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Early risk assessment in pediatric and adult household contacts of confirmed tuberculosis cases by novel diagnostic tests (ERASE-TB): protocol for a prospective, non-interventional, longitudinal, multi-country cohort study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-060985
Article Type:	Protocol
Date Submitted by the Author:	11-Jan-2022
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Keywords:	Tuberculosis < INFECTIOUS DISEASES, Diagnostic microbiology < INFECTIOUS DISEASES, RESPIRATORY MEDICINE (see Thoracic Medicine)

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3 1 **Early risk assessment in pediatric and adult household contacts of confirmed**
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5 2 **tuberculosis cases by novel diagnostic tests (ERASE-TB): protocol for a**
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7 3 **prospective, non-interventional, longitudinal, multi-country cohort study**
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26 Word count: 3,254
27

28 **Keywords:** *Mycobacterium tuberculosis*, diagnostics, cohort study, household contacts, WHO
29 END TB strategy, ERASE-TB
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30 **ABSTRACT**

31 **Introduction**

32 The World Health Organization (WHO) End-TB Strategy calls for the development of novel
33 diagnostics to detect tuberculosis earlier and more accurately. Better diagnostics, together with
34 tools to predict disease progression are critical for achieving WHO END-TB targets. The Early
35 Risk Assessment in TB contactS by new diagnostic tEsts (ERASE-TB) study aims to evaluate
36 novel diagnostics and testing algorithms for early tuberculosis diagnosis and accurate
37 prediction of disease progression among household contacts exposed to confirmed index
38 cases in Mozambique, Tanzania and Zimbabwe.

40 **Methods and analysis**

41 A total of 2,100 household contacts (HHCs) (aged ≥ 10 years) of adults with microbiologically-
42 confirmed pulmonary tuberculosis will be recruited and followed up at 6-month intervals for 18
43 to 24 months. At each time-point a WHO symptom screen and digital chest-radiograph (dCXR)
44 will be performed, and blood and urine samples collected. Individuals screening positive (WHO
45 symptom screen or dCXR) will be requested to provide sputum for Xpert MTB/Rif Ultra. At
46 baseline, HHCs will also be screened for HIV, diabetes (HbA1c), chronic lung disease
47 (spirometry), hypertension and anaemia. Study outcomes will be co-prevalent tuberculosis
48 (diagnosed at enrollment), incident tuberculosis (diagnosed during follow-up) or no
49 tuberculosis at completion of follow up. Novel diagnostics will be validated using fresh and
50 biobanked samples with a nested case control design. Cases are defined as HHCs diagnosed
51 with tuberculosis (for early diagnosis) or with incident tuberculosis (for prediction of
52 progression) and will be matched by age, sex and country to HHCs who remain healthy
53 (controls). Statistical analyses will include assessment of diagnostic accuracy by constructing
54 receiver operating curves and calculation of sensitivity and specificity.

56 **Ethics and dissemination**

1
2
3 57 ERASE-TB has been approved by regulatory and ethical committees in each African country
4
5 58 and by each partner organisation. Consent, with additional assent for participants <18 years,
6
7 59 is voluntary. Attestation by impartial witnesses is sought in case of illiteracy. Confidentiality of
8
9 60 participants is being maintained throughout. Study findings will be presented at scientific
10
11 61 conferences and published in peer-reviewed international journals.
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16 63 **Trial registration number**

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18 64 NCT04781257
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3 65 **Strengths and limitations of this study**

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5 66 **Strengths**

- 6
7 67 • Recruitment of highly infectious index cases aimed at maximising the number of
8 tuberculosis (TB) diagnoses in the household contact (HHCs) cohort.
9
10 68
11 69 • Sequencing of *Mycobacterium tuberculosis* isolates from both index cases and HHCs
12 allows confirmation of household transmission and thus determination of timing of the
13 transmission event; resulting in more precise estimates of new test sensitivity
14 compared to population-based cohorts with unknown timing of infection.
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16 71
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19
20 73 • Large sample size across three southern African countries with high HIV prevalence;
21 including adolescents will ensure study findings are generalisable to the clinically
22 relevant population at high risk of TB compared to studies focused on adults only.
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28 77 **Limitations**

- 29
30 78 • Despite the large cohort of HHCs, the number of diagnosed TB cases will be small,
31 limiting the power of the study and sub-group analyses such as by age and HIV status.
32
33 79
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35 80 • Geographically limited to sub-Saharan Africa, therefore results may not be
36 generalisable to other populations, including those with lower HIV prevalence such as
37 in South-East Asia or the Americas.
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83 INTRODUCTION

84 Tuberculosis (TB) remains a leading global public health problem, with an estimated 10 million
85 new cases and 1.5 million deaths globally in 2020 [1]. In 2014, the World Health Assembly
86 approved the World Health Organization (WHO) End-TB Strategy, aiming for a 90% reduction
87 in TB incidence and 95% reduction in TB deaths by 2035 [2]. However, in 2019, three million
88 TB cases ('the missing millions') remained undiagnosed and untreated globally, resulting in
89 potentially avoidable morbidity, mortality and onward transmission. The Covid-19 pandemic
90 has resulted in a large decrease in the number of people newly diagnosed with TB and
91 reported. This has increased the diagnostic gap by a further 1.3 million, resulting in an
92 estimated 4.2 million undiagnosed TB cases in 2020 [3]. Also, for the first time in a decade TB
93 deaths have risen, from an estimated 1.4 million in 2019 to 1.5 million in 2020, as a result of
94 reduced access to and provision of essential TB services including diagnostics during the
95 Covid-19 pandemic.

96
97 Without an efficacious and safe vaccine, early detection and containment are the main tools
98 to interrupt transmission and successfully control TB. Similar to SARS-CoV2, asymptomatic
99 spreading of *M.tuberculosis* and subclinical but infectious disease states are a major concern
100 in the control of airborne infectious diseases [4]. Early and accurate identification of persons
101 with TB, combined with identification of those at risk of progression to TB and provision of
102 targeted preventive treatment are critical to reducing TB-associated morbidity and mortality,
103 and preventing onward transmission.

104
105 Currently available diagnostics such as sputum microscopy, mycobacterial culture and nucleic
106 acid amplification tests are based on direct pathogen detection, thus requiring a high
107 mycobacterial load; they therefore predominately target advanced TB when onward
108 transmission and significant lung damage has occurred [5,6]. Further, for many patients with
109 minimal or no symptoms, expectoration of high-quality sputum specimens remains

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3 110 challenging, limiting the accuracy of sputum-based tests. The same holds true for young
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5 111 children and people living with HIV.
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9 113 The **Early Risk Assessment in TB Contacts by new diagnostic tests** (ERASE-TB) study aims
10
11 114 to fill this diagnostic gap by evaluating new sputum and non-sputum-based TB diagnostics for
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13 115 early TB detection (before onward transmission occurs), as well as tools for more accurate
14
15 116 prediction of TB progression to allow for targeted preventive therapy.
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19 20 118 **METHODS AND ANALYSES**

21 22 119 **Study objectives**

23
24 120 ERASE-TB's primary objectives are (I) to determine the sensitivity and specificity of novel
25
26 121 diagnostics to detect TB, in particular asymptomatic or minimally symptomatic TB; (II) to
27
28 122 evaluate novel diagnostics for detection of likely TB progression; and (III) to enhance the
29
30 123 performance of novel diagnostics by simulating testing algorithms coupled with individual risk
31
32 124 estimates from a mathematical model. The secondary objectives are (I) to determine the TB
33
34 125 prevalence among household contacts (HHCs) of infectious TB index cases (ICs) at baseline
35
36 126 and during a 18-24 months follow-up; (II) to establish a biorepository of cryopreserved
37
38 127 specimens from HHCs for future development and validation of diagnostic tests; and (III) to
39
40 128 assess the association of selected chronic disease conditions and TB among HHCs.
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44 45 130 **Study endpoints**

46
47 131 The study's primary endpoints are the presence or development of TB among HHCs with the
48
49 132 following possible scenarios of (I) prevalent symptomatic TB at baseline, (II) incident TB during
50
51 133 follow-up, and (III) remained healthy until study completion. An endpoint review committee will
52
53 134 review the data and case classification before finalization.
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58 136 Through the sequencing of *Mycobacterium tuberculosis* (*Mtb*) isolates, cases of co-prevalent
59
60 137 or incident TB will be classified either as secondary, infected by the source case – the timepoint

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3 138 of infection will be known; or as infected by another, unknown source of infection, with an
4
5 139 unknown timepoint of infection.
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9 141 **Recruitment sites**

11 142 Recruitment of ICs and HHCs at selected primary healthcare facilities and communities has
12
13 143 commenced in Harare, Zimbabwe in March 2021, Maputo, Mozambique in August 2021, and
14
15 144 Mbeya, Tanzania in September 2021. Partners of the ERASE-TB consortium are illustrated in
16
17 145 Figure 1. All three countries have a high TB incidence ranging from 100 to 499/100,000
18
19 146 population [1] and HIV prevalence among adults aged 15 years and older of 5% to 20% [7].
20
21 147 The African research institutions have established collaborations with their respective National
22
23 148 Tuberculosis Programs ensuring referral and appropriate follow-up of TB patients. Figure 2
24
25 149 illustrates the geographic location of research institutions, healthcare facilities where
26
27 150 recruitment is taking place, demographic characteristics of study populations, and estimates
28
29 151 on TB incidence and HIV prevalence [8–15].
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32 152

34 153 **Study design**

36 154 ERASE-TB is a non-interventional, longitudinal, prospective cohort study among HHCs aged
37
38 155 ≥ 10 years exposed to highly infectious pulmonary TB ICs aged ≥ 18 years. Eligibility criteria
39
40 156 are detailed in Figure 3 and the study design is shown in Figure 4. TB ICs are eligible if the
41
42 157 bacterial load in their sputum is at least at the “medium” level according to Xpert MTB/RIF or
43
44 158 Xpert MTB/RIF Ultra, and they have received less than seven daily doses of anti-TB treatment
45
46 159 before enrollment. This maximises the likelihood of culturing and storing *Mtb* isolates. The total
47
48 160 study duration will be 36 months. This includes 12-months enrollment of ICs and HHCs, and
49
50 161 18- to 24-months follow-ups of HHCs. Follow-up ends when a HHCs withdraws from the study,
51
52 162 is lost to follow-up, dies, or is diagnosed with TB and referred for treatment. Scheduled or
53
54 163 unscheduled unwell visits can be conducted physically and/or telephonically in case of
55
56 164 abnormal finding e.g. by abnormal dCXR, or when a participant feels unwell inbetween
57
58 165 scheduled follow-up visits.
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Procedures***TB index cases***

Following informed consent obtained, a questionnaire is administered to collect socio-demographic information, TB risk factors, and the medical history of TB, HIV and other diseases. Two spontaneous sputum samples are obtained, of which one is for mycobacterial culturing and one for storage for performing retrospectively Molecular Bacterial Load Assay (MBLA) to quantify viable *Mtb* by 16S rRNA [6]; an alternative means to quantify expectorated bacterial load for an estimate of infectiousness. Both liquid and solid mycobacterial cultures are performed on decontaminated sputum samples, with all *Mtb* isolates stored at -80 degrees for future DNA extraction and whole genome sequencing. A questionnaire on symptom duration and TB risk factors is also administered.

178

Household key informant

At baseline, a household key informant (either the TB index case or one of the household contacts) is identified and asked to answer questions of a household questionnaire that collects socio-economic elements like structure of the house or flat, income and household assets, and covariates possibly associated with risk of TB infection, e.g., windows/air exchange, presence of comorbid conditions, and risk factors like the source of cooking energy, and properties of the household kitchen.

186

Household contacts

Informed consent is obtained from all eligible adult HHCs. For HHC <18 years of age, the guardian is asked to provide informed consent, with assent also sought from children dependent on local guidance. At baseline a questionnaire is administered collecting information on socioeconomic and demographic characteristics, past medical history of TB, HIV and other diseases, exposure risk factors, smoking and alcohol history. The physical examination includes height, weight, mid-upper arm circumference and blood pressure

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3 194 measurement. In addition, all HHCs are offered free HIV testing according to the National
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5 195 Guidelines. All people with confirmed HIV infection will have CD4 counts performed and be
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7 196 referred for TB preventive therapy. Those not yet on antiretroviral therapy (ART) and those
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9 197 who interrupted ART are referred for ART at local services.
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13 199 Point of care HbA1c (A1cCare, SD Biosensor, Gyeonggi-do, Republic of Korea) and
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15 200 haemoglobin (Hemocue 301+, Hemocue, Angelholm, Sweden) tests and spirometry (including
16
17 201 pre- and post-bronchodilation with inhaled salbutamol) are performed at baseline or the six-
18
19 202 month visit. HHCs who did not take up HIV testing or other screening at baseline are offered
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21 203 these tests at each study visit. Any HHCs with test results requiring treatment or further
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23 204 investigations are referred for respective services.
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27 206 HHCs are screened for TB using the WHO symptom questionnaire and a digital chest-
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29 207 radiograph (dCXR), reviewed by a clinical officer. dCXRs are not performed in pregnant HHCs.
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31 208 HHCs with a positive WHO symptom screening and abnormal dCXR are asked to provide
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33 209 sputum samples for TB investigations i.e., for GeneXpert and mycobacterial culture. Those
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35 210 with negative symptom screen and normal dCXR are asked to provide a spontaneous sputum
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37 211 sample for storage (with sputum induction performed if required).
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41 213 At baseline, urine, serum, plasma, whole blood (native, and with RNA preservation in
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43 214 PAXgene® tubes [BD Biosciences, NJ, USA]) are stored. A finger-prick sample is taken and
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45 215 investigated using the Xpert TB Host Response RUO Prototype cartridge (Cepheid,
46
47 216 Sunnyvale, CA, USA). T-cell Activation Marker Tuberculosis (TAM-TB) assay and Interferon
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49 217 Gamma Release Assay (IGRA; STANDARDTM F TB-Feron FIA (IFN-gamma; SD Biosensor,
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51 218 Republic of Korea), are performed on fresh venous blood. In Tanzania and Mozambique,
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53 219 storage of peripheral blood mononuclear cells for later characterization of the TB-specific
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55 220 immune response is also performed.
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3 222 Procedures for follow-up and unwell visits are similar to those at baseline. Measurement of
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5 223 HIV status, haemoglobin, HbA1c, spirometry, CD4 count and IGRA testing are not performed
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7 224 at follow-up visits, unless not done previously. At the last scheduled visit all HHCs not known
8
9 225 to have HIV are re-offered HIV testing and a spontaneous or induced sputum sample is stored
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11 226 for all participants.
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15 228 ***Household contacts screening positive for TB symptoms and/or with a DCXR***
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17 229 ***suggestive of TB***

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20 230 HHCs screening positive for symptoms and/or those with DCXRs suggestive of TB are asked
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22 231 for a sputum sample, which is investigated using Xpert MTB/RIF Ultra (Cepheid). If this sample
23
24 232 is positive for *Mtb* (including a trace result), a minimum of two additional sputum samples are
25
26 233 investigated, following decontamination, with Xpert MTB/RIF Ultra, solid and liquid culture.
27
28 234 Isolates stored from these cultures will be sequenced for matching with the IC isolates in order
29
30 235 to verify intra-household transmission. Sputum induction is performed for those unable to
31
32 236 provide a spontaneous sputum sample. HHCs with microbiologically confirmed TB are referred
33
34 237 for TB treatment to the National TB Programme.
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39 239 ***Patient and public involvement***

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41 240 The ERASE-TB study sites have established Community Advisory Boards, which are voices
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43 241 of communities, people affected, and study participants, providing a strategic link between the
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45 242 communities and the study team. Community Advisory Boards meet regularly and provide
46
47 243 feedback on design, procedures and conduct of the study. They will also be closely involved
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49 244 in the dissemination of study results. In addition to the Community Advisory Boards, each study
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51 245 site conducts community engagement activities focused on young people with the aim to foster
52
53 246 interest in science and research, specifically in the field of respiratory diseases/illness. This
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55 247 includes close partnership with schools and universities. Furthermore, planned qualitative
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57 248 research will specifically aim to understand the perceptions of HHCs with regards to TB
58
59 249 diagnostics and screening.
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251 Sample size

252 An estimated 800 to 900 TB-confirmed ICs are required for the subsequent enrollment of an
253 anticipated 2,100 HHCs, i.e., 700 HHCs per country. Loss to follow-up of HHCs is estimated
254 to be 10%. A total of 64 HHCs (3%) are estimated to be diagnosed with TB during the study
255 period, based on previous active case finding studies among HHCs [16].

