food questions a Questions What is the birth date of the child? In day/month old is this child?) NOTE FOR INTERVIEWERS (Screening question to verify that the date of birth	Response         / year (How many months       Birth Date:	

# PART II: CHILD BIOCHEMICAL MEASUREMENT

(Y OR N)	
PL03 Code for Laboratory Technician:	Lab Tech Name
<b>PL04</b> BLUE TOP TUBE (METAL FREE) Not collected =00.0 Refused = 77.7	ML.
PL05 PURPLE TOP TUBE (EDTA) Not collected =00.0 Refused = 77.7	
PLO6 RED TOP TUBE (EDTA) Not collected =00.0 Refused = 77.7	
PL07 Date blood sample taken (Ethiopian Day/Month/Year)	Date:/ Day / Month / Year
PL08 TIME BLOOD DRAW (Ethiopian time)	Blood draw: Hour Minute
PL09 When did you eat your most recent meal (food (Ethiopian time)	)?:: Hour Minute
PL10 MALARIA RESULTS (RDK)	NEGATIVE0 POSITIVE P FALCIPARUM1
	Positive P VIVAX2 INVALID3
PL11 FEVER in last 24 HR?	NO0 YES1
PL12 HEMOGLOBIN RESULTS	g/dL
you can provide this now, we appreciate it. If not no INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL:	I, we would like to collect a stool sample from your child. ow, we can come back to pick up the sample at a later tim ool. We would like the freshest stool you can give us. Plea
PL13 STOOL COLLECTED?	NO0
PL14 Date stool sample taken (Ethiopian Day/Month/Year)	Date:/ Day / Month / Year
PL15 TIME: STOOL COLLECTED (Ethiopian time)	

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	Hour	Minute
<b>PL16</b> TIME: STOOL PASSED, Ethiopian time (as recorded on cup)		:
	Hour	Minute
PL17 Time Blood centrifuged (Ethiopian time)	:	
	Hour	Minute

Thank you for completing this interview.

# INTERVIEWER'S OBSERVATIONS TO BE FILLED IN AFTER COMPLETING INTERVIEW

COMMENTS	ABOUT	RESPO	NDFNT:
COMMENTS	ADOUT		

# SCHOOL AGE CHILDREN 6-12 YEARS ETHIOPIAN FOOD AND NUTRITION STRATEGY BASELINE SURVEY 2020/21 Biochemical and Health Related Data Collection Tool

IDENTIFICATION	
SG01. CLUSTER NUMBER:	
SG02. HH NUMBER:	4
<b>SG03</b> . RESPONDENT LINE NUMBER: (SHOULD BE MOTHER/CAREGIVER)	
SG04 SCHOOL CHILD LINE NUMBER	

In general, for children 6-10 years of age: get parental report (ask the questions of the caretaker and enter the child's name into the parentheses) For children 11-12 years of age who are present and can provide information: get self-report (ask

questions directly of the child and enter "you" or "yourself" into the parentheses)

# PART I: CHILD HEALTH RELATED QUESTIONS

No.	QUESTION	CODING CATEGORIES		SKIP
<b>S1</b>	How old are you/is your child?	Years		
	(Verify that the age is the same age as written on the household listing)			
<b>S2</b>	Have you/ your child ever attended school?	No	00	<b>00 →</b> 54
		Yes	01	
<b>S</b> 3	What is the highest level of school (name of child) completed?	None0		
		Primary1		

# PART II: CHILD BIOCHEMICAL MEASUREMENT

Verbal consent given for: SL01 Blood SL02 Urine	sLO3 Stool
0= No OR 1= yes	
SL04 Phlebotomist Code	
SL5 BLUE TOP TUBE (METAL FREE)	
Did not work =00.0	ML.
Refused = 77.7	•
SL6 PURPLE TOP TUBE (EDTA)	
Did not work =00.0	ML.
Refused = 77.7	
SL7 REDTOP TUBE (EDTA)	
Did not work =00.0	ML.
Refused = 77.7	
<b>SL8</b> DATE BLOOD SAMPLE TAKEN (Ethiopian calendar)	Date://
	Day / Month / Year
SL9 TIME BLOOD DRAW (Ethiopian time)	Blood draw::
	Hour Minute
SL10 When did you eat your most recent meal (food)?	Last Meal Eaten : :
(Ethiopian time)	
	Hour Minute
<b>SL11</b> FEVER in last 24 HR? (Since same time yesterday)	No00
	Yes01
SL12 MALARIA RESULTS (RDK)	NEGATIVE00
	POSITIVE P falciparum01
	POSITIVE P vivax02
	INVALID03

