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Vaccine Preventable Disease Seroprevalence In a Nationwide Assessment of Timor-Leste (VASINA-TL) - study protocol for a population-representative cross-sectional serosurvey

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Data availability: This protocol does not report results. All relevant data are within the paper and its supporting information files.

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ABSTRACT

Introduction: Historic disruption in health infrastructure combined with data from a recent vaccine coverage survey suggests there are likely significant immunity gaps to vaccine preventable diseases and high risk of outbreaks in Timor-Leste. Community-based serological surveillance is an important tool to augment understanding of population-level immunity achieved through vaccine coverage and/or derived from prior infection.

Methods and analysis: This national population-representative serosurvey will take a three-stage cluster sample and aims to include 5600 individuals above one year of age. Serum samples will be collected by phlebotomy and analysed for measles immunoglobulin G (IgG), rubella IgG, severe acute respiratory syndrome coronavirus-2 anti-spike protein IgG, hepatitis B surface antibody and hepatitis B core antigen using commercially available chemiluminescent immunoassays or enzyme-linked immunosorbent assays. In addition to crude prevalence estimates and to account for differences in Timor-Leste's age structure, we will calculate stratified age-standardised prevalence estimates, using Asia in 2013 as the standard population. Additionally, this survey will derive a national asset of serum and dried blood spot samples which can be used for further investigation of infectious disease sero-epidemiology and/or validation of existing and novel serological assays for infectious diseases.

Ethics and dissemination: Ethical approval has been obtained from the Research Ethics and Technical Committee of the Instituto Nacional da Saúde,Timor-Leste and the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research, Australia. Co-designing this study with Timor-Leste Ministry-of-Health and other relevant partner organisations will allow immediate translation of findings into public health policy (which may include changes to routine immunisation service delivery and/or plans for supplementary immunisation activities).

STRENGTHS AND LIMITATIONS OF THIS STUDY

This project is one of very few large-scale, community-based, population-representative serosurveys to be conducted in low-middle income countries.

It will provide accurate seroprevalence estimates for multiple vaccine-preventable diseases, which will immediately inform public health policy and support an ongoing programme of vaccine research in Timor-Leste and the surrounding region.

A national asset of bio-banked serum samples will be derived, which can be used in cross-sectional and prospective studies of infectious disease epidemiology, including those which evaluate disease control interventions.

Diverse, remote communities across Timor-Leste will be visited, with primary sample analysis occurring at the National Health Laboratory in Timor-Leste. Therefore, fieldwork and laboratory-related logistical challenges will need to be overcome.

INTRODUCTION

The Democratic Republic of Timor-Leste (Timor-Leste) achieved independence in 2002. It is a half-island nation located between Australia and Indonesia with a population of 1.3 million people. The Expanded Program on Immunisation (EPI) began *circa* 1989 when Timor-Leste was still an Indonesian province. In 1999 there was significant disruption of healthcare infrastructure, including the near cessation of routine vaccine delivery. After independence was regained in 2002, the EPI was reinstated as part of a national vaccination programme, initially with single-dose measles vaccine. Hepatitis B vaccination in infancy (three doses) was introduced by 2007. Birth dose hepatitis B vaccine was introduced in 2016, along with combined measles-rubella (MR) vaccination (two doses). Vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) began in adults in April 2021 and children above 12 years of age in October 2021.

The most comprehensive recent assessment of routine childhood vaccination coverage in Timor-Leste was a survey undertaken in 2018 which used a combination of maternal history and vaccination card review to confirm doses of vaccines given in the first and second years of life. This study found variable uptake between different vaccines and across geographic regions, and highlighted the need for further investigation of population immunity to vaccine-preventable diseases (VPDs, see table 1).[1]

<Table 1 here>

For many pathogens, including measles, rubella, hepatitis B and SARS-CoV-2, specific immunoglobulin G (IgG) antibodies can be detected in the blood for many years, and sometimes lifelong, following infection or vaccination. In some cases, a specific quantity and/or quality of antibody in individuals' sera has been associated with protection from infection upon subsequent exposure. Presence above antibody cut-off levels can in some contexts infer protection, although how much such levels correlate with protection, varies on a range of factors.[2,3] Nonetheless, community-based serological surveillance is an

important tool to augment understanding of population level immunity achieved through vaccine coverage over many years and/or immunity to VPDs derived from prior infection.[4] The results of serosurveys can be used to guide supplementary immunisation activities (SIAs), and tailor routine immunisation service delivery. There have been no previous community-based studies estimating VPD seroprevalence in Timor-Leste.

This paper describes the protocol for a first and comprehensive national population-representative serosurvey of multiple VPDs. The survey is also designed to derive a national asset of serum and dried blood spot (DBS) samples which can be used for further investigation of infectious disease sero-epidemiology in Timor-Leste.

METHODS AND ANALYSIS

Aim: To determine the seroprevalence of measles, rubella, hepatitis B and SARS-CoV-2 among individuals of different age-strata in Timor-Leste.

Design: Population-representative, national cross-sectional serological survey.

Setting: Recruitment and data collection will occur in the community (within households) across Timor-Leste. Laboratory analysis will occur at Laboratório Nacional da Saúde (LNS) in Dili, Timor-Leste.

Sampling methods: Timor-Leste is made up of 12 municipalities and one Special Region (*Região Administrativa Especial de Oecusse Ambeno*), some of which are divided into sub-municipalities (*Posto administrativos)*. Atauro is a 14th municipality but through legacy is included as part of the Municipality of Dili. Each (sub-)municipality is divided into *sucos* (villages), which are further divided into *aldeias* (hamlets). In 2015, a national census took place in Timor-Leste. All households in the country were visited in-person, assigned a household number and global positioning (GPS) coordinates, and grouped into 2320

'enumeration areas' (EAs, the boundaries of which roughly correspond to those of each aldeia, see Figure 1).

<Figure 1 here>

A three-stage cluster random sample will be taken. First, a pre-specified number of EAs will be randomly selected from all EAs in the country, with probability proportionate to municipality population. Second, within each participating EA, a pre-specified number of households will be randomly selected from all households in that EA. Third, all occupants at participating households who meet eligibility criteria will be invited into the study.

A household will be defined as a dwelling unit that consists of a person or a group of related or unrelated persons, who live together in the same dwelling unit or informal shelter, who are considered as one unit and share a cooking area.

Eligibility criteria: Household members will be eligible to participate if they are ≥1 year of age and they (or their parent/guardian) provide consent to participate in the study. Individuals who report current illness which is compatible with coronavirus disease (COVID-19), and those who report any of the following conditions will be excluded:

- Needle phobia

- Anaemia

- Skin condition affecting phlebotomy sites

- Bleeding disorder

Additionally, individuals who cannot communicate verbally in Tetum, Portuguese or English will be excluded.

Sample size: The sample size estimation and the proposed parameters are described in Table 2. Age groups of 1-4, 5-14, 15-24, 25-40, and >40 years have been considered as separate strata, for a range of reasons, with some including:

- Measles in children under 5 years of age because they are most likely to suffer serious sequalae from infection when compared to other age groups[5] and between 5-14 years of age because outbreaks can occur and/or amplify in schools and other settings where groups of children from different households congregate.
- Rubella in women 15-40 years of age because future pregnancies may be at risk of congenital rubella syndrome (CRS). It is anticipated that rubella virus is circulating in Timor-Leste as there has only been recent introduction of rubella vaccination and there is very little surveillance for CRS.
- SARS-CoV-2 in children 1-12 years of age because seropositivity is likely to represent naturally acquired infection as this group are not eligible for vaccination in Timor-Leste, and so it will give an indication of the extent of local transmission which has occurred.
- Hepatitis B (surface antibody, HBsAb and core antibody, HBcAb) in children under 5 and 5-14 years of age because hepatitis B birth vaccination was introduced approximately 5 years ago and comparison of these groups will give an indication of uptake.

The required sample size for this multi-stage survey (householders within households within EAs) will need to power seroprevalence estimates with a precision of 6%. For the first step of this determination and assuming 50% seroprevalence, a simple random sample of 280 will be required for each of the five age strata. Using an intraclass correlation of 0.333, which accounts for dependence among households and households at the second and third stages, respectively, this sample size was then adjusted by the design effect of four in step 2. Thus the required effective sample size for each stratum was calculated to be 1120. As non-response is expected to be minimal, there was no need to adjust this figure by a non-response factor. Lastly, and considering the five strata, the required sample size is estimated to be 5600.

The formula for the sample size calculation is:

$$N=(p)^{2}/\Delta^{2} *(1-p)*[1+(n-1)*\rho]*K$$

Where,

Δ = precision estimate

p = seroprevalence estimate

n = mean number of householders in a household estimate

ρ = intraclass correlation coefficient estimate

K = number of age strata

<Table 2 here>

Fieldwork procedures: Municipalities will be visited sequentially depending on various logistical considerations including weather and road conditions, availability of staff, vehicles, and accommodation, and local municipal and health leader preference.

First, the study team leader will make a 'coordination visit' to the municipality during which they will explain the study procedures, receive permission to conduct the study, and discuss travel routes and gaining access to each EA with the following individuals:

- Municipality Administrator (at Municipality Office; one per municipality)
- Director of Municipality Health Service (at Municipality Health Service Office; one per municipality)
- Sub-Municipality Administrator (at Administrative Post Offices; one for each sub-municipality being visited)
- Head of Community Health Centre (at Community Health Centre Office; one for each sub-municipality being visited)
- Chief of Suco (at Suco Office;1 for every suco being visited)
- Commander of Police in Suco (at Suco Police Station; one per suco being visited, at the discretion of the Chief of Suco)

- Chief of Aldeia (at Aldeia Office; one for every aldeia being visited)

If any of these individuals are not available in-person during the coordination visit attempts will be made to contact them by telephone or through WhatsApp.

Secondly, a 'study visit' will be made by a whole study team, consisting of a team leader (usually non-clinical), three research nurses, and two drivers. Additionally, at the discretion of the Municipality Administrator, Sub-Municipality Administrator and/or Head of Community Health Centre, one or two local government representatives and/or one or two Community Health Centre representatives may join the study visits. It is anticipated that these individuals will primarily observe study procedures. Any involvement in participant recruitment, data collection or sample collection will be directly supervised by the appropriate study team member.

Navigation and maps: Selected households will be identified using electronic tablets which will have GPS capability and Google Earth® software installed. Keyhole Markup Language (KML) files with GPS coordinates for all selected households in each EA will be pre-loaded onto the tablets, such that they can be used without mobile/internet connectivity. KML files are generated in QGIS. Each household location is verified using a Google Satellite base map. Study teams will also carry printed colour copies of bespoke maps for each EA, which will show the location of households in relation to roads, paths, and landmarks. These will be produced using ArcMap™ 10.4.1 and will include ESRI imagery base map, showing the location of main roads and the selected households coded by letters. The standard operating procedure (SOP) for location of households is shown in appendix 1.

Data collection: Study teams will approach the household occupants and introduce themselves. The occupants will be asked to identify an (acting) head-of-household. If this individual is not available (or if no occupants are present), the study team will arrange to return twice to the household, and at least once on a separate day until a head-of-household is present.

Data will be collected using structured interview-questionnaires. Reponses will be entered into electronic tablets which will have REDCap® installed. This is a secure web platform for building and managing online databases which allows offline data entry[6]. Questionnaires data will be uploaded to the REDCap® secure server hosted at Menzies School of Health Research, Charles Darwin University, Darwin, Australia.

Three bespoke data collection tools have been developed (see appendices 2-4):

- Housenold questionnaire. This will be completed first. Demographic data on all household occupants (whether they are present at the time or not), will be collected by interviewing the head-of-household.
- Participant questionnaire. This will be completed if/when any household occupants agree to participate. Each will be assigned a unique identification number (participant ID number) and relevant demographic, clinical and vaccine-related data will be collected. Participants will not be asked to provide written documentation of vaccines received because a low proportion of participants in the recent vaccine coverage survey had retained this.[1]
- Unable to complete questionnaire. This will only be completed if the household questionnaire cannot be completed (i.e. if a head-of-household was not present or not willing to provide demographic data after 3 household visits). The reason for non-completion will be recorded in free-text.

Sample collection and handling: Research nurses with training and experience in adult and paediatric phlebotomy will collect primary blood samples using appropriate infection prevention control procedures and safe management of sharps. Participants >5 years of age will undergo venepuncture using either a standard hypodermic or a winged butterfly needle with a syringe attached. Venous blood will then be injected directly into a gel serum separator tube (SST). Participants between 1-5 years of age (and those who do not consent to venepuncture but provide consent for a finger prick) may undergo capillary blood sampling through finger prick technique, in which case drops of blood will be applied directly to a

paediatric gel SST. The method used will be determined on a case-by-case basis by the research nurse in the field. Table 3 shows sample volumes and collection techniques for participants in different age groups.

<Table 3 here>

Primary blood samples will be kept at ambient temperature out of direct sunlight and allowed to clot for a maximum of eight hours (i.e. one day of fieldwork). They will then undergo centrifugation at 1,500 RCF for ten minutes and the resulting separated serum samples will be kept at 4 degrees Celsius using a portable refrigerator with battery power backup. They will be transported to LNS within five days of sample collection and will undergo primary serological analysis within two weeks of sample collection.

A secondary dried blood spot (DBS) sample will be created: For participants who undergo venepuncture, the last 300-500μL of venous blood in the syringe will be injected onto Whatman 903 filter paper marked with three 12mm diameter circles. For participants who undergo finger-prick, additional drops of capillary blood will be applied directly from the finger to the filter paper. Once the circles are saturated with blood (typically using 100-150μL blood for each circle), the filter paper will be dried at ambient temperature out of direct sunlight for four hours, then placed alongside a desiccant sachet into a plastic zip lock bag. The SOP for data and sample collection is shown in appendix 5.

Sample analysis: Primary (serum) samples from all participants will be tested at LNS for rubella IgG (quantitative; considered positive if >10 IU/mL), SARS-CoV-2 anti-spike IgG (qualitative), hepatitis B core antibody (HBcAb, qualitative) and hepatitis B surface antibody (HBsAb, quantitative, considered positive if >10 mIU/mL) using Ortho Clinic Diagnostics® chemiluminescent assays on the Vitros ECiQ® platform, and for measles IgG using the Eurimmun® ELISA assay (quantitative, positive if >120IU/L). For quantitative assays, serological cut-offs which have been most commonly shown to correlate with protection from infection and/or those which are conventionally used in serosurveys and/or assessment of

immunity have been chosen.[7–9] Where data are somewhat conflicting, there is lack of consensus supporting a correlate-of-protection, or considerable inter-assay variability in quantitative determination has been observed, secondary (exploratory) analyses using alternative cut-offs may also be undertaken (for example 200IU/L and/or 250IU/L for measles IgG).[3]

Additionally, samples from participants residing in Dili Municipality (excluding Atauro) will be tested for hepatitis B surface antigen (HBsAg, qualitative). This marker denotes active hepatitis B infection which may have significant health implications and will therefore only be tested in Dili municipality where there is a hepatology clinic in which participants can receive further assessment and follow-up. Any samples which are positive for HBsAg will also be tested for hepatitis B envelope antigen (HBeAg, qualitative) and hepatitis B envelope antibody (HBeAb, qualitative) testing using Ortho Clinic Diagnostics chemiluminescent assays on the Vitros ECiQ platform, and hepatitis B viral load (HBVL, quantitative) using the Cepheid® assay on the GeneXpert platform. All testing will be carried out according to manufacturers' instructions and cited serological cut-off values. For qualitative assays, samples with borderline/indeterminate results will be considered negative, apart from HBsAg where the test will be repeated and both results reviewed alongside other hepatitis B results by an appropriately qualified clinical member of the research team who will decide whether the participant should be referred to the hepatology clinic for repeat sampling and clinical assessment.

Assays have been chosen based on their previous performance in seroprevalence studies, immediate availability for shipment to Timor-Leste, and local laboratory expertise in operating these types of assays (with ongoing capacity building for serological testing in LNS). The measles IgG assay is quantitative and calibrated against a World Health Organisation (WHO) Standard (NIBSC, Anti-Measles serum, 3rd International Standard 97/648). It showed acceptable performance when assessed in a recent study of concordance between commercially available assays (concordance for samples with

positive/negative status = 90%/100%)[10]. The rubella IgG assay is quantitative and calibrated against a Centers for Disease Control and Prevention (CDC) standard (Low Titer Rubella Standard) and a World Health Organization (WHO) standard (1st International Rubella IgG Standard). While concerns around standardisation of rubella IgG assays are noted[11], this assay showed acceptable performance when assessed in a recent study of concordance between automated immunoassays (concordance for samples with negative/positive status = 90.6%/91.1%)[12]. The hepatitis B surface antibody assay is quantitative and showed high sensitivity (97.1%) and specificity (97.9%) when evaluated in a panel of sera from healthcare workers and patients[13]. The SARS-CoV-2 anti-spike IgG assay has high sensitivity (93.3%, >21 days post infection) and specificity (100%), which compares favourably to many other available immunoassays[14], and has been used in several serological surveillance studies[15–17].

Provision of results to participants: The majority of testing in this study will be for antibodies against VPDs (either IgG or total antibody). Results will therefore only indicate whether an individual has been previously infected and/or vaccinated against each disease at some time in the past and will not provide information on current infection. While seronegative participants may be at risk of future infections (and may benefit from vaccination), individual notification of results and provision of vaccines to all seronegative study participants is not considered feasible in this large cross-sectional study. Instead, all participants will be advised of the benefits of routine vaccination and immunisation clinics in their area, as well as on any forthcoming SIAs which may occur as a result of this study.

In addition to antibody tests, serum from participants within Dili Municipality (excluding Atauro) will be tested for HBsAg. This marker denotes active hepatitis B infection which may have significant health implications and will therefore only be tested in Dili municipality where there is a hepatology clinic in which participants can be seen. Participants who test positive for HBsAg will be contacted by telephone to discuss their results and will be offered assessment including biochemical and radiological investigation of liver function and

consideration of antiviral treatment in-line with international clinical guidelines[18]. This approach has been successful and has been acceptable to participants in a smaller serological surveillance study including hepatitis B testing among healthcare workers in Timor-Leste[19].