256

257 Novel test candidates

258 A range of novel test candidates targeted at pathogen detection or identification of host
259 responses to *Mtb* are being applied, either in real-time (for all participants) or retrospectively
260 (in a case-control design). Whilst a number of novel test candidates have been pre-specified,
261 the ERASE-TB biobanking processes allow for addition of further candidate tests to be
262 evaluated on stored samples as they become available.

263

264 DCXRs offer good sensitivity for diagnosis of pulmonary TB. However, high inter- and intra-
265 investigator variability, and lack of trained interpreters present a barrier to implementation in
266 many high-TB burden settings. Computer-aided interpretation systems, such as CAD4TB
267 (Delft Imaging, Hertogenbosch, Netherlands) and qXR (Qure.ai, India) may increase image-
268 reading capacity, with good performance, and serve, therefore, as a systematic screening tool
269 to identify individuals in need of confirmatory TB tests [17,18].

270

271 Xpert MTB/RIF Ultra is a nucleic acid amplification test for *Mtb* with a lower limit of detection
272 compared to the previous Xpert MTB/RIF generation, and, therefore, conferring higher
273 sensitivity in paucibacillary specimens. This, however, comes at the expense of specificity,
274 particularly in high TB incidence settings, resulting in 'false positives' [19]. WHO guidelines
275 recommend Xpert MTB/RIF Ultra for TB diagnosis among adults and children acknowledging
276 that further evaluation, particularly of the role of Xpert MTB/RIF Ultra for TB screening, is
277 needed [20,21].

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5 279 FLOW-TB is an advanced enzyme-linked immunosorbent assay for the detection of *Mtb*
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7 280 lipoarabinomannan (a mycobacterial cell wall component) in urinary specimens with results
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9 281 available within 65 minutes [22].
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13 283 The T-cell activation marker-TB assay (TAM-TB) detects *Mtb*-specific CD4 T-cells through *in-*
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15 284 *vitro* antigen stimulation with *Mtb*-derived peptides, i.e., from ESAT-6 and CFP-10, followed by
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17 285 flow cytometry. TAM-TB discriminated latent *Mtb* infection from TB in freshly collected blood
18
19 286 with 83% sensitivity and 96-98% specificity in previous studies. Further, TAM-TB may detect
20
21 287 early TB disease progression up to 9 months prior to the identification of *Mtb* in sputum [23–
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23 288 25].
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28 290 Multiple transcriptomic signatures, capturing the host response to TB, have been described as
29
30 291 promising candidate tests for earlier TB diagnosis (up to two years before microbiological
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32 292 diagnosis). An individual patient data meta-analysis suggested equivalent performance of
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34 293 eight signatures, with 25-40% sensitivity and 92-95% specificity 0-24 months before TB
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36 294 diagnosis. Diagnostic accuracy of each signature improved as the interval between testing and
37
38 295 microbiological TB diagnosis shortened [26]. Several signatures have been developed into
39
40 296 polymerase chain reaction (PCR)-based assays to facilitate real-time implementation: the
41
42 297 recent CORTIS trial reported sensitivity of 48% and specificity of 75% for incipient TB for the
43
44 298 RISK-11 signature [27]. Cepheid have developed a 3-transcript TB score into a fully automated
45
46 299 in-cartridge PCR assay performed on finger-prick blood using the Xpert platform (Xpert TB
47
48 300 Host Response RUO Prototype cartridge). This cartridge will be evaluated using freshly
49
50 301 collected specimens in ERASE-TB; storage of RNA-stabilised blood samples also allows for
51
52 302 retrospective evaluation of additional transcriptomic signatures in our cohort [28,29]
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57 304 An alternative approach to capture the host response to TB is through protein-based biomarker
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59 305 signatures. Candidate tests in this category include a serum- or plasma-based multiplex assay

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3 306 assessing 13 protein biomarkers (CRP, procalcitonin[30], sTREM-1[31,32], angiopoietin-
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5 307 2[33,34], interleukin-6[35], TRAIL[36] and IP-10[37]) that is being developed by the London
6
7 308 School of Hygiene and Tropical Medicine; in addition, a seven biomarker signature is under
8
9 309 development as a point-of-care test for TB diagnosis, with 94% sensitivity and 73% specificity
10
11 310 detected in previous work [38].
12

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14 311

15 312 **Statistical analyses**

16
17 313 Baseline characteristics and analytical data will be summarized using descriptive statistics
18
19 314 inclusive of mean, median, range, standard deviation, and absolute as well as relative
20
21 315 frequencies depending on the nature of data. A logistic regression model will be used to identify
22
23 316 characteristics of TB among ICs, households and HHCs that are predictive of incident TB.
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25 317 From the study database, we will simulate algorithms of different tests to obtain the testing
26
27 318 combination with the best accuracy. We will couple tests with a mathematical model that
28
29 319 quantifies the risk of infection and/or disease to enhance predictive performance. The reporting
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31 320 of the development of the prediction model will follow the Transparent Reporting of a
32
33 321 multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) Initiative [39].
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39 323 The validation of novel diagnostic tests for detecting TB will be analysed as a 1:4 matched
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41 324 nested case-control study with HHCs diagnosed with TB at baseline and during follow-up
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43 325 serving as cases, and HHCs who do not develop TB during follow-up as controls; controls will
44
45 326 be matched for site, age, sex, HIV status and other risk factors for developing TB. Sensitivity
46
47 327 and specificity of novel tests will be determined using pre-existing positive/negative cut-offs
48
49 328 where these exist; and receiver operating curves (ROC) constructed with area under the ROC
50
51 329 curve calculated. For tests aiming to identify individuals at high risk of TB in the future, only
52
53 330 HHCs who are diagnosed with TB during follow-up will serve as cases (i.e. those diagnosed
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55 331 with TB at baseline will be excluded). Stored samples from all timepoints will be retrieved and
56
57 332 diagnostic accuracy (i.e. sensitivity and specificity) of the novel test determined at different
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3 333 time-points before TB diagnosis. The decision of assigning the “active TB” endpoints to
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5 334 participants will be blinded from the new test results to avoid inclusion bias.

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9 336 **Data management**

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11 337 All source data will be kept confidential in secured locations with restricted access by
12
13 338 authorized personnel only inclusive of monitors, auditors and reviewers of ethical and
14
15 339 regulatory committees in line with applicable data privacy regulations. Each participant is
16
17 340 asked to consent to this handling of the data, and is assigned a pseudonymous identification
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19 341 number that is used throughout the study on all source data.

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24 343 Accurate documentation of paper-based and electronic source data, e.g., original records and
25
26 344 certified copies of original records, progress notes, screening logs, and recorded data from
27
28 345 automated instruments, will be maintained. The pseudonymized clinical data captured on
29
30 346 paper-based Case Report Forms will be entered at the sites into a database using the web-
31
32 347 based Clinical Data Management System of OpenClinica (OpenClinica LLC, Waltham, MA,
33
34 348 USA). The study specific database has been built, maintained and hosted by the LMU Klinikum
35
36 349 on a centralized secure server. Data modifications and necessary corrections performed in the
37
38 350 database also within the context of double data entry will be documented and tracked in audit
39
40 351 trails. Data quality and plausibility are assured by a series of pre-programmed edit and range
41
42 352 checks in OpenClinica. Further validation checks are programmed in Stata (Statacorp, College
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44 353 Station, TX, USA) with extracts of the database and electronically received data, e.g.,
45
46 354 spirometry, dCXR and laboratory, will be integrated into analyses of datasets.

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51 356 **Monitoring**

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53 357 Assigned study monitors will visit the sites at regular intervals physically and/or virtually in
54
55 358 addition to frequent day-to-day communication. Close follow-up on all study-related aspects
56
57 359 will be performed to ascertain compliance with standards of Good Clinical Practice, the
58
59 360 Declaration of Helsinki, and other local and national regulatory guidelines inclusive of

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3 361 guidelines for infection prevention and control of airborne-transmitted diseases, e.g., social
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5 362 distancing in well-ventilated spaces, and wearing of personal protective equipment. In
6
7 363 particular, monitors that support designated study personnel are responsible to verify (I)
8
9 364 adequacy of the study personnels' qualifications and facilities, (II) accuracy of informed
10
11 365 consent procedures and patient eligibility, (III) adherence to the study protocol, (IV) protection
12
13 366 of rights and well-being of participants, (V) adherence to infection prevention and control
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15 367 measures (VI) accuracy and completeness of study documents and other study-related
16
17 368 records, and (VII) maintenance of source documents.
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20 369

21 22 370 **Ethics and dissemination**

23
24 371 The study protocol and informed consent/assent documents have been approved by regulatory
25
26 372 and ethical committees of the participating institutions. This includes the Medical Research
27
28 373 Council in Zimbabwe, the National Health Research Ethics Committee in Tanzania, the
29
30 374 National Bioethics for Health Committee in Mozambique, and the ethical committees of London
31
32 375 School of Hygiene & Tropical Medicine, United Kingdom, and the medical faculty of the
33
34 376 Ludwig-Maximilians-Universität München, Germany.
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39 378 Adult ICs and HHCs are asked for written informed consent prior to their participation.
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41 379 Underage HHCs are asked for assent in addition to obtaining the consent of their legal
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43 380 guardians/parents; with ages for assent depending on local guidance. In case of illiteracy, the
44
45 381 participant is asked to give its consent by fingerprint while an adult impartial, literate witness
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47 382 present during the entire consent procedure signs the consent on behalf. All participants have
48
49 383 the right to withdraw from the study at any time. Findings derived from ERASE-TB will be
50
51 384 presented at scientific conferences, and published in peer-reviewed international journals.
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55 56 386 **Current study status**

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58 387 The recruitment of ICs and HHCs is in progress in Zimbabwe, Mozambique and Tanzania
59
60 388 since March, August and September 2021, respectively. The follow-up of HHCs is anticipated

389 to be completed in March, August and September 2023 in Zimbabwe, Mozambique and
390 Tanzania, respectively; laboratory analyses are estimated to be performed by December 2024.

391

392 **Author contributions**

393 The study proposal and protocol were written by NH, KK with scientific input from CK, TM, CG,
394 JM. ETM, UP, DB, AM wrote the initial manuscript with scientific input on the database and
395 data management section from FR, TA. NH, KK critically reviewed the initial draft of the
396 manuscript. KH, GK, CJC, ENN, TA, provided critical feedback on the manuscript. All authors
397 have read and approved the final version of the manuscript.

398

399 **Funding statement**

400 ERASE-TB is part of the EDCTP2 programme supported by the European Union (grant
401 number RIA2018D-2508-ERASE-TB), the German Center for Infection Research (DZIF) grant
402 number: 02.710 and the Swedish Research Council (220-23602). CJC is funded by the
403 Wellcome Trust (203905/Z/16/Z). Cepheid, Inc., and SD Biosensor provided test kits and
404 analyzers at no cost to the Consortium.

405

406 **Acknowledgments**

407 We are grateful to the study personnel from the Biomedical Research and Training Institute
408 and the Zvitambo Research Institute, Zimbabwe, the Instituto Nacional de Saúde,
409 Mozambique, and the National Institute for Medical Research - Mbeya Medical Research
410 Centre, Tanzania for their exceptional efforts and contributions, which made this research
411 possible.

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37 433 **Competing interests statement**

38
39 434 None declared.

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43 436 **Disclaimer**

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45 437 Not applicable.
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3 575 **Figure legend**

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5 576 **Figure 1.** The ERASE-TB consortium

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7 577 Abbreviations: ERASE-TB=Early Risk Assessment in TB contactS by new diagnostic tEsts

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11 579 **Figure 2.** Location and characteristics of ERASE-TB study sites

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13 580 Notes: The location of each study site is indicated by a red asterix. Source data used within
14
15 581 this figure are taken from the references [7,8,41,42,9–15,40].

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17 582 Abbreviations: ERASE=Early Risk Assessment in TB contactS by new diagnostic tEsts;
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19 583 TB=tuberculosis; HIV=human immunodeficiency virus

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24 585 **Figure 3.** Eligibility criteria and schedules of events for index cases and household contacts

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26 586 Notes: **A**=depending on the time point of study enrollment and consequently on the duration
27
28 587 available for follow-up, i.e. 18 or 24 months, the follow-up visit at 24 months \pm 30 days may be
29
30 588 conditional; **B**=the follow-up visit by phone may be conducted after the last scheduled follow-
31
32 589 up visit at 18 months \pm 30 days or 24 months \pm 30 days to assess whether symptoms suggestive
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34 590 of TB have occurred, TB diagnosis has been made or anti-TB treatment has been initiated;
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36 591 **C**=unwell visits by phone or on-site may be conducted between scheduled follow-up visits if a
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38 592 participant presents at a recruitment healthcare facility with signs and symptoms suggestive of
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40 593 TB; **D**=coached spontaneous or induced sputum collection for storage at scheduled follow-up
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42 594 visit at 18 months \pm 30 days or 24 months \pm 30 days, and for repetition of HIV testing if tested
43
44 595 negative at baseline; **E**=coached spontaneous or induced sputum collection upon the decision
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46 596 of the investigating team for testing by Xpert MTB/RIF Ultra if participant presents with signs
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48 597 and symptoms suggestive of TB; **F**=coached spontaneous or induced sputum collection in
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50 598 case of Xpert MTB/RIF Ultra positivity or strong clinical suspicion of TB for repetition of the
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52 599 Xpert MTB/RIF Ultra; **G**=in case of HIV positivity to be followed by the assessment of CD4
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54 600 counts; **H**=CXR to be conducted at an unscheduled on-site unwell visit upon the decision of
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56 601 the investigating team depending on the nature of symptoms reported, and the time elapsed
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58 602 since the last CXR including its findings; **I**=not to be conducted among pregnant women;

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3 603 **J**=stored venous blood includes 6mL EDTA blood for whole blood and plasma, 4mL serum
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5 604 and 2.5mL PAXgene blood, all samples will be deep frozen for retrospective testing using new
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7 605 diagnostics as described in text; **K**=in case the evaluation of symptoms of a participant unable
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9 606 to present at a recruitment healthcare facility is required an unscheduled on-site or home visit
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11 607 will be arranged by phone, the resolution of symptoms can alternatively be addressed by
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13 608 phone; **L**=collection of PBMC at baseline and follow-up visit at 6 months \pm 30 days is optional,
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15 609 thus will not be performed at each participating site and for each participant; **M**=in case the
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17 610 evaluation of symptoms of a participant unable to present at a recruitment healthcare facility
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19 611 is required or doubtful if required an unscheduled unwell visit by phone will be arranged, the
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21 612 resolution of symptoms can alternatively be addressed by phone; **N**=spirometry and/or
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23 613 diabetes (HbA1c) will be performed at scheduled follow-up visits at 6 months \pm 30 days, 12
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25 614 months \pm 30 days and 18 or 24 months \pm 30 days if required or not performed at baseline,
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27 615 anaemia (Hb) will be performed at baseline and scheduled follow-up visits at 6 months \pm 30
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29 616 days, 12 months \pm 30 days and 18 or 24 months \pm 30 days if possible; **O**=blood pressure
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31 617 measurement will be performed at baseline and scheduled follow-up visits at 6 months \pm 30
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33 618 days, 12 months \pm 30 days and 18 or 24 months \pm 30 days; **P**=WGS to be performed once *Mtb*
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35 619 infection is confirmed and an isolate could be recovered.