g/dL

2	
3 4 5	SL13 HEMOGLOBIN RESULTS
6 7 8 9	SL14 Is that finger prick or ver
10 11 12 13	In order to determine if you sample. If you can provide that a later time.
14 15	SL15 Urine collected?
16 17 18	SL16 Blood in urine RESULTS
19 20	SL17 Stool collected?
21 22 23 24	<b>SL18</b> Date and <b>t</b> ime when sto respondent (as recorded on c
24 25 26 27	SL19 Date stool sample taken
28 29 30	<b>SL20</b> Time when stool collector (Ethiopian time)
31 32 33 34	SL21 TIME BLOOD centrifuged
35 36 37 38	Thank the respondent and tell
39 40 41 42	TO COMMENTS:
43 44	
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<b>L14</b> Is that finger prick or venous sample taken?	Finger prick00
	Venous01
n order to determine if you have blood in urine or wori	ms in stool we would like to collect a urine and stool
ample. If you can provide this now, we appreciate it.	If not now, we can come back to pick up the sample
at a later time.	
L15 Urine collected?	No00
	yes01
SL16 Blood in urine RESULTS	Negative00
$\mathbf{O}$	positive01
SL17 Stool collected?	No00
	yes01
<b>L18</b> Date and time when stool passed by the	Date:// and : :
espondent (as recorded on cup) (Ethiopian time)	
	Day / Month /Year Hour Minute
<b>L19</b> Date stool sample taken (Ethiopian calendar)	Date://
	Day / Month / Year
<b>SL20</b> Time when stool collected from the respondent	:
Ethiopian time)	
	Hour Minute
<b>5L21</b> TIME BLOOD centrifuged (Ethiopian time)	: :
×	
	Hour Minute

ne respondent and tell them that the lab team will be arriving later.

# **INTERVIEWER'S OBSERVATIONS** TO BE FILLED IN AFTER COMPLETING INTERVIEW

# ADELESCENT GIRLS (10-19 YEARS) ETHIOPIAN FOOD AND NUTRITION STRATEGY BASELINE SURVEY 2020/21 Biochemical and Health Related Data Collection Tool

IDENTIFICATION			
HH00. CLUSTER (EA) NAME	HH01. CLUSTER NUMBER:		
HH02. HH NUMBER:	HH03. RESIDENCE (RURAL=1, URBAN=2):		
HH04. RESPONDENT LINE NUMBER: (SHOULD BE MOTHER/CAREGIVER)	HH05 WOMEN LINE NUMBER		
HH06. INTERVIEWER NAME	HH07. TEAM LEADER, NAME:		
CODE:	CODE:		
HH08. SUPERVISOR NAME:			
CODE			

# PART I: HEALTH RELATED QUESTIONS

I would like to ask you some health and food questions about yourself. Fill or Circle the correct answer

No.	Question	Coding categories	Skip
1	How old are you? (verify that the age is the same age as written on the household listing)	Years	
2	Have you been diagnosed with anemia in the past six months?	No0 Yes1	
<u>3</u>	Do you smoke? (do not include the powder and chew type)	Don't know98 No0 - Yes1	

#### PART II: ADOLESCENT BIOCHEMICAL MEASUREMENT

Consent given for: 0= No or 1= Yes	AG <b>01</b> Blood	AG <b>L02</b> Stool	
AG03 BLUE TOP TUBE Did not work =00.0	(METAL FREE)		ML.

# Page 131 of 151

AG04 PURPLE TOP TUBE (EDTA)	
Did not work =00.0	ML.
Refused = 77.7	
AG05 REDTOP TUBE (EDTA)	
Did not work =00.0	ML.
Refused = 77.7	
AG06 Date blood sample taken (Ethiopian calendar)	Date://
	David Marth ( ) Year
AG07 TIME BLOOD DRAW (Ethiopian time)	Day / Month / Year Blood draw :
AGO TIME BLOOD DRAW (Ethiopian time)	
	Hour Minute
AG08 When did you eat your most recent meal (food)?	
(Ethiopian date and time)	
	Date /Month/ Year Hour Minute
AG09 Is it Finger prick or venous blood sample taken?	01 Finger prick
rees is it miger prick of venous slood sumple taken.	
	02 Venous
AG09 MALARIA RESULTS (RDT)	NEGATIVE
	POSITIVE P falciparum
	POSITIVE P vivax
	POSITIVE FOR BOTH P falciparum and P vivax
	INVALID
	04
AG10 HEMOGLOBIN RESULTS	g/dL
	•
In order to determine if you have worms in the stool we	would like to collect a stool sample. If you can prov
this now, we appreciate it. If not now, we can come back	to pick up the sample at a later time.
INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL:	
For stool: We will return tomorrow to pick up your stool.	We would like the fresh stool you can give us. Please
1 1 7	
one cup to collect the first stool you pass.	
	No
one cup to collect the first stool you pass.	No yes
one cup to collect the first stool you pass.	
one cup to collect the first stool you pass.           AG11 Stool collected?	yes
one cup to collect the first stool you pass.           AG11 Stool collected?	yes
one cup to collect the first stool you pass.           AG11 Stool collected?	yes Date:/ Day / Month / Year
one cup to collect the first stool you pass.          AG11 Stool collected?         AG12 Date stool sample taken (Ethiopian calendar)	yes Date:/ Day / Month / Year
one cup to collect the first stool you pass.          AG11 Stool collected?         AG12 Date stool sample taken (Ethiopian calendar)         AG13 Time when stool passed by the respondent (as recording the respondent for the	yes Date:/ Day / Month / Year
one cup to collect the first stool you pass.          AG11 Stool collected?         AG12 Date stool sample taken (Ethiopian calendar)         AG13 Time when stool passed by the respondent (as recording the respondent for the	yes           Date:        /           Day / Month / Year           'ded on cup)        :           Hour         Minute
one cup to collect the first stool you pass.          AG11 Stool collected?         AG12 Date stool sample taken (Ethiopian calendar)         AG13 Time when stool passed by the respondent (as record (Ethiopian time)	yes           Date:        /           Day / Month / Year           'ded on cup)        :           Hour         Minute