Sample storage: Primary (serum) samples will be stored at -80 degrees Celsius and secondary (DBS) samples will be stored at 4 degrees Celsius at LNS for 10 years. These may undergo additional serological analyses to further investigate infectious disease sero-epidemiology in Timor-Leste and/or validating existing and novel serological assays for infectious diseases, pending successful funding application and appropriate ethical approval.

Fieldworker training: Field workers will undergo one week of formal in-person training in study procedures. Days 1-2 will be classroom based and will include sessions on 'the study protocol', 'field team composition' (structure, members, responsibilities), 'logistics, technology and map reading', 'recruitment and consent', and 'collection of data using interview questionnaires'. Days 3-5 will be practical and will include demonstrations and training in adult and paediatric phlebotomy and finger-prick techniques, infection prevention control procedures, and the use of personal protective equipment (PPE). These skills will be assessed formatively throughout the training and summatively using pre- and post- session assessments. Training will be delivered by PA, JF, NSSF and/or JY who are clinicians with experience of epidemiological and clinical research in Timor-Leste.

Laboratory team training: Laboratory training will occur in the Serology Department of LNS. Training will be delivered by PA and TO who have significant experience with ELISA and chemiluminescent techniques, including in Timor-Leste. The focus of training will be on assay verification and quality assurance, as well as procedures for sample processing, analysis and storage.

Data storage and handling: Field data will be stored in the REDCap® secure server hosted at Menzies School of Health Research, Charles Darwin University, Darwin, Australia, until

analysis. Laboratory data (i.e. serology worksheets and results) will be stored on the password-protected LNS laboratory information system (SchuyLab®) until analysis. Deidentified field and laboratory datasets will be downloaded and stored as password-protected databases on computer(s) at Menzies School of Health Research, Timor-Leste Office, Dili, Timor-Leste, where they will be linked using participant ID numbers and analysed. Only named investigators who are working directly on this project will have access to data.

Statistical analysis plan: Primary data analysis will occur at the end of the study, once all fieldwork is complete and all samples have been analysed. Interim analyses may also occur upon reasonable request from the Timor-Leste MoH or other partner organisation. As a multi-stage sampling survey design will be used to select participants, sampling weights will be calculated at each stage. These weights will reflect a participant's inverse probability of selection at a particular stage, be it at the EA level, the household level or the householder level. Furthermore, these weights will subject to both non-response adjustment and finite population correction. Measures of prevalence will be age-standardised using the standard population for Asia given by the International Network for the Demographic PNGIMR 2018, Papua New Guinea MIS 2016-2017.[20] To account for this design, the 'svy' data commands in Stata (version 16, StataCorp, College Station, TX, USA) will be used for ally analyses. Characteristics of participants will be summarised using weighted descriptive statistics. Frequencies and proportions will be used to describe categorical distributions, whilst means and standard deviations will be used to describe continuous variables. In the presence of non-normality, medians and interquartile ranges will be reported. Univariable and multivariable binary logistic regression will be undertaken to model age, the independent variables of primary interest with the five VPD outcomes. In addition to this variable, other variables known to be risk factors of VPD, such as sex and travel history, will be subjected to a manual backward stepwise procedure. Variables with a p-value ≥ 0.20 will not be retained. The Hosmer-Lemeshow test will be used to test the goodness of fit of each multivariable

model. A p-value < 0.05 will be considered statistically significant with Odds Ratios (OR), 95% confidence intervals (CI) and p-values calculated for age and sex.

ETHICS AND DISSEMINATION

Informed consent: Each prospective participant will receive a participant information sheet which will be printed in English and Tetum. They will also be provided with a verbal explanation of the study rationale and procedures. This will include potential risks and benefits of sample collection, specific tests which their sample will undergo, the fact that they will not receive notification of any results (with the exception of a positive HBsAg, tested in Dili Municipality only) and the possibility that their sample will undergo additional analyses for evidence of communicable diseases during the next 10 years. They will be given up to 30 minutes to ask questions and decide whether they wish to participate, and will then provide informed, written consent by signing a consent form. For individuals under 16 years of age, verbal assent will be sought, in addition to written consent from their parent or guardian. This study has received ethical approval from the Research Ethics and Technical Committee of the Instituto Nacional da Saúde,Timor-Leste (Reference: 875 MS-INS/DGE/IX/2021) and the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research, Australia (Reference: 2021-4064).

Protocol amendments: Any modifications to the protocol which may impact on the conduct of the study will be documented in a formal protocol amendment and approved by both Research Ethics Committees prior to implementation of the changes. The Research Ethics Committees will also be notified of any minor corrections/clarifications or administrative changes to the protocol, which will be documented in a protocol amendment letter.

Adverse events: Data on adverse events will be collected throughout the study, with participants (or their parent/guardian) being informed of the risks of phlebotomy (including bruising, bleeding and infection), how to recognise these, and how to contact the study team

if they occur. Adverse events will be reported to the Principal Investigator. In cases where infection or any other serious adverse event has occurred, the Principle Investigator will conduct a review of the study visit and decide whether any phlebotomy retraining or change in practice is required and/or whether recruitment to the study should be paused.

Strengths and limitations: This project will produce accurate, nationally representative seroprevalence data for multiple VPDs and relevant age-groups, which has not been achieved in Timor-Leste previously. It has been co-designed by investigators at Menzies School of Health Research (Timor-Leste Office), the National Centre for Immunisation Research and Surveillance (Australia), the MoH (Timor-Leste), LNS (Timor-Leste), and the WHO (Timor-Leste Office) according to local research and public health priorities. This will allow immediate translation of findings into public health policy (including potentially changes to routine immunisation service delivery and/or plans for SIAs). Additionally, the survey will derive a national asset of serum and dried blood spot (DBS) samples which can be used for further investigation of infectious disease sero-epidemiology in Timor-Leste and/or validating existing and novel serological assays for infectious diseases.[21–25] Engagement with local administrative and health leaders and maximisation of participant choice and welfare have been central to the design, including ensuring all individuals diagnosed with active hepatitis B during the study have access to appropriate further investigation and follow-up.

Risks and limitations include the ongoing global outbreak of SARS-CoV-2 (which may delay/prohibit study visits), disruption of supply of field and laboratory consumables to Timor-Leste (which may delay/increase the cost of laboratory analysis), natural disasters such as flooding, and potential unwillingness of individuals to participate in provision of data and/or samples (which may affect recruitment, potentially disproportionately among children).

Dissemination / knowledge transition plan: After each interim analyses, results will be shared with Timor-Leste MoH partners in the form of an oral presentation and in a written report. Following completion of the study, results will be shared with Timor-Leste MoH, other partner organisations, and local administrative and health leaders for EAs where the study

took place (Municipality Administrators, Directors of Municipality Health Services, Sub-Municipality Administrators, Heads of Community Health Centres, Chiefs of Sucos, Chief of Aldeia), in the form of a written report. Results will also be submitted for publication in peer-reviewed journals and presented at relevant international conferences.

CONCLUSION

Historic disruption in health infrastructure including to the delivery of vaccines combined with data from a recent vaccine coverage survey suggests there are likely significant immunity gaps to VPDs and high risk of outbreaks in Timor-Leste. Targeted seroprevalence studies including healthcare workers in Timor-Leste have identified lower than expected seropositivity against measles, high seropositivity against SARS-CoV-2 and a high prevalence of active hepatitis B infection.[19,26] This study will fill a crucial gap in understanding of national population immunity against VPDs and will guide routine immunisation service delivery, plans for SIAs, and an ongoing programme of vaccine research in Timor-Leste.

AUTHOR CONTRIBUTIONS

PA, SLS, NM, SA, NS, F, FNM, NSSF, KM, JY and JRF conceived the study. PA, SLS, NM, MYT, SA, ADKD, NS, LA, CF, FNM, CAG reviewed existing literature and performed situation analysis. PA, MYT, SA, VS, LA, CAG, ICB determined the fieldwork procedures and designed data collection tools. PA, NG, TO, NS, ES, LA, ICB determined laboratory procedures. PA, MD, ADKD, NS, NSSF drafted the data and statistical analysis plan. NM, MYT, SA, NS, CF, FNM, CAG planned community engagement, obtained ethical approval and lead other regulatory aspects of the study. All authors reviewed and commented on the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests for this study.

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Table 1: Routine Timor-Leste vaccination schedule in 2022 and estimated coverage in 2018

Vaccine	Recommended age of administration	Introduced	Crude estimated coverage (2018 national vaccine coverage survey) (95%CI)*
BCG	At birth	Pre 1999	94.7% (91.7%-97.0%)
HepB0		2016	66.2% (58.5%-73.0%)
bOPV0		2016	80.4% (74.0%-86.0%)
DTwP-Hib-HepB1	6 weeks	2007	91.8% (87.8%-95.0%)
bOPV1		201	91.8% (87.8%-95.0%)
RV1		2019	Not part of routine vaccination in 2018
DTwP-Hib-HepB2	10 weeks	2007	87.4% (82.6%-91.0%)
bOPV2		2016	87.8% (83.0%-91.0%)
RV2		2019	Not part of routine vaccination in 2018
DTwP-Hib-HepB3	14 weeks	2007	83.3% (78.0%-87.0%)
bOPV3		2016	83.3% (78.0%-87.0%)
RV3		2019	Not part of routine vaccination in 2018
IPV		2016	80.6% (74.1%-86.0%)
MR1	9 months	2016	77.3% (71.5%-82.0%)
DTwP4	18 months	2016	54.8% (46.5%-63.0%)
MR2		2016	54.4% (46.1%-62.0%)
DT	6 year or school entry	2016	Not measured in 2018 survey
TT1-5	Unimmunised pregnant women	Pre 1999	68.2% (62.4%-74.0%)
SARS-CoV-2	Adults and children above 12 years of age	Adults: Apr 2021 Children Oct 2021	Not part of routine vaccination in 2018

Abbreviations: BCG = bacillus Calmette-Guérin; HepB = hepatitis B; bOPV0 = bivalent oral polio vaccine; DTwP-Hib-HepB = diphtheria, tetanus, pertussis, hepatitis B and Haemophilus influenzae type B; RV = rotavirus vaccine; IPV = inactivated poliovirus vaccine; MR = measles and rubella vaccine; DT - diphtheria and tetanus vaccine; TT - tetanus toxoid vaccine.

*crude coverage is defined as all doses, whether valid or not, by any documented evidence or verbal history at the time of the survey.

Table 2: Parameters for the cluster survey sample size calculation (for national age-stratified seroprevalence estimates for all VPDs)

Parameter	Value used
Number of strata (age groups)	5
Expected seroprevalence* (p)	50%
Desired precision level (d)	6%
Alpha error	5%
Z value	1.96
Estimated sample size (simple random)	280
Target number of respondents per cluster	10
Intra-cluster correlation coefficient	0.333
Coefficient of variation	0.3
Design effect	4
Non-response rate	15%
Sample size per age strata† (excluding non-responders)	1120
Total sample size (excluding non-responders)	5600

*There are no nationally representative historic data to indicate VPD seroprevalence in Timor-Leste. Therefore, expected seroprevalence has been set at 50% for all VPDs, to ensure the study is sufficiently powered
†Age strata are: 1-4, 5-14, 15-24, 25-40, and 40+ years

Table 3: Sample volumes and collection techniques for primary sample collection by age group

Age group	Method of blood collection	Equipment	Collection container	Target sample volume
1-5 years	Venepuncture*	23G butterfly needle, venous blood aspirated into 5ml syringe	5ml SST tube	5ml
	Finger prick*	Lancet, capillary blood drops transferred directly into collection tube	2ml paediatric SST tube	2ml
6-15 years	Venepuncture	21-23G needle or 23G butterfly needle, venous blood aspirated into 5ml syringe	5ml SST tube	5ml
Adults	Venepuncture	21-23G needle, venous blood aspirated into 10ml syringe	2x 5ml SST tube	10ml

Abbreviations: SST = serum separator tube

*Determined in field by phlebotomist on case-by-case basis

APPENDICIES

Appendix 1: Standard operating procedure for location of households

Appendix 2: Household questionnaire

Appendix 3: Participant questionnaire

Appendix 4: Did not complete questionnaire

Appendix 5: Standard operating procedure for data and sample collection

FIGURE LEGENDS

Figure 1: Map of Timor-Leste showing enumeration areas (EAs) within municipalities

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Mr Trevor Clifford

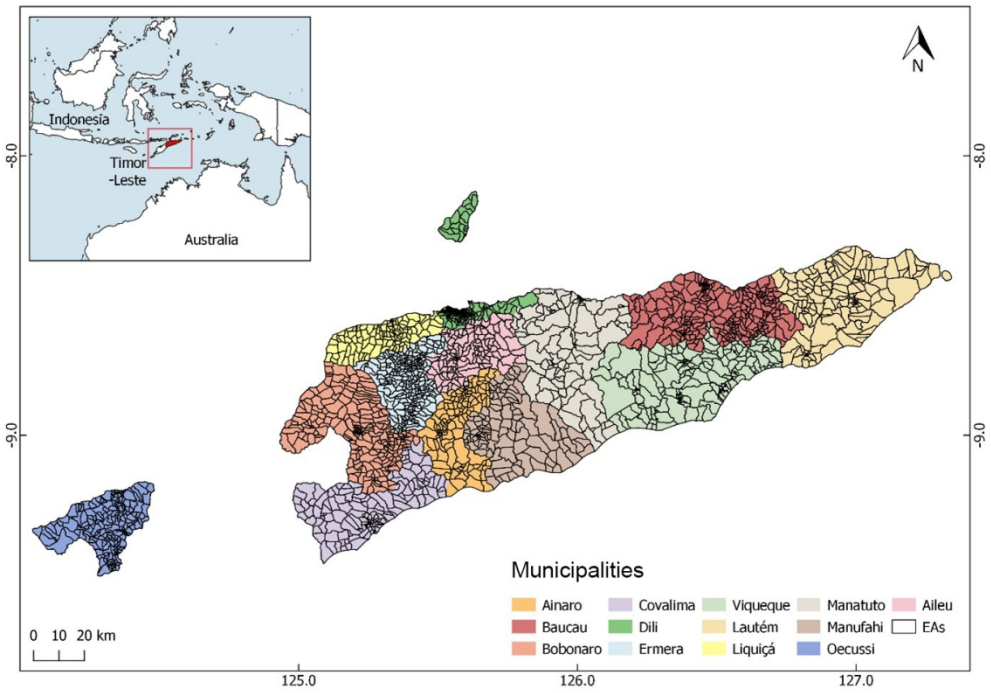

Figure 1: Map of Timor-Leste showing enumeration areas (EAs) within municipalities

159x112mm (220 x 220 DPI)

VASINA fieldwork SOP – location of households

1. **Navigate to household using GPS device and printed maps**
   - Make sure you identify the exact house (do not accept another household nearby)

2. **If at least one adult household member is present, move to *Data and Sample Collection SOP***

3. **If the household was found, but there are no household members present (or if one or more household members are missing):**
   - Arrange and conduct a second and third visit. At least one of these should be on a different day.
   - If there are no household members present on all three visits, complete the *'Unable to Complete Questionnaire'* on RedCap
   - Do not find another household to replace this one

4. **If the household was found, but it looks derelict**
   - Fill out the *'Unable to Complete Questionnaire'* on RedCap
   - Choose the nearest occupied house and offer study participation to these occupants instead. If there are two equidistant houses, choose the one on the Left. When collecting data for the new household, add "B" to the end of the household number (e.g. "12345**B**")

5. **If the household cannot be found**
   - Fill out the *'Unable to Complete Questionnaire'* on RedCap
   - Make sure you enter the reason that the household cannot be found
   - Do not find another household to replace this one

Household Questionnaire

Record ID	_____
Household number Númeru Uma-kain	_____
How many people currently live in this household? Ema nain hira mak agora dadauk hela iha uma-kain ida-ne'e?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ 7 ◯ 8 ◯ 9 ◯ 10 ◯ 11 ◯ 12 ◯ 13 ◯ 14 ◯ 15 ◯ 16 ◯ 17 ◯ 18 ◯ 19 ◯ 20 (If the household has more than 20 mebers, fill out a second Household Questionnaire / Karik iha uma-kain ida nia membru barak liu ema 20, preenxe fali iha kuestionariu uma-kain segundu)
Gender of household member 1 Jeneru membru uma-kain 1	◯ Male / Mane ◯ Female / Feto
Age of household member 1 Idade membru uma-kain 1	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 1 Númeru ID estudu nian ba membru uma-kain 1	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 2 Jeneru membru uma-kain 2	◯ Male / Mane ◯ Female / Feto

Age of household member 2 Idade membru uma-kain 2	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 2 Númeru ID estudu nian ba membru uma-kain 2	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 3 Jeneru membru uma-kain 3	◯ Male / Mane ◯ Female / Feto
Age of household member 3 Idade membru uma-kain 3	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 3 Númeru ID estudu nian ba membru uma-kain 3	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 4 Jeneru membru uma-kain 4	◯ Male / Mane ◯ Female / Feto
Age of household member 4 Idade membru uma-kain 4	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 4 Númeru ID estudu nian ba membru uma-kain 4	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 5 Jeneru membru uma-kain 5	◯ Male / Mane ◯ Female / Feto
Age of household member 5 Idade membru uma-kain 5	_____ (If unsure, please estimate age (to the nearest 10 years))

Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 5 Númeru ID estudu nian ba membru uma-kain 5	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 6 Jeneru membru uma-kain 6	◯ Male / Mane ◯ Female / Feto
Age of household member 6 Idade membru uma-kain 6	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 6 Númeru ID estudu nian ba membru uma-kain 6	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 7 Jeneru membru uma-kain 7	◯ Male / Mane ◯ Female / Feto
Age of household member 7 Idade membru uma-kain 7	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 7 Númeru ID estudu nian ba membru uma-kain 7	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 8 Jeneru membru uma-kain 8	◯ Male / Mane ◯ Female / Feto
Age of household member 8 Idade membru uma-kain 8	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)

Study ID number of household member 8 Númeru ID estudu nian ba membru uma-kain 8	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 9 Jeneru membru uma-kain 9	◯ Male / Mane ◯ Female / Feto
Age of household member 9 Idade membru uma-kain 9	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 9 Númeru ID estudu nian ba membru uma-kain 9	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 10 Jeneru membru uma-kain 10	◯ Male / Mane ◯ Female / Feto
Age of household member 10 Idade membru uma-kain 10	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 10 Númeru ID estudu nian ba membru uma-kain 10	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 11 Jeneru membru uma-kain 11	◯ Male / Mane ◯ Female / Feto
Age of household member 11 Idade membru uma-kain 11	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 11 Númeru ID estudu nian ba membru uma-kain 11	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)