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39 620 Abbreviations: TB=tuberculosis; WHO=World Health Organization; HIV=human
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41 621 immunodeficiency virus; WGS=whole genome sequencing; MBLA=molecular bacterial load
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43 622 assay; CXR=chest radiograph; IGRA=interferon gamma release assay; PBMC=peripheral
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45 623 blood mononuclear cell; TAM-TB=T- cell activation marker tuberculosis; MTB=*Mycobacterium*
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47 624 *tuberculosis*; RIF=rifampicin, Hb=haemoglobin; HbA1c=glycated hemoglobin;
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49 625 LAM=lioparabinomannan; CD4=cluster of differentiation 4

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52 53 627 **Figure 4.** Study design

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55 628 Abbreviations: HHC=household contact; TB=tuberculosis; IC=index case; WHO=World
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57 629 Health Organization; CXR= chest radiograph; SS=symptom score; MTB=*Mycobacterium*
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59 630 *tuberculosis*; RIF=rifampicin; pos=positive; CT=computer tomography; FU=follow-up

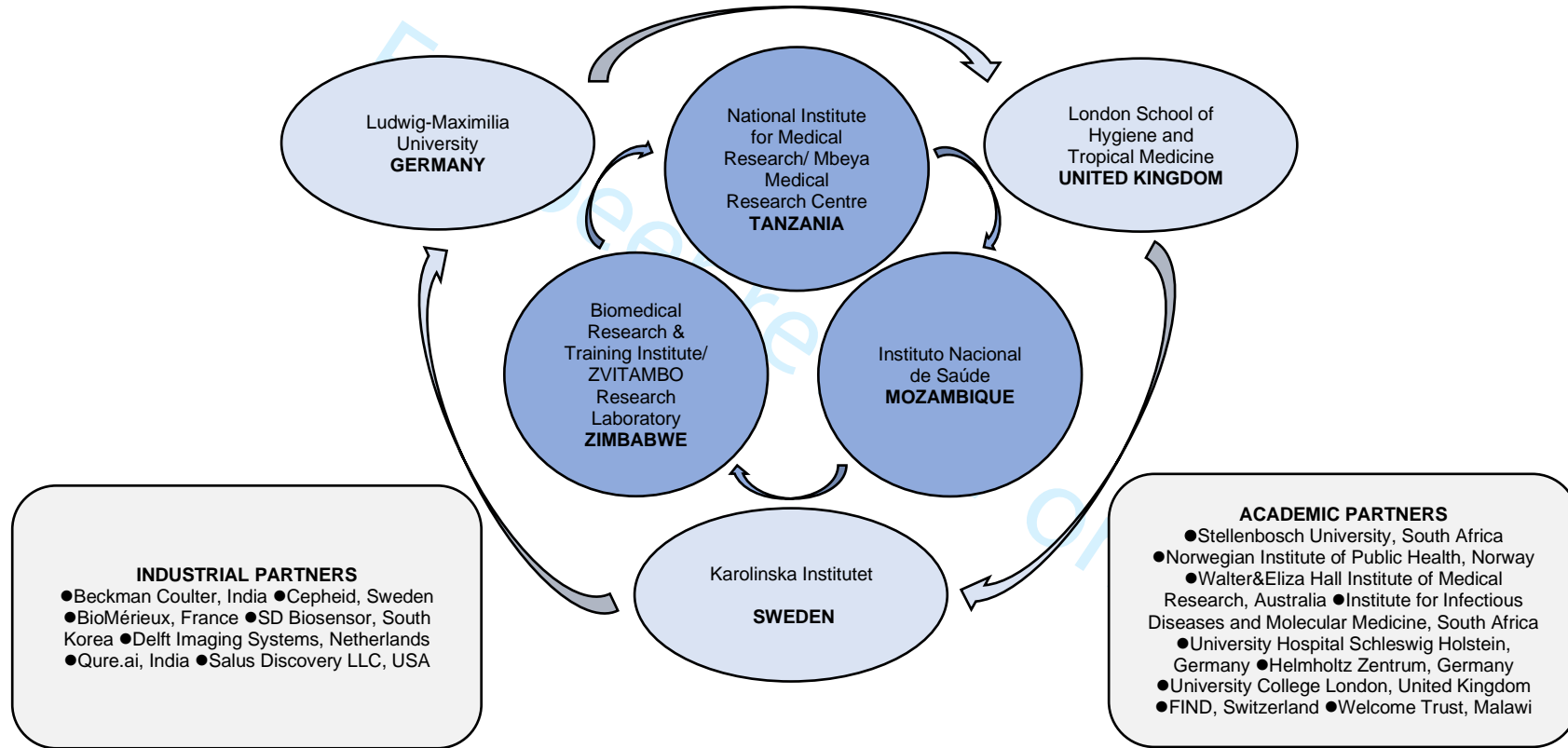


Figure 1. The ERASE-TB consortium

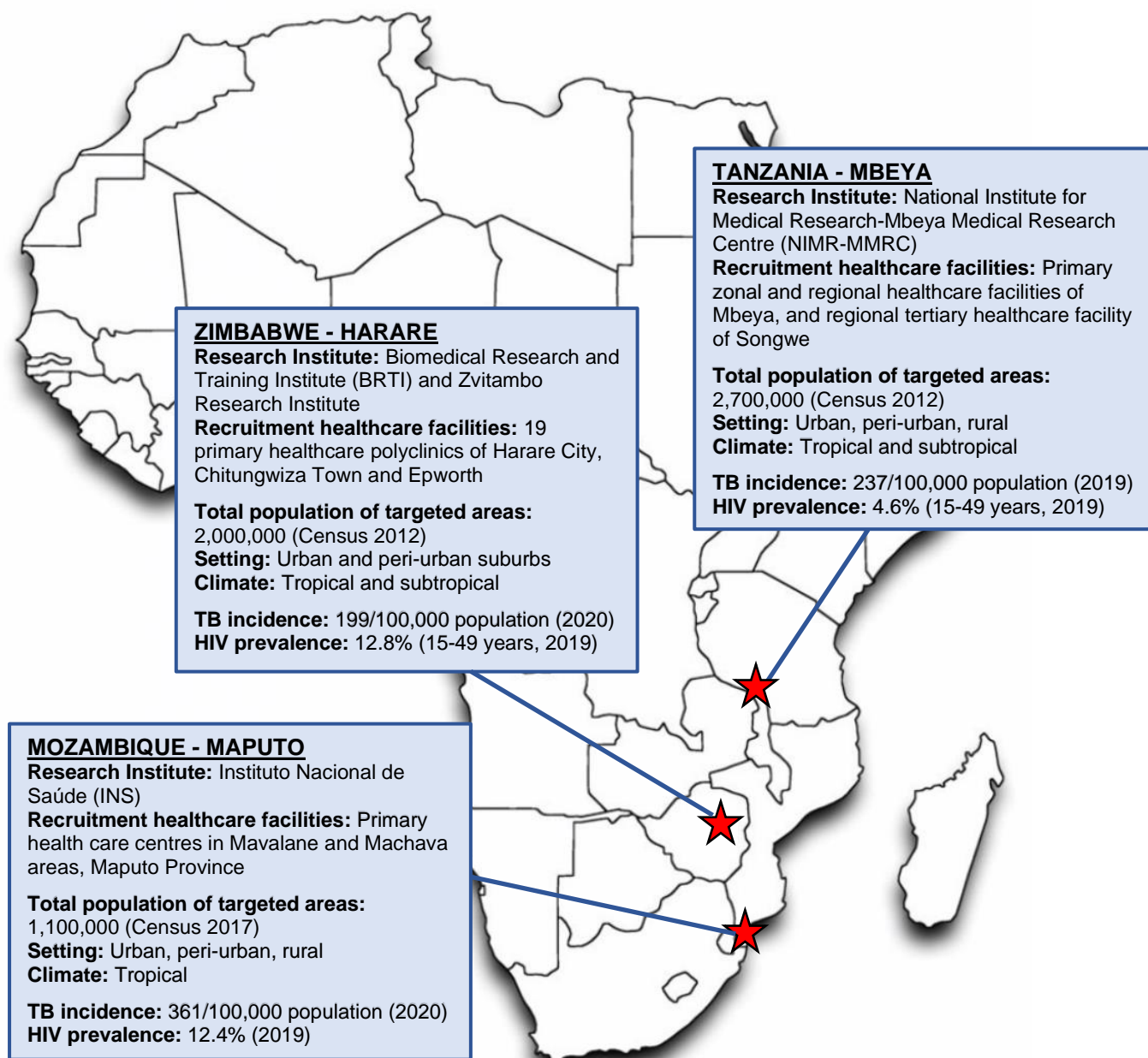


Figure 2. Location and characteristics of ERASE-TB study sites

INDEX CASE

HOUSEHOLD CONTACT

INCLUSION CRITERIA

- Aged ≥18 years
- Living with at least another person aged ≥10 years in the same household with a firm unchanged address for 6 months
- Pulmonary TB diagnosis within 4 weeks prior to enrolment
- Spontaneous sputum production
- Positive sputum smear, i.e. >+2 positivity by Ziehl-Neelsen stain or Auramine-O stain OR ≥+1 positivity on WHO symptom scale OR ≥medium positivity by GeneXpert (if no microscopy performed)
- <7 daily doses of anti-TB treatment since diagnosis
- Voluntary written informed consent obtained

EXCLUSION CRITERIA

- Treatment for TB within the past 30 days OR on current TB treatment OR on current preventive TB therapy (if HIV negative) at the time of enrolment



INCLUSION CRITERIA

- Aged ≥10 years
- Substantial exposure, i.e. spending ≥3 nights/week over the past 4 weeks in the same household as the index case,
- Voluntary written informed consent or parent/guardian consent & assent in case of minors obtained



EVENTS AT BASELINE/SCREENING VISIT

- Administration of socio-demographic questionnaire
 - Assessment of vital signs, medical history
 - Digital CXR (if available)^I
- Specimen investigations**
- Sputum: culture, storage, WGS, MBLA

EVENTS AT BASELINE/ ±14days of SCREENING VISIT

- Administration of socio-demographic questionnaire
 - Assessment vital signs, medical history
 - Screening for diabetes (HbA1c)^N, high blood pressure^O, anaemia (Hb)^N, WHO TB symptoms
 - Digital CXR^I, spirometry^N
- Specimen investigations**
- Sputum: Xpert MTB/RIF Ultra^E, culture^F, storage, WGS^P
 - Blood: HIV/CD4 counts^G, Cepheid host response, TAM-TB, IGRA SD Biosensor, PBMC^L, storage^J
 - Urine: dipstick/LAM, storage



EVENTS AT UNSCHEDULED UNWELL VISIT(S)
(phone/on-site)^{C,K,M}

- Assessment of vital signs, medical history
 - Screening for high blood pressure, WHO TB symptoms,
 - Digital CRX^{H,I}
- Specimen investigations**
- Sputum: Xpert MTB/RIF Ultra, culture, storage, WGS^P
 - Blood: Cepheid host response, TAM-TB, storage^J
 - Urine: dipstick/LAM, storage

EVENTS AT UN-SCHEDULED FOLLOW-UP VISIT(S)^B (phone)

- Assessment of medical history
 - Screening for WHO TB symptoms
- Specimen investigations**
- None

EVENTS AT 6months ±30days/ 12months ±30days/ 18months ±30days/ 24months ±30days^A FOLLOW-UP VISITS

- Assessment of vital signs, medical history
 - Screening for high blood pressure, WHO TB symptoms
 - Digital CXR^I, spirometry^N
- Specimen investigations**
- Sputum: Xpert MTB/RIF Ultra^E, culture^F, storage^D, WGS^P
 - Blood: Cepheid host response, TAM-TB, PBMC^L, storage
 - Urine: dipstick/LAM, storage^J



Optional

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Figure 3. Eligibility criteria and schedules of events for index cases and household contacts

For peer review only

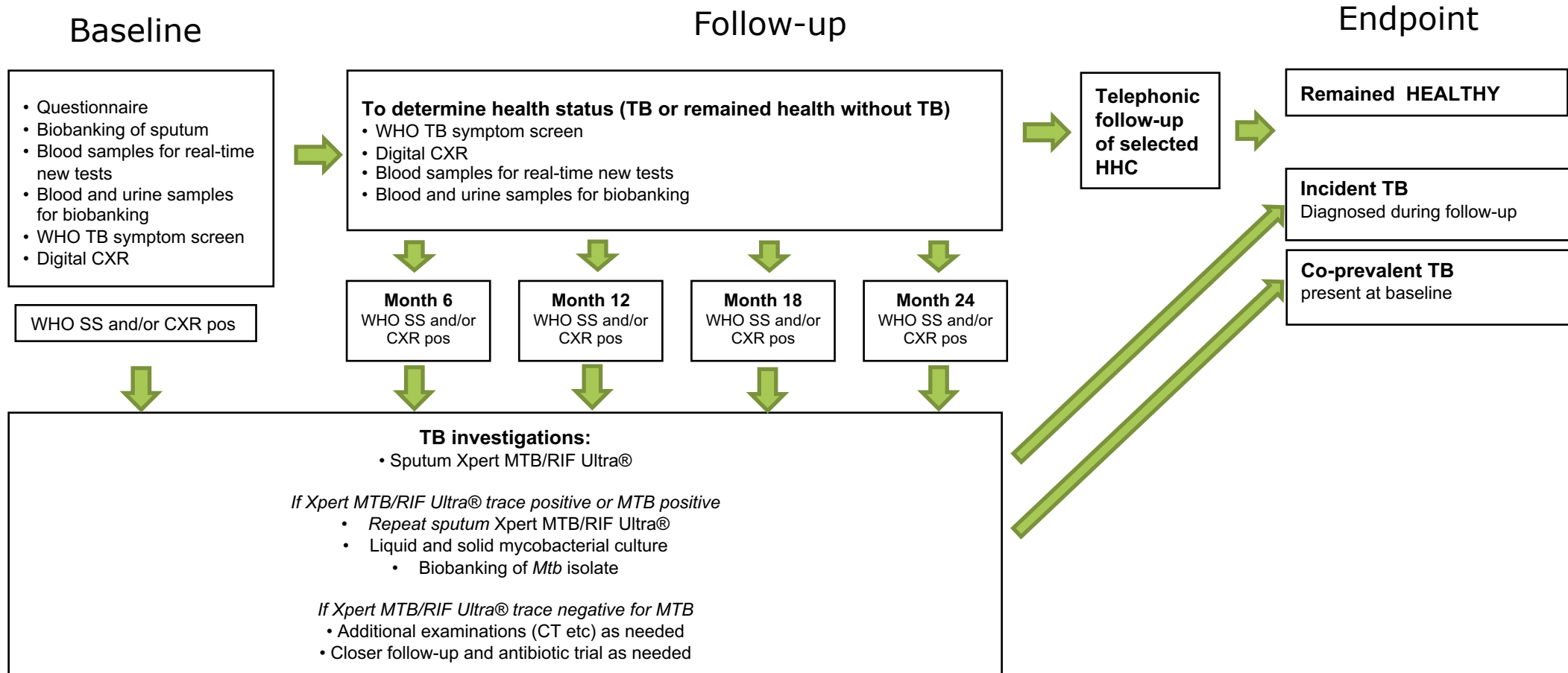


Figure 4. Study design.

BMJ Open

Early risk assessment in pediatric and adult household contacts of confirmed tuberculosis cases by novel diagnostic tests (ERASE-TB): protocol for a prospective, non-interventional, longitudinal, multi-country cohort study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-060985.R1
Article Type:	Protocol
Date Submitted by the Author:	31-May-2022
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Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Diagnostics, Infectious diseases

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Keywords:	Tuberculosis < INFECTIOUS DISEASES, Diagnostic microbiology < INFECTIOUS DISEASES, RESPIRATORY MEDICINE (see Thoracic Medicine)

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Manuscripts

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3 1 **Early risk assessment in pediatric and adult household contacts of confirmed**
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5 2 **tuberculosis cases by novel diagnostic tests (ERASE-TB): protocol for a**
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7 3 **prospective, non-interventional, longitudinal, multi-country cohort study**
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9 4

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56 23 § Contributed equally

57 24 # Contributed equally
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52 26 Word count: 3,668

56 28 **Keywords:** *Mycobacterium tuberculosis*, diagnostics, cohort study, household contacts, WHO
57
58 29 END TB strategy, ERASE-TB

30 **ABSTRACT**

31 **Introduction**

32 The World Health Organization (WHO) End-TB Strategy calls for the development of novel
33 diagnostics to detect tuberculosis earlier and more accurately. Better diagnostics, together with
34 tools to predict disease progression are critical for achieving WHO END-TB targets. The Early
35 Risk Assessment in TB contactS by new diagnostic tEsts (ERASE-TB) study aims to evaluate
36 novel diagnostics and testing algorithms for early tuberculosis diagnosis and accurate
37 prediction of disease progression among household contacts exposed to confirmed index
38 cases in Mozambique, Tanzania and Zimbabwe.

40 **Methods and analysis**

41 A total of 2,100 household contacts (HHCs) (aged ≥ 10 years) of adults with microbiologically-
42 confirmed pulmonary tuberculosis will be recruited and followed up at 6-month intervals for 18
43 to 24 months. At each time-point a WHO symptom screen and digital chest-radiograph (dCXR)
44 will be performed, and blood and urine samples collected. Individuals screening positive (WHO
45 symptom screen or dCXR) will be requested to provide sputum for Xpert MTB/Rif Ultra. At
46 baseline, HHCs will also be screened for HIV, diabetes (HbA1c), chronic lung disease
47 (spirometry), hypertension and anaemia. Study outcomes will be co-prevalent tuberculosis
48 (diagnosed at enrollment), incident tuberculosis (diagnosed during follow-up) or no
49 tuberculosis at completion of follow up. Novel diagnostics will be validated using fresh and
50 biobanked samples with a nested case control design. Cases are defined as HHCs diagnosed
51 with tuberculosis (for early diagnosis) or with incident tuberculosis (for prediction of
52 progression) and will be matched by age, sex and country to HHCs who remain healthy
53 (controls). Statistical analyses will include assessment of diagnostic accuracy by constructing
54 receiver operating curves and calculation of sensitivity and specificity.