BMJ Open

		Ηοι	ır Minute	
AG15	FIME BLOOD centrifuged (Ethiopian time)			
		Hou	: ur Minute	
			in Minute	
	OBSE	RVATIONS		
		AFTER COMPLETING INT	ERVIEW	
CON	MMENTS:			
	O,			
		CTIVE AGE 15-49 YEAR C		
	ETHIOPIAN FOOD AND NUTRITION Biochemical and Health		-	
DENTIFIC				
IHOO CI	.USTER (EA) NAME		HH01. CLUSTER NUMBER:	
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<b>ноз</b> ни	HNUMBER:	HH03. RESIDENCE (RURAL=1,		
1102.111		URBAN=2):		
	SPONDENT LINE NUMBER:	HH05 WOMEN LINE	NUMBER	
SHOULD	BE MOTHER/CAREGIVER)			
IH06. IN	TERVIEWER NAME	HH07. TEAM LEADER, NAME:		
COI	DE:	CODE:		
IH08. SL	IPERVISOR NAME:		77	
COI	DE			
DAR	T I: HEALTH RELATED QUESTIONS			
S.N	QUESTION		Response	SKIP
1	How old are you?			
(verify that the age is the same age as written on the household Years		Years		
Now	<i>listing</i> ) I would like to ask you some questions about	vour health I will first a	k you about the last f	5 months
2	Have you been diagnosed with anemia in the		No	
				50
				56

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		Yes1 Don't know98
2	Have you been ill with malaria in the past 2 weeks?	No0
		Yes1 Don't know98
3	Do you smoke? (do not include the powder and chew type)	No0
4	Are you currently lactating?	Yes1 No0
		Yes1
5	During that last pregnancy (that resulted in a live birth) did you have difficulty with your vision at night ("Dafent" night blindness in local	No0
	language)?	Yes1 Don't know98
6	In the first two months after delivery, did you receive a	No0
	vitamin A dose (like this)?	Yes1 Don't know
	SHOW THE CAPSULE	

# PART II: WOMEN BIOCHEMICAL MEASUREMENT

SHOW THE CAPSULE	2.
PART II: WOMEN BIOCHEMICAL MEASUREMEN	
	.02 Urine WL03 Stool
WL4 BLUE TOP TUBE (METAL FREE) Did not work =00.0 Refused = 77.7 Pregnant = 99.9	ML.
WL5 PURPLE TOP TUBE (EDTA) Did not work =00.0 Refused = 77.7 Pregnant = 99.9	ML.
WL6 REDTOP TUBE (EDTA) Did not work =00.0 Refused = 77.7 Pregnant = 99.9	ML.
WL7 Date blood sample taken (Ethiopian calend	dar) Date://

	Day / Month / Year
WL8 TIME BLOOD DRAW (Ethiopian time)	Blood draw : :
• •	
	Hour Minute
WL9 When did you eat your most recent meal (food)?	· · _ · _ · _ · _ · _ · _ · _ · _ ·
(Ethiopian date and time)	
	Date /Month/ Year Hour Minute
WL 10 Finger prick or venous sample taken?	01 Finger prick
	02 Venous
WL11 MALARIA RESULTS (RDT)	NEGATIVE
	POSITIVE P falciparum         0           POSITIVE P vivax         02
	POSITIVE FOR BOTH P falciparum and P vivax
	03
	INVALID
	04
WL12 HEMOGLOBIN RESULTS	
stool sample. If you can provide this now, we appreciate at a later time. INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL: For stool: We will return tomorrow to pick up your stool.	
stool sample. If you can provide this now, we appreciate at a later time. INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL: For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass.	we would like the fresh stool you can give us. Please
<ul> <li>stool sample. If you can provide this now, we appreciate at a later time.</li> <li>INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL:</li> <li>For stool: We will return tomorrow to pick up your stool.</li> <li>use one cup to collect the first stool you pass.</li> <li>For urine: We will return tomorrow to pick up your urine.</li> </ul>	e it. If not now, we can come back to pick up the same we would like the fresh stool you can give us. Please
stool sample. If you can provide this now, we appreciate at a later time. INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL: For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass.	We would like the fresh stool you can give us. Please
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stool sample. If you can provide this now, we appreciate at a later time. INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL: For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass. For urine: We will return tomorrow to pick up your urine. WL13 Urine collected? WL14 RESULTS (blood in urine)	e it. If not now, we can come back to pick up the same         We would like the fresh stool you can give us. Please         No
<ul> <li>stool sample. If you can provide this now, we appreciate at a later time.</li> <li>INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL:</li> <li>For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass.</li> <li>For urine: We will return tomorrow to pick up your urine.</li> <li>WL13 Urine collected?</li> <li>WL14 RESULTS (blood in urine)</li> <li>Ask the women if she is Menstruating (Don't test if the women is in Menstruation)</li> </ul>	e it. If not now, we can come back to pick up the same         We would like the fresh stool you can give us. Please         No01         No01         Negative
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<ul> <li>stool sample. If you can provide this now, we appreciate at a later time.</li> <li>INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL:</li> <li>For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass.</li> <li>For urine: We will return tomorrow to pick up your urine.</li> <li>WL13 Urine collected?</li> <li>WL14 RESULTS (blood in urine)</li> <li>Ask the women if she is Menstruating (Don't test if the women is in Menstruation)</li> </ul>	e it. If not now, we can come back to pick up the sam         We would like the fresh stool you can give us. Please         No01         0       yes        01         Negative
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<ul> <li>stool sample. If you can provide this now, we appreciate at a later time.</li> <li>INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL:</li> <li>For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass.</li> <li>For urine: We will return tomorrow to pick up your urine.</li> <li>WL13 Urine collected?</li> <li>WL14 RESULTS (blood in urine)</li> <li>Ask the women if she is Menstruating</li> <li>(Don't test if the women is in Menstruation)</li> <li>WL15 Stool collected?</li> <li>WL16 Date stool sample taken (Ethiopian calendar)</li> <li>WL17 Time when stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent (as recompared to the stool passed by the respondent to the stool passed by the respondent (as recompared to the stool passed by the respondent to t</li></ul>	e it. If not now, we can come back to pick up the same         We would like the fresh stool you can give us. Please         No00         0       yes
<ul> <li>stool sample. If you can provide this now, we appreciate at a later time.</li> <li>INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL:</li> <li>For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass.</li> <li>For urine: We will return tomorrow to pick up your urine.</li> <li>WL13 Urine collected?</li> <li>WL14 RESULTS (blood in urine)</li> <li>Ask the women if she is Menstruating</li> <li>(Don't test if the women is in Menstruation)</li> <li>WL15 Stool collected?</li> <li>WL16 Date stool sample taken (Ethiopian calendar)</li> </ul>	e it. If not now, we can come back to pick up the same         We would like the fresh stool you can give us. Please         No00         0       yes
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<pre>stool sample. If you can provide this now, we appreciate at a later time. INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL: For stool: We will return tomorrow to pick up your stool. use one cup to collect the first stool you pass. For urine: We will return tomorrow to pick up your urine. WL13 Urine collected? WL14 RESULTS (blood in urine) Ask the women if she is Menstruating (Don't test if the women is in Menstruation) WL15 Stool collected? WL16 Date stool sample taken (Ethiopian calendar) WL17 Time when stool passed by the respondent (as record) (Ethiopian time)</pre>	e it. If not now, we can come back to pick up the same         We would like the fresh stool you can give us. Please         No00       yes         0       yes