Gender of household member 12 Jeneru membru uma-kain 12	◯ Male / Mane ◯ Female / Feto
Age of household member 12 Idade membru uma-kain 12	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 12 Númeru ID estudu nian ba membru uma-kain 12	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 13 Jeneru membru uma-kain 13	◯ Male / Mane ◯ Female / Feto
Age of household member 13 Idade membru uma-kain 13	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 13 Númeru ID estudu nian ba membru uma-kain 13	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 14 Jeneru membru uma-kain 14	◯ Male / Mane ◯ Female / Feto
Age of household member 14 Idade membru uma-kain 14	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 14 Númeru ID estudu nian ba membru uma-kain 14	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 15 Jeneru membru uma-kain 15	◯ Male / Mane ◯ Female / Feto

Age of household member 15 Idade membru uma-kain 15	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 15 Númeru ID estudu nian ba membru uma-kain 15	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 16 Jeneru membru uma-kain 16	◯ Male / Mane ◯ Female / Feto
Age of household member 16 Idade membru uma-kain 16	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 16 Númeru ID estudu nian ba membru uma-kain 16	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 17 Jeneru membru uma-kain 17	◯ Male / Mane ◯ Female / Feto
Age of household member 17 Idade membru uma-kain 17	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 17 Númeru ID estudu nian ba membru uma-kain 17	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 18 Jeneru membru uma-kain 18	◯ Male / Mane ◯ Female / Feto
Age of household member 18 Idade membru uma-kain 18	_____ (If unsure, please estimate age (to the nearest 10 years))

Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 18 Númeru ID estudu nian ba membru uma-kain 18	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 19 Jeneru membru uma-kain 19	◯ Male / Mane ◯ Female / Feto
Age of household member 19 Idade membru uma-kain 19	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 19 Númeru ID estudu nian ba membru uma-kain 19	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 20 Jeneru membru uma-kain 20	◯ Male / Mane ◯ Female / Feto
Age of household member 20 Idade membru uma-kain 20	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 20 Númeru ID estudu nian ba membru uma-kain 20	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
At any time in the past 12 months, has anyone sprayed the interior walls of your house? Durante fulan 12 liu-bá, iha ema ruma mai rega ita boot nia moru/uma nia didin lolon (parte laran), ka lae?	☐ Yes / Sim ☐ No / Lae ☐ Don't know / hatene
What do you use to stop being bitten by mosquitoes at night time? Saida mak ita bo'ot uza hodi prevene susuk labele tata iha tempu kalan?	☐ Sprays / Rega ho aimoruk susuk nian ☐ Coils / Ai-moruk susuk nian (sunu) ☐ Bednets / Moskiteiru ☐ Other / Seluk ☐ None / La iha

Please specify

Favor espesifika

Individual Participant Questionnaire

Record ID _____

Demographic information

Informasaun Demográfiku

Household number

Númeru Uma-kain _____

Study ID number

Númeru ID estudu _____ (Use number on sample sticker / Uza númeru iha amostra nia sticker)

Name of interviewer

Naran entrevistador _____

Date of interview

Data halao entrevista _____

First name

Naran prinsipal _____

Family name

Naran Familia _____

Gender

Jeneru
◯ Female / Feto
◯ Male / Mane
◯ Other/Unknown / Seluk/La hatene

Date of birth

Data moris _____

Age

Idade _____ (Only need to fill if date-of-birth is not known / Presiza atu preenxe deit karik la hatene data moris)

Phone number(s)

Numeru Telemovel _____

Email address

_____

Nationality Nasionalidade	◯ Timorese / Timor oan ◯ Other / seluk
Specify Espesifika	_____
Have you traveled outside Timor-Leste (including West Timor, NTT) within the last 6 MONTHS? Iha fulan 6 ikus liu, ita bo'ot halao viajen ba rai-liur ka lae (inklui nasaun viziño Indonesia nia provinsia, NTT)?	◯ Yes / Sim ◯ No / Lae ◯ Don't know / La hatene
Where did you travel (in the last 6 months)? Ita bo'ot ba iha rai ne'ebé? (iha fulan 6 ikus liu)	◯ West Timor (NTT) ◯ Other / Seluk
Please specify the country (or countries) you went to (in the last 6 months) Favor espesifika nasaun sira ne'ebé mak ita bo'ot viajem ba ona (iha fulan 6 ikus liu)	_____
Have you ever traveled outside Timor-Leste in your lifetime (but MORE THAN 6 MONTHS AGO)? This includes travel to West Timor (NTT). Ita bo'ot halao ona viajem fora husi Timor-Leste, durante vida moris? (maibe husi fulan 6 liu-bá kotuk)? Ida ne'e inklui viajem provinsia NTT	◯ Yes / Sim ◯ No / Lae ◯ Don't know / La hatene
Where did you travel (MORE THAN 6 MONTHS AGO)? Ita bo'ot viajen ba iha rai ne'ebé (FULAN 6 IKUS LIU BA KOTUK)?	◯ West Timor (NTT) ◯ Other / Seluk
Please specify the country (or countries) you went to MORE THAN 6 MONTHS AGO Favor espesifika nasaun sira ne'ebé mak ita bo'ot ba ona Iha FULAN 6 IKUS LIU BA KOTUK	_____

Questions about febrile illness within the last 6 months

Pergunta konaba moras isin manas iha fulan 6 ikus liu

Have you been unwell with a FEVER within the last 6 months? Ita bo'ot sente moras ho ISIN MANAS iha fulan 6 ikus liu nia laran ka lae?	◯ Yes / Sim ◯ No / Lae ◯ Don't know / La hatene

When you had this illness, did you seek any medical care? Iha momentu ita bo'ot hetan moras ida-ne'e, ita bo'ot ba konsulta iha fasilidade saúde ka lae?	◯ Yes / Sim ◯ No / Lae ◯ Don't know / La hatene (Includes medical clinic, pharmacy, hospital etc. / Inklui klinika mediku, farmasia, hospital, etc.)
When you sought medical care with fever in the last 6 months, did you get diagnosed by a healthcare professional with any of the following infections? Wainhira ita bo'ot buka tratamentu husi mediku sira ho isin manas iha fulan 6 ikus liu, ita bo'ot hetan diagnostiku husi professional saúde ho infeksaun hirak tuir mai ne'e ka lae?	☐ COVID-19 ☐ Dengue / Denge ☐ Malaria ☐ Other / Seluk ☐ No specific diagnosis was made / La halo diagnostiku espesifiku
When you were diagnosed with COVID-19, how was this diagnosis made? Wainhira ita bo'ot diagnoza ho COVID-19, oinsa mak hetan diagnoza ida ne'e?	◯ I was tested for COVID-19 and the test was POSITIVE / Hau halo teste ba COVID-19 no teste nia rezultadu POZITIVU ◯ I did NOT receive a positive test, but I was diagnosed with COVID-19 anyway / Hau la hetan teste pozitivu, maibe nafatin diagnoza hau ho COVID-19 ◯ I don't know if I was tested or not / Hau la hatene karik hau teste ona ou seidauk
When you were diagnosed with DENGUE, how was this diagnosis made? Wainhira ita bo'ot diagnoza ho DENGE, oinsa mak hetan diagnoza ida ne'e?	◯ I was tested for DENGUE and the test was POSITIVE / Hau halo teste ba DENGE no teste nia rezultadu POZITIVU ◯ I did NOT receive a positive test, but I was diagnosed with DENGUE anyway / Hau la hetan teste pozitivu, maibe nafatin diagnoza hau ho DENGE ◯ I don't know if I was tested or not / Hau la hatene karik hau teste ona ou seidauk
When you were diagnosed with MALARIA, how was this diagnosis made? Wainhira ita bo'ot diagnoza ho MALARIA, oinsa mak hetan diagnoza ida ne'e?	◯ I was tested for MALARIA and the test was POSITIVE / Hau halo teste ba MALARIA no teste nia rezultadu POZITIVU ◯ I did NOT receive a positive test, but I was diagnosed with MALARIA anyway / Hau la hetan teste pozitivu, maibe nafatin diagnoza hau ho MALARIA ◯ I don't know if I was tested or not / Hau la hatene karik hau teste ona ou seidauk

Questions about PREVIOUS illness and vaccination

Have you ever been diagnosed with COVID-19 by a healthcare worker in your life (even MORE THAN 6 MONTHS AGO)? Antes ne'e doutor sira diagnoza ona ita bo'ot ho COVID-19 ka lae, iha ita bo'ot nia moris (BELE LIU HUSI FULAN 6 BA KOTUK)	◯ Yes / Sim ◯ No / Lae ◯ Don't know / La hatene (Answer 'yes', even if a COVID diagnosis within 6 months was reported in previous answers / Hatán 'Sim', maske diagnoza ho COVID-19 iha fulan 6 nia laran ne'e relata ona iha resposta anterior)
When did you get diagnosed with COVID-19? Ita bo'ot hetan diagnostiku ho COVID-19 ne'e iha loron saida?	_____ (Enter approximate date if unsure, leave blank if unknown / Hatama data tuir hanoin karik la serteza, husik mamuk deit karik la hatene)

Have you ever been diagnosed by a healthcare worker with DENGUE in your life (even MORE THAN 6 MONTHS AGO)? Antes ne'e doutor sira diagnoza ona ita bo'ot ho DENGE ka lae, iha ita bo'ot nia moris (BELE LIU HUSI FULAN 6 BA KOTUK)?	◯ Yes / Sim ◯ No / Lae ◯ Don't know / La hatene (Answer 'yes', even if a DENGUE diagnosis within 6 months was reported in previous answers / Hatán 'Sim', maske diagnoza ho DENGE iha fulan 6 nia laran ne'e relata ona iha resposta anterior)
Have you ever been diagnosed by a healthcare worker with MALARIA in your life (even MORE THAN 6 MONTHS AGO)? Antes ne'e doutor sira diagnoza ona ita bo'ot ho MALARIA ka lae, iha ita bo'ot nia moris (BELE LIU HUSI FULAN 6 BA KOTUK)?	◯ Yes / Sim ◯ No / Lae ◯ Don't know / La hatene (Answer 'yes', even if a MALARIA diagnosis within 6 months was reported in previous answers / Hatán 'Sim', maske kuandu diagnoza ho MALARIA iha fulan 6 nia laran ne'e relata ona iha resposta anterior)
Have you received vaccination for COVID-19? Ita bo'ot simu ona vasinasaun ba COVID-19?	◯ Yes / Sim ◯ No / Lae
Number of doses Numeru dose	◯ 1 ◯ 2 ◯ 3 ◯ More than 3 / Liu husi 3
When was your FIRST COVID-19 vaccine? Ita bo'ot nia vasina COVID-19 primeiru ne'e simu iha loron saida?	_____ (Enter approximate date if unsure, leave blank if unknown / Hatama data tuir hanoin karik la serteza, husik mamuk deit karik la hatene)
Which type of COVID-19 vaccine was your FIRST dose? Vasina COVID-19 nia tipu ida ne'ebé mak ita bo'ot simu ba dose primeiru?	☐ AstraZenica ☐ Sinovac ☐ Pfizer ☐ Other / Seluk ☐ Don't know / La hatene
When was your SECOND COVID-19 vaccine Ita bo'ot nia vasina COVID-19 segundu ne'e simu iha loron saida?	_____ (Enter approximate date if unsure, leave blank if unknown / Hatama data tuir hanoin karik la serteza, husik mamuk deit karik la hatene)
Which type of COVID-19 vaccine was your SECOND dose? Vasina COVID-19 nia tipu ida ne'ebé mak ita bo'ot simu ba dose segundu?	☐ AstraZenica ☐ Sinovac ☐ Pfizer ☐ Other / Seluk ☐ Don't know / La hatene
When was your THIRD COVID-19 vaccine Ita bo'ot nia vasina COVID-19 terseiru ne'e simu iha loron saida?	_____ (Enter approximate date if unsure, leave blank if unknown / Hatama data tuir hanoin karik la serteza, husik mamuk deit karik la hatene)

Which type of COVID-19 vaccine was your THIRD dose? Vasina COVID-19 nia tipu ida ne'ebé mak ita bo'ot simu ba dose terseiru?	☐ AstraZenica ☐ Sinovac ☐ Pfizer ☐ Other / Seluk ☐ Don't know / La hatene
If you have had more than three COVID-19 vaccines, when was your MOST RECENT dose? Karik ita bo'ot simu vasina COVID-19 barak liu dala tolu, entaun ita bo'ot nia dose ikus liu ne'e simu iha loron saida?	_____ (Enter approximate date if unsure, leave blank if unknown / Hatama data tuir hanoin karik la serteza, husik mamuk deit karik la hatene)
If you have had more than three doses of COVID vaccines, which type have you recieved for your MOST RECENT DOSE? Karik ita bo'ot simu vasina COVID-19 ne'e barak liu dala tolu, tipu vasina ida ne'ebé mak ita bo'ot simu ikus liu?	☐ AstraZenica ☐ Sinovac ☐ Pfizer ☐ Other / Seluk ☐ Don't know / La hatene
Do you wish to be vaccinated for COVID-19? Ita bo'ot hakarak atu simu vasina ba COVID-19?	◯ Yes / Sim ◯ No / Lae ◯ Don't know / La hatene ◯ Not applicable - too young to answer this question / La aplikavel - sei kiik seidauk bele hatán ba pergunta ida ne'e (Only for participants who have not received any doses of COVID-19 vaccines / pergunta ida ne'e husu deit ba partisipante sira ne'ebé mak seidauk simu vasina COVID-19 nia dose ruma)
Why have you not received a SECOND dose of COVID-19 vaccine? Tamba saida mak ita bo'ot seidauk simu vasina COVID-19 nia dose segundu?	◯ It is less than 3 months since my first dose, therefore my second dose is not due yet / Seidauk to'o fulan 3, desde hau nia vasina dose primeiru, tamba ne'e hau nia dose segundu nia tempu seidauk to'o ◯ It is more than 3 months since my first dose, but I have not been offered a second dose yet / Tempu liu tiha ona fulan 3 desde hau nia dose primeiru, maibe hau seidauk hetan dose segundu ◯ I got COVID-19 infection after my first dose, therefore I don't think I need another dose / Hau hetan infeksaun COVID-19 depois de simu dose primeiru, tamba ne'e hau hanoin hau la presiza dose seluk tan ◯ I don't want to have another dose / Hau lakoi atu simu dose seluk tan ◯ Other / Seluk (Only asked to participants who have received just one dose of COVID-19 vaccine / Husu deit ba partisipante sira ne'ebé mak simu ona vasina COVID-19 nia dose primeiru)
Why don't you want another dose? Tamba saida mak ita bo'ot lakoi simu dose segundu?	_____
Please specify Favor espesifika	_____

Unable to Complete Questionnaire

Record ID _____

Fill this form if Household Questionnaire cannot be filled out (i.e. no data can be collected from the household)

Household number _____

Why could no data be collected?

- ◯ Household occupied but head-of-household does not wish to fill out Household Questionnaire
- ◯ Household looks occupied but no adult household members were present during all three visits
- ◯ Household looks derelict
- ◯ Household has been demolished
- ◯ Cannot access household

Please state the reason you couldn't find the household _____

VASINA fieldwork SOP – data and sample collection

For each household, do the following:

1. **Introduce research team to head-of-household and other household member(s)**
   - Explain study, using patient information sheet
   - Wear mask and visor for this

2. **Conduct *Household Questionnaire* on RedCap with head-of-household**
   - Fill this out even if no household members give consent for individual data or samples to be collected (if this is the case, head-of-household should still fill consent form)
   - If head-of-household does not wish to fill *Household Questionnaire* on RedCap, fill *Unable to Complete Questionnaire* on RedCap

3. **Each time you enrol an individual, give them a sticker with their Study ID number on it**
   - Children less than 1 year old should not be enrolled and do not need to be assigned a Study ID number (but their details should still be included on the *Household Questionnaire*)
   - For individuals who are not currently present, assign a study ID number, but keep his/her sticker until the next visit

For each participant, do the following:

4. **Receive written informed consent (signed form)**
   - For children, receive written informed consent from one of their parents
   - Also receive 'verbal assent' from the child

5. **Conduct *Individual Participant Questionnaire* on RedCap**

6. **Don PPE**

7. **Phlebotomy (maximum x2 attempts)**
   - Adults: use needle and syringe to collect 10ml (x2 tubes)
   - Children: use needle and syringe or butterfly to collect 5ml (x1 tube)
   - Also place x9-12 drops onto filter paper to make three blood spots (*see Dried Blood Spot SOP*).
   - Make sure blood tubes and dried blood spots are clearly labelled with stickers

8. **Finger-prick blood sample (where phlebotomy failed)**
   - Collect 2mls of blood drops into paediatric serum tubes
   - Collect x9-12 drops onto filter paper to make three blood spots.
   - Make sure blood tubes and dried blood spots are clearly labelled with stickers

9. **Store samples**
   - Put labelled blood samples into cool box

- Allow blood spot to dry for 4 hours, put into zip-lock bag with desiccant sachet. Put this into cool box

10. **Equipment disposal**
    - Place all sharps into sharps container
    - Place all other phlebotomy equipment into clinical waste bags
    - Dengue RDT can be disposed of, after good-quality picture and interpretation is done

11. **Thank household member(s) and make sure they have a copy of the participant information sheet**

12. **Make sure gloves and apron are changed and hands are washed/alcohol gelled before moving onto next participant**

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Vaccine Preventable Disease Seroprevalence In a Nationwide Assessment of Timor-Leste (VASINA-TL) - study protocol for a population-representative cross-sectional serosurvey

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Vaccine Preventable Disease Seroprevalence In a Nationwide Assessment of Timor-Leste (VASINA-TL) - study protocol for a population-representative cross-sectional serosurvey

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ABSTRACT

Introduction: Historic disruption in health infrastructure combined with data from a recent vaccine coverage survey suggests there are likely significant immunity gaps to vaccine preventable diseases and high risk of outbreaks in Timor-Leste. Community-based serological surveillance is an important tool to augment understanding of population-level immunity achieved through vaccine coverage and/or derived from prior infection.