56 **Ethics and dissemination**

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2
3 57 ERASE-TB has been approved by regulatory and ethical committees in each African country
4
5 58 and by each partner organisation. Consent, with additional assent for participants <18 years,
6
7 59 is voluntary. Attestation by impartial witnesses is sought in case of illiteracy. Confidentiality of
8
9 60 participants is being maintained throughout. Study findings will be presented at scientific
10
11 61 conferences and published in peer-reviewed international journals.
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16 63 **Study registration number**

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3 65 **Strengths**
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- 5 66 • Recruitment of highly infectious index cases aimed at maximising the number of
6 tuberculosis (TB) diagnoses in the household contact (HHCs) cohort.
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9 68 • Sequencing of *Mycobacterium tuberculosis* isolates from both index cases and HHCs
10 allows confirmation of household transmission and thus determination of timing of the
11 transmission event; resulting in more precise estimates of new test sensitivity
12 compared to population-based cohorts with unknown timing of infection.
13 70
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15 71
16 72 • Large sample size across three southern African countries with high HIV prevalence;
17 including adolescents will ensure study findings are generalisable to the clinically
18 relevant population at high risk of TB compared to studies focused on adults only.
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26 76 **Limitations**
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- 28 77 • Despite the large cohort of HHCs, the number of diagnosed TB cases will be small,
29 limiting the power of the study and sub-group analyses such as by age and HIV status.
30 78
31
32 79 • Geographically limited to sub-Saharan Africa, therefore results may not be
33 generalisable to other populations, including those with lower HIV prevalence such as
34 in South-East Asia or the Americas.
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82 INTRODUCTION

83 Tuberculosis (TB) remains a leading global public health problem, with an estimated 10 million
84 new cases and 1.5 million deaths globally in 2020 [1]. In 2014, the World Health Assembly
85 approved the World Health Organization (WHO) End-TB Strategy, aiming for a 90% reduction
86 in TB incidence and 95% reduction in TB deaths by 2035 [2]. However, in 2019, three million
87 TB cases ('the missing millions') remained undiagnosed and untreated globally, resulting in
88 potentially avoidable morbidity, mortality and onward transmission. The Covid-19 pandemic
89 has resulted in a large decrease in the number of people newly diagnosed with TB and
90 reported. This has increased the diagnostic gap by a further 1.3 million, resulting in an
91 estimated 4.2 million undiagnosed TB cases in 2020 [3]. Also, for the first time in a decade TB
92 deaths have risen, from an estimated 1.4 million in 2019 to 1.5 million in 2020, as a result of
93 reduced access to and provision of essential TB services including diagnostics during the
94 Covid-19 pandemic.

95
96 Without an efficacious and safe vaccine, early detection and containment are the main tools
97 to interrupt transmission and successfully control TB. Similar to SARS-CoV2, asymptomatic
98 spreading of *M.tuberculosis* and subclinical but infectious disease states are a major concern
99 in the control of airborne infectious diseases [4]. Early and accurate identification of persons
100 with TB, combined with identification of those at risk of progression to TB and provision of
101 targeted preventive treatment are critical to reducing TB-associated morbidity and mortality,
102 and preventing onward transmission.

103
104 Currently available diagnostics such as sputum microscopy, mycobacterial culture and nucleic
105 acid amplification tests are based on direct pathogen detection, thus requiring a high
106 mycobacterial load; they therefore predominately target advanced TB when onward
107 transmission and significant lung damage has occurred [5,6]. Further, for many patients with
108 minimal or no symptoms, expectoration of high-quality sputum specimens remains

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3 109 challenging, limiting the accuracy of sputum-based tests. The same holds true for young
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5 110 children and people living with HIV.
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9 112 The **Early Risk Assessment in TB Contacts by new diagnostic tests** (ERASE-TB) study aims
10
11 113 to fill this diagnostic gap by evaluating new sputum and non-sputum-based TB diagnostics for
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13 114 early TB detection (before onward transmission occurs), as well as tools for more accurate
14
15 115 prediction of TB progression to allow for targeted preventive therapy.
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19 20 117 **METHODS AND ANALYSES**

21 22 118 **Study objectives**

23
24 119 ERASE-TB's primary objectives are (I) to determine the sensitivity and specificity of novel
25
26 120 diagnostics to detect TB, in particular asymptomatic or minimally symptomatic TB; (II) to
27
28 121 evaluate novel diagnostics for detection of likely TB progression; and (III) to enhance the
29
30 122 performance of novel diagnostics by simulating testing algorithms coupled with individual risk
31
32 123 estimates from a mathematical model. The secondary objectives are (I) to determine the TB
33
34 124 prevalence among household contacts (HHCs) of infectious TB index cases (ICs) at baseline
35
36 125 and during a 18-24 months follow-up; (II) to establish a biorepository of cryopreserved
37
38 126 specimens from HHCs for future development and validation of diagnostic tests; and (III) to
39
40 127 assess the association of selected chronic disease conditions and TB among HHCs.
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44 45 129 **Study endpoints**

46
47 130 The study's primary endpoints are the presence or development of TB among HHCs with the
48
49 131 following possible scenarios of (I) prevalent symptomatic TB at baseline, (II) incident TB during
50
51 132 follow-up, and (III) remained healthy until study completion. An endpoint review committee will
52
53 133 review the data and case classification before finalization.
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58 135 Through the sequencing of *Mycobacterium tuberculosis* (*Mtb*) isolates, cases of co-prevalent
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60 136 or incident TB will be classified either as secondary, infected by the source case – the timepoint

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3 137 of infection will be known; or as infected by another, unknown source of infection, with an
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5 138 unknown timepoint of infection.
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9 140 **Recruitment sites**

11 141 Recruitment of ICs and HHCs at selected primary healthcare facilities and communities has
12
13 142 commenced in Harare, Zimbabwe in March 2021, Maputo, Mozambique in August 2021, and
14
15 143 Mbeya, Tanzania in September 2021. Partners of the ERASE-TB consortium are illustrated in
16
17 144 Figure 1. All three countries have a high TB incidence ranging from 100 to 499/100,000
18
19 145 population [1] and HIV prevalence among adults aged 15 years and older of 5% to 20% [7].
20
21 146 The African research institutions have established collaborations with their respective National
22
23 147 Tuberculosis Programs ensuring referral and appropriate follow-up of TB patients. Figure 2
24
25 148 illustrates the geographic location of research institutions, healthcare facilities where
26
27 149 recruitment is taking place, demographic characteristics of study populations, and estimates
28
29 150 on TB incidence and HIV prevalence [8–15].
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32 151

34 152 **Study design**

36 153 ERASE-TB is a non-interventional, longitudinal, prospective cohort study among HHCs aged
37
38 154 ≥ 10 years exposed to highly infectious pulmonary TB ICs aged ≥ 18 years. Eligibility criteria
39
40 155 are detailed in Figure 3 and the study design is shown in Figure 4. TB ICs are eligible if the
41
42 156 bacterial load in their sputum is at least at the “medium” level according to Xpert MTB/RIF or
43
44 157 Xpert MTB/RIF Ultra, and they have received less than seven daily doses of anti-TB treatment
45
46 158 before enrollment. This maximises the likelihood of culturing and storing *Mtb* isolates. The total
47
48 159 study duration will be 36 months. This includes 12-months enrollment of ICs and HHCs, and
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50 160 18- to 24-months follow-ups of HHCs. Follow-up ends when a HHCs withdraws from the study,
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52 161 is lost to follow-up, dies, or is diagnosed with TB and referred for treatment. Scheduled or
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54 162 unscheduled unwell visits can be conducted physically and/or telephonically in case of
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56 163 abnormal finding e.g. by abnormal dCXR, or when a participant feels unwell inbetween
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58 164 scheduled follow-up visits.
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Procedures***TB index cases***

Following informed consent obtained, a questionnaire is administered to collect socio-demographic information, TB risk factors, and the medical history of TB, HIV and other diseases. Two spontaneous sputum samples are obtained, of which one is for mycobacterial culturing and one for storage for performing retrospectively Molecular Bacterial Load Assay (MBLA) to quantify viable *Mtb* by 16S rRNA [6]; an alternative means to quantify expectorated bacterial load for an estimate of infectiousness. Both liquid and solid mycobacterial cultures are performed on decontaminated sputum samples, with all *Mtb* isolates stored at -80 degrees for future DNA extraction and whole genome sequencing. A questionnaire on symptom duration and TB risk factors is also administered.

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Household key informant

At baseline, a household key informant (either the TB index case or one of the household contacts) is identified and asked to answer questions of a household questionnaire that collects socio-economic elements like structure of the house or flat, income and household assets, and covariates possibly associated with risk of TB infection, e.g., windows/air exchange, presence of comorbid conditions, and risk factors like the source of cooking energy, and properties of the household kitchen.

185

Household contacts

Informed consent is obtained from all eligible adult HHCs. For HHC <18 years of age, the guardian is asked to provide informed consent, with assent also sought from children dependent on local guidance. At baseline a questionnaire is administered collecting information on socioeconomic and demographic characteristics, past medical history of TB, HIV and other diseases, exposure risk factors, smoking and alcohol history. The physical examination includes height, weight, mid-upper arm circumference and blood pressure

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3 193 measurement. In addition, all HHCs are offered free HIV testing according to the National
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5 194 Guidelines. All people with confirmed HIV infection will have CD4 counts performed and be
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7 195 referred for TB preventive therapy. Those not yet on antiretroviral therapy (ART) and those
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9 196 who interrupted ART are referred for ART at local services.
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13 198 Point of care HbA1c (A1cCare, SD Biosensor, Gyeonggi-do, Republic of Korea) and
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15 199 haemoglobin (Hemocue 301+, Hemocue, Angelholm, Sweden) tests and spirometry (including
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17 200 pre- and post-bronchodilation with inhaled salbutamol) are performed at baseline or the six-
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19 201 month visit. HHCs who did not take up HIV testing or other screening at baseline are offered
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21 202 these tests at each study visit. Any HHCs with test results requiring treatment or further
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23 203 investigations are referred for respective services.
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28 205 HHCs are screened for TB using the WHO symptom questionnaire and a digital chest-
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30 206 radiograph (dCXR), reviewed by a clinical officer. dCXRs are not performed in pregnant HHCs.
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32 207 HHCs with a positive WHO symptom screening and abnormal dCXR are asked to provide
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34 208 sputum samples for TB investigations i.e., for GeneXpert and mycobacterial culture. Those
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36 209 with negative symptom screen and normal dCXR are asked to provide a spontaneous sputum
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38 210 sample for storage (with sputum induction performed if required).
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43 212 At baseline, urine, serum, plasma, whole blood (native, and with RNA preservation in
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45 213 PAXgene® tubes [BD Biosciences, NJ, USA]) are stored. A finger-prick sample is taken and
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47 214 investigated using the Xpert TB Host Response RUO Prototype cartridge (Cepheid,
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49 215 Sunnyvale, CA, USA). T-cell Activation Marker Tuberculosis (TAM-TB) assay and Interferon
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51 216 Gamma Release Assay (IGRA; STANDARDTM F TB-Feron FIA (IFN-gamma; SD Biosensor,
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53 217 Republic of Korea), are performed on fresh venous blood. In Tanzania and Mozambique,
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55 218 storage of peripheral blood mononuclear cells for later characterization of the TB-specific
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57 219 immune response is also performed.
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3 221 Procedures for follow-up and unwell visits are similar to those at baseline. Measurement of
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5 222 HIV status, haemoglobin, HbA1c, spirometry, CD4 count and IGRA testing are not performed
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7 223 at follow-up visits, unless not done previously. At the last scheduled visit all HHCs not known
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9 224 to have HIV are re-offered HIV testing and a spontaneous or induced sputum sample is stored
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11 225 for all participants.
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16 227 ***Household contacts screening positive for TB symptoms and/or with a DCXR***
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18 228 ***suggestive of TB***

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20 229 HHCs screening positive for symptoms and/or those with DCXRs suggestive of TB are asked
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22 230 for a sputum sample, which is investigated using Xpert MTB/RIF Ultra (Cepheid). If this sample
23
24 231 is positive for *Mtb* (including a trace result), a minimum of two additional sputum samples are
25
26 232 investigated, following decontamination, with Xpert MTB/RIF Ultra, solid and liquid culture.
27
28 233 Isolates stored from these cultures will be sequenced for matching with the IC isolates in order
29
30 234 to verify intra-household transmission. Sputum induction is performed for those unable to
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32 235 provide a spontaneous sputum sample. HHCs with microbiologically confirmed TB are referred
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34 236 for TB treatment to the National TB Programme.
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39 238 ***Patient and public involvement***

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41 239 The ERASE-TB study sites have established Community Advisory Boards, which are voices
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43 240 of communities, people affected, and study participants, providing a strategic link between the
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45 241 communities and the study team. Community Advisory Boards meet regularly and provide
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47 242 feedback on design, procedures and conduct of the study. They will also be closely involved
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49 243 in the dissemination of study results. In addition to the Community Advisory Boards, each study
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51 244 site conducts community engagement activities focused on young people with the aim to foster
52
53 245 interest in science and research, specifically in the field of respiratory diseases/illness. This
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55 246 includes close partnership with schools and universities. Furthermore, planned qualitative
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57 247 research will specifically aim to understand the perceptions of HHCs with regards to TB
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59 248 diagnostics and screening.
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5 **250 Sample size**

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7 251 An estimated 800 to 900 TB-confirmed ICs are required for the subsequent enrollment of an
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9 252 anticipated 2,100 HHCs, i.e., 700 HHCs per country. Loss to follow-up of HHCs is estimated
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11 253 to be 10%. A total of 64 HHCs (3%) are estimated to be diagnosed with TB during the study
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13 254 period, based on previous active case finding studies among HHCs [16]. Validation for
14
15 255 subclinical and early TB will include incident (n=49) and co-prevalent TB cases (n=15).
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17 256 Validation for detection of incipient *M.tb* infection will include samples of participants with
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19 257 incident TB (n=49) matched 1:4 to samples of participants without TB (n=196). For tests
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21 258 diagnosing incipient *M.tb* infection sensitivities of 73% and 82% would be detected with a
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23 259 precision of 59-85% and 68-91% respectively. For specificities of 92% and 94% the confidence
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25 260 intervals would be 87-95% and 90-97%.

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30 **262 Novel test candidates**

31
32 263 A range of novel test candidates targeted at pathogen detection or identification of host
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34 264 responses to *Mtb* are being applied, either in real-time (for all participants) or retrospectively
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36 265 (in a case-control design). Whilst a number of novel test candidates have been pre-specified,
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38 266 the ERASE-TB biobanking processes allow for addition of further candidate tests to be
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40 267 evaluated on stored samples as they become available.

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45 269 DCXRs offer good sensitivity for diagnosis of pulmonary TB. However, high inter- and intra-
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47 270 investigator variability, and lack of trained interpreters present a barrier to implementation in
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49 271 many high-TB burden settings. Computer-aided interpretation systems, such as CAD4TB
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51 272 (Delft Imaging, Hertogenbosch, Netherlands) and qXR (Qure.ai, India) may increase image-
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53 273 reading capacity, with good performance, and serve, therefore, as a systematic screening tool
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55 274 to identify individuals in need of confirmatory TB tests [17,18].