	Hour Minute
WL19 TIME BLOOD centrifuged (Ethiopian time)	
	· · · · · · · · · · · · · · · · · · ·
	Hour Minute
<u>OBSERVA</u>	LIONS
	COMPLETING INTERVIEW
COMMENTS:	

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# Supplementary consent 1

1. Informed Consent Form for Household Head

# Ethiopian Food and Nutrition Strategy (FNS) Baseline Survey

Investigator(s): Dr.Masresha Tessema (PI), Meseret W/Yohannes, Dr. Meron Girma, Alemnesh Petros, Dr Aregash Samuel, Arnaud Laillou, Stanley Chitekwe, Kaleab Baye, Ramadhani Noor, Anne Sophie Donze and other co-authors

You are being invited to take part in this research because you are head of household. There are about [16596] households taking part in this research. We will ask you about household characteristics, and socio-economic status of your household. We will also assess dietary intake, anthropometric status, nutrition sensitive and specific indicators and micronutrient status of your household member.

# Box 1. Taking part in this research is voluntary

You **can refuse** to take part in this study.

□You **can withdraw** your participation from the study at any time

# Information related to the study

The FNS baseline survey will be conducted in the 12 regions of Ethiopia. The study population will be children age 0-59 months having caregivers/mothers, school-age children (6-12 years), adolescent girls (10-19 years), reproductive-age women (15-49 years), pregnant and lactating women, and household head. The indicators that will be collected for the survey will be dietary intake, anthropometric status, nutrition sensitive and specific indicators and micronutrient status.

The expected possible adverse effects: there is no adverse effect by participating in this study

The objective of this research: to produce information on anthropometric status, dietary

intakes, and micronutrient status of different population groups in Ethiopia, and assess the

coverage of direct and indirect nutrition interventions.

Study design: A population-based, cross-sectional design

The schedule of the study: The study will be conducted from July, 2021 to April, 2023

# Foreseeable risks and expected benefits arising from participation in the study

Foreseeable risks	Expected benefits
Risks to study participants for involvement in the	The findings of the study will help the ministry of
coverage survey are low. There may be risks associated	health and other stakeholders engaged in nutrition

with COVID pandemic. Interviewers will be tr	rained to to improve and/or design appropriate heal
minimize this risk and will use appropriate pr	
measures.	
Occurrences that may take place during the st Occurrences	udy period How to manage
	n such a case, we would respect the volunteer's decision
	withdraw and also get a clear understanding of the reasor heir withdrawal
At the end of the study, you will not be	e receiving any financial benefits, but will get your resu
height, weight, mid upper arm and wais	st circumference measurements, anemia and goiter stat
time you spent and participation.	
All data collected from the study will be k	ept confidential. If you have any questions related to the
you may contact directly Dr. Masresha Te	essema who is the project PI.
The contact persons	
1. Dr. Masresha Tessema Tel. [+251 9197	782082] E-mail: [masresha88@gmail.com] or
2. [Mr. Ibrahim Kedir] Tel. [+251 9119571	61] EPHI'S IRB
	Certificate of Consent
I have read the foregoing information. I	have an I confirm that the participant was giver
opportunity to ask questions and all m	ny quest opportunity to ask questions about the study
have been answered to my satisfa	
volunteer to give consent to participate in	
research study	voluntarily
Printed name of the participant	
	Printed name of the person taking the consen
Signature of the participant	
	Signature of the person taking the consent
Date	Date
day/month/year	day/month/year