Methods and analysis: This national population-representative serosurvey will take a three-stage cluster sample and aims to include 5600 individuals above one year of age. Serum samples will be collected by phlebotomy and analysed for measles immunoglobulin G (IgG), rubella IgG, severe acute respiratory syndrome coronavirus-2 anti-spike protein IgG, hepatitis B surface antibody and hepatitis B core antigen using commercially available chemiluminescent immunoassays or enzyme-linked immunosorbent assays. In addition to crude prevalence estimates and to account for differences in Timor-Leste's age structure, stratified age-standardised prevalence estimates will be calculated, using Asia in 2013 as the standard population. Additionally, this survey will derive a national asset of serum and dried blood spot samples which can be used for further investigation of infectious disease sero-epidemiology and/or validation of existing and novel serological assays for infectious diseases.

Ethics and dissemination: Ethical approval has been obtained from the Research Ethics and Technical Committee of the Instituto Nacional da Saúde,Timor-Leste and the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research, Australia. Co-designing this study with Timor-Leste Ministry-of-Health and other relevant partner organisations will allow immediate translation of findings into public health policy (which may include changes to routine immunisation service delivery and/or plans for supplementary immunisation activities).

STRENGTHS AND LIMITATIONS OF THIS STUDY

Strengths include:

- This serosurvey uses a three-stage cluster sample strategy to ensure it includes a representative individuals >1 year of age in Timor-Leste
- Serum samples will be analysed using validated assays and internationally recognised serological cut-offs which relate to VPD correlates of protection
- All procedures will be undertaken in Timor-Leste, representing an opportunity for local research and laboratory capacity building

Limitations include:

- Information on possible immunosuppressive conditions or treatments which may impact on serological responses to infection and vaccination will not be collected
- Serological targets for most pathogens do not distinguish between serological responses to infection and vaccination

INTRODUCTION

The Democratic Republic of Timor-Leste (Timor-Leste) achieved independence in 2002. It is a half-island nation located between Australia and Indonesia with a population of 1.3 million people. The Expanded Program on Immunisation (EPI) began *circa* 1989 when Timor-Leste was still an Indonesian province. In 1999 there was significant disruption of healthcare infrastructure, including the near cessation of routine vaccine delivery. After independence was regained in 2002, the EPI was reinstated as part of a national vaccination programme, initially with single-dose measles vaccine. Hepatitis B vaccination in infancy (three doses) was introduced by 2007. Birth dose hepatitis B vaccine was introduced in 2016, along with combined measles-rubella (MR) vaccination (two doses). Vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) began in adults in April 2021 and children above 12 years of age in October 2021.

The most comprehensive recent assessment of routine childhood vaccination coverage in Timor-Leste was a survey undertaken in 2018 which used a combination of maternal history and vaccination card review to confirm doses of vaccines given in the first and second years of life. This study found variable uptake between different vaccines and across geographic regions, large differences between 'valid' and 'crude' estimated vaccine coverage and highlighted the need for further investigation of population immunity to vaccine-preventable diseases (VPDs, see table 1).[1]

If you have room – be great to mention limitations of vaccine coverage surveys (which I increasing think are rubbish, and better to invest the money and time into serosurveys) – including limitation of various methods of vaccine coverage reporting (inaccuracy of verbal report, incompleteness of written records) and limitation of using receipt of a vaccine as proxy for immunity, in contexts where cold chain may not be reliable. Attached a paper to the email which compares serosurvey results to administrative data and vaccine coverage survey data in Ethiopia.

<Table 1 here>

For many pathogens, including measles, rubella, hepatitis B and SARS-CoV-2, specific immunoglobulin G (IgG) antibodies can be detected in the blood for many years, and sometimes lifelong, following infection or vaccination. In some cases, a specific quantity and/or quality of antibody in individuals' sera has been associated with protection from infection upon subsequent exposure. Presence above antibody cut-off levels can in some contexts infer protection, although how much such levels correlate with protection, varies on a range of factors.[2,3] Nonetheless, community-based serological surveillance is an important tool to augment understanding of population level immunity achieved through vaccine coverage over many years and/or immunity to VPDs derived from prior infection.[4] The results of serosurveys can be used to guide supplementary immunisation activities (SIAs), and tailor routine immunisation service delivery. There have been no previous community-based studies estimating VPD seroprevalence in Timor-Leste. However, targeted seroprevalence studies including healthcare workers in Timor-Leste have identified lower than expected seropositivity against measles, high seropositivity against SARS-CoV-2 and a high prevalence of active hepatitis B infection.[5,6]

This paper describes the protocol for a first and comprehensive national population-representative serosurvey of multiple VPDs. The survey is also designed to derive a national asset of serum and dried blood spot (DBS) samples which can be used for further investigation of infectious disease sero-epidemiology in Timor-Leste.

METHODS AND ANALYSIS

Aim: To determine the seroprevalence of measles, rubella, hepatitis B and SARS-CoV-2 among individuals of different age-strata in Timor-Leste.

Design: Population-representative, national cross-sectional serological survey.

Setting: Recruitment and data collection will occur in the community (within households) across Timor-Leste. Laboratory analysis will occur at Laboratório Nacional da Saúde (LNS) in Dili, Timor-Leste.

Sampling methods: Timor-Leste is made up of 12 municipalities and one Special Region (*Região Administrativa Especial de Oecusse Ambeno*), some of which are divided into sub-municipalities (*Posto administrativos)*. Atauro is a 14th municipality but through legacy is included as part of the Municipality of Dili. Each (sub-)municipality is divided into *sucos* (villages), which are further divided into *aldeias* (hamlets). In 2015, a national census took place in Timor-Leste. All households in the country were visited in-person, assigned a household number and global positioning (GPS) coordinates, and grouped into 2320 'enumeration areas' (EAs, the boundaries of which roughly correspond to those of each aldeia, see Figure 1).

<Figure 1 here>

A three-stage cluster random sample will be taken. First, a pre-specified number of EAs will be randomly selected from all EAs in the country, with probability proportionate to municipality population. Second, within each participating EA, a pre-specified number of households will be randomly selected from all households in that EA. Third, all occupants at participating households who meet eligibility criteria will be invited into the study.

A household will be defined as a dwelling unit that consists of a person or a group of related or unrelated persons, who live together in the same dwelling unit or informal shelter, who are considered as one unit and share a cooking area.

Eligibility criteria: Household members will be eligible to participate if they are ≥1 year of age and they (or their parent/guardian) provide consent to participate in the study. Individuals who report current illness which is compatible with coronavirus disease (COVID-19), and those who report any of the following conditions will be excluded:

- Needle phobia

- Anaemia

- Skin condition affecting phlebotomy sites

- Bleeding disorder

Additionally, individuals who cannot communicate verbally in Tetum, Portuguese or English will be excluded. Individuals under 1 year of age were excluded because maternal transfer of antibodies may affect interpretation of serology results and because this age group is more challenging to sample.

Sample size: A sample size was calculated with reference to the World Health Organization Reference Manual for Vaccination Coverage Cluster Surveys.[7] The investigator group considered which age groups would represent the most important sub-categories for estimating vaccine-preventable disease seroprevalence. This process took into account the local vaccine programme history (so serological findings can be related to estimated uptake), existing data on vaccine coverage including the referenced vaccine coverage survey (so that data from each source can be triangulated), programmatic considerations relating to potential intervention(s) in case immunity-gaps are identified, and various logistical and technical considerations (for example excluding individuals <1 year of age because the maternal transfer of antibodies would affect the interpretation and because this age group is more challenging to sample).

VPD seroprevalence estimates which were considered of particular importance to specific age-strata include the following:

- Measles in children under 5 years of age because they are most likely to suffer serious sequelae from infection when compared to other age groups[8] and between 5-14 years of age because outbreaks can occur and/or amplify in schools and other settings where groups of children from different households congregate.

- Rubella in women 15-40 years of age because future pregnancies may be at risk of congenital rubella syndrome (CRS). It is anticipated that the rubella virus is circulating in Timor-Leste as there has only been a recent introduction of rubella vaccination and there is very little surveillance for CRS.
- SARS-CoV-2 in children 1-12 years of age because seropositivity is likely to represent naturally acquired infection as this group are not eligible for vaccination in Timor-Leste, which will indicate the extent of local transmission which has occurred.
- Hepatitis B (surface antibody, HBsAb and core antibody, HBcAb) in children under 5 and 5-14 years of age because hepatitis B birth vaccination was introduced approximately 5 years ago and comparison of these groups will give an indication of uptake.

Therefore, each of the following age groups was considered separate strata to provide age-specific seroprevalence results of sufficient precision: 1-4, 5-14, 15-24, 25-40, and 41+ years.

An effective sample size was calculated (i.e. the sample size required if undertaking a simple random sample) of 280 for each stratum. This used the World Health Organization Reference Manual for Vaccination Coverage Cluster Surveys[7] and was based upon an expected seroprevalence of 50% (because this provided the most conservative estimate) and a precision of +/- 6% (because this was precise enough to adequately inform decision-making and small enough to provide a feasible sample size for the financial and human resources available) for the 95% confidence interval.

Without local data on which to confidently base estimates of design effect, a conservative design effect of 4 was estimated, to ensure a sufficient sample to provide precise results. This provided a sample size for each strata of 1120 individuals, and a total survey sample size of 5600.

Based on national census data from 2015 the average number of individuals in each household was 5.7.[9] Population projections for 2021 estimated the proportion of individuals in Timor-Leste belonging to each age strata to be 9.6%, 24.0%, 21.9%, 20.2% and 21.5%, respectively. As such, the expected number of required households to sample sufficient individuals from the smallest age strata (1-4 years) was calculated as:

1120 / (0.096 *5.7) = 2047

Household response rate was assumed to be 85%,based on consensus opinion of local investigators who had been involved in previous community surveys in Timor-Leste. The number of households which will be targeted is therefore calculated as:

2047/0.85 = 2408

This lead to 112 EAs being selected (with probability proportionate to municipality population), and 23 households being randomly selected from each EA.

Fieldwork procedures: Municipalities will be visited sequentially depending on various logistical considerations including weather and road conditions, availability of staff, vehicles, and accommodation, and local municipal and health leader preference.

First, the study team leader will make a 'coordination visit' to the municipality during which they will explain the study procedures, receive permission to conduct the study, and discuss travel routes and gaining access to each EA with the following individuals:

- Municipality Administrator (at Municipality Office; one per municipality)
- Director of Municipality Health Service (at Municipality Health Service Office; one per municipality)
- Sub-Municipality Administrator (at Administrative Post Offices; one for each sub-municipality being visited)
- Head of Community Health Centre (at Community Health Centre Office; one for each sub-municipality being visited)

- Chief of Suco (at Suco Office;1 for every suco being visited)
- Commander of Police in Suco (at Suco Police Station; one per suco being visited, at the discretion of the Chief of Suco)
- Chief of Aldeia (at Aldeia Office; one for every aldeia being visited)

If any of these individuals are not available in-person during the coordination visit attempts will be made to contact them by telephone or through WhatsApp.

Secondly, a 'study visit' will be made by a whole study team, consisting of a team leader (usually non-clinical), three research nurses, and two drivers. Additionally, at the discretion of the Municipality Administrator, Sub-Municipality Administrator and/or Head of Community Health Centre, one or two local government representatives and/or one or two Community Health Centre representatives may join the study visits. It is anticipated that these individuals will primarily observe study procedures. Any involvement in participant recruitment, data collection or sample collection will be directly supervised by the appropriate study team member.

Navigation and maps: Selected households will be identified using electronic tablets which will have GPS capability and Google Earth® software installed. Keyhole Markup Language (KML) files with GPS coordinates for all selected households in each EA will be pre-loaded onto the tablets, such that they can be used without mobile/internet connectivity. KML files are generated in QGIS. Each household location is verified using a Google Satellite base map. Study teams will also carry printed colour copies of bespoke maps for each EA, which will show the location of households in relation to roads, paths, and landmarks. These will be produced using ArcMap™ 10.4.1 and will include ESRI imagery base map, showing the location of main roads and the selected households coded by letters. The standard operating procedure (SOP) for location of households is shown in appendix 1.

Data collection: Study teams will approach the household occupants and introduce themselves. The occupants will be asked to identify an (acting) head-of-household. If this

individual is not available (or if no occupants are present), the study team will arrange to return twice to the household, and at least once on a separate day until a head-of-household is present.

Data will be collected using structured interview-questionnaires. Reponses will be entered into electronic tablets which will have REDCap® installed. This is a secure web platform for building and managing online databases which allows offline data entry[10]. Questionnaires data will be uploaded to the REDCap® secure server hosted at Menzies School of Health Research, Charles Darwin University, Darwin, Australia.

Three bespoke data collection tools have been developed (see appendices 2-4):

- Household questionnaire. This will be completed first. Demographic data on all household occupants (whether they are present at the time or not), will be collected by interviewing the head-of-household.
- Participant questionnaire. This will be completed if/when any household occupants agree to participate. Each will be assigned a unique identification number (participant ID number) and relevant demographic, clinical and vaccine-related data will be collected. Participants will not be asked to provide written documentation of vaccines received because a low proportion of participants in the recent vaccine coverage survey had retained this.[1]
- Unable to complete questionnaire. This will only be completed if the household questionnaire cannot be completed (i.e. if a head-of-household was not present or not willing to provide demographic data after 3 household visits). The reason for non-completion will be recorded in free-text.

Sample collection and handling: Research nurses with training and experience in adult and paediatric phlebotomy will collect primary blood samples using appropriate infection prevention control procedures and safe management of sharps. Participants >5 years of age will undergo venepuncture using either a standard hypodermic or a winged butterfly

needle with a syringe attached. Venous blood will then be injected directly into a gel serum separator tube (SST). Participants between 1-5 years of age (and those who do not consent to venepuncture but provide consent for a finger prick) may undergo capillary blood sampling through finger prick technique, in which case drops of blood will be applied directly to a paediatric gel SST. The method used will be determined on a case-by-case basis by the research nurse in the field. Table 2 shows sample volumes and collection techniques for participants in different age groups.

<Table 2 here>

Primary blood samples will be kept at ambient temperature out of direct sunlight and allowed to clot for a maximum of eight hours (i.e. one day of fieldwork). They will then undergo centrifugation at 1,500 RCF for ten minutes and the resulting separated serum samples will be kept at 4 degrees Celsius using a portable refrigerator with battery power backup. They will be transported to LNS within five days of sample collection and will undergo primary serological analysis within two weeks of sample collection.

A secondary dried blood spot (DBS) sample will be created: For participants who undergo venepuncture, the last 300-500µL of venous blood in the syringe will be injected onto Whatman 903 filter paper marked with three 12mm diameter circles. For participants who undergo finger-prick, additional drops of capillary blood will be applied directly from the finger to the filter paper. Once the circles are saturated with blood (typically using 100-150µL blood for each circle), the filter paper will be dried at ambient temperature out of direct sunlight for four hours, then placed alongside a desiccant sachet into a plastic zip lock bag. The SOP for data and sample collection is shown in appendix 5.

Sample analysis: Primary (serum) samples from all participants will be tested at LNS for rubella IgG (quantitative; considered positive if >10 IU/mL), SARS-CoV-2 anti-spike IgG (qualitative), hepatitis B core antibody (HBcAb, qualitative) and hepatitis B surface antibody (HBsAb, quantitative, considered positive if >10 mIU/mL) using Ortho Clinic Diagnostics®

chemiluminescent assays on the Vitros ECiQ® platform, and for measles IgG using the Eurimmun® ELISA assay (quantitative, positive if >120IU/L). For quantitative assays, serological cut-offs which have been most commonly shown to correlate with protection from infection and/or those which are conventionally used in serosurveys and/or assessment of immunity have been chosen.[11–13] Where data are somewhat conflicting, there is lack of consensus supporting a correlate-of-protection, or considerable inter-assay variability in quantitative determination has been observed, secondary (exploratory) analyses using alternative cut-offs may also be undertaken (for example 200IU/L and/or 250IU/L for measles IgG).[3]

Additionally, samples from participants residing in Dili Municipality (excluding Atauro) will be tested for hepatitis B surface antigen (HBsAg, qualitative). This marker denotes active hepatitis B infection which may have significant health implications and will therefore only be tested in Dili municipality where there is a hepatology clinic in which participants can receive further assessment and follow-up. Any samples which are positive for HBsAg will also be tested for hepatitis B envelope antigen (HBeAg, qualitative) and hepatitis B envelope antibody (HBeAb, qualitative) testing using Ortho Clinic Diagnostics chemiluminescent assays on the Vitros ECiQ platform, and hepatitis B viral load (HBVL, quantitative) using the Cepheid® assay on the GeneXpert platform. All testing will be carried out according to manufacturers' instructions and cited serological cut-off values. For qualitative assays, samples with borderline/indeterminate results will be considered negative, apart from HBsAg where the test will be repeated and both results reviewed alongside other hepatitis B results by an appropriately qualified clinical member of the research team who will decide whether the participant should be referred to the hepatology clinic for repeat sampling and clinical assessment.

Assays have been chosen based on their previous performance in seroprevalence studies, immediate availability for shipment to Timor-Leste, and local laboratory expertise in operating these types of assays (with ongoing capacity building for serological testing in

LNS). The measles IgG assay is quantitative and calibrated against a World Health Organisation (WHO) Standard (NIBSC, Anti-Measles serum, 3rd International Standard 97/648). It showed acceptable performance when assessed in a recent study of concordance between commercially available assays (concordance for samples with positive/negative status = 90%/100%)[14]. The rubella IgG assay is quantitative and calibrated against a Centers for Disease Control and Prevention (CDC) standard (Low Titer Rubella Standard) and a World Health Organization (WHO) standard (1st International Rubella IgG Standard). While concerns around standardisation of rubella IgG assays are noted[15], this assay showed acceptable performance when assessed in a recent study of concordance between automated immunoassays (concordance for samples with negative/positive status = 90.6%/91.1%)[16]. The hepatitis B surface antibody assay is quantitative and showed high sensitivity (97.1%) and specificity (97.9%) when evaluated in a panel of sera from healthcare workers and patients[17]. The SARS-CoV-2 anti-spike IgG assay has high sensitivity (93.3%, >21 days post infection) and specificity (100%), which compares favourably to many other available immunoassays[18], and has been used in several serological surveillance studies[19–21].