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3 276 Xpert MTB/RIF Ultra is a nucleic acid amplification test for *Mtb* with a lower limit of detection
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5 277 compared to the previous Xpert MTB/RIF generation, and, therefore, conferring higher
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7 278 sensitivity in paucibacillary specimens. This, however, comes at the expense of specificity,
8
9 279 particularly in high TB incidence settings, resulting in 'false positives' [19]. WHO guidelines
10
11 280 recommend Xpert MTB/RIF Ultra for TB diagnosis among adults and children acknowledging
12
13 281 that further evaluation, particularly of the role of Xpert MTB/RIF Ultra for TB screening, is
14
15 282 needed [20,21].
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20 284 FLOW-TB is an advanced enzyme-linked immunosorbent assay for the detection of *Mtb*
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22 285 lipoarabinomannan (a mycobacterial cell wall component) in urinary specimens with results
23
24 286 available within 65 minutes [22].
25

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28 288 The T-cell activation marker-TB assay (TAM-TB) detects *Mtb*-specific CD4 T-cells through *in-*
29
30 289 *vitro* antigen stimulation with *Mtb*-derived peptides, i.e., from ESAT-6 and CFP-10, followed by
31
32 290 flow cytometry. TAM-TB discriminated latent *Mtb* infection from TB in freshly collected blood
33
34 291 with 83% sensitivity and 96-98% specificity in previous studies. Further, TAM-TB may detect
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36 292 early TB disease progression up to 9 months prior to the identification of *Mtb* in sputum [23–
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38 293 25].
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43 295 Multiple transcriptomic signatures, capturing the host response to TB, have been described as
44
45 296 promising candidate tests for earlier TB diagnosis (up to two years before microbiological
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47 297 diagnosis). An individual patient data meta-analysis suggested equivalent performance of
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49 298 eight signatures, with 25-40% sensitivity and 92-95% specificity 0-24 months before TB
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51 299 diagnosis. Diagnostic accuracy of each signature improved as the interval between testing and
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53 300 microbiological TB diagnosis shortened [26]. Several signatures have been developed into
54
55 301 polymerase chain reaction (PCR)-based assays to facilitate real-time implementation: the
56
57 302 recent CORTIS trial reported sensitivity of 48% and specificity of 75% for incipient TB for the
58
59 303 RISK-11 signature [27]. Cepheid have developed a 3-transcript TB score into a fully automated

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3 304 in-cartridge PCR assay performed on finger-prick blood using the Xpert platform (Xpert TB
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5 305 Host Response RUO Prototype cartridge). This cartridge will be evaluated using freshly
6
7 306 collected specimens in ERASE-TB; storage of RNA-stabilised blood samples also allows for
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9 307 retrospective evaluation of additional transcriptomic signatures in our cohort [28,29]
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13 309 An alternative approach to capture the host response to TB is through protein-based biomarker
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15 310 signatures. Candidate tests in this category include a serum- or plasma-based multiplex assay
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17 311 assessing 13 protein biomarkers (CRP, procalcitonin[30], sTREM-1[31,32], angiopoietin-
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19 312 2[33,34], interleukin-6[35], TRAIL[36] and IP-10[37]) that is being developed by the London
20
21 313 School of Hygiene and Tropical Medicine; in addition, a seven biomarker signature is under
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23 314 development as a point-of-care test for TB diagnosis, with 94% sensitivity and 73% specificity
24
25 315 detected in previous work [38].
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30 317 **Statistical analyses**

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32 318 Baseline characteristics and analytical data will be summarized using descriptive statistics
33
34 319 inclusive of mean, median, range, standard deviation, and absolute as well as relative
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36 320 frequencies depending on the nature of data. A logistic regression model will be used to identify
37
38 321 characteristics of TB among ICs, households and HHCs that are predictive of incident TB.
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40 322 From the study database, we will simulate algorithms of different tests to obtain the testing
41
42 323 combination with the best accuracy. We will couple tests with a mathematical model that
43
44 324 quantifies the risk of infection and/or disease to enhance predictive performance. The reporting
45
46 325 of the development of the prediction model will follow the Transparent Reporting of a
47
48 326 multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) Initiative [39].
49

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53 328 The validation of novel diagnostic tests for detecting TB will be analysed as a 1:4 matched
54
55 329 nested case-control study with HHCs diagnosed with TB at baseline and during follow-up
56
57 330 serving as cases, and HHCs who do not develop TB during follow-up as controls; controls will
58
59 331 be matched for site, age, sex, HIV status and other risk factors for developing TB. Sensitivity

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3 332 and specificity of novel tests will be determined using pre-existing positive/negative cut-offs
4
5 333 where these exist [40]; and receiver operating curves (ROC) constructed with area under the
6
7 334 ROC curve calculated. For tests aiming to identify individuals at high risk of TB in the future,
8
9 335 only HHCs who are diagnosed with TB during follow-up will serve as cases (i.e. those
10
11 336 diagnosed with TB at baseline will be excluded). Stored samples from all timepoints will be
12
13 337 retrieved and diagnostic accuracy (i.e. sensitivity and specificity) of the novel test determined
14
15 338 at different time-points before TB diagnosis. The decision of assigning the “active TB”
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17 339 endpoints to participants will be blinded from the new test results to avoid inclusion bias.
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21 341 **Data management**

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24 342 All source data will be kept confidential in secured locations with restricted access by
25
26 343 authorized personnel only inclusive of monitors, auditors and reviewers of ethical and
27
28 344 regulatory committees in line with applicable data privacy regulations. Each participant is
29
30 345 asked to consent to this handling of the data, and is assigned a pseudonymous identification
31
32 346 number that is used throughout the study on all source data.
33
34
35 347

36
37 348 Accurate documentation of paper-based and electronic source data, e.g., original records and
38
39 349 certified copies of original records, progress notes, screening logs, and recorded data from
40
41 350 automated instruments, will be maintained. The pseudonymized clinical data captured on
42
43 351 paper-based Case Report Forms will be entered at the sites into a database using the web-
44
45 352 based Clinical Data Management System of OpenClinica (OpenClinica LLC, Waltham, MA,
46
47 353 USA). The study specific database has been built, maintained and hosted by the LMU Klinikum
48
49 354 on a centralized secure server. Data modifications and necessary corrections performed in the
50
51 355 database also within the context of double data entry will be documented and tracked in audit
52
53 356 trails. Data quality and plausibility are assured by a series of pre-programmed edit and range
54
55 357 checks in OpenClinica. Further validation checks are programmed in Stata (Statacorp, College
56
57 358 Station, TX, USA) with extracts of the database and electronically received data, e.g.,
58
59 359 spirometry, dCXR and laboratory, will be integrated into analyses of datasets.
60

360

361 Monitoring

362 Assigned study monitors will visit the sites at regular intervals physically and/or virtually in
363 addition to frequent day-to-day communication. Close follow-up on all study-related aspects
364 will be performed to ascertain compliance with standards of Good Clinical Practice, the
365 Declaration of Helsinki, and other local and national regulatory guidelines inclusive of
366 guidelines for infection prevention and control of airborne-transmitted diseases, e.g., social
367 distancing in well-ventilated spaces, and wearing of personal protective equipment. In
368 particular, monitors that support designated study personnel are responsible to verify (I)
369 adequacy of the study personnels' qualifications and facilities, (II) accuracy of informed
370 consent procedures and patient eligibility, (III) adherence to the study protocol, (IV) protection
371 of rights and well-being of participants, (V) adherence to infection prevention and control
372 measures (VI) accuracy and completeness of study documents and other study-related
373 records, and (VII) maintenance of source documents.

374

375 Ethics and dissemination

376 The study protocol and informed consent/assent documents have been approved by regulatory
377 and ethical committees of the participating institutions [Medical Research Council in Zimbabwe
378 (MRCZ/A/2618), the National Health Research Ethics Committee in Tanzania (TMDA-
379 WEB0021/CTR/0004/03), the National Bioethics Committee for Health in Mozambique
380 (541/CNBS/21), and the ethical committees of London School of Hygiene & Tropical Medicine,
381 United Kingdom (22522-2), and the medical faculty of the Ludwig-Maximilians-Universität
382 München, Germany (20-0771)].

383

384 Adult ICs and HHCs are asked for written informed consent prior to their participation.
385 Underage HHCs are asked for assent in addition to obtaining the consent of their legal
386 guardians/parents; with ages for assent depending on local guidance. In case of illiteracy, the
387 participant is asked to give its consent by fingerprint while an adult impartial, literate witness

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3 388 present during the entire consent procedure signs the consent on behalf. All participants have
4
5 389 the right to withdraw from the study at any time. Findings derived from ERASE-TB will be
6
7 390 presented at scientific conferences, and published in peer-reviewed international journals.
8
9 391

10 391

11 392 **Current study status**

12
13 393 The recruitment of ICs and HHCs is in progress in Zimbabwe, Mozambique and Tanzania
14
15 394 since March, August and September 2021, respectively. The follow-up of HHCs is anticipated
16
17 395 to be completed in March, August and September 2023 in Zimbabwe, Mozambique and
18
19 396 Tanzania, respectively; laboratory analyses are estimated to be performed by December 2024.
20
21

22 397

23 398 **Author contributions**

24
25
26 399 The study proposal and protocol were written by NH, KK with scientific input from CK, TM, CG,
27
28 400 JM. ETM, UP, DB, AM wrote the initial manuscript with scientific input on the database and
29
30 401 data management section from FR, TA. NH, KK critically reviewed the initial draft of the
31
32 402 manuscript. KH, GK, CJC, ENN, TA, provided critical feedback on the manuscript. All authors
33
34 403 have read and approved the final version of the manuscript.
35
36

37 404

38 405 **Funding statement**

39
40
41 406 ERASE-TB is part of the EDCTP2 programme supported by the European Union (grant
42
43 407 number RIA2018D-2508-ERASE-TB), the German Center for Infection Research (DZIF) grant
44
45 408 number: 02.710 and the Swedish Research Council (220-23602). CJC is funded by the
46
47 409 Wellcome Trust (203905/Z/16/Z). Cepheid, Inc., and SD Biosensor provided test kits and
48
49 410 analyzers at no cost to the Consortium.
50
51

52 411

53 412 **Acknowledgments**

54
55
56 413 We are grateful to the study personnel from the Biomedical Research and Training Institute
57
58 414 and the Zvitambo Research Institute, Zimbabwe, the Instituto Nacional de Saúde,
59
60 415 Mozambique, and the National Institute for Medical Research - Mbeya Medical Research

1
2
3 416 Centre, Tanzania for their exceptional efforts and contributions, which made this research
4
5 417 possible.

6
7 418

8
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50
51 439 **Competing interests statement**

52
53 440 None declared.

54
55 441

56
57 442 **Disclaimer**

58
59 443 Not applicable.

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3 444 **References**
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3 584 **Figure legend**

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5 585 **Figure 1.** The ERASE-TB consortium

6
7 586 Abbreviations: ERASE-TB=Early Risk Assessment in TB contactS by new diagnostic tEsts

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11 588 **Figure 2.** Location and characteristics of ERASE-TB study sites

12
13 589 Notes: The location of each study site is indicated by a red asterix. Source data used within
14
15 590 this figure are taken from the references [7,8,42,43,9–15,41].

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18 591 Abbreviations: ERASE=Early Risk Assessment in TB contactS by new diagnostic tEsts;
19
20 592 TB=tuberculosis; HIV=human immunodeficiency virus

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24 594 **Figure 3.** Eligibility criteria and schedules of events for index cases and household contacts

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26 595 Notes: **A**=depending on the time point of study enrollment and consequently on the duration
27
28 596 available for follow-up, i.e. 18 or 24 months, the follow-up visit at 24 months ± 30 days may be

29
30 597 conditional; **B**=the follow-up visit by phone may be conducted after the last scheduled follow-
31
32 598 up visit at 18 months ± 30 days or 24 months ± 30 days to assess whether symptoms suggestive

33
34 599 of TB have occurred, TB diagnosis has been made or anti-TB treatment has been initiated;

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36 600 **C**=unwell visits by phone or on-site may be conducted between scheduled follow-up visits if a
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38 601 participant presents at a recruitment healthcare facility with signs and symptoms suggestive of

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40 602 TB; **D**=coached spontaneous or induced sputum collection for storage at scheduled follow-up
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42 603 visit at 18 months ± 30 days or 24 months ± 30 days, and for repetition of HIV testing if tested

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44 604 negative at baseline; **E**=coached spontaneous or induced sputum collection upon the decision
45
46 605 of the investigating team for testing by Xpert MTB/RIF Ultra if participant presents with signs

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48 606 and symptoms suggestive of TB; **F**=coached spontaneous or induced sputum collection in
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50 607 case of Xpert MTB/RIF Ultra positivity or strong clinical suspicion of TB for repetition of the

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52 608 Xpert MTB/RIF Ultra; **G**=in case of HIV positivity to be followed by the assessment of CD4
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54 609 counts; **H**=CXR to be conducted at an unscheduled on-site unwell visit upon the decision of

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56 610 the investigating team depending on the nature of symptoms reported, and the time elapsed
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58 611 since the last CXR including its findings; **I**=not to be conducted among pregnant women;

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3 612 **J**=stored venous blood includes 6mL EDTA blood for whole blood and plasma, 4mL serum
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5 613 and 2.5mL PAXgene blood, all samples will be deep frozen for retrospective testing using new
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7 614 diagnostics as described in text; **K**=in case the evaluation of symptoms of a participant unable
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9 615 to present at a recruitment healthcare facility is required an unscheduled on-site or home visit
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11 616 will be arranged by phone, the resolution of symptoms can alternatively be addressed by
12
13 617 phone; **L**=collection of PBMC at baseline and follow-up visit at 6 months \pm 30 days is optional,
14
15 618 thus will not be performed at each participating site and for each participant; **M**=in case the
16
17 619 evaluation of symptoms of a participant unable to present at a recruitment healthcare facility
18
19 620 is required or doubtful if required an unscheduled unwell visit by phone will be arranged, the
20
21 621 resolution of symptoms can alternatively be addressed by phone; **N**=spirometry and/or
22
23 622 diabetes (HbA1c) will be performed at scheduled follow-up visits at 6 months \pm 30 days, 12
24
25 623 months \pm 30 days and 18 or 24 months \pm 30 days if required or not performed at baseline,
26
27 624 anaemia (Hb) will be performed at baseline and scheduled follow-up visits at 6 months \pm 30
28
29 625 days, 12 months \pm 30 days and 18 or 24 months \pm 30 days if possible; **O**=blood pressure
30
31 626 measurement will be performed at baseline and scheduled follow-up visits at 6 months \pm 30
32
33 627 days, 12 months \pm 30 days and 18 or 24 months \pm 30 days; **P**=WGS to be performed once *Mtb*
34
35 628 infection is confirmed and an isolate could be recovered.

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38
39 629 Abbreviations: TB=tuberculosis; WHO=World Health Organization; HIV=human
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41 630 immunodeficiency virus; WGS=whole genome sequencing; MBLA=molecular bacterial load
42
43 631 assay; CXR=chest radiograph; IGRA=interferon gamma release assay; PBMC=peripheral
44
45 632 blood mononuclear cell; TAM-TB=T- cell activation marker tuberculosis; MTB=*Mycobacterium*
46
47 633 *tuberculosis*; RIF=rifampicin, Hb=haemoglobin; HbA1c=glycated hemoglobin;
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49 634 LAM=lioparabinomannan; CD4=cluster of differentiation 4

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51 635

52 636 **Figure 4.** Study design

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54
55 637 Abbreviations: HHC=household contact; TB=tuberculosis; IC=index case; WHO=World
56
57 638 Health Organization; CXR= chest radiograph; SS=symptom score; MTB=*Mycobacterium*
58
59 639 *tuberculosis*; RIF=rifampicin; pos=positive; CT=computer tomography; FU=follow-up