2. Informed Consent Form for Women of Reproductive Age

# Ethiopian Food and Nutrition Strategy (FNS) Baseline Survey Investigator(s): Dr.Masresha Tessema (PI), Meseret W/Yohannes, Dr. Meron Girma, Alemnesh Petros, Dr. Aregash Samuel, Arnaud Laillou, Stanley Chitekwe, Kaleab Baye, Ramadhani Noor, Anne Sophie Donze and other co-authors You are being invited to take part in this research because you are women of reproductive age. There are [16596] households taking part in this research. We will assess your dietary intake, anthropometric status, nutrition sensitive and specific indicators and micronutrient status Box 1. Taking part in this research is voluntary You **can refuse** to take part in this study. You **can withdraw** your participation from the study at any time Information related to the study The FNS baseline survey will be conducted in the 12 regions of Ethiopia. The study population will be children age 0-59 months having caregivers/mothers, school-age children (6-12 years), adolescent girls (10-19 years), reproductive-age women (15-49 years), pregnant and lactating women, and household head. The indicators that will be collected for the survey will be dietary intake, anthropometric status, nutrition sensitive and specific indicators and micronutrient status. The expected possible adverse effects: there is no adverse effect by participating in this study The objective of this research: to produce information on anthropometric status, dietary intakes, and micronutrient status of different population groups in Ethiopia, and assess the coverage of direct and indirect nutrition interventions. Study design: A population-based, cross-sectional design The schedule of the study: The study will be conducted from July, 2021 to April, 2023 Foreseeable risks and expected benefits arising from participation in the study **Foreseeable risks Expected benefits** The findings of the study will help the ministry of Risks to study participants for involvement in the coverage survey are low. There may be risks associated health and other stakeholders engaged in nutrition with COVID pandemic. Interviewers will be trained to to improve and/or design appropriate health and minimize this risk and will use appropriate prevention nutrition intervention programs in the country. measures.

urrences	How to ma	anage
ndrawal of volunteers from the study	withdraw a	ase, we would respect the volunteer's decision to and also get a clear understanding of the reason for Irawal
At the end of the study, you will not		g any financial benefits, but will get your results f
height, weight, mid upper arm and wa	aist circumf	erence measurements, anemia and goiter status f
time you spent and participation.		
All data collected from the study will be	e kept confi	dential. If you have any questions related to the stu
you may contact directly Dr. Masresha	Tessema wh	o is the project PI.
The contact persons		
1. Dr. Masresha Tessema Tel. [+251 91	9782082] E-	mail: [masresha88@gmail.com] or
2. [Mr. Ibrahim Kedir] Tel. [+251 91195]	7161] EPHI'	s IRB
	Certificate	of Consent
I have read the foregoing information.	I have an	I confirm that the participant was given ar
opportunity to ask questions and all	my quest	opportunity to ask questions about the study and
have been answered to my satis	faction.	all questions have been answered correctly.
		confirm that the consent has been giver
research study		voluntarily
		0
Printed name of the participant		Printed name of the person taking the consent
		Thinked have of the person taking the consent
		Signature of the person taking the consent
		Date
day/month/year		day/month/year
	At the end of the study, you will not height, weight, mid upper arm and w time you spent and participation. All data collected from the study will be you may contact directly Dr. Masresha <b>The contact persons</b> 1. Dr. Masresha Tessema Tel. [+251 91 2. [Mr. Ibrahim Kedir] Tel. [+251 91195] I have read the foregoing information. opportunity to ask questions and all have been answered to my satis volunteer to give consent to participate	In such a c         withdrawa         At the end of the study, you will not be receiving         height, weight, mid upper arm and waist circumf         time you spent and participation.         All data collected from the study will be kept confid         you may contact directly Dr. Masresha Tessema wh         The contact persons         1. Dr. Masresha Tessema Tel. [+251 919782082] E-         2. [Mr. Ibrahim Kedir] Tel. [+251 911957161] EPHI'         Certificate         I have read the foregoing information. I have an         opportunity to ask questions and all my quest         have been answered to my satisfaction. I         volunteer to give consent to participate in this         research study

# 3. Informed Consent Form for Pregnant Women

#### Ethiopian Food and Nutrition Strategy (FNS) Baseline Survey

**Investigator(s):** Dr.Masresha Tessema (PI), Meseret W/Yohannes, Dr. Meron Girma, Alemnesh Petros, Dr Aregash Samuel, Arnaud Laillou, Stanley Chitekwe, Kaleab Baye, Ramadhani Noor, Anne Sophie Donze and other co-authors

You are being invited to take part in this research because you are pregnant women. There are [16596] households taking part in this research. We will assess your, anthropometric status, nutrition sensitive and specific indicators and anemia status

#### Box 1. Taking part in this research is voluntary

You **can refuse** to take part in this study.