Provision of results to participants: The majority of testing in this study will be for antibodies against VPDs (either IgG or total antibody). Results will therefore only indicate whether an individual has been previously infected and/or vaccinated against each disease at some time in the past and will not provide information on current infection. While seronegative participants may be at risk of future infections (and may benefit from vaccination), individual notification of results and provision of vaccines to all seronegative study participants is not considered feasible in this large cross-sectional study. Instead, all participants will be advised of the benefits of routine vaccination and immunisation clinics in their area, as well as on any forthcoming SIAs which may occur as a result of this study.

In addition to antibody tests, serum from participants within Dili Municipality (excluding Atauro) will be tested for HBsAg. This marker denotes active hepatitis B infection which may

have significant health implications and will therefore only be tested in Dili municipality where there is a hepatology clinic in which participants can be seen. Participants who test positive for HBsAg will be contacted by telephone to discuss their results and will be offered assessment including biochemical and radiological investigation of liver function and consideration of antiviral treatment in-line with international clinical guidelines[22]. This approach has been successful and has been acceptable to participants in a smaller serological surveillance study including hepatitis B testing among healthcare workers in Timor-Leste[6].

Sample storage: Primary (serum) samples will be stored at -80 degrees Celsius and secondary (DBS) samples will be stored at 4 degrees Celsius at LNS for 10 years. These may undergo additional serological analyses to further investigate infectious disease sero-epidemiology in Timor-Leste and/or validating existing and novel serological assays for infectious diseases, pending successful funding application and appropriate ethical approval.

Fieldworker training: Field workers will undergo one week of formal in-person training in study procedures. Days 1-2 will be classroom based and will include sessions on 'the study protocol', 'field team composition' (structure, members, responsibilities), 'logistics, technology and map reading', 'recruitment and consent', and 'collection of data using interview questionnaires'. Days 3-5 will be practical and will include demonstrations and training in adult and paediatric phlebotomy and finger-prick techniques, infection prevention control procedures, and the use of personal protective equipment (PPE). These skills will be assessed formatively throughout the training and summatively using pre- and post- session assessments. Training will be delivered by PA, JF, NSSF and/or JY who are clinicians with experience of epidemiological and clinical research in Timor-Leste.

Laboratory team training: Laboratory training will occur in the Serology Department of LNS. Training will be delivered by PA and TO who have significant experience with ELISA and chemiluminescent techniques, including in Timor-Leste. The focus of training will be on

assay verification and quality assurance, as well as procedures for sample processing, analysis and storage.

Data storage and handling: Field data will be stored in the REDCap® secure server hosted at Menzies School of Health Research, Charles Darwin University, Darwin, Australia, until analysis. Laboratory data (i.e. serology worksheets and results) will be stored on the password-protected LNS laboratory information system (SchuyLab®) until analysis. Deidentified field and laboratory datasets will be downloaded and stored as password-protected databases on computer(s) at Menzies School of Health Research, Timor-Leste Office, Dili, Timor-Leste, where they will be linked using participant ID numbers and analysed. Only named investigators who are working directly on this project will have access to data.

Statistical analysis plan: Primary data analysis will occur at the end of the study, once all fieldwork is complete and all samples have been analysed. Interim analyses may also occur upon reasonable request from the Timor-Leste MoH or other partner organisation. As a multi-stage sampling survey design will be used to select participants, sampling weights will be calculated at each stage. These weights will reflect a participant's inverse probability of selection at a particular stage, be it at the EA level, the household level or the householder level. Furthermore, these weights will subject to both non-response adjustment and finite population correction. Measures of prevalence will be age-standardised using the standard population for Asia given by the International Network for the Demographic PNGIMR 2018, Papua New Guinea MIS 2016-2017.[23] To account for this design, the 'svy' data commands in Stata (version 16, StataCorp, College Station, TX, USA) will be used for ally analyses. Characteristics of participants will be summarised using weighted descriptive statistics. Frequencies and proportions will be used to describe categorical distributions, whilst means and standard deviations will be used to describe continuous variables. In the presence of non-normality, medians and interquartile ranges will be reported. Univariable and multivariable binary logistic regression will be undertaken to model age, the independent

variables of primary interest with the five VPD outcomes. In addition to this variable, other variables known to be risk factors of VPD, such as sex and travel history, will be subjected to a manual backward stepwise procedure. Variables with a p-value ≥ 0.20 will not be retained. The Hosmer-Lemeshow test will be used to test the goodness of fit of each multivariable model. A p-value < 0.05 will be considered statistically significant with Odds Ratios (OR), 95% confidence intervals (CI) and p-values calculated for age and sex.

Timing: This study will begin in January 2022 and is expected to end in December 2023.

Patient and public involvement: Engagement with members of the public, local administrative and health leaders has been central to the design of this study. It has resulted in procedures which will maximise participant choice and ensure any clinically relevant diagnoses made during the study are followed-up.

ETHICS AND DISSEMINATION

Informed consent: Each prospective participant will receive a participant information sheet which will be printed in English and Tetum (appendices 6 and 7). They will also be provided with a verbal explanation of the study rationale and procedures. This will include potential risks and benefits of sample collection, specific tests which their sample will undergo, the fact that they will not receive notification of any results (with the exception of a positive HBsAg, tested in Dili Municipality only) and the possibility that their sample will undergo additional analyses for evidence of communicable diseases during the next 10 years. They will be given up to 30 minutes to ask questions and decide whether they wish to participate, and will then provide informed, written consent by signing a consent form (appendices 6 and 7). For individuals under 16 years of age, verbal assent will be sought, in addition to written consent from their parent or guardian. This study has received ethical approval from the Research Ethics and Technical Committee of the Instituto Nacional da Saúde,Timor-Leste (Reference: 875 MS-INS/DGE/IX/2021) and the Human Research Ethics Committee of the

Northern Territory Department of Health and Menzies School of Health Research, Australia (Reference: 2021-4064).

Protocol amendments: Any modifications to the protocol which may impact on the conduct of the study will be documented in a formal protocol amendment and approved by both Research Ethics Committees prior to implementation of the changes. The Research Ethics Committees will also be notified of any minor corrections/clarifications or administrative changes to the protocol, which will be documented in a protocol amendment letter.

Adverse events: Data on adverse events will be collected throughout the study, with participants (or their parent/guardian) being informed of the risks of phlebotomy (including bruising, bleeding and infection), how to recognise these, and how to contact the study team if they occur. Adverse events will be reported to the Principal Investigator. In cases where infection or any other serious adverse event has occurred, the Principle Investigator will conduct a review of the study visit and decide whether any phlebotomy retraining or change in practice is required and/or whether recruitment to the study should be paused.

Strengths and limitations: This project will produce accurate, nationally representative seroprevalence data for multiple VPDs and relevant age-groups, which has not been achieved in Timor-Leste previously. It has been co-designed by investigators at Menzies School of Health Research (Timor-Leste Office), the National Centre for Immunisation Research and Surveillance (Australia), the MoH (Timor-Leste), LNS (Timor-Leste), and the WHO (Timor-Leste Office) according to local research and public health priorities. This will allow immediate translation of findings into public health policy (including potentially changes to routine immunisation service delivery and/or plans for SIAs). Additionally, the survey will derive a national asset of serum and dried blood spot (DBS) samples which can be used for further investigation of infectious disease sero-epidemiology in Timor-Leste and/or validating existing and novel serological assays for infectious diseases.[24–28] Engagement with local administrative and health leaders and maximisation of participant choice and welfare have

been central to the design, including ensuring all individuals diagnosed with active hepatitis B during the study have access to appropriate further investigation and follow-up.

Limitations include the collection of only a small number of VPD-related clinical variables. This decision was taken because the investigator group felt that conducting prolonged interview questionnaires including potentially sensitive health information may make some potential participants feel uncomfortable, and as such ease-of-questionnaire-administration and survey acceptability +/- uptake was prioritised. However, interpretation of serology can be affected by underlying immunosuppressive conditions (for example), and therefore absence of these data may affect study findings. The prevalence of such conditions in Timor-Leste is likely to be low, and therefore only to be a minor limitation to our study: Prevalence of HIV in Timor-Leste is estimated to be 0.2% in those aged 15-49 years, and chemotherapy for malignant conditions (except for corticosteroids) is largely unavailable. Another limitation is the exclusion of individuals who report current illness which is compatible with COVID-19. It is possible that this could lead to underestimation of SARS-CoV-2 seroprevalence. However, since acute illness is relatively short lived (<2 weeks for the majority of people) when compared to the duration of anti-S seropositivity (typically many months), this effect will likely be low, but will depend on the timing of fieldwork in relation to any local outbreaks of SARS-CoV-2 (i.e. the prevalence of acute infection in relation to overall seroprevalence, at the time of survey). A final limitation relates to the choice of serological targets, which (except for hepatitis B) will not differentiate vaccine- from infection-derived immunity. For example, SARS-CoV-2 anti-S antibodies will be tested, but not anti-nucleocapsid antibodies. This decision was taken because targets related to population immunity (i.e. those correlating with protection) were considered most important, regardless of its source, and additional targets would be costly. Additionally, some whole-virus vaccines will likely be used in Timor-Leste, which cannot be differentiated with such methods.

Risks include the ongoing global outbreak of SARS-CoV-2 (which may delay/prohibit study visits), disruption of supply of field and laboratory consumables to Timor-Leste (which may delay/increase the cost of laboratory analysis), natural disasters such as flooding, and potential unwillingness of individuals to participate in provision of data and/or samples (which may affect recruitment, potentially disproportionately among children).

Dissemination / knowledge transition plan: After each interim analyses, results will be shared with Timor-Leste MoH partners in the form of an oral presentation and in a written report. Following completion of the study, results will be shared with Timor-Leste MoH, other partner organisations, and local administrative and health leaders for EAs where the study took place (Municipality Administrators, Directors of Municipality Health Services, Sub-Municipality Administrators, Heads of Community Health Centres, Chiefs of Sucos, Chief of Aldeia), in the form of a written report. Results will also be submitted for publication in peer-reviewed journals and presented at relevant international conferences.

AUTHOR CONTRIBUTIONS

PA, SLS, NM, SA, NS, CF, FNM, NSSF, KM, JY and JRF conceived the study. PA, SLS, NM, MYT, SA, ADKD, NS, LA, CF, FNM, CAG reviewed existing literature and performed situation analysis. PA, MYT, SA, VS, LA, CAG, ICB determined the fieldwork procedures and designed data collection tools. PA, NG, TO, NS, ES, LA, ICB determined laboratory procedures. PA, MD, ADKD, NS, NSSF drafted the data and statistical analysis plan. NM, MYT, SA, NS, CF, FNM, CAG planned community engagement, obtained ethical approval and lead other regulatory aspects of the study. All authors reviewed and commented on the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests for this study.

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Table 1: Routine Timor-Leste vaccination schedule in 2022 and estimated coverage in 2018

Vaccine	Recommended age of administration	Introduced	Crude estimated coverage (2018 national vaccine coverage survey) (95%CI)* (%)	Valid estimated coverage (2018 national vaccine coverage survey) (95%CI)** (%)
BCG	At birth	Pre 1999	94.7 (91.7-97.0)	68.8 (61.3-75.0)
HepB0		2016	66.2 (58.5-73.0)	55.0 (46.9-63.0)
bOPV0		2016	80.4 (74.0-86.0)	60.8 (52.2-69.0)
DTwP-Hib-HepB1	6 weeks	2007	91.8 (87.8-95.0)	74.6 (68.4-80.0)
bOPV1		2016	91.8 (87.8-95.0)	75.5 (69.4-81.0)
RV1		2019	Not part of routine vaccination in 2018	
DTwP-Hib-HepB2	10 weeks	2007	87.4 (82.6-91.0)	72.8 (66.7-78.0)
bOPV2		2016	87.8 (83.0-91.0)	73.2 (66.9-79.0)
RV2		2019	Not part of routine vaccination in 2018	
DTwP-Hib-HepB3	14 weeks	2007	83.3 (78.0-87.0)	75.0 (68.8-80.0)
bOPV3		2016	83.3 (78.0-87.0)	75.3 (69.0-81.0)
RV3		2019	Not part of routine vaccination in 2018	
IPV		2016	80.6 (74.1-86.0)	71.5 (64.6-77.0)
MR1	9 months	2016	77.3 (71.5-82.0)	60.5 (54.0-67.0)
DTwP4	18 months	2016	54.8 (46.5-63.0)	16.0 (12.1-21.0)
MR2		2016	54.4 (46.1-62.0)	12.5 (9.0-17.0)
DT	6 year or school entry	2016	Not measured in 2018 survey	
TT1-5	Unimmunised pregnant women	Pre 1999	68.2 (62.4-74.0)	

SARS-CoV-2	Adults and children above 12 years of age	Adults: Apr 2021 Children Oct 2021	Not part of routine vaccination in 2018	

Abbreviations: BCG = bacillus Calmette-Guérin; HepB = hepatitis B; bOPV0 = bivalent oral polio vaccine; DTwP-Hib-HepB = diphtheria, tetanus, pertussis, hepatitis B and Haemophilus influenzae type B; RV = rotavirus vaccine; IPV = inactivated poliovirus vaccine; MR = measles and rubella vaccine; DT - diphtheria and tetanus vaccine; TT - tetanus toxoid vaccine.

*crude coverage is defined as all doses, whether valid or not, by any documented evidence or verbal history at the time of the survey.

**valid coverage includes only the doses of vaccines that were given on or after the minimum date of eligibility and requires a vaccination record (either home based or health facility) with a documented date.

Table 2: Sample volumes and collection techniques for primary sample collection by age group

Age group	Method of blood collection	Equipment	Collection container	Target sample volume
1-5 years	Venepuncture*	23G butterfly needle, venous blood aspirated into 5ml syringe	5ml SST tube	5ml
	Finger prick*	Lancet, capillary blood drops transferred directly into collection tube	2ml paediatric SST tube	2ml
6-15 years	Venepuncture	21-23G needle or 23G butterfly needle, venous blood aspirated into 5ml syringe	5ml SST tube	5ml
Adults	Venepuncture	21-23G needle, venous blood aspirated into 10ml syringe	2x 5ml SST tube	10ml

Abbreviations: SST = serum separator tube

*Determined in field by phlebotomist on case-by-case basis

APPENDICIES

Appendix 1: Standard operating procedure for location of households

Appendix 2: Household questionnaire

Appendix 3: Participant questionnaire

Appendix 4: Did not complete questionnaire

Appendix 5: Standard operating procedure for data and sample collection

Appendix 6: Participant information sheet and consent form (within Dili)

Appendix 7: Participant information sheet and consent form (outside Dili)

FIGURE LEGENDS

Figure 1: Map of Timor-Leste showing enumeration areas (EAs) within municipalities

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Mr Trevor Clifford

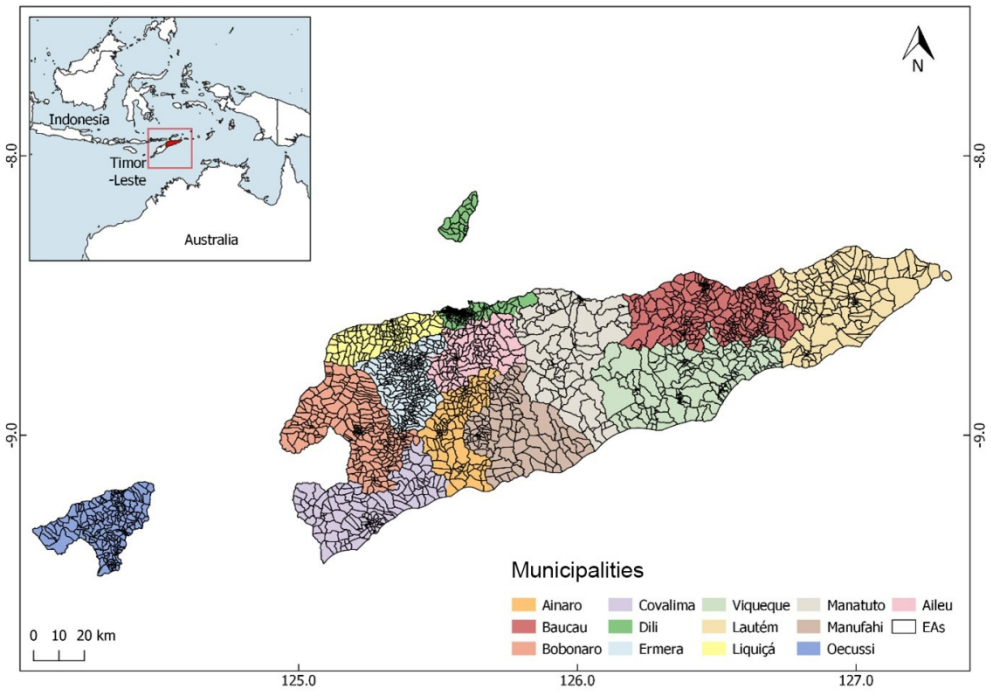

Figure 1: Map of Timor-Leste showing enumeration areas (EAs) within municipalities

159x112mm (220 x 220 DPI)

VASINA fieldwork SOP – location of households

1. **Navigate to household using GPS device and printed maps**
   - Make sure you identify the exact house (do not accept another household nearby)

2. **If at least one adult household member is present, move to *Data and Sample Collection SOP***

3. **If the household was found, but there are no household members present (or if one or more household members are missing):**
   - Arrange and conduct a second and third visit. At least one of these should be on a different day.
   - If there are no household members present on all three visits, complete the *'Unable to Complete Questionnaire'* on RedCap
   - Do not find another household to replace this one

4. **If the household was found, but it looks derelict**
   - Fill out the *'Unable to Complete Questionnaire'* on RedCap
   - Choose the nearest occupied house and offer study participation to these occupants instead. If there are two equidistant houses, choose the one on the Left. When collecting data for the new household, add "B" to the end of the household number (e.g. "12345**B**")

5. **If the household cannot be found**
   - Fill out the *'Unable to Complete Questionnaire'* on RedCap
   - Make sure you enter the reason that the household cannot be found
   - Do not find another household to replace this one