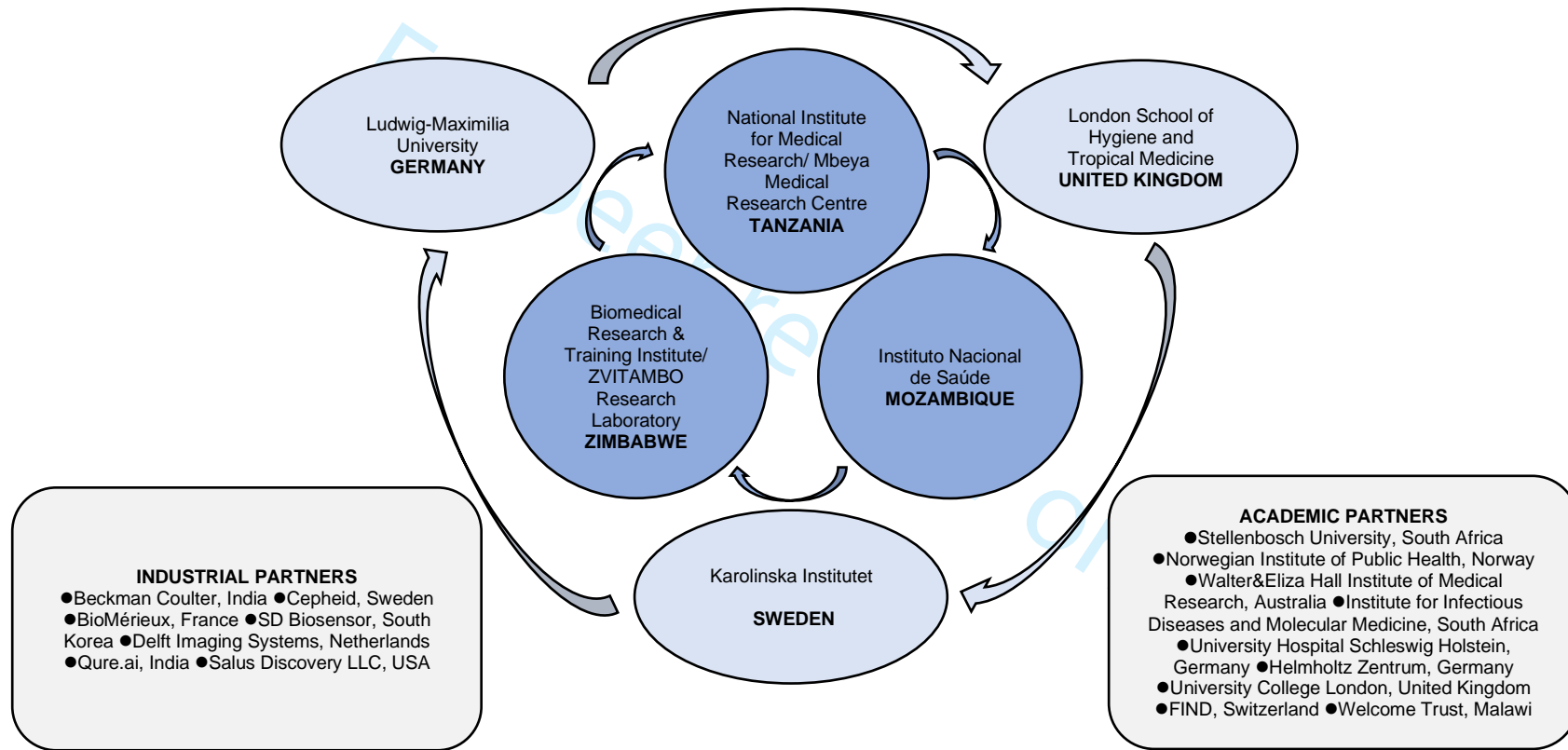


Figure 1. The ERASE-TB consortium

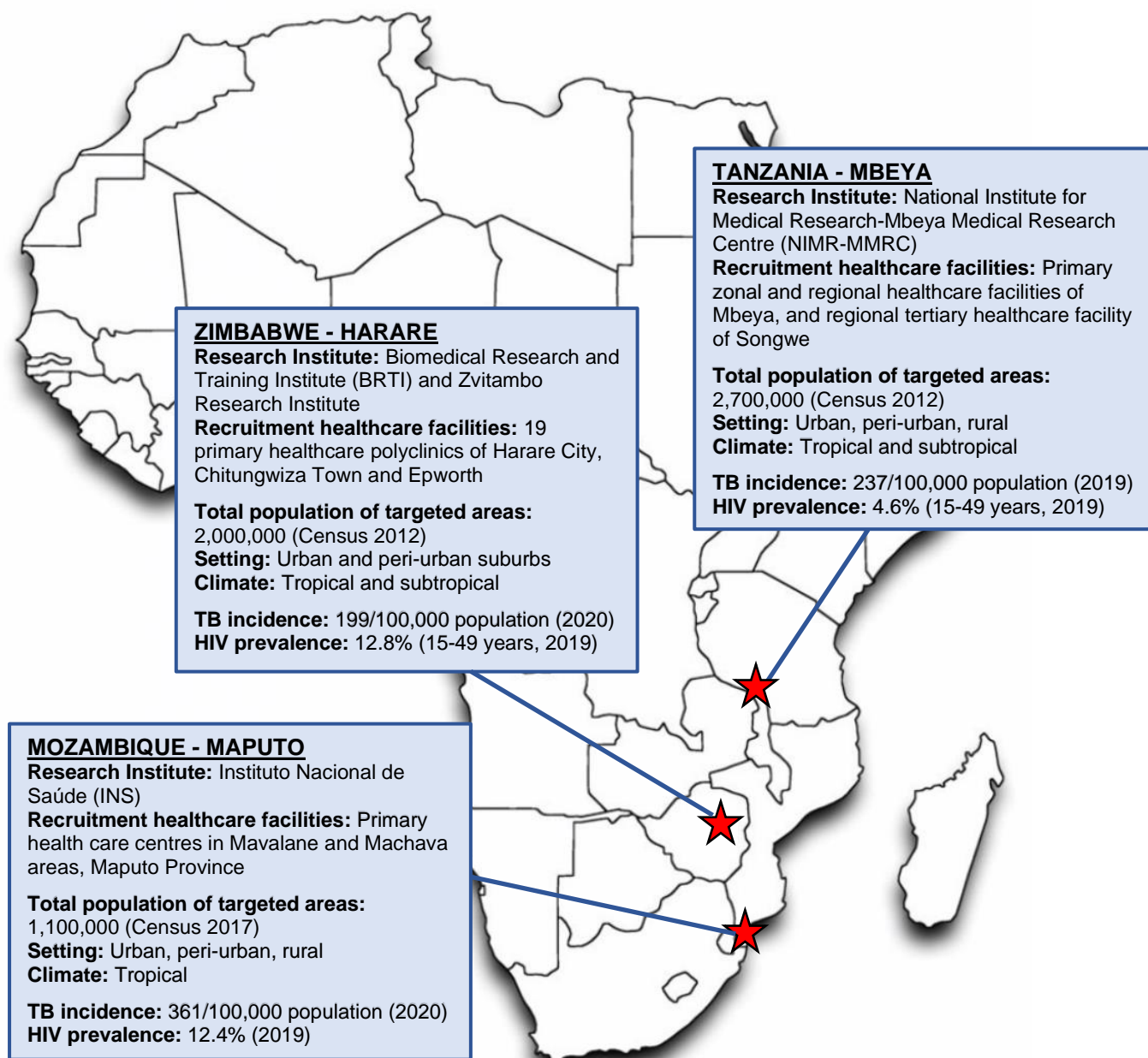


Figure 2. Location and characteristics of ERASE-TB study sites

INDEX CASE

HOUSEHOLD CONTACT

INCLUSION CRITERIA

- Aged ≥18 years
- Living with at least another person aged ≥10 years in the same household with a firm unchanged address for 6 months
- Pulmonary TB diagnosis within 4 weeks prior to enrolment
- Spontaneous sputum production
- Positive sputum smear, i.e. >+2 positivity by Ziehl-Neelsen stain or Auramine-O stain OR ≥+1 positivity on WHO symptom scale OR ≥medium positivity by GeneXpert (if no microscopy performed)
- <7 daily doses of anti-TB treatment since diagnosis
- Voluntary written informed consent obtained

EXCLUSION CRITERIA

- Treatment for TB within the past 30 days OR on current TB treatment OR on current preventive TB therapy (if HIV negative) at the time of enrolment



INCLUSION CRITERIA

- Aged ≥10 years
- Substantial exposure, i.e. spending ≥3 nights/week over the past 4 weeks in the same household as the index case,
- Voluntary written informed consent or parent/guardian consent & assent in case of minors obtained

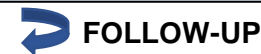


EVENTS AT BASELINE/SCREENING VISIT

- Administration of socio-demographic questionnaire
 - Assessment of vital signs, medical history
 - Digital CXR (if available)^I
- Specimen investigations**
- Sputum: culture, storage, WGS, MBLA

EVENTS AT BASELINE/ ±14days of SCREENING VISIT

- Administration of socio-demographic questionnaire
 - Assessment vital signs, medical history
 - Screening for diabetes (HbA1c)^N, high blood pressure^O, anaemia (Hb)^N, WHO TB symptoms
 - Digital CXR^I, spirometry^N
- Specimen investigations**
- Sputum: Xpert MTB/RIF Ultra^E, culture^F, storage, WGS^P
 - Blood: HIV/CD4 counts^G, Cepheid host response, TAM-TB, IGRA SD Biosensor, PBMC^L, storage^J
 - Urine: dipstick/LAM, storage



EVENTS AT UNSCHEDULED UNWELL VISIT(S)
(phone/on-site)^{C,K,M}

- Assessment of vital signs, medical history
 - Screening for high blood pressure, WHO TB symptoms,
 - Digital CRX^{H,I}
- Specimen investigations**
- Sputum: Xpert MTB/RIF Ultra, culture, storage, WGS^P
 - Blood: Cepheid host response, TAM-TB, storage^J
 - Urine: dipstick/LAM, storage

EVENTS AT UN-SCHEDULED FOLLOW-UP VISIT(S)^B (phone)

- Assessment of medical history
 - Screening for WHO TB symptoms
- Specimen investigations**
- None

EVENTS AT 6months ±30days/ 12months ±30days/ 18months ±30days/ 24months ±30days^A FOLLOW-UP VISITS

- Assessment of vital signs, medical history
 - Screening for high blood pressure, WHO TB symptoms
 - Digital CXR^I, spirometry^N
- Specimen investigations**
- Sputum: Xpert MTB/RIF Ultra^E, culture^F, storage^D, WGS^P
 - Blood: Cepheid host response, TAM-TB, PBMC^L, storage
 - Urine: dipstick/LAM, storage^J



Optional

Optional

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Figure 3. Eligibility criteria and schedules of events for index cases and household contacts

For peer review only

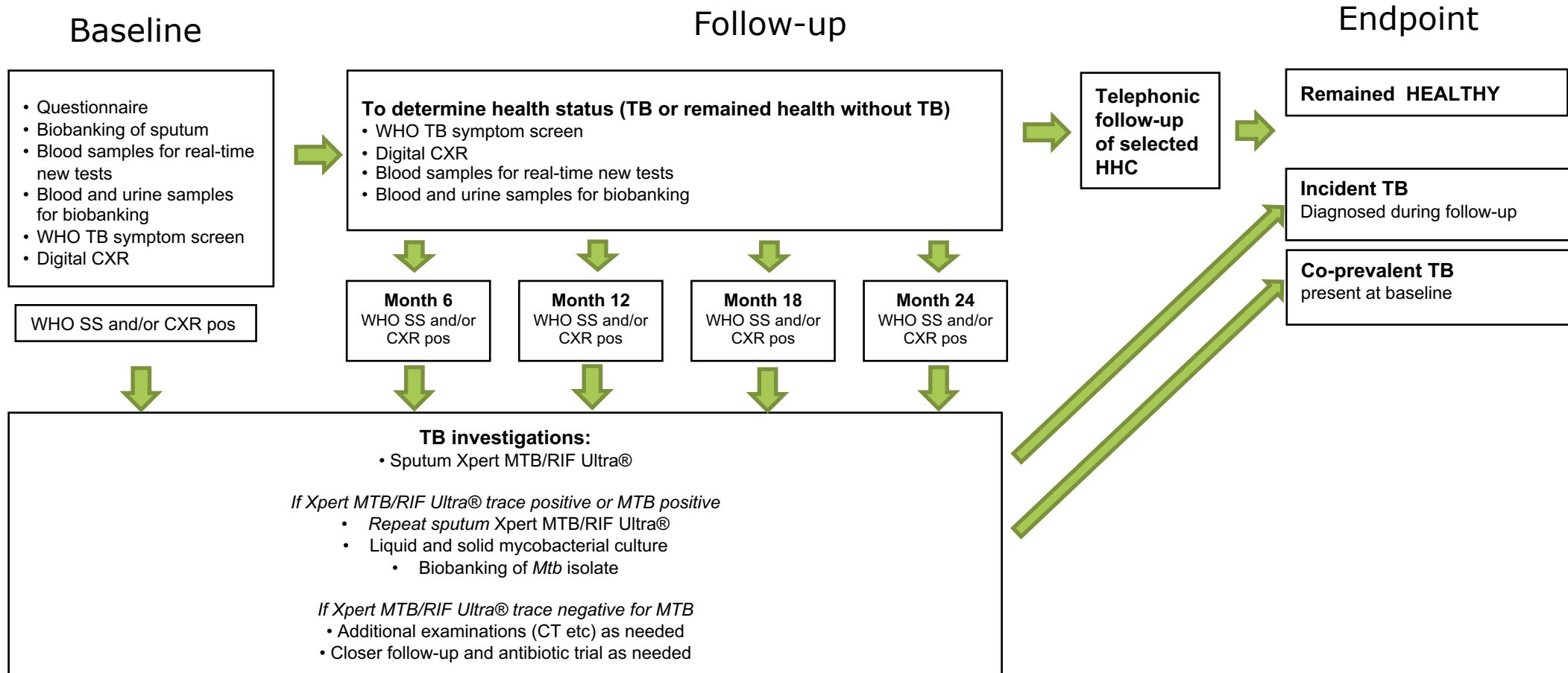


Figure 4. Study design.

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2-3
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	5-6
	4	Study objectives and hypotheses	6
METHODS			
<i>Study design</i>	5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	6-7
<i>Participants</i>	6	Eligibility criteria	7 (Figure 3)
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	7 (Figure 3)
	8	Where and when potentially eligible participants were identified (setting, location and dates)	7
	9	Whether participants formed a consecutive, random or convenience series	7, 11
<i>Test methods</i>	10a	Index test, in sufficient detail to allow replication	8-10, 11-13
	10b	Reference standard, in sufficient detail to allow replication	8-10, 11-13
	11	Rationale for choosing the reference standard (if alternatives exist)	8-10, 11-13
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	13-14
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	13-14
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	13-14
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	13-14
<i>Analysis</i>	14	Methods for estimating or comparing measures of diagnostic accuracy	13-14
	15	How indeterminate index test or reference standard results were handled	13-14
	16	How missing data on the index test and reference standard were handled	14
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	13-14
	18	Intended sample size and how it was determined	11
RESULTS			
<i>Participants</i>	19	Flow of participants, using a diagram	8-10 (Figure 3)
	20	Baseline demographic and clinical characteristics of participants	8-10 (Figure 3)
	21a	Distribution of severity of disease in those with the target condition	
	21b	Distribution of alternative diagnoses in those without the target condition	
	22	Time interval and any clinical interventions between index test and reference standard	
<i>Test results</i>	23	Cross-tabulation of the index test results (or their distribution) by the results of the reference standard	
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	13-14
	25	Any adverse events from performing the index test or the reference standard	
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	3
	27	Implications for practice, including the intended use and clinical role of the index test	5-6
OTHER INFORMATION			
	28	Registration number and name of registry	2
	29	Where the full study protocol can be accessed	
	30	Sources of funding and other support; role of funders	16

STARD 2015

AIM

STARD stands for “Standards for Reporting Diagnostic accuracy studies”. This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition**. This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test**. A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross-tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross-tabulation (sometimes referred to as the contingency or “2x2” table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on <http://www.equator-network.org/reporting-guidelines/stard>.



BMJ Open

Early risk assessment in pediatric and adult household contacts of confirmed tuberculosis cases by novel diagnostic tests (ERASE-TB): protocol for a prospective, non-interventional, longitudinal, multi-country cohort study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-060985.R2
Article Type:	Protocol
Date Submitted by the Author:	23-Jun-2022
Complete List of Authors:	Marambire, Edson; Biomedical Research and Training Institute, Banze, Denise; Instituto Nacional de Saúde Mfinanga, Alfred; Mbeya Medical Research Centre Mutsvangwa, Junior; Biomedical Research and Training Institute Mbunda, Theodora ; NIMR-Mbeya Medical Research Programme Ntinginya, Nyanda; 6. National Institute of Medical Research-Mbeya Medical Research Centre Celso, Khosa; Instituto Nacional de Saúde Kallenius, Gunilla; Karolinska Institutet Calderwood, Claire J.; London School of Hygiene and Tropical Medicine Faculty of Infectious and Tropical Diseases Geldmacher, Christof; University Hospital, Division of Infectious Diseases and Tropical Medicine, LMU Munich ; German Center for Infection Research, Partner site Munich Held, Kathrin; University Hospital, Division of Infectious and Tropical Medicine, LMU Munich ; German Center for Infection Research, Partner site Munich Appalarowthu, Tejaswi; University Hospital, Division of Infectious Diseases and Tropical Medicine, LMU Munich; German Center for Infection Research, Partner site Munich Rieß, Friedrich; University Hospital, Division of Infectious Diseases and Tropical Medicine, LMU Munich; German Center for Infection Research, Partner site Munich Panzner, Ursula; University Hospital, Division of Infectious Diseases and Tropical Medicine, LMU Munich; German Center for Infection Research, Partner site Munich Heinrich, Norbert; University Hospital, Division of Infectious Diseases and Tropical Medicine, LMU Munich; German Center for Infection Research, Partner site Munich Kranzer, Katharina; London School of Hygiene and Tropical Medicine Faculty of Infectious and Tropical Diseases; Biomedical Research and Training Institute
Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Diagnostics, Infectious diseases

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Keywords:	Tuberculosis < INFECTIOUS DISEASES, Diagnostic microbiology < INFECTIOUS DISEASES, RESPIRATORY MEDICINE (see Thoracic Medicine)

SCHOLARONE™
Manuscripts

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3 1 **Early risk assessment in pediatric and adult household contacts of confirmed**
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5 2 **tuberculosis cases by novel diagnostic tests (ERASE-TB): protocol for a**
6
7 3 **prospective, non-interventional, longitudinal, multi-country cohort study**
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9 4

10 5 Edson Tawanda Marambire^{1*}, Denise Banze^{2§}, Alfred Mfinanga^{3§}, Junior Mutsvangwa¹, Theodora
11 6 Mbunda³, Elias N. Nyanda³, Khosa Celso², Gunilla Kallenius⁴, Claire J. Calderwood⁵, Christof
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13 8 Heinrich^{6,7#}, Katharina Kranzer^{1,5,6#}, on behalf of the ERASE-TB Consortium
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23 § Contributed equally

24 # Contributed equally
25

26 Word count: 3,668
27

28 **Keywords:** *Mycobacterium tuberculosis*, diagnostics, cohort study, household contacts, WHO
29 END TB strategy, ERASE-TB
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30 **ABSTRACT**

31 **Introduction**

32 The World Health Organization (WHO) End-TB Strategy calls for the development of novel
33 diagnostics to detect tuberculosis earlier and more accurately. Better diagnostics, together with
34 tools to predict disease progression are critical for achieving WHO END-TB targets. The Early
35 Risk Assessment in TB contactS by new diagnostic tEsts (ERASE-TB) study aims to evaluate
36 novel diagnostics and testing algorithms for early tuberculosis diagnosis and accurate
37 prediction of disease progression among household contacts exposed to confirmed index
38 cases in Mozambique, Tanzania and Zimbabwe.