□You can withdraw your participation from the study at any time

#### Information related to the study

The FNS baseline survey will be conducted in the 12 regions of Ethiopia. The study population will be children age 0-59 months having caregivers/mothers, school-age children (6-12 years), adolescent girls (10-19 years), reproductive-age women (15-49 years), pregnant and lactating women, and household head. The indicators that will be collected for the survey will be dietary intake, anthropometric status, nutrition sensitive and specific indicators and micronutrient status.

The expected possible adverse effects: There is no adverse effect by participating in this study

**The objective of this research:** to produce information on anthropometric status, dietary intakes, and micronutrient status of different population groups in Ethiopia, and assess the coverage of direct and indirect nutrition interventions.

Study design: a population-based, cross-sectional design	
The schedule of the study: The study will be conducted from July, 2021 to April, 2023	
Foreseeable risks and expected benefits arising from participation in the study	

Foreseeable risks	Expected benefits
Risks to study participants for involvement in the	The findings of the study will help the ministry of
coverage survey are low. There may be risks	health and other stakeholder engaged in nutrition
associated with COVID pandemic. Interviewers	to improve and/or design appropriate health and
will be trained to minimize this risk and will use	nutrition intervention programs in the country.
appropriate prevention measures.	

#### Occurrences that may take place during the study period

Occurrences	How to manage
Withdrawal of volunteers from the study	In such a case, we would respect the volunteer's
	decision to withdraw and also get a clear
	understanding of the reason for their withdrawal
At the end of the study, you will not be receiving	g any financial benefits, but will get your results for
height, weight, mid upper arm circumference me	easurements, anemia and goiter status for time you
spent and participation.	
All data collected from the study will be kept confid	dential. If you have any questions related to the study
you may contact directly Dr. Masresha Tessema wh	no is the PI
The contact persons	
1. Dr. Masresha Tessema	
Tel. [+251 919782082] E-mail: [masresha88@gmail	l.com]
2. [Mr. Ibrahim Kedir] Tel. [+251 911957161]	
Certificate	of Consent
I have read the foregoing information. I have an	I confirm that the participant was given an
opportunity to ask questions and all my quest	opportunity to ask questions about the study and
have been answered to my satisfaction. I	all questions have been answered correctly. I
volunteer to give consent to participate in this	confirm that the consent has been given
research study	voluntarily
Printed name of the participant	0.
	Drinted name of the parson taking the concept
Signature of the participant	Printed name of the person taking the consent
Date	
day/month/year	Signature of the person taking the consent Date
	day/month/year

# 4. Informed Consent Form for Preschool Child

Ethiopian Food and Nutrition Strategy (FNS) Baseline Survey Investigator(s): Dr.Masresha Tessema (PI), Meseret W/Yohannes, Dr. Meron Girma, Alemnesh Petros, Dr

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

**BMJ** Open

Aregash Samuel, Arnaud Laillou, Stanley Chitekwe, Kaleab Baye, Ramadhani Noor, Anne Sophie Donze and

You are being invited to take part in this research because you are either a mother or caregiver who has

a child under the age of 5 years (0-59 months). There are [16596] households taking part in this research. We would collect a sample of your child's dietary information, blood, urine and stool. And, we will also measure your child's height/ length, weight, and mid upper arm circumference. Finally we will assess, your child's eye for bitot spot Box 1. Taking part in this research is voluntary You can refuse to take part in this study. You can withdraw your participation from the study at any time Information related to the study The FNS baseline survey will be conducted in the 12 regions of Ethiopia. The study population will be children age 0-59 months having caregivers/mothers, school-age children 6-12 years), adolescent girls (10-19 years), reproductive-age women (15-49 years), pregnant and lactating women, and household head. The indicators that will be collected for the survey will be dietary intake, anthropometric status, nutrition sensitive and specific indicators and micronutrient status. The expected possible adverse effects: there is no adverse effect by participating in this study The objective of this research: to produce information on anthropometric status, dietary intakes, and micronutrient status of different population groups in Ethiopia, and assess the coverage of direct and indirect nutrition interventions. Study design: a population-based, cross-sectional design The schedule of the study: The study will be conducted from July, 2021 to April, 2023 Foreseeable risks and expected benefits arising from participation in the study **Foreseeable risks** Expected benefits Risks to study participants for involvement in the The findings of the study will help the ministry of coverage survey are low. There may be risks health and other stakeholder engaged in nutrition to associated with COVID pandemic. Interviewers will improve and/or design appropriate health and be trained to minimize this risk and will use nutrition intervention programs in the country. appropriate prevention measures. Occurrences that may take place during the study period Occurrences How to manage

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other co-authors

Nithdrawal of volunteers from the study	in such a case, we would respect the volunteer
	decision to withdraw and also get a clea
	understanding of the
	reason for their withdrawal
At the end of the study, you will not be receiv	ing any financial benefits, but will get your results for
height/length, weight, mid upper arm circumfere	ence measurements, and anemia for time you spent and
participation.	
All data collected from the study will be kept con	fidential. If you have any questions related to the study
you may contact directly Dr. Masresha Tessema	who is the project principal investigator
The contact persons	
1. Dr. Masresha Tessema	
Tel. [+251 919782082] E-mail: [masresha88@gm	ail.com]
2. [Mr. Ibrahim Kedir] Tel. [+251 911957161] EPH	ll's IRB
Certificat	te of Consent
I have read the foregoing information. I have a	n I confirm that the participant was given an
opportunity to ask questions and all my ques	t opportunity to ask questions about the study and
have been answered to my satisfaction.	I all questions have been answered correctly. I
volunteer to give consent to participate in this	confirm that the consent has been given
research study	voluntarily
	0
Printed name of the participant	
Signature of the participant's parent or guardian	Printed name of the person taking the consent
Date	
day/month/year	Signature of the person taking the consent Date
	day/month/year