Household Questionnaire

Record ID	_____
Household number Númeru Uma-kain	_____
How many people currently live in this household? Ema nain hira mak agora dadauk hela iha uma-kain ida-ne'e?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ 7 ◯ 8 ◯ 9 ◯ 10 ◯ 11 ◯ 12 ◯ 13 ◯ 14 ◯ 15 ◯ 16 ◯ 17 ◯ 18 ◯ 19 ◯ 20 (If the household has more than 20 mebers, fill out a second Household Questionnaire / Karik iha uma-kain ida nia membru barak liu ema 20, preenxe fali iha kuestionariu uma-kain segundu)
Gender of household member 1 Jeneru membru uma-kain 1	◯ Male / Mane ◯ Female / Feto
Age of household member 1 Idade membru uma-kain 1	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 1 Númeru ID estudu nian ba membru uma-kain 1	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 2 Jeneru membru uma-kain 2	◯ Male / Mane ◯ Female / Feto

Age of household member 2 Idade membru uma-kain 2	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 2 Númeru ID estudu nian ba membru uma-kain 2	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 3 Jeneru membru uma-kain 3	◯ Male / Mane ◯ Female / Feto
Age of household member 3 Idade membru uma-kain 3	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 3 Númeru ID estudu nian ba membru uma-kain 3	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 4 Jeneru membru uma-kain 4	◯ Male / Mane ◯ Female / Feto
Age of household member 4 Idade membru uma-kain 4	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 4 Númeru ID estudu nian ba membru uma-kain 4	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 5 Jeneru membru uma-kain 5	◯ Male / Mane ◯ Female / Feto
Age of household member 5 Idade membru uma-kain 5	_____ (If unsure, please estimate age (to the nearest 10 years))

Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 5 Númeru ID estudu nian ba membru uma-kain 5	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 6 Jeneru membru uma-kain 6	◯ Male / Mane ◯ Female / Feto
Age of household member 6 Idade membru uma-kain 6	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 6 Númeru ID estudu nian ba membru uma-kain 6	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 7 Jeneru membru uma-kain 7	◯ Male / Mane ◯ Female / Feto
Age of household member 7 Idade membru uma-kain 7	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 7 Númeru ID estudu nian ba membru uma-kain 7	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 8 Jeneru membru uma-kain 8	◯ Male / Mane ◯ Female / Feto
Age of household member 8 Idade membru uma-kain 8	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)

Study ID number of household member 8 Númeru ID estudu nian ba membru uma-kain 8	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 9 Jeneru membru uma-kain 9	◯ Male / Mane ◯ Female / Feto
Age of household member 9 Idade membru uma-kain 9	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 9 Númeru ID estudu nian ba membru uma-kain 9	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 10 Jeneru membru uma-kain 10	◯ Male / Mane ◯ Female / Feto
Age of household member 10 Idade membru uma-kain 10	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 10 Númeru ID estudu nian ba membru uma-kain 10	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 11 Jeneru membru uma-kain 11	◯ Male / Mane ◯ Female / Feto
Age of household member 11 Idade membru uma-kain 11	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 11 Númeru ID estudu nian ba membru uma-kain 11	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)

Gender of household member 12 Jeneru membru uma-kain 12	◯ Male / Mane ◯ Female / Feto
Age of household member 12 Idade membru uma-kain 12	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 12 Númeru ID estudu nian ba membru uma-kain 12	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 13 Jeneru membru uma-kain 13	◯ Male / Mane ◯ Female / Feto
Age of household member 13 Idade membru uma-kain 13	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 13 Númeru ID estudu nian ba membru uma-kain 13	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 14 Jeneru membru uma-kain 14	◯ Male / Mane ◯ Female / Feto
Age of household member 14 Idade membru uma-kain 14	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 14 Númeru ID estudu nian ba membru uma-kain 14	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 15 Jeneru membru uma-kain 15	◯ Male / Mane ◯ Female / Feto

Age of household member 15 Idade membru uma-kain 15	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 15 Númeru ID estudu nian ba membru uma-kain 15	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 16 Jeneru membru uma-kain 16	◯ Male / Mane ◯ Female / Feto
Age of household member 16 Idade membru uma-kain 16	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 16 Númeru ID estudu nian ba membru uma-kain 16	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 17 Jeneru membru uma-kain 17	◯ Male / Mane ◯ Female / Feto
Age of household member 17 Idade membru uma-kain 17	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 17 Númeru ID estudu nian ba membru uma-kain 17	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 18 Jeneru membru uma-kain 18	◯ Male / Mane ◯ Female / Feto
Age of household member 18 Idade membru uma-kain 18	_____ (If unsure, please estimate age (to the nearest 10 years))

Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 18 Númeru ID estudu nian ba membru uma-kain 18	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 19 Jeneru membru uma-kain 19	◯ Male / Mane ◯ Female / Feto
Age of household member 19 Idade membru uma-kain 19	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 19 Númeru ID estudu nian ba membru uma-kain 19	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
Gender of household member 20 Jeneru membru uma-kain 20	◯ Male / Mane ◯ Female / Feto
Age of household member 20 Idade membru uma-kain 20	_____ (If unsure, please estimate age (to the nearest 10 years))
Adult or child?	◯ Adult ◯ Child (Only fill this out if age cannot be estimated)
Study ID number of household member 20 Númeru ID estudu nian ba membru uma-kain 20	_____ (Only fill if he/she chooses to participate / Preenxe deit kuandu nia hili atu partisipa)
At any time in the past 12 months, has anyone sprayed the interior walls of your house? Durante fulan 12 liu-bá, iha ema ruma mai rega ita boot nia moru/uma nia didin lolon (parte laran), ka lae?	☐ Yes / Sim ☐ No / Lae ☐ Don't know / hatene
What do you use to stop being bitten by mosquitoes at night time? Saida mak ita bo'ot uza hodi prevene susuk labele tata iha tempu kalan?	☐ Sprays / Rega ho aimoruk susuk nian ☐ Coils / Ai-moruk susuk nian (sunu) ☐ Bednets / Moskiteiru ☐ Other / Seluk ☐ None / La iha

Please specify

Favor espesifika

Individual Participant Questionnaire

Record ID	_____

Demographic information

Informasaun Demográfiku

Household number Númeru Uma-kain	_____
Study ID number Númeru ID estudu	_____ (Use number on sample sticker / Uza númeru iha amostra nia sticker)
Name of interviewer Naran entrevistador	_____
Date of interview Data halao entrevista	_____
First name Naran prinsipal	_____
Family name Naran Familia	_____
Gender Jeneru	◯ Female / Feto ◯ Male / Mane ◯ Other/Unknown / Seluk/La hatene
Date of birth Data moris	_____
Age Idade	_____ (Only need to fill if date-of-birth is not known / Presiza atu preenxe deit karik la hatene data moris)
Phone number(s) Numeru Telemovel	_____
Email address	_____

Nationality Nasionalidade	◯ Timorese / Timor oan ◯ Other / seluk
Specify Espesifika	_____
Have you traveled outside Timor-Leste (including West Timor, NTT) within the last 6 MONTHS? Iha fulan 6 ikus liu, ita bo'ot halao viajen ba rai-liur ka lae (inklui nasaun viziño Indonesia nia provinsia, NTT)?	◯ Yes / Sim ◯ No / Lae ◯ Don't know / La hatene
Where did you travel (in the last 6 months)? Ita bo'ot ba iha rai ne'ebé? (iha fulan 6 ikus liu)	◯ West Timor (NTT) ◯ Other / Seluk
Please specify the country (or countries) you went to (in the last 6 months) Favor espesifika nasaun sira ne'ebé mak ita bo'ot viajem ba ona (iha fulan 6 ikus liu)	_____
Have you ever traveled outside Timor-Leste in your lifetime (but MORE THAN 6 MONTHS AGO)? This includes travel to West Timor (NTT). Ita bo'ot halao ona viajem fora husi Timor-Leste, durante vida moris? (maibe husi fulan 6 liu-bá kotuk)? Ida ne'e inklui viajem provinsia NTT	◯ Yes / Sim ◯ No / Lae ◯ Don't know / La hatene
Where did you travel (MORE THAN 6 MONTHS AGO)? Ita bo'ot viajen ba iha rai ne'ebé (FULAN 6 IKUS LIU BA KOTUK)?	◯ West Timor (NTT) ◯ Other / Seluk
Please specify the country (or countries) you went to MORE THAN 6 MONTHS AGO Favor espesifika nasaun sira ne'ebé mak ita bo'ot ba ona Iha FULAN 6 IKUS LIU BA KOTUK	_____

Questions about febrile illness within the last 6 months

Pergunta konaba moras isin manas iha fulan 6 ikus liu

Have you been unwell with a FEVER within the last 6 months? Ita bo'ot sente moras ho ISIN MANAS iha fulan 6 ikus liu nia laran ka lae?	◯ Yes / Sim ◯ No / Lae ◯ Don't know / La hatene

When you had this illness, did you seek any medical care? Iha momentu ita bo'ot hetan moras ida-ne'e, ita bo'ot ba konsulta iha fasilidade saúde ka lae?	◯ Yes / Sim ◯ No / Lae ◯ Don't know / La hatene (Includes medical clinic, pharmacy, hospital etc. / Inklui klinika mediku, farmasia, hospital, etc.)
When you sought medical care with fever in the last 6 months, did you get diagnosed by a healthcare professional with any of the following infections? Wainhira ita bo'ot buka tratamentu husi mediku sira ho isin manas iha fulan 6 ikus liu, ita bo'ot hetan diagnostiku husi professional saúde ho infeksaun hirak tuir mai ne'e ka lae?	☐ COVID-19 ☐ Dengue / Denge ☐ Malaria ☐ Other / Seluk ☐ No specific diagnosis was made / La halo diagnostiku espesifiku
When you were diagnosed with COVID-19, how was this diagnosis made? Wainhira ita bo'ot diagnoza ho COVID-19, oinsa mak hetan diagnoza ida ne'e?	◯ I was tested for COVID-19 and the test was POSITIVE / Hau halo teste ba COVID-19 no teste nia rezultadu POZITIVU ◯ I did NOT receive a positive test, but I was diagnosed with COVID-19 anyway / Hau la hetan teste pozitivu, maibe nafatin diagnoza hau ho COVID-19 ◯ I don't know if I was tested or not / Hau la hatene karik hau teste ona ou seidauk
When you were diagnosed with DENGUE, how was this diagnosis made? Wainhira ita bo'ot diagnoza ho DENGE, oinsa mak hetan diagnoza ida ne'e?	◯ I was tested for DENGUE and the test was POSITIVE / Hau halo teste ba DENGE no teste nia rezultadu POZITIVU ◯ I did NOT receive a positive test, but I was diagnosed with DENGUE anyway / Hau la hetan teste pozitivu, maibe nafatin diagnoza hau ho DENGE ◯ I don't know if I was tested or not / Hau la hatene karik hau teste ona ou seidauk
When you were diagnosed with MALARIA, how was this diagnosis made? Wainhira ita bo'ot diagnoza ho MALARIA, oinsa mak hetan diagnoza ida ne'e?	◯ I was tested for MALARIA and the test was POSITIVE / Hau halo teste ba MALARIA no teste nia rezultadu POZITIVU ◯ I did NOT receive a positive test, but I was diagnosed with MALARIA anyway / Hau la hetan teste pozitivu, maibe nafatin diagnoza hau ho MALARIA ◯ I don't know if I was tested or not / Hau la hatene karik hau teste ona ou seidauk

Questions about PREVIOUS illness and vaccination

Have you ever been diagnosed with COVID-19 by a healthcare worker in your life (even MORE THAN 6 MONTHS AGO)? Antes ne'e doutor sira diagnoza ona ita bo'ot ho COVID-19 ka lae, iha ita bo'ot nia moris (BELE LIU HUSI FULAN 6 BA KOTUK)	◯ Yes / Sim ◯ No / Lae ◯ Don't know / La hatene (Answer 'yes', even if a COVID diagnosis within 6 months was reported in previous answers / Hatán 'Sim', maske diagnoza ho COVID-19 iha fulan 6 nia laran ne'e relata ona iha resposta anterior)
When did you get diagnosed with COVID-19? Ita bo'ot hetan diagnostiku ho COVID-19 ne'e iha loron saida?	_____ (Enter approximate date if unsure, leave blank if unknown / Hatama data tuir hanoin karik la serteza, husik mamuk deit karik la hatene)

Have you ever been diagnosed by a healthcare worker with DENGUE in your life (even MORE THAN 6 MONTHS AGO)? Antes ne'e doutor sira diagnoza ona ita bo'ot ho DENGE ka lae, iha ita bo'ot nia moris (BELE LIU HUSI FULAN 6 BA KOTUK)?	◯ Yes / Sim ◯ No / Lae ◯ Don't know / La hatene (Answer 'yes', even if a DENGUE diagnosis within 6 months was reported in previous answers / Hatán 'Sim', maske diagnoza ho DENGE iha fulan 6 nia laran ne'e relata ona iha resposta anterior)
Have you ever been diagnosed by a healthcare worker with MALARIA in your life (even MORE THAN 6 MONTHS AGO)? Antes ne'e doutor sira diagnoza ona ita bo'ot ho MALARIA ka lae, iha ita bo'ot nia moris (BELE LIU HUSI FULAN 6 BA KOTUK)?	◯ Yes / Sim ◯ No / Lae ◯ Don't know / La hatene (Answer 'yes', even if a MALARIA diagnosis within 6 months was reported in previous answers / Hatán 'Sim', maske kuandu diagnoza ho MALARIA iha fulan 6 nia laran ne'e relata ona iha resposta anterior)
Have you received vaccination for COVID-19? Ita bo'ot simu ona vasinasaun ba COVID-19?	◯ Yes / Sim ◯ No / Lae
Number of doses Numeru dose	◯ 1 ◯ 2 ◯ 3 ◯ More than 3 / Liu husi 3
When was your FIRST COVID-19 vaccine? Ita bo'ot nia vasina COVID-19 primeiru ne'e simu iha loron saida?	_____ (Enter approximate date if unsure, leave blank if unknown / Hatama data tuir hanoin karik la serteza, husik mamuk deit karik la hatene)
Which type of COVID-19 vaccine was your FIRST dose? Vasina COVID-19 nia tipu ida ne'ebé mak ita bo'ot simu ba dose primeiru?	☐ AstraZenica ☐ Sinovac ☐ Pfizer ☐ Other / Seluk ☐ Don't know / La hatene
When was your SECOND COVID-19 vaccine Ita bo'ot nia vasina COVID-19 segundu ne'e simu iha loron saida?	_____ (Enter approximate date if unsure, leave blank if unknown / Hatama data tuir hanoin karik la serteza, husik mamuk deit karik la hatene)
Which type of COVID-19 vaccine was your SECOND dose? Vasina COVID-19 nia tipu ida ne'ebé mak ita bo'ot simu ba dose segundu?	☐ AstraZenica ☐ Sinovac ☐ Pfizer ☐ Other / Seluk ☐ Don't know / La hatene
When was your THIRD COVID-19 vaccine Ita bo'ot nia vasina COVID-19 terseiru ne'e simu iha loron saida?	_____ (Enter approximate date if unsure, leave blank if unknown / Hatama data tuir hanoin karik la serteza, husik mamuk deit karik la hatene)

Which type of COVID-19 vaccine was your THIRD dose? Vasina COVID-19 nia tipu ida ne'ebé mak ita bo'ot simu ba dose terseiru?	☐ AstraZenica ☐ Sinovac ☐ Pfizer ☐ Other / Seluk ☐ Don't know / La hatene
If you have had more than three COVID-19 vaccines, when was your MOST RECENT dose? Karik ita bo'ot simu vasina COVID-19 barak liu dala tolu, entaun ita bo'ot nia dose ikus liu ne'e simu iha loron saida?	_____ (Enter approximate date if unsure, leave blank if unknown / Hatama data tuir hanoin karik la serteza, husik mamuk deit karik la hatene)
If you have had more than three doses of COVID vaccines, which type have you recieved for your MOST RECENT DOSE? Karik ita bo'ot simu vasina COVID-19 ne'e barak liu dala tolu, tipu vasina ida ne'ebé mak ita bo'ot simu ikus liu?	☐ AstraZenica ☐ Sinovac ☐ Pfizer ☐ Other / Seluk ☐ Don't know / La hatene
Do you wish to be vaccinated for COVID-19? Ita bo'ot hakarak atu simu vasina ba COVID-19?	◯ Yes / Sim ◯ No / Lae ◯ Don't know / La hatene ◯ Not applicable - too young to answer this question / La aplikavel - sei kiik seidauk bele hatán ba pergunta ida ne'e (Only for participants who have not received any doses of COVID-19 vaccines / pergunta ida ne'e husu deit ba partisipante sira ne'ebé mak seidauk simu vasina COVID-19 nia dose ruma)
Why have you not received a SECOND dose of COVID-19 vaccine? Tamba saida mak ita bo'ot seidauk simu vasina COVID-19 nia dose segundu?	◯ It is less than 3 months since my first dose, therefore my second dose is not due yet / Seidauk to'o fulan 3, desde hau nia vasina dose primeiru, tamba ne'e hau nia dose segundu nia tempu seidauk to'o ◯ It is more than 3 months since my first dose, but I have not been offered a second dose yet / Tempu liu tiha ona fulan 3 desde hau nia dose primeiru, maibe hau seidauk hetan dose segundu ◯ I got COVID-19 infection after my first dose, therefore I don't think I need another dose / Hau hetan infeksaun COVID-19 depois de simu dose primeiru, tamba ne'e hau hanoin hau la presiza dose seluk tan ◯ I don't want to have another dose / Hau lakoi atu simu dose seluk tan ◯ Other / Seluk (Only asked to participants who have received just one dose of COVID-19 vaccine / Husu deit ba partisipante sira ne'ebé mak simu ona vasina COVID-19 nia dose primeiru)
Why don't you want another dose? Tamba saida mak ita bo'ot lakoi simu dose segundu?	_____
Please specify Favor espesifika	_____

Unable to Complete Questionnaire

Record ID _____

Fill this form if Household Questionnaire cannot be filled out (i.e. no data can be collected from the household)

Household number _____

Why could no data be collected?