40 **Methods and analysis**

41 A total of 2,100 household contacts (HHCs) (aged ≥ 10 years) of adults with microbiologically-
42 confirmed pulmonary tuberculosis will be recruited and followed up at 6-month intervals for 18
43 to 24 months. At each time-point a WHO symptom screen and digital chest-radiograph (dCXR)
44 will be performed, and blood and urine samples collected. Individuals screening positive (WHO
45 symptom screen or dCXR) will be requested to provide sputum for Xpert MTB/Rif Ultra. At
46 baseline, HHCs will also be screened for HIV, diabetes (HbA1c), chronic lung disease
47 (spirometry), hypertension and anaemia. Study outcomes will be co-prevalent tuberculosis
48 (diagnosed at enrollment), incident tuberculosis (diagnosed during follow-up) or no
49 tuberculosis at completion of follow up. Novel diagnostics will be validated using fresh and
50 biobanked samples with a nested case control design. Cases are defined as HHCs diagnosed
51 with tuberculosis (for early diagnosis) or with incident tuberculosis (for prediction of
52 progression) and will be matched by age, sex and country to HHCs who remain healthy
53 (controls). Statistical analyses will include assessment of diagnostic accuracy by constructing
54 receiver operating curves and calculation of sensitivity and specificity.

56 **Ethics and dissemination**

1
2
3 57 ERASE-TB has been approved by regulatory and ethical committees in each African country
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5 58 and by each partner organisation. Consent, with additional assent for participants <18 years,
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7 59 is voluntary. Attestation by impartial witnesses is sought in case of illiteracy. Confidentiality of
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9 60 participants is being maintained throughout. Study findings will be presented at scientific
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11 61 conferences and published in peer-reviewed international journals.
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16 63 **Study registration number**

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65 **Strengths and limitations of this study**

- 66 • Recruitment of highly infectious index cases aimed at maximising the number of
67 tuberculosis (TB) diagnoses in the household contact (HHCs) cohort.
- 68 • Sequencing of *Mycobacterium tuberculosis* isolates from both index cases and HHCs
69 allows confirmation of household transmission and thus determination of timing of the
70 transmission event; resulting in more precise estimates of new test sensitivity
71 compared to population-based cohorts with unknown timing of infection.
- 72 • Large sample size across three southern African countries with high HIV prevalence;
73 including adolescents will ensure study findings are generalisable to the clinically
74 relevant population at high risk of TB compared to studies focused on adults only.
- 75 • Despite the large cohort of HHCs, the number of diagnosed TB cases will be small,
76 limiting the power of the study and sub-group analyses such as by age and HIV status.
- 77 • Geographically limited to sub-Saharan Africa, therefore results may not be
78 generalisable to other populations, including those with lower HIV prevalence such as
79 in South-East Asia or the Americas.

80 INTRODUCTION

81 Tuberculosis (TB) remains a leading global public health problem, with an estimated 10 million
82 new cases and 1.5 million deaths globally in 2020 [1]. In 2014, the World Health Assembly
83 approved the World Health Organization (WHO) End-TB Strategy, aiming for a 90% reduction
84 in TB incidence and 95% reduction in TB deaths by 2035 [2]. However, in 2019, three million
85 TB cases ('the missing millions') remained undiagnosed and untreated globally, resulting in
86 potentially avoidable morbidity, mortality and onward transmission. The Covid-19 pandemic
87 has resulted in a large decrease in the number of people newly diagnosed with TB and
88 reported. This has increased the diagnostic gap by a further 1.3 million, resulting in an
89 estimated 4.2 million undiagnosed TB cases in 2020 [3]. Also, for the first time in a decade TB
90 deaths have risen, from an estimated 1.4 million in 2019 to 1.5 million in 2020, as a result of
91 reduced access to and provision of essential TB services including diagnostics during the
92 Covid-19 pandemic.

93
94 Without an efficacious and safe vaccine, early detection and containment are the main tools
95 to interrupt transmission and successfully control TB. Similar to SARS-CoV2, asymptomatic
96 spreading of *M.tuberculosis* and subclinical but infectious disease states are a major concern
97 in the control of airborne infectious diseases [4]. Early and accurate identification of persons
98 with TB, combined with identification of those at risk of progression to TB and provision of
99 targeted preventive treatment are critical to reducing TB-associated morbidity and mortality,
100 and preventing onward transmission.

101
102 Currently available diagnostics such as sputum microscopy, mycobacterial culture and nucleic
103 acid amplification tests are based on direct pathogen detection, thus requiring a high
104 mycobacterial load; they therefore predominately target advanced TB when onward
105 transmission and significant lung damage has occurred [5,6]. Further, for many patients with
106 minimal or no symptoms, expectoration of high-quality sputum specimens remains

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3 107 challenging, limiting the accuracy of sputum-based tests. The same holds true for young
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5 108 children and people living with HIV.
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9 110 The **Early Risk Assessment in TB Contacts by new diagnostic tests** (ERASE-TB) study aims
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11 111 to fill this diagnostic gap by evaluating new sputum and non-sputum-based TB diagnostics for
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13 112 early TB detection (before onward transmission occurs), as well as tools for more accurate
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15 113 prediction of TB progression to allow for targeted preventive therapy.
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19 20 115 **METHODS AND ANALYSES**

21 22 116 **Study objectives**

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24 117 ERASE-TB's primary objectives are (I) to determine the sensitivity and specificity of novel
25
26 118 diagnostics to detect TB, in particular asymptomatic or minimally symptomatic TB; (II) to
27
28 119 evaluate novel diagnostics for detection of likely TB progression; and (III) to enhance the
29
30 120 performance of novel diagnostics by simulating testing algorithms coupled with individual risk
31
32 121 estimates from a mathematical model. The secondary objectives are (I) to determine the TB
33
34 122 prevalence among household contacts (HHCs) of infectious TB index cases (ICs) at baseline
35
36 123 and during a 18-24 months follow-up; (II) to establish a biorepository of cryopreserved
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38 124 specimens from HHCs for future development and validation of diagnostic tests; and (III) to
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40 125 assess the association of selected chronic disease conditions and TB among HHCs.
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44 45 127 **Study endpoints**

46
47 128 The study's primary endpoints are the presence or development of TB among HHCs with the
48
49 129 following possible scenarios of (I) prevalent symptomatic TB at baseline, (II) incident TB during
50
51 130 follow-up, and (III) remained healthy until study completion. An endpoint review committee will
52
53 131 review the data and case classification before finalization.
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58 133 Through the sequencing of *Mycobacterium tuberculosis* (*Mtb*) isolates, cases of co-prevalent
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60 134 or incident TB will be classified either as secondary, infected by the source case – the timepoint

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3 135 of infection will be known; or as infected by another, unknown source of infection, with an
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5 136 unknown timepoint of infection.
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8 9 138 **Recruitment sites**

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11 139 Recruitment of ICs and HHCs at selected primary healthcare facilities and communities has
12
13 140 commenced in Harare, Zimbabwe in March 2021, Maputo, Mozambique in August 2021, and
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15 141 Mbeya, Tanzania in September 2021. Partners of the ERASE-TB consortium are illustrated in
16
17 142 Figure 1. All three countries have a high TB incidence ranging from 100 to 499/100,000
18
19 143 population [1] and HIV prevalence among adults aged 15 years and older of 5% to 20% [7].
20
21 144 The African research institutions have established collaborations with their respective National
22
23 145 Tuberculosis Programs ensuring referral and appropriate follow-up of TB patients. Figure 2
24
25 146 illustrates the geographic location of research institutions, healthcare facilities where
26
27 147 recruitment is taking place, demographic characteristics of study populations, and estimates
28
29 148 on TB incidence and HIV prevalence [8–15].
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32 149

33 34 150 **Study design**

35
36 151 ERASE-TB is a non-interventional, longitudinal, prospective cohort study among HHCs aged
37
38 152 ≥ 10 years exposed to highly infectious pulmonary TB ICs aged ≥ 18 years. Eligibility criteria
39
40 153 are detailed in Figure 3 and the study design is shown in Figure 4. TB ICs are eligible if the
41
42 154 bacterial load in their sputum is at least at the “medium” level according to Xpert MTB/RIF or
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44 155 Xpert MTB/RIF Ultra, and they have received less than seven daily doses of anti-TB treatment
45
46 156 before enrollment. This maximises the likelihood of culturing and storing *Mtb* isolates. The total
47
48 157 study duration will be 36 months. This includes 12-months enrollment of ICs and HHCs, and
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50 158 18- to 24-months follow-ups of HHCs. Follow-up ends when a HHCs withdraws from the study,
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52 159 is lost to follow-up, dies, or is diagnosed with TB and referred for treatment. Scheduled or
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54 160 unscheduled unwell visits can be conducted physically and/or telephonically in case of
55
56 161 abnormal finding e.g. by abnormal dCXR, or when a participant feels unwell inbetween
57
58 162 scheduled follow-up visits.
59
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163

164 Procedures**165 TB index cases**

166 Following informed consent obtained, a questionnaire is administered to collect socio-
167 demographic information, TB risk factors, and the medical history of TB, HIV and other
168 diseases. Two spontaneous sputum samples are obtained, of which one is for mycobacterial
169 culturing and one for storage for performing retrospectively Molecular Bacterial Load Assay
170 (MBLA) to quantify viable *Mtb* by 16S rRNA [6]; an alternative means to quantify expectorated
171 bacterial load for an estimate of infectiousness. Both liquid and solid mycobacterial cultures
172 are performed on decontaminated sputum samples, with all *Mtb* isolates stored at -80 degrees
173 for future DNA extraction and whole genome sequencing. A questionnaire on symptom
174 duration and TB risk factors is also administered.

175

176 Household key informant

177 At baseline, a household key informant (either the TB index case or one of the household
178 contacts) is identified and asked to answer questions of a household questionnaire that collects
179 socio-economic elements like structure of the house or flat, income and household assets, and
180 covariates possibly associated with risk of TB infection, e.g., windows/air exchange, presence
181 of comorbid conditions, and risk factors like the source of cooking energy, and properties of
182 the household kitchen.

183

184 Household contacts

185 Informed consent is obtained from all eligible adult HHCs. For HHC <18 years of age, the
186 guardian is asked to provide informed consent, with assent also sought from children
187 dependent on local guidance. At baseline a questionnaire is administered collecting
188 information on socioeconomic and demographic characteristics, past medical history of TB,
189 HIV and other diseases, exposure risk factors, smoking and alcohol history. The physical
190 examination includes height, weight, mid-upper arm circumference and blood pressure

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3 191 measurement. In addition, all HHCs are offered free HIV testing according to the National
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5 192 Guidelines. All people with confirmed HIV infection will have CD4 counts performed and be
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7 193 referred for TB preventive therapy. Those not yet on antiretroviral therapy (ART) and those
8
9 194 who interrupted ART are referred for ART at local services.
10

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13 196 Point of care HbA1c (A1cCare, SD Biosensor, Gyeonggi-do, Republic of Korea) and
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15 197 haemoglobin (Hemocue 301+, Hemocue, Angelholm, Sweden) tests and spirometry (including
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17 198 pre- and post-bronchodilation with inhaled salbutamol) are performed at baseline or the six-
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19 199 month visit. HHCs who did not take up HIV testing or other screening at baseline are offered
20
21 200 these tests at each study visit. Any HHCs with test results requiring treatment or further
22
23 201 investigations are referred for respective services.
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27 203 HHCs are screened for TB using the WHO symptom questionnaire and a digital chest-
28
29 204 radiograph (dCXR), reviewed by a clinical officer. dCXRs are not performed in pregnant HHCs.
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31 205 HHCs with a positive WHO symptom screening and abnormal dCXR are asked to provide
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33 206 sputum samples for TB investigations i.e., for GeneXpert and mycobacterial culture. Those
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35 207 with negative symptom screen and normal dCXR are asked to provide a spontaneous sputum
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37 208 sample for storage (with sputum induction performed if required).
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41 210 At baseline, urine, serum, plasma, whole blood (native, and with RNA preservation in
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43 211 PAXgene® tubes [BD Biosciences, NJ, USA]) are stored. A finger-prick sample is taken and
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45 212 investigated using the Xpert TB Host Response RUO Prototype cartridge (Cepheid,
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47 213 Sunnyvale, CA, USA). T-cell Activation Marker Tuberculosis (TAM-TB) assay and Interferon
48
49 214 Gamma Release Assay (IGRA; STANDARDTM F TB-Feron FIA (IFN-gamma; SD Biosensor,
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51 215 Republic of Korea), are performed on fresh venous blood. In Tanzania and Mozambique,
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53 216 storage of peripheral blood mononuclear cells for later characterization of the TB-specific
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55 217 immune response is also performed.
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3 219 Procedures for follow-up and unwell visits are similar to those at baseline. Measurement of
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5 220 HIV status, haemoglobin, HbA1c, spirometry, CD4 count and IGRA testing are not performed
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7 221 at follow-up visits, unless not done previously. At the last scheduled visit all HHCs not known
8
9 222 to have HIV are re-offered HIV testing and a spontaneous or induced sputum sample is stored
10
11 223 for all participants.
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13 224

15 225 ***Household contacts screening positive for TB symptoms and/or with a DCXR***
16
17 226 ***suggestive of TB***

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20 227 HHCs screening positive for symptoms and/or those with DCXRs suggestive of TB are asked
21
22 228 for a sputum sample, which is investigated using Xpert MTB/RIF Ultra (Cepheid). If this sample
23
24 229 is positive for *Mtb* (including a trace result), a minimum of two additional sputum samples are
25
26 230 investigated, following decontamination, with Xpert MTB/RIF Ultra, solid and liquid culture.
27
28 231 Isolates stored from these cultures will be sequenced for matching with the IC isolates in order
29
30 232 to verify intra-household transmission. Sputum induction is performed for those unable to
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32 233 provide a spontaneous sputum sample. HHCs with microbiologically confirmed TB are referred
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34 234 for TB treatment to the National TB Programme.
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39 236 ***Patient and public involvement***

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41 237 The ERASE-TB study sites have established Community Advisory Boards, which are voices
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43 238 of communities, people affected, and study participants, providing a strategic link between the
44
45 239 communities and the study team. Community Advisory Boards meet regularly and provide
46
47 240 feedback on design, procedures and conduct of the study. They will also be closely involved
48
49 241 in the dissemination of study results. In addition to the Community Advisory Boards, each study
50
51 242 site conducts community engagement activities focused on young people with the aim to foster
52
53 243 interest in science and research, specifically in the field of respiratory diseases/illness. This
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55 244 includes close partnership with schools and universities. Furthermore, planned qualitative
56
57 245 research will specifically aim to understand the perceptions of HHCs with regards to TB
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59 246 diagnostics and screening.
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5 248 **Sample size**

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7 249 An estimated 800 to 900 TB-confirmed ICs are required for the subsequent enrollment of an
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9 250 anticipated 2,100 HHCs, i.e., 700 HHCs per country. Loss to follow-up of HHCs is estimated
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11 251 to be 10%. A total of 64 HHCs (3%) are estimated to be diagnosed with TB during the study
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13 252 period, based on previous active case finding studies among HHCs [16]. Validation for
14
15 253 subclinical and early TB will include incident (n=49) and co-prevalent TB cases (n=15).
16
17 254 Validation for detection of incipient *M.tb* infection will include samples of participants with
18
19 255 incident TB (n=49) matched 1:4 to samples of participants without TB (n=196). For tests
20
21 256 diagnosing incipient *M.tb* infection sensitivities of 73% and 82% would be detected with a
22
23 257 precision of 59-85% and 68-91% respectively. For specificities of 92% and 94% the confidence
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25 258 intervals would be 87-95% and 90-97%.