# 5. Informed Consent Form for School Age Children

#### Ethiopian Food and Nutrition Strategy (FNS) Baseline Survey

**Investigator(s):** Dr.Masresha Tessema (PI), Meseret W/Yohannes, Dr. Meron Girma, Alemnesh Petros, Dr Aregash Samuel, Arnaud Laillou, Stanley Chitekwe, Kaleab Baye, Ramadhani Noor, Anne Sophie Donze and other co-authors

You are being invited to take part in this research because you are either a mother or caregiver who has a school-age child. Among children 6 - 12 years, we will collect your child's blood, urine and stool.

#### Box 1. Taking part in this research is voluntary

□You can refuse to take part in this study.

You **can withdraw** your participation from the study at any time

#### Information related to the study

The FNS baseline survey will be conducted in the 12 regions of Ethiopia. The study population will be children age 0-59 months having caregivers/mothers, school-age children (6-12 years), adolescent girls (10-19 years), reproductive-age women (15-49 years), pregnant and lactating women, household head. The indicators that will be collected for the survey will be dietary intake, anthropometric status, nutrition sensitive and specific indicators and micronutrient status.

The expected possible adverse effects: there is no adverse effect by participating in this study

**The objective of this research:** to produce information on anthropometric status, dietary intakes, and micronutrient status of different population groups in Ethiopia, and assess the coverage of direct and indirect nutrition interventions.

Study design: a population-based, cross-sectional design

The schedule of the study: The study will be conducted from July, 2021 to April, 2023

Foreseeable risks and expected benefits arising from participation in the study

Foreseeable risks	Expected benefits	
Risks to study participants for involvement in the	The findings of the study will help the ministry of	
coverage survey are low. There may be risks	health and other stakeholder engaged in nutrition	
associated with COVID pandemic. Interviewers	to improve and/or design appropriate health and	
will be trained to minimize this risk and will use	nutrition intervention programs in the country.	
appropriate prevention measures.	1	
Occurrences that may take place during the study period		
Occurrences	How to manage	

Withdrawal of voluntoors from the study	In such a case, we would respect the valuateor
Withdrawal of volunteers from the study	In such a case, we would respect the volunteer
	decision to withdraw and also get a clea
	understanding of the reason for their withdrawal
At the end of the study, you will not be receiving a	ny financial benefits, but you will get your <b>anemia</b> ar
goiter status for time you spent and participation.	
All data collected from the study will be kept confi	dential. If you have any questions related to the stud
you may contact directly Dr. Masresha Tessema wh	no is the project's PI
The contact persons	
1. Dr. Masresha Tessema	
Tel. [+251 919782082] E-mail: [masresha88@gmail	l.com]
2. [Mr. Ibrahim Kedir] Tel. [+251 911957161] EPHI	's IRB
Certificate	of Consent
I have read the foregoing information. I have an	I confirm that the participant was given a
opportunity to ask questions and all my quest	opportunity to ask questions about the study and
have been answered to my satisfaction. I	all questions have been answered correctly.
volunteer to give consent to participate in this 🦷	confirm that the consent has been give
research study	voluntarily
Printed name of the participant	
Signature of the participant's parent or guardian	Printed name of the person taking the consent
Date	Signature of the person taking the consent Date
day/month/year	
	day/month/year

# 6. Assent form for Adolescent Girls (10-19 years)

**BMJ** Open

Ethiopian Food and Nutrition Strategy (FNS) Baseline Survey
Investigator(s): Dr.Masresha Tessema (PI), Meseret W/Yohannes, Dr. Meron Girma, Alemnesh Petros, Dr
Aregash Samuel, Arnaud Laillou, Stanley Chitekwe, Kaleab Baye, Ramadhani Noor, Anne Sophie Donze and
other co-authors
You are being invited to take part in this research because you are Adolescent girl. There are [16596]
households taking part in this research. We will measure your dietary information (for those adolescent
girls aged 15-17 years), information related to nutrition -sensitive and nutrition-specific practices, blood,

and stool, we will also measure your height, weight, and mid upper arm and waist circumference and your goiter status

# Box 1. Taking part in this research is voluntary

You **can refuse** to take part in this study.

You **can withdraw** your participation from the study at any time

# Information related to the study

The FNS baseline survey will be conducted in the 12 regions of Ethiopia. The study population will be children age 0-59 months having caregivers/mothers, school-age children (6-12 years), adolescent girls (10-19 years), reproductive-age women (15-49 years), pregnant and lactating women, and household head. The indicators that will be collected for the survey will be dietary intake, anthropometric status, nutrition sensitive and specific indicators and micronutrient status

The expected possible adverse effects: There is no adverse effect by participating in this study

**The objective of this research:** to produce information on anthropometric status, dietary intakes, and micronutrient status of different population groups in Ethiopia, and assess the coverage of direct and indirect nutrition interventions.