- ◯ Household occupied but head-of-household does not wish to fill out Household Questionnaire
- ◯ Household looks occupied but no adult household members were present during all three visits
- ◯ Household looks derelict
- ◯ Household has been demolished
- ◯ Cannot access household

Please state the reason you couldn't find the household _____

VASINA fieldwork SOP – data and sample collection

For each household, do the following:

1. **Introduce research team to head-of-household and other household member(s)**
   - Explain study, using patient information sheet
   - Wear mask and visor for this

2. **Conduct *Household Questionnaire* on RedCap with head-of-household**
   - Fill this out even if no household members give consent for individual data or samples to be collected (if this is the case, head-of-household should still fill consent form)
   - If head-of-household does not wish to fill *Household Questionnaire* on RedCap, fill *Unable to Complete Questionnaire* on RedCap

3. **Each time you enrol an individual, give them a sticker with their Study ID number on it**
   - Children less than 1 year old should not be enrolled and do not need to be assigned a Study ID number (but their details should still be included on the *Household Questionnaire*)
   - For individuals who are not currently present, assign a study ID number, but keep his/her sticker until the next visit

For each participant, do the following:

4. **Receive written informed consent (signed form)**
   - For children, receive written informed consent from one of their parents
   - Also receive 'verbal assent' from the child

5. **Conduct *Individual Participant Questionnaire* on RedCap**

6. **Don PPE**

7. **Phlebotomy (maximum x2 attempts)**
   - Adults: use needle and syringe to collect 10ml (x2 tubes)
   - Children: use needle and syringe or butterfly to collect 5ml (x1 tube)
   - Also place x9-12 drops onto filter paper to make three blood spots (*see Dried Blood Spot SOP*).
   - Make sure blood tubes and dried blood spots are clearly labelled with stickers

8. **Finger-prick blood sample (where phlebotomy failed)**
   - Collect 2mls of blood drops into paediatric serum tubes
   - Collect x9-12 drops onto filter paper to make three blood spots.
   - Make sure blood tubes and dried blood spots are clearly labelled with stickers

9. **Store samples**
   - Put labelled blood samples into cool box

- Allow blood spot to dry for 4 hours, put into zip-lock bag with desiccant sachet. Put this into cool box

10. **Equipment disposal**
    - Place all sharps into sharps container
    - Place all other phlebotomy equipment into clinical waste bags
    - Dengue RDT can be disposed of, after good-quality picture and interpretation is done

11. **Thank household member(s) and make sure they have a copy of the participant information sheet**

12. **Make sure gloves and apron are changed and hands are washed/alcohol gelled before moving onto next participant**

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Appendix 1: Participant information sheet: Participants within Dili

Local Study Coordinator Name, National Health Laboratory, Dili, Phone: TBC; Email: TBC

SEROLOGICAL STUDY OF VACCINE-PREVENTABLE DISEASES IN TIMOR-LESTE

This is for you to keep / Ida ne'e fó ba ita bo'ot atu rai

You are being invited to take part in a study about vaccine-preventable diseases, which include COVID-19, Hepatitis B, Measles, Rubella and Dengue.

Participation: Taking part in this project is entirely voluntary. You can withdraw from the study at any time. You do not have to explain why you want to withdraw from the study, and there are no negative consequences if you withdraw. If you have any questions, please discuss them with members of our team.

Partisipasaun: Partisipa iha projetu ida ne'e kompletamente voluntariu. Ita bo'ot bele sai husi estudu iha kualker tempu. Ita bo'ot la presiza atu esplika razaun tamba saida, no sei la iha konsekuensia negativu karik ita bo'ot sai. Karik ita bo'ot iha kualker pergunta, favor bele hato'o ba membru husi ami nia ekipa.

What are vaccine preventable diseases? Vaccine preventable diseases are infectious diseases which can be prevented through vaccination. These include COVID-19, Hepatitis B, Measles, Rubella and Dengue.

COVID-19 is the disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is a type of coronavirus. The virus was first identified in December 2019 and is new for humans. Coronavirus is spread from person to person mostly by droplets and contaminated surfaces. Coronavirus can cause both mild and severe disease, especially in older people and people with medical conditions. Some people with coronavirus may have no symptoms. Common symptoms of coronavirus are fever and cough. Other symptoms include headache, sore throat, tiredness, shortness of breath, sore muscles, loss of taste and smell, chills and vomiting.

COVID-19 started spreading in Timor-Leste in February 2021, and vaccines have been available since April 2021.

Hepatitis B is a virus which is spread by contact with blood. This usually occurs from mother to child (during childbirth), but can also occur by contaminated needles or blood transfusions. In some people the virus causes a short infection, the goes away. In other people, the virus causes a long-lasting infection, which can cause damage to the liver over time. Vaccines are given to new-born babies in Timor-Leste, to prevent them from getting Hepatitis B.

Measles and rubella are infections which can cause outbreaks in children. They usually cause fever and rash, and can occasionally cause serious illness. Vaccines are given to young children to prevent them from getting measles and rubella.

Dengue is a virus spread by mosquitoes which can cause illness in children and adults. Usually it causes fever with headache and rash, sometimes the illness can be serious. Currently vaccines are not used against dengue in Timor-Leste.

DRAFT

Who is organising the project? The study is a collaboration between:

Se mak organiza projetu ida ne'e? Estudu ida ne'e kolaborasaun entre:

- Ministry of Health, Timor-Leste / Ministeriu da Saúde, Timor-Leste
- Menzies School of Health Research, Australia
- National Health Laboratory, Dili / Laboratóriu Nasional Saúde, Dili
- National Centre for Immunisation Research and Surveillance, Australia / Sentru Nasional ba Peskiza no Vigilansia Imunizasaun, Australia

Approval for this project. This study has been approved by the National Institute of Health, Timor-Leste (Instituto Nacional da Saúde; INS) and the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research in Australia.

Aprovasaun ba projetu ida ne'e. Estudu ida ne'e hetan ona aprovasaun husi Institutu Nasional da Saúde (INS), Timor-Leste no Human Research Ethics Committee of the Northern Territory Department of Health no Menzies School of Health Research iha Australia.

Informed Consent Information / Informasaun Konsentimentu ne'ebé mak Informadu

Section 1: Purpose of the study.

The main purpose of this study is to see how many people in Timor-Leste have antibodies against different vaccine-preventable diseases. If a person has antibodies to a particular infection, it means they have either been exposed in the past, or have been vaccinated. It sometimes means that the person has protection against the infection ('immunity').

We will test the blood for antibodies against COVID-19, hepatitis B, measles, rubella and dengue. These tests will be done in the laboratory in Dili (National Health Laboratory)

Approximately 5600 people in Timor-Leste will be invited to participate in the project.

Section 2: Procedures. For each person, we will:

Seksaun 2: Prosedimentu. Ba kada partisipante, ami sei:

- Ask some **QUESTIONS** including name, age, gender, contact details, occupation, vaccination details.
- Take a **BLOOD SAMPLE** (5mL). The blood samples will be used to test for previous exposure or vaccination against COVID-19, hepatitis B, measles, rubella and dengue. The blood sample will also be used to test for hepatitis B infection, and to validate check the accuracy of different tests.
- Take a **DRIED BLOOD SPOT** (3 drops of blood). This will be made by applying drops of blood to a small piece of filter paper, then drying.
- The **blood samples may also be used for validating different tests, and for potential future use to answer medical research questions relating to communicable or non-communicable diseases in Timor-Leste**. All results will be de-identified, and only grouped results will be reported. Anonymized blood samples will be stored at the National Health Laboratory for 10 years before being disposed of.

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Section 3: Duration of Procedures. Collection of the blood sample will take less than 10 minutes. The questionnaire should take about 5 minutes to complete and can be answered in Tetun or English.

Seksaun 3: Durasaun ba Prosedimentu. Koleksaun ba amostra ran sei uza tempu pelu menus minutu 10. Kuestionariu sei uza tempu durante minutu 5 hodi kompleta, no bele resposta uza lian Tetun ou Ingles.

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Section 4: Potential benefit to you and others. / Seksaun 4: Benefisiu potensial ba ita bo'ot no ema seluk.

The blood tests for COVID-19, measles, rubella and dengue provides evidence of past infection or vaccination, but do not test for current infection. There is no need for any treatment if these tests are positive. The blood test for hepatitis B will check for active infection. If this is positive, you will be offered assessment and management (which may include treatment) at the hepatology clinic at Hospital Nacional Guido Valadares (NHGV).

The results of the study will provide important and useful information for Timor-Leste on the success of managing patients with COVID-19 in health care facilities without the transmission of coronavirus transmission.

Section 5: Discomforts and Risks. The potential risks of collecting a venous blood sample include temporary discomfort from the needle, bruising, bleeding, and very rarely, infection. Experienced doctors, nurses, or laboratory technicians will take the blood samples. The risk of problems is very small. In the unlikely event of any significant injury because of the blood collection procedure, the study team and/or the Ministry of Health will arrange necessary medical treatment without financial cost. However, no financial compensation will be given in case of any study-related injury.

Seksaun 5: Deskonfortus no Risku. Potensia ba risku husi kolekta ran venozu sei inklui mos deskonfortu temporariu husi daun, kulit mean (bubu), ran sai, no infeksaun raramente. Doutor, enfermeiru/a, ou tekniku laboratoriu sira ne'ebé mak iha ona esperiensia mak sei foti amostra ran. Risku ba problema ne'e kiik tebes. Karik mosu kualker prejuizu signifikante relasiona ho prosedimentu ba koleksaun ran nian, ekipa estudu/Ministeriu Saude sei aranja tratamentu medikal ne'ebé mak nesesariu sem kustu finansial. No entantu, sei la iha kompensasaun finansial ba kualker injuria ne'ebé mak mosu relasiona ho prosesu estudu.

If you are found to have active hepatitis B, you will be referred to the hepatology clinic and you will be offered further tests to see if any treatment is required.

Section 6: Costs for participation. Taking part in the project will cost nothing apart from your time.

Seksaun 6: Kustu ba partisipasaun. Partisipasaun iha projetu ida ne'e, sei la kobre kustu ruma exeptu ita bo'ot nia tempu.

Section 7: Compensation for participation. You will not receive any payments for being part of this project.

Seksaun 7: Kompensasaun ba Partisipasaun. Ita bo'ot sei la simu kualker pagamentu ba partisipasaun iha projetu ida ne'e.

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Section 9: Voluntary participation. Taking part in this project is your choice, and you will have the right to stop at any time. If individuals or communities decide not to participate or decide to stop taking part at any time, there will be no penalties.

Seksaun 9: Partisipasaun Voluntariu. Partisipa iha projetu ida ne'e ita bo'ot mak hili, no ita bo'ot sei

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iha direitu atu hapara/sai iha kualker tempu. Karik individual sira ou komunidade deside atu la partisipa ou deside atu sai husi partisipasaun iha kualker tempu, ida ne'e sei la iha penalidade ruma.

Section 10: Delivery of results. If your blood test shows that you have active hepatitis B, you will be contacted and offered assessment and management (which may include treatment) at the hepatology clinic at Hospital Nacional Guido Valadares (NHGV).

You will not be contacted with results of the other blood tests (COVID-19, measles, rubella and dengue antibodies). This is because we are planning on testing 5600 people, the tests may take several months to complete, and it would be very difficult to contact everyone. Additionally, these tests are not for active infections, so there is no need for any treatment if they are positive.

Results for the whole study will be written-up and may be published. It may be used by the Ministry of Health to plan additional vaccines and other disease-control activities in Timor-Leste. During this whole process, individual results will remain strictly confidential.

Section 11: Privacy and confidentiality. A unique code will be used to identify and link the questionnaire data and blood sample results. Study records and completed questionnaires will be reviewed, stored, and analyzed on a computer at the Menzies office in Dili and will be kept in secured areas and password protected computers. Blood samples collected for laboratory testing will be labeled with the unique code and securely stored at the National Health Laboratory in Dili, Timor-Leste. The list that matches participants' names with unique codes will be kept in a password protected file on a computer in the Menzies office in Dili. No personally identifiable information will be shared in any project reports, publication, or presentations. We will keep each individual's participation in this project confidential to the extent permitted by law. However, it is possible that other people may become aware of their participation in this study, e.g. the following people/groups may inspect project records:

- The Instituto Nacional da Saúde; INS and the Ministry of Health, Timor-Leste
- Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research in Australia

Seksaun 6: Privasidade no Konfidensialidade. Sei uza kodigu uniku hodi identifika no liga dadus husi kuestionariu no rezultadu amostra ran. Registrus no kuestionariu ne'ebé kompleta ona husi estudu sei reviza, armazena, no halo analizasaun uza komputador iha Menzies nia office Dili, no sei rai iha area seguru no iha komputador ne'ebé mak proteje uza password. Amostra ran ne'ebé mak koletadu hodi halo teste iha laboratoriu sei tau rotulagem/label ho kodigu uniku no seguramente sei rai iha Laboratoriu Nasional Saude, Dili Timor-Leste. Lista ne'ebé mak kombinadu ho partisipante nia naran ho kodigu uniku ne'e sei rai iha arkivu 1 protektidu ho password iha komputador iha Menzies nia Office, Dili. Kualker informasaun personal ne'ebé mak identifikavel sei la kompartilha iha kualker projetu nia relatoriu, publikasaun, ou aprezentasaun sira. Ami sei mantein kada individual nia partisipasaun iha projetu ida ne'e konfidensial tuir lei haruka. No entantu, iha posibilidade ba ema balu atu notifika konaba ita bo'ot sira nia partisipasaun iha estudu ida ne'e, por ezemplu ema/grupu hirak hanesan tuir mai ne'e sei bele halo inspeksaun ba projetu nia registrus:

- Instituto Nacional da Saúde (INS) no Ministeriu Saude, Timor-Leste
- Human Research Ethics Committee of the Northern Territory Department of Health no Menzies School of Health Research, iha Australia

Some of these records could contain information that tells them who participated in the project. However, the results of blood test and answers to the questionnaires will not be linked to this information. Your test results, including the hepatitis B results will not be shared with any person outside the study team.

Husi registrasaun sira ne'e balun sei inklui informasaun ne'ebé mak sei informa konaba partisipante sira iha projetu ida ne'e. No entantu, rezultadu teste ran nian no resposta iha kuestionariu sira, sei la

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inklui iha informasaun refere. Ita bo'ot nia rezultadu teste, inklui rezultadu hepatitis B sei la kompartilha ho kualker ema ne'ebé mak laos ekipa estudu.

Section 12: Contact information for questions or concerns. You have the right to ask any questions you may have about this study. If you have questions, complaints or concerns or believe you may have developed an injury related to this study, please contact Local Study Coordinator Name, National Health Laboratory, Dili, Phone: TBC; Email: TBC. If you have questions regarding your rights as a study participant or concerns regarding the ethical conduct of the project, please contact Secretario ethiku, Komisaun etiku peskija komite tekniku INS (Ph +670 7708 7665) or the Northern Territory Department of Health and Menzies School of Health Research Human Research Ethics Committee (Phone +618 8946 8600; Email ethics@menzies.edu.au). If you have any medical problems associated with this study, such as a pain from where your blood was collected or feeling unwell, please contact the above phone numbers or attend your local hospital or clinic if urgent.

Seksaun 12: Informasaun kontaktu karik iha pergunta ou preokupasaun ruma. Ita bo'ot iha direitu hodi husu kualker pergunta ne'ebé mak relasiona ho estudu ida ne'e. Karik ita bo'ot iha pergunta, reklamasaun ou preokupasaun ou karik ita bo'ot hetan injuria relasiona ho estudu ida ne'e, favor kontaktu Local Study Coordinator Name, National Health Laboratory, Dili, Phone: TBC; Email: TBC. Karik ita bo'ot iha pergunta relasiona ho ita bo'ot nia direitu nudar partisipante ou preokupasaun relasiona ho projetu nia étika konduta, favor kontaktu Sekretariu étiku, komisaun etika peskija komite tekniku INS (Ph +670 7708 7665) ou Northern Territory Department of Health no Menzies School of Health Research Human Research Ethics Committee (telemovel +618 8946 8600; Email ethics@menzies.edu.au). Karik ita bo'ot iha kualker problema mediku ne'ebé mak asosiadu ho estudu ida ne'e, por ezemplu hanesan moras iha fatin hasai ran ou sente la saudavel, favor kontaktu ba numeru ne'ebé mensiona iha leten ou konsulta iha hospital lokal ou klinika sira karik urgente.

Section 13: Consent to participate in project. Before making the decision about participating in this study you should have:

- Discussed this project with a member of the study team
- Read and understood the information in this form
- Had the opportunity to ask any questions that you may have
- Had time to consider whether to take part
- Information about who to contact in case you have any further questions or problems

Seksaun 13: konsentimentu atu partisipa iha projetu. Antes foti desizaun atu partisipa iha estudu ida ne'e ita bo'ot tenke:

- Diskute uluk konaba projetu ida ne'e ho membru husi ekipa estudu nian
- Le'e no komprende informasaun iha formulariu ida ne'e
- Iha oportunidade hodi husu kualker pergunta ne'ebé mak ita bo'ot iha
- Iha tempu hodi hanoin antes deside atu partisipa
- Infomasaun konaba se mak ita bo'ot atu kontaktu, kuandu ita bo'ot iha tan pergunta ou problema ruma.

Section 14: Ethics Committee Clearance. The ethical aspects of this research have been approved by the National Institute of Health, Timor-Leste (Instituto Nacional da Saúde; INS) and the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research in Australia (Protocol HREC-2021-4064). If you have any concerns about this research, please contact: Secretario ethiku, Komisaun etiku peskija komite tekniku INS (Ph +670 7708 7665) or the Northern Territory Department of Health and Menzies School of Health Research Human Research Ethics Committee (Phone +618 8946 8600; Email ethics@menzies.edu.au).

Seksaun 14: Lisensa Komite Étika. Aspektu etikal husi peskiza ida ne'e hetan ona aprovasaun husi Institutu Nasional Saude Timor-Leste (INS) no Human Research Ethics Committee of the Northern Territory Department of Health no Menzies School of Health Research, iha Australia (Protokolu HREC-2021-4064). Karik ita bo'ot iha kualker preokupasaun konaba peskiza ida ne'e, favor kontaktu: Sekretariu Étiku, Komisaun Étiku Peskija Komite Tekniku INS (Telemovel +670 7708 7665) ou the Northern Territory Department of Health no Menzies School of Health Research Human Research Ethics Committee (Telemovel +618 8946 8600; Email: ethics@menzies.edu.au).