The schedule of the study: The study will be conducted	from July, 2021 to April, 2023
Foreseeable risks and expected benefits arising from p	articipation in the study
Foreseeable risks	Expected benefits
Risks to study participants for involvement in the coverage survey are low. There may be risks associated with COVID pandemic. Interviewers will be trained to minimize this risk and will use appropriate prevention measures.	The findings of the study will help the ministry of health and other stakeholder engaged in nutrition to improve and/or design appropriate health and nutrition intervention programs in the country.

Occurrences that may take place during the stud	y period
Occurrences	How to manage
Withdrawal of volunteers from the study	In such a case, we would respect the volu
	decision to withdraw and also get a
	understanding of the reason for their withdr
At the end of the study, you will not be receivin	ng any financial benefits, but will get your res
height, weight, mid upper arm and waist circum	ference measurements, anemia and goiter st
time you spent and participation.	
All data collected from the study will be kept conf	idential. If you have any questions related to th
you may contact directly Dr. Masresha Tessema w	ho is the project's PI
The contact persons	
1. Dr. Masresha Tessema	
Tel. [+251 919782082] E-mail: [masresha88@gma	il.com]
2. [Mr. Ibrahim Kedir] Tel. [+251 911957161] EPH	l's IRB
Certificat	e of Assent
I have read the foregoing information. I have an	I confirm that the participant was give
opportunity to ask questions and all my quest	opportunity to ask questions about the stud
have been answered to my satisfaction. I	all questions have been answered corre
volunteer to give assent to participate in this	confirm that the assent has been given volu
research study	
	0
Printed name of the participant	
	Printed name of the person taking the assen
Signature of the participant	
Date	Signature of the person taking the assent Da
day/month/year	day/month/year

STROBE Statement—checklist of items that should be included in reports of observational studies

	ltem No	Recommendation	Reported on page #
Title and	1	(a) Indicate the study's design with a commonly used term in the	Title p.1; Abstract p.2
abstract		title or the abstract	3
		(b) Provide in the abstract an informative and balanced summary of	
		what was done and what was found	
Introduction			
Introduction	4	Explain the scientific background and rationale for the investigation being reported	Introduction
/rationale			p.3-4
Objectives	5	State specific objectives, including any prespecified hypotheses	Background p.5 last statements
Methods		O,	
Study design	5	Present key elements of study design early in the paper	Methods (data source) p.4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Methods (data source) p.5
Participants	5	(a) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	Methods (participants) p.5-8
		(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed Case-control study—For matched studies, give matching criteria and the number of controls per case	
Variables	5	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Methods (all outcome and exposure variables are listed) p.6-8
Data sources/ measurement	NA	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Methods (methods of measurement indicated in) p.5-8
Bias	10	Describe any efforts to address potential sources of bias	Methods (data quality indicated) p.10-11
Study size	5-6	Explain how the study size was arrived at	Methods (data source,statistical

		analysis) p.5
Quantitative 10 variables	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Methods (measuremen and statistica analysis sections) p. 8 9
Statistical 10 methods	( <i>a</i> ) Describe all statistical methods, including those used to control for confounding	Methods (analysis section) p. 8-
	(b) Describe any methods used to examine subgroups and interactions	
	(c) Explain how missing data were addressed	
	(d) Cohort study—If applicable, explain how loss to follow-up was addressed	
	Case-control study—If applicable, explain how matching of cases	
	and controls was addressed	
	Cross-sectional study—If applicable, describe analytical methods	
	taking account of sampling strategy	
	( <u>e</u> ) Describe any sensitivity analyses	

Participants	5*	(a) Report numbers of individuals at each stage of study—eg numbers	NA
·		potentially eligible, examined for eligibility, confirmed eligible,	
		included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive	14*	(a) Give characteristics of study participants (eg demographic, clinical,	NA
data		social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable	
		of interest	
		(c) Cohort study—Summarise follow-up time (eg, average and total	
		amount)	
Outcome data	15*	Cohort study—Report numbers of outcome events or summary	NA
		measures over time	
		Case-control study—Report numbers in each exposure category, or	
		summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or	
		summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted	NA
		estimates and their precision (eg, 95% confidence interval). Make	
		clear which confounders were adjusted for and why they were	
		included	
		(b) Report category boundaries when continuous variables were	
		categorized	
		(c) If relevant, consider translating estimates of relative risk into	
		absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and	NA
		interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	p. 9-10
Limitations	19	Discuss limitations of the study, taking into account sources of	Discussion
		potential bias or imprecision. Discuss both direction and magnitude of	(strengths
		any potential bias	and
			weaknesses
			of the
			study) p.10-
			11
Interpretation	20	Give a cautious overall interpretation of results considering	Discussion
	-	objectives, limitations, multiplicity of analyses, results from similar	(interpretation
		studies, and other relevant evidence	of findings in
			the context o
			existing
			research,
			meaning of

			p.9-11 implication
Generalisability	21	Discuss the generalisability (external validity) of the study results	Discussion (strengths ar weakness of study) p.10-1
Other informati	on		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Funding p.12
		arately for cases and controls in case-control studies and, if applicable, for other provided and cross-sectional studies.	or exposed and
http://www.ann	als.org	ites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Intern ;/, and Epidemiology at http://www.epidem.com/). Information on the S	
http://www.ann	als.org		