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Appendix 2: Consent form

Apendise 2: Formulariu Konsentimentu

Local Study Coordinator Name, National Health Laboratory, Dili, Phone: TBC; Email: TBC

SEROLOGICAL STUDY OF VACCINE-PREVENTABLE DISEASES IN TIMOR-LESTE

Informed Consent /Assent Form

This means you can say NO /

PRINCIPAL INVESTIGATOR / INVESTIGADOR PRINSIPAL: Dr Nelson Martins

STUDY COLLABORATORS / KOLABORADORES BA ESTUDU:

- Ministry of Health (MOH), Timor-Leste / Ministeriu da Saúde, Timor-Leste
- Menzies School of Health Research, Australia
- National Health Laboratory, Dili, Timor-Leste / Laboratoriu Nasional Saude, Dili, Timor-Leste
- National Centre for Immunisation Research and Surveillance, Australia / Sentru Nasional ba Peskiza no Vigilansia Imunizasaun, Australia

I understand the aim of this research study is **to improve understanding of the number of people who have been exposed and/or vaccinated against infectious diseases in Timor-Leste.** Details of the study have been explained to me, and I have been provided with a written information sheet and given the opportunity to ask questions.

I acknowledge that: / Hau rekonhese katak:

- Any risks and possible effects of having a **venous blood test** have been explained to my satisfaction;
  Kualker risku no efeitu ne'ebé mak posivel atu hetan tamba **teste ran venozu** nian, esplika ona mai hau nia satisfasaun;
- Taking part in this study is voluntary and I am aware that I can stop taking part at any time without explanation or prejudice and to withdraw any unprocessed data I have provided;
  Partisipasaun iha estudu ida ne'e voluntariu no hau hatene katak hau bele hapara hau nia partisipasaun iha kualker tempu sem explikasaun ou prekonseitu, no dada fila fali hau nia dadus ne'ebé mak seidauk prosesa
- Any information I give will be kept strictly confidential and that no names will be used to identify me in this study without my approval.
  Kualker informasaun ne'ebé mak hau fó, sei rai estreitamente konfidensial no sei la uza kualker naran hodi identifika hau iha estudu ida ne'e sem hau nia aprovasaun

By providing my name and signature, I agree to:

Liu husi fó hau nia naran no asinatura, hau konkorda atu:

A venous blood test for past exposure or vaccination against COVID-19	☐ **Yes** **Sim**	☐ **No** **Lae**
A venous blood test for past exposure or vaccination against measles Teste ran venozu hodi hatene hau nia imunidade ba Sarampo	☐ **Yes** **Sim**	☐ **No** **Lae**

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A venous blood test for past exposure or vaccination against rubella Teste ran venozu hodi hatene hau nia imunidade ba rubella		**Yes** **Sim**		**No** **Lae**
A blood test for past exposure to dengue				
A venous blood test for past exposure to hepatitis B and active infection with hepatitis B Teste ran venozu hodi hatene hau nia imunidade ba hepatitis B no infeksaun		**Yes** **Sim**		**No** **Lae**
My blood being used to make a 'dried blood spot'				
The anonymised blood sample and 'dried blood spot' being stored for 10 years and being used to validate tests for infections, and potentially tested for other communicable or non-communicable diseases		**Yes** **Sim**		**No** **Lae**
Completing a questionnaire with the help of a research assistant Kompleta kuestinariu ho ajuda husi asistente peskiza nian		**Yes** **Sim**		**No** **Lae**
Being contacted again about vaccine-preventable diseases		**Yes** **Sim**		**No** **Lae**
Being contacted again about my hepatitis B results		**Yes** **Sim**		**No** **Lae**

Name of participant / Naran partisipante: *(printed)*	**Unique identifier: / Identifikador uniku:**
Signature of participant or parent/guardian:	**Date / Data:**
Name of witness/interpreter: / Naran testemunha/tradutor: *(printed)*	**Signature of witness/interpreter: / Asinatura husi testemunha/tradutor:**
Name of researcher / Naran peskizador: *(printed)*	**Signature of researcher / Asinatura husi peskizador:**

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Appendix 3: Participant information sheet: Participants outside Dili

Local Study Coordinator Name, National Health Laboratory, Dili, Phone: TBC; Email: TBC

SEROLOGICAL STUDY OF VACCINE-PREVENTABLE DISEASES IN TIMOR-LESTE

This is for you to keep / Ida ne'e fó ba ita bo'ot atu rai

You are being invited to take part in a study about vaccine-preventable diseases, which include COVID-19, Hepatitis B, Measles, Rubella and Dengue.

Participation: Taking part in this project is entirely voluntary. You can withdraw from the study at any time. You do not have to explain why you want to withdraw from the study, and there are no negative consequences if you withdraw. If you have any questions, please discuss them with members of our team.

Partisipasaun: Partisipa iha projetu ida ne'e kompletamente voluntariu. Ita bo'ot bele sai husi estudu iha kualker tempu. Ita bo'ot la presiza atu esplika razaun tamba saida, no sei la iha konsekuensia negativu karik ita bo'ot sai. Karik ita bo'ot iha kualker pergunta, favor bele hato'o ba membru husi ami nia ekipa.

What are vaccine preventable diseases? Vaccine preventable diseases are infectious diseases which can be prevented through vaccination. These include COVID-19, Hepatitis B, Measles, Rubella and Dengue.

COVID-19 is the disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is a type of coronavirus. The virus was first identified in December 2019 and is new for humans. Coronavirus is spread from person to person mostly by droplets and contaminated surfaces. Coronavirus can cause both mild and severe disease, especially in older people and people with medical conditions. Some people with coronavirus may have no symptoms. Common symptoms of coronavirus are fever and cough. Other symptoms include headache, sore throat, tiredness, shortness of breath, sore muscles, loss of taste and smell, chills and vomiting.

COVID-19 started spreading in Timor-Leste in February 2021, and vaccines have been available since April 2021.

Hepatitis B is a virus which is spread by contact with blood. This usually occurs from mother to child (during childbirth), but can also occur by contaminated needles or blood transfusions. In some people the virus causes a short infection, the goes away. In other people, the virus causes a long-lasting infection, which can cause damage to the liver over time. Vaccines are given to new-born babies in Timor-Leste, to prevent them from getting Hepatitis B.

Measles and rubella are infections which can cause outbreaks in children. They usually cause fever and rash, and can occasionally cause serious illness. Vaccines are given to young children to prevent them from getting measles and rubella.

Dengue is a virus spread by mosquitoes which can cause illness in children and adults. Usually it causes fever with headache and rash, sometimes the illness can be serious. Currently vaccines are not used against dengue in Timor-Leste.

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Who is organising the project? The study is a collaboration between:

Se mak organiza projetu ida ne'e? Estudu ida ne'e kolaborasaun entre:

- Ministry of Health, Timor-Leste / Ministeriu da Saúde, Timor-Leste
- Menzies School of Health Research, Australia
- National Health Laboratory, Dili / Laboratóriu Nasional Saúde, Dili
- National Centre for Immunisation Research and Surveillance, Australia / Sentru Nasional ba Peskiza no Vigilansia Imunizasaun, Australia

Approval for this project. This study has been approved by the National Institute of Health, Timor-Leste (Instituto Nacional da Saúde; INS) and the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research in Australia.

Aprovasaun ba projetu ida ne'e. Estudu ida ne'e hetan ona aprovasaun husi Institutu Nasional da Saúde (INS), Timor-Leste no Human Research Ethics Committee of the Northern Territory Department of Health no Menzies School of Health Research iha Australia.

Informed Consent Information / Informasaun Konsentimentu ne'ebé mak Informadu

Section 1: Purpose of the study.

The main purpose of this study is to see how many people in Timor-Leste have antibodies against different vaccine-preventable diseases. If a person has antibodies to a particular infection, it means they have either been exposed in the past, or have been vaccinated. It sometimes means that the person has protection against the infection ('immunity').

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You will not be contacted with results of the blood tests. This is because we are planning on testing 5600 people, the tests may take several months to complete, and it would be very difficult to contact

everyone. Additionally, these tests are not for active infections, so there is no need for any treatment if they are positive.

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- The Instituto Nacional da Saúde; INS and the Ministry of Health, Timor-Leste
- Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research in Australia

Seksaun 6: Privasidade no Konfidensialidade. Sei uza kodigu uniku hodi identifika no liga dadus husi kuestionariu no rezultadu amostra ran. Registrus no kuestionariu ne'ebé kompleta ona husi estudu sei reviza, armazena, no halo analizasaun uza komputador iha Menzies nia office Dili, no sei rai iha area seguru no iha komputador ne'ebé mak proteje uza password. Amostra ran ne'ebé mak koletadu hodi halo teste iha laboratoriu sei tau rotulagem/label ho kodigu uniku no seguramente sei rai iha Laboratoriu Nasional Saude, Dili Timor-Leste. Lista ne'ebé mak kombinadu ho partisipante nia naran ho kodigu uniku ne'e sei rai iha arkivu 1 protektidu ho password iha komputador iha Menzies nia Office, Dili. Kualker informasaun personal ne'ebé mak identifikavel sei la kompartilha iha kualker projetu nia relatoriu, publikasaun, ou aprezentasaun sira. Ami sei mantein kada individual nia partisipasaun iha projetu ida ne'e konfidensial tuir lei haruka. No entantu, iha posibilidade ba ema balu atu notifika konaba ita bo'ot sira nia partisipasaun iha estudu ida ne'e, por ezemplu ema/grupu hirak hanesan tuir mai ne'e sei bele halo inspeksaun ba projetu nia registrus:

- Instituto Nacional da Saúde (INS) no Ministeriu Saude, Timor-Leste
- Human Research Ethics Committee of the Northern Territory Department of Health no Menzies School of Health Research, iha Australia

Some of these records could contain information that tells them who participated in the project. However, the results of blood test and answers to the questionnaires will not be linked to this information. Your test results, including the hepatitis B results will not be shared with any person outside the study team.

Husi registrasaun sira ne'e balun sei inklui informasaun ne'ebé mak sei informa konaba partisipante sira iha projetu ida ne'e. No entantu, rezultadu teste ran nian no resposta iha kuestionariu sira, sei la inklui iha informasaun refere. Ita bo'ot nia rezultadu teste, inklui rezultadu hepatitis B sei la kompartilha ho kualker ema ne'ebé mak laos ekipa estudu.

Section 12: Contact information for questions or concerns. You have the right to ask any questions you may have about this study. If you have questions, complaints or concerns or believe you may have developed an injury related to this study, please contact Local Study Coordinator Name, National Health Laboratory, Dili, Phone: TBC; Email: TBC. If you have questions regarding your rights as a study participant or concerns regarding the ethical conduct of the project, please contact Secretario ethiku,

Komisaun etiku peskija komite tekniku INS (Ph +670 7708 7665) or the Northern Territory Department of Health and Menzies School of Health Research Human Research Ethics Committee (Phone +618 8946 8600; Email ethics@menzies.edu.au). If you have any medical problems associated with this study, such as a pain from where your blood was collected or feeling unwell, please contact the above phone numbers or attend your local hospital or clinic if urgent.

Seksaun 12: Informasaun kontaktu karik iha pergunta ou preokupasaun ruma. Ita bo'ot iha direitu hodi husu kualker pergunta ne'ebé mak relasiona ho estudu ida ne'e. Karik ita bo'ot iha pergunta, reklamasaun ou preokupasaun ou karik ita bo'ot hetan injuria relasiona ho estudu ida ne'e, favor kontaktu Local Study Coordinator Name, National Health Laboratory, Dili, Phone: TBC; Email: TBC. Karik ita bo'ot iha pergunta relasiona ho ita bo'ot nia direitu nudar partisipante ou preokupasaun relasiona ho projetu nia étika konduta, favor kontaktu Sekretariu étiku, komisaun etika peskija komite tekniku INS (Ph +670 7708 7665) ou Northern Territory Department of Health no Menzies School of Health Research Human Research Ethics Committee (telemovel +618 8946 8600; Email ethics@menzies.edu.au). Karik ita bo'ot iha kualker problema mediku ne'ebé mak asosiadu ho estudu ida ne'e, por ezemplu hanesan moras iha fatin hasai ran ou sente la saudavel, favor kontaktu ba numeru ne'ebé mensiona iha leten ou konsulta iha hospital lokal ou klinika sira karik urgente.

Section 13: Consent to participate in project. Before making the decision about participating in this study you should have:

- Discussed this project with a member of the study team
- Read and understood the information in this form
- Had the opportunity to ask any questions that you may have
- Had time to consider whether to take part
- Information about who to contact in case you have any further questions or problems

Seksaun 13: konsentimentu atu partisipa iha projetu. Antes foti desizaun atu partisipa iha estudu ida ne'e ita bo'ot tenke:

- Diskute uluk konaba projetu ida ne'e ho membru husi ekipa estudu nian
- Le'e no komprende informasaun iha formulariu ida ne'e
- Iha oportunidade hodi husu kualker pergunta ne'ebé mak ita bo'ot iha
- Iha tempu hodi hanoin antes deside atu partisipa
- Infomasaun konaba se mak ita bo'ot atu kontaktu, kuandu ita bo'ot iha tan pergunta ou problema ruma.

Section 14: Ethics Committee Clearance. The ethical aspects of this research have been approved by the National Institute of Health, Timor-Leste (Instituto Nacional da Saúde; INS) and the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research in Australia (Protocol HREC-2021-4064). If you have any concerns about this research, please contact: Secretario ethiku, Komisaun etiku peskija komite tekniku INS (Ph +670 7708 7665) or the Northern Territory Department of Health and Menzies School of Health Research Human Research Ethics Committee (Phone +618 8946 8600; Email ethics@menzies.edu.au).

Seksaun 14: Lisensa Komite Étika. Aspektu etikal husi peskiza ida ne'e hetan ona aprovasaun husi Institutu Nasional Saude Timor-Leste (INS) no Human Research Ethics Committee of the Northern Territory Department of Health no Menzies School of Health Research, iha Australia (Protokolu HREC-2021-4064). Karik ita bo'ot iha kualker preokupasaun konaba peskiza ida ne'e, favor kontaktu: Sekretariu Étiku, Komisaun Étiku Peskija Komite Tekniku INS (Telemovel +670 7708 7665) ou the Northern Territory Department of Health no Menzies School of Health Research Human Research Ethics Committee (Telemovel +618 8946 8600; Email: ethics@menzies.edu.au).

DRAFT

DRAFT

Appendix 4: Consent form

Apendise 2: Formulariu Konsentimentu

Local Study Coordinator Name, National Health Laboratory, Dili, Phone: TBC; Email: TBC

SEROLOGICAL STUDY OF VACCINE-PREVENTABLE DISEASES IN TIMOR-LESTE

Informed Consent /Assent Form

This means you can say NO /

PRINCIPAL INVESTIGATOR / INVESTIGADOR PRINSIPAL: Dr Nelson Martins

STUDY COLLABORATORS / KOLABORADORES BA ESTUDU:

- Ministry of Health (MOH), Timor-Leste / Ministeriu da Saúde, Timor-Leste
- Menzies School of Health Research, Australia
- National Health Laboratory, Dili, Timor-Leste / Laboratoriu Nasional Saude, Dili, Timor-Leste
- National Centre for Immunisation Research and Surveillance, Australia / Sentru Nasional ba Peskiza no Vigilansia Imunizasaun, Australia

I understand the aim of this research study is **to improve understanding of the number of people who have been exposed and/or vaccinated against infectious diseases in Timor-Leste.** Details of the study have been explained to me, and I have been provided with a written information sheet and given the opportunity to ask questions.

I acknowledge that: / Hau rekonhese katak:

- Any risks and possible effects of having a **venous blood test** have been explained to my satisfaction;
  Kualker risku no efeitu ne'ebé mak posivel atu hetan tamba **teste ran venozu** nian, esplika ona mai hau nia satisfasaun;
- Taking part in this study is voluntary and I am aware that I can stop taking part at any time without explanation or prejudice and to withdraw any unprocessed data I have provided;
  Partisipasaun iha estudu ida ne'e voluntariu no hau hatene katak hau bele hapara hau nia partisipasaun iha kualker tempu sem explikasaun ou prekonseitu, no dada fila fali hau nia dadus ne'ebé mak seidauk prosesa
- Any information I give will be kept strictly confidential and that no names will be used to identify me in this study without my approval.
  Kualker informasaun ne'ebé mak hau fó, sei rai estreitamente konfidensial no sei la uza kualker naran hodi identifika hau iha estudu ida ne'e sem hau nia aprovasaun

By providing my name and signature, I agree to:

Liu husi fó hau nia naran no asinatura, hau konkorda atu:

A venous blood test for past exposure or vaccination against COVID-19	☐ **Yes** **Sim**	☐ **No** **Lae**
A venous blood test for past exposure or vaccination against measles Teste ran venozu hodi hatene hau nia imunidade ba Sarampo	☐ **Yes** **Sim**	☐ **No** **Lae**

A venous blood test for past exposure or vaccination against rubella Teste ran venozu hodi hatene hau nia imunidade ba rubella		**Yes** **Sim**		**No** **Lae**
A blood test for past exposure to dengue				
A venous blood test for past exposure to or vaccination against hepatitis B Teste ran venozu hodi hatene hau nia imunidade ba hepatitis B no infeksaun		**Yes** **Sim**		**No** **Lae**
My blood being used to make a 'dried blood spot'		**Yes** **Sim**		**No** **Lae**
The anonymised blood sample being stored for 10 years and being used to validate tests for infections, and potentially tested for other communicable or non-communicable diseases		**Yes** **Sim**		**No** **Lae**
Completing a questionnaire with the help of a research assistant Kompleta kuestinariu ho ajuda husi asistente peskiza nian		**Yes** **Sim**		**No** **Lae**
Being contacted again about vaccine-preventable diseases		**Yes** **Sim**		**No** **Lae**

Name of participant / Naran partisipante: *(printed)*	**Unique identifier: / Identifikador uniku:**
Signature of participant or parent/guardian:	**Date / Data:**
Name of witness/interpreter: / Naran testemunha/tradutor: *(printed)*	**Signature of witness/interpreter: / Asinatura husi testemunha/tradutor:**
Name of researcher / Naran peskizador: *(printed)*	**Signature of researcher / Asinatura husi peskizador:**