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30 260 **Novel test candidates**

31
32 261 A range of novel test candidates targeted at pathogen detection or identification of host
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34 262 responses to *Mtb* are being applied, either in real-time (for all participants) or retrospectively
35
36 263 (in a case-control design). Whilst a number of novel test candidates have been pre-specified,
37
38 264 the ERASE-TB biobanking processes allow for addition of further candidate tests to be
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40 265 evaluated on stored samples as they become available.

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45 267 DCXRs offer good sensitivity for diagnosis of pulmonary TB. However, high inter- and intra-
46
47 268 investigator variability, and lack of trained interpreters present a barrier to implementation in
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49 269 many high-TB burden settings. Computer-aided interpretation systems, such as CAD4TB
50
51 270 (Delft Imaging, Hertogenbosch, Netherlands) and qXR (Qure.ai, India) may increase image-
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53 271 reading capacity, with good performance, and serve, therefore, as a systematic screening tool
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55 272 to identify individuals in need of confirmatory TB tests [17,18].

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3 274 Xpert MTB/RIF Ultra is a nucleic acid amplification test for *Mtb* with a lower limit of detection
4
5 275 compared to the previous Xpert MTB/RIF generation, and, therefore, conferring higher
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7 276 sensitivity in paucibacillary specimens. This, however, comes at the expense of specificity,
8
9 277 particularly in high TB incidence settings, resulting in 'false positives' [19]. WHO guidelines
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11 278 recommend Xpert MTB/RIF Ultra for TB diagnosis among adults and children acknowledging
12
13 279 that further evaluation, particularly of the role of Xpert MTB/RIF Ultra for TB screening, is
14
15 280 needed [20,21].
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19
20 282 FLOW-TB is an advanced enzyme-linked immunosorbent assay for the detection of *Mtb*
21
22 283 lipoarabinomannan (a mycobacterial cell wall component) in urinary specimens with results
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24 284 available within 65 minutes [22].
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27
28 286 The T-cell activation marker-TB assay (TAM-TB) detects *Mtb*-specific CD4 T-cells through *in-*
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30 287 *vitro* antigen stimulation with *Mtb*-derived peptides, i.e., from ESAT-6 and CFP-10, followed by
31
32 288 flow cytometry. TAM-TB discriminated latent *Mtb* infection from TB in freshly collected blood
33
34 289 with 83% sensitivity and 96-98% specificity in previous studies. Further, TAM-TB may detect
35
36 290 early TB disease progression up to 9 months prior to the identification of *Mtb* in sputum [23–
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38 291 25].
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43 293 Multiple transcriptomic signatures, capturing the host response to TB, have been described as
44
45 294 promising candidate tests for earlier TB diagnosis (up to two years before microbiological
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47 295 diagnosis). An individual patient data meta-analysis suggested equivalent performance of
48
49 296 eight signatures, with 25-40% sensitivity and 92-95% specificity 0-24 months before TB
50
51 297 diagnosis. Diagnostic accuracy of each signature improved as the interval between testing and
52
53 298 microbiological TB diagnosis shortened [26]. Several signatures have been developed into
54
55 299 polymerase chain reaction (PCR)-based assays to facilitate real-time implementation: the
56
57 300 recent CORTIS trial reported sensitivity of 48% and specificity of 75% for incipient TB for the
58
59 301 RISK-11 signature [27]. Cepheid have developed a 3-transcript TB score into a fully automated

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3 302 in-cartridge PCR assay performed on finger-prick blood using the Xpert platform (Xpert TB
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5 303 Host Response RUO Prototype cartridge). This cartridge will be evaluated using freshly
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7 304 collected specimens in ERASE-TB; storage of RNA-stabilised blood samples also allows for
8
9 305 retrospective evaluation of additional transcriptomic signatures in our cohort [28,29]
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11 306
12
13 307 An alternative approach to capture the host response to TB is through protein-based biomarker
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15 308 signatures. Candidate tests in this category include a serum- or plasma-based multiplex assay
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17 309 assessing 13 protein biomarkers (CRP, procalcitonin [30], sTREM-1 [31,32], angiotensin-2
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19 310 [33,34], interleukin-6 [35], TRAIL [36] and IP-10 [37]) that is being developed by the London
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21 311 School of Hygiene and Tropical Medicine; in addition, a seven biomarker signature is under
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23 312 development as a point-of-care test for TB diagnosis, with 94% sensitivity and 73% specificity
24
25 313 detected in previous work [38].
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31 315 **Statistical analyses**

32 316 Baseline characteristics and analytical data will be summarized using descriptive statistics
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34 317 inclusive of mean, median, range, standard deviation, and absolute as well as relative
35
36 318 frequencies depending on the nature of data. A logistic regression model will be used to identify
37
38 319 characteristics of TB among ICs, households and HHCs that are predictive of incident TB.
39
40 320 From the study database, we will simulate algorithms of different tests to obtain the testing
41
42 321 combination with the best accuracy. We will couple tests with a mathematical model that
43
44 322 quantifies the risk of infection and/or disease to enhance predictive performance. The reporting
45
46 323 of the development of the prediction model will follow the Transparent Reporting of a
47
48 324 multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) Initiative [39].
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52
53 326 The validation of novel diagnostic tests for detecting TB will be analysed as a 1:4 matched
54
55 327 nested case-control study with HHCs diagnosed with TB at baseline and during follow-up
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57 328 serving as cases, and HHCs who do not develop TB during follow-up as controls; controls will
58
59 329 be matched for site, age, sex, HIV status and other risk factors for developing TB. Sensitivity

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3 330 and specificity of novel tests will be determined using pre-existing positive/negative cut-offs
4
5 331 where these exist [40]; and receiver operating curves (ROC) constructed with area under the
6
7 332 ROC curve calculated. For tests aiming to identify individuals at high risk of TB in the future,
8
9 333 only HHCs who are diagnosed with TB during follow-up will serve as cases (i.e. those
10
11 334 diagnosed with TB at baseline will be excluded). Stored samples from all timepoints will be
12
13 335 retrieved and diagnostic accuracy (i.e. sensitivity and specificity) of the novel test determined
14
15 336 at different time-points before TB diagnosis. The decision of assigning the “active TB”
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17 337 endpoints to participants will be blinded from the new test results to avoid inclusion bias.
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21 22 339 **Data management**

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24 340 All source data will be kept confidential in secured locations with restricted access by
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26 341 authorized personnel only inclusive of monitors, auditors and reviewers of ethical and
27
28 342 regulatory committees in line with applicable data privacy regulations. Each participant is
29
30 343 asked to consent to this handling of the data, and is assigned a pseudonymous identification
31
32 344 number that is used throughout the study on all source data.
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36
37 346 Accurate documentation of paper-based and electronic source data, e.g., original records and
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39 347 certified copies of original records, progress notes, screening logs, and recorded data from
40
41 348 automated instruments, will be maintained. The pseudonymized clinical data captured on
42
43 349 paper-based Case Report Forms will be entered at the sites into a database using the web-
44
45 350 based Clinical Data Management System of OpenClinica (OpenClinica LLC, Waltham, MA,
46
47 351 USA). The study specific database has been built, maintained and hosted by the LMU Klinikum
48
49 352 on a centralized secure server. Data modifications and necessary corrections performed in the
50
51 353 database also within the context of double data entry will be documented and tracked in audit
52
53 354 trails. Data quality and plausibility are assured by a series of pre-programmed edit and range
54
55 355 checks in OpenClinica. Further validation checks are programmed in Stata (Statacorp, College
56
57 356 Station, TX, USA) with extracts of the database and electronically received data, e.g.,
58
59 357 spirometry, dCXR and laboratory, will be integrated into analyses of datasets.
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5 **359 Monitoring**

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7 360 Assigned study monitors will visit the sites at regular intervals physically and/or virtually in
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9 361 addition to frequent day-to-day communication. Close follow-up on all study-related aspects
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11 362 will be performed to ascertain compliance with standards of Good Clinical Practice, the
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13 363 Declaration of Helsinki, and other local and national regulatory guidelines inclusive of
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15 364 guidelines for infection prevention and control of airborne-transmitted diseases, e.g., social
16
17 365 distancing in well-ventilated spaces, and wearing of personal protective equipment. In
18
19 366 particular, monitors that support designated study personnel are responsible to verify (I)
20
21 367 adequacy of the study personnels' qualifications and facilities, (II) accuracy of informed
22
23 368 consent procedures and patient eligibility, (III) adherence to the study protocol, (IV) protection
24
25 369 of rights and well-being of participants, (V) adherence to infection prevention and control
26
27 370 measures (VI) accuracy and completeness of study documents and other study-related
28
29 371 records, and (VII) maintenance of source documents.

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35 **373 Ethics and dissemination**

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37 374 The study protocol and informed consent/assent documents have been approved by regulatory
38
39 375 and ethical committees of the participating institutions [Medical Research Council in Zimbabwe
40
41 376 (MRCZ/A/2618), the National Health Research Ethics Committee in Tanzania (TMDA-
42
43 377 WEB0021/CTR/0004/03), the National Bioethics Committee for Health in Mozambique
44
45 378 (541/CNBS/21), and the ethical committees of London School of Hygiene & Tropical Medicine,
46
47 379 United Kingdom (22522-2), and the medical faculty of the Ludwig-Maximilians-Universität
48
49 380 München, Germany (20-0771)].

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53 382 Adult ICs and HHCs are asked for written informed consent prior to their participation.
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55 383 Underage HHCs are asked for assent in addition to obtaining the consent of their legal
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57 384 guardians/parents; with ages for assent depending on local guidance. In case of illiteracy, the
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59 385 participant is asked to give its consent by fingerprint while an adult impartial, literate witness

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3 386 present during the entire consent procedure signs the consent on behalf. All participants have
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5 387 the right to withdraw from the study at any time. Findings derived from ERASE-TB will be
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7 388 presented at scientific conferences, and published in peer-reviewed international journals.
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11 390 **Current study status**

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13 391 The recruitment of ICs and HHCs is in progress in Zimbabwe, Mozambique and Tanzania
14
15 392 since March, August and September 2021, respectively. The follow-up of HHCs is anticipated
16
17 393 to be completed in March, August and September 2023 in Zimbabwe, Mozambique and
18
19 394 Tanzania, respectively; laboratory analyses are estimated to be performed by December 2024.
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22 395

23 24 396 **Author contributions**

25
26 397 The study proposal and protocol were written by NH, KK with scientific input from CK, TM, CG,
27
28 398 JM. ETM, UP, DB, AM wrote the initial manuscript with scientific input on the database and
29
30 399 data management section from FR, TA. NH, KK critically reviewed the initial draft of the
31
32 400 manuscript. KH, GK, CJC, ENN, TA, provided critical feedback on the manuscript. All authors
33
34 401 have read and approved the final version of the manuscript.
35
36
37 402

38 39 403 **Funding statement**

40
41 404 ERASE-TB is part of the EDCTP2 programme supported by the European Union (grant
42
43 405 number RIA2018D-2508-ERASE-TB), the German Center for Infection Research (DZIF) grant
44
45 406 number: 02.710 and the Swedish Research Council (220-23602). CJC is funded by the
46
47 407 Wellcome Trust (203905/Z/16/Z). Cepheid, Inc., and SD Biosensor provided test kits and
48
49 408 analyzers at no cost to the Consortium.
50
51
52 409

53 54 410 **Acknowledgments**

55
56 411 We are grateful to the study personnel from the Biomedical Research and Training Institute
57
58 412 and the Zvitambo Research Institute, Zimbabwe, the Instituto Nacional de Saúde,
59
60 413 Mozambique, and the National Institute for Medical Research - Mbeya Medical Research

1
2
3 414 Centre, Tanzania for their exceptional efforts and contributions, which made this research
4
5 415 possible.

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49
50 437 **Competing interests statement**

51
52 438 None declared.

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55
56 440 **Disclaimer**

57
58 441 Not applicable.

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3 582 **Figure legend**

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5 583 **Figure 1.** The ERASE-TB consortium

6
7 584 Abbreviations: ERASE-TB=Early Risk Assessment in TB contactS by new diagnostic tEsts

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11 586 **Figure 2.** Location and characteristics of ERASE-TB study sites

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13 587 Notes: The location of each study site is indicated by a red asterix. Source data used within
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15 588 this figure are taken from the references [7,8,41–43,9–15].

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17 589 Abbreviations: ERASE=Early Risk Assessment in TB contactS by new diagnostic tEsts;
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19 590 TB=tuberculosis; HIV=human immunodeficiency virus

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24 592 **Figure 3.** Eligibility criteria and schedules of events for index cases and household contacts

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26 593 Notes: **A**=depending on the time point of study enrollment and consequently on the duration
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28 594 available for follow-up, i.e. 18 or 24 months, the follow-up visit at 24 months \pm 30 days may be
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30 595 conditional; **B**=the follow-up visit by phone may be conducted after the last scheduled follow-
31
32 596 up visit at 18 months \pm 30 days or 24 months \pm 30 days to assess whether symptoms suggestive
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34 597 of TB have occurred, TB diagnosis has been made or anti-TB treatment has been initiated;
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36 598 **C**=unwell visits by phone or on-site may be conducted between scheduled follow-up visits if a
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38 599 participant presents at a recruitment healthcare facility with signs and symptoms suggestive of
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40 600 TB; **D**=coached spontaneous or induced sputum collection for storage at scheduled follow-up
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42 601 visit at 18 months \pm 30 days or 24 months \pm 30 days, and for repetition of HIV testing if tested
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44 602 negative at baseline; **E**=coached spontaneous or induced sputum collection upon the decision
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46 603 of the investigating team for testing by Xpert MTB/RIF Ultra if participant presents with signs
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48 604 and symptoms suggestive of TB; **F**=coached spontaneous or induced sputum collection in
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50 605 case of Xpert MTB/RIF Ultra positivity or strong clinical suspicion of TB for repetition of the
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52 606 Xpert MTB/RIF Ultra; **G**=in case of HIV positivity to be followed by the assessment of CD4
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54 607 counts; **H**=CXR to be conducted at an unscheduled on-site unwell visit upon the decision of
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56 608 the investigating team depending on the nature of symptoms reported, and the time elapsed
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58 609 since the last CXR including its findings; **I**=not to be conducted among pregnant women;

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3 610 **J**=stored venous blood includes 6mL EDTA blood for whole blood and plasma, 4mL serum
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5 611 and 2.5mL PAXgene blood, all samples will be deep frozen for retrospective testing using new
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7 612 diagnostics as described in text; **K**=in case the evaluation of symptoms of a participant unable
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9 613 to present at a recruitment healthcare facility is required an unscheduled on-site or home visit
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11 614 will be arranged by phone, the resolution of symptoms can alternatively be addressed by
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13 615 phone; **L**=collection of PBMC at baseline and follow-up visit at 6 months \pm 30 days is optional,
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15 616 thus will not be performed at each participating site and for each participant; **M**=in case the
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17 617 evaluation of symptoms of a participant unable to present at a recruitment healthcare facility
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19 618 is required or doubtful if required an unscheduled unwell visit by phone will be arranged, the
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21 619 resolution of symptoms can alternatively be addressed by phone; **N**=spirometry and/or
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23 620 diabetes (HbA1c) will be performed at scheduled follow-up visits at 6 months \pm 30 days, 12
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25 621 months \pm 30 days and 18 or 24 months \pm 30 days if required or not performed at baseline,
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27 622 anaemia (Hb) will be performed at baseline and scheduled follow-up visits at 6 months \pm 30
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29 623 days, 12 months \pm 30 days and 18 or 24 months \pm 30 days if possible; **O**=blood pressure
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31 624 measurement will be performed at baseline and scheduled follow-up visits at 6 months \pm 30
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33 625 days, 12 months \pm 30 days and 18 or 24 months \pm 30 days; **P**=WGS to be performed once *Mtb*
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35 626 infection is confirmed and an isolate could be recovered.

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38
39 627 Abbreviations: TB=tuberculosis; WHO=World Health Organization; HIV=human
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41 628 immunodeficiency virus; WGS=whole genome sequencing; MBLA=molecular bacterial load
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43 629 assay; CXR=chest radiograph; IGRA=interferon gamma release assay; PBMC=peripheral
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45 630 blood mononuclear cell; TAM-TB=T- cell activation marker tuberculosis; MTB=*Mycobacterium*
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47 631 *tuberculosis*; RIF=rifampicin, Hb=haemoglobin; HbA1c=glycated hemoglobin;
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49 632 LAM=lioparabinomannan; CD4=cluster of differentiation 4

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51 633

52 634 **Figure 4.** Study design

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54
55 635 Abbreviations: HHC=household contact; TB=tuberculosis; IC=index case; WHO=World
56
57 636 Health Organization; CXR= chest radiograph; SS=symptom score; MTB=*Mycobacterium*
58
59 637 *tuberculosis*; RIF=rifampicin; pos=positive; CT=computer tomography; FU=follow-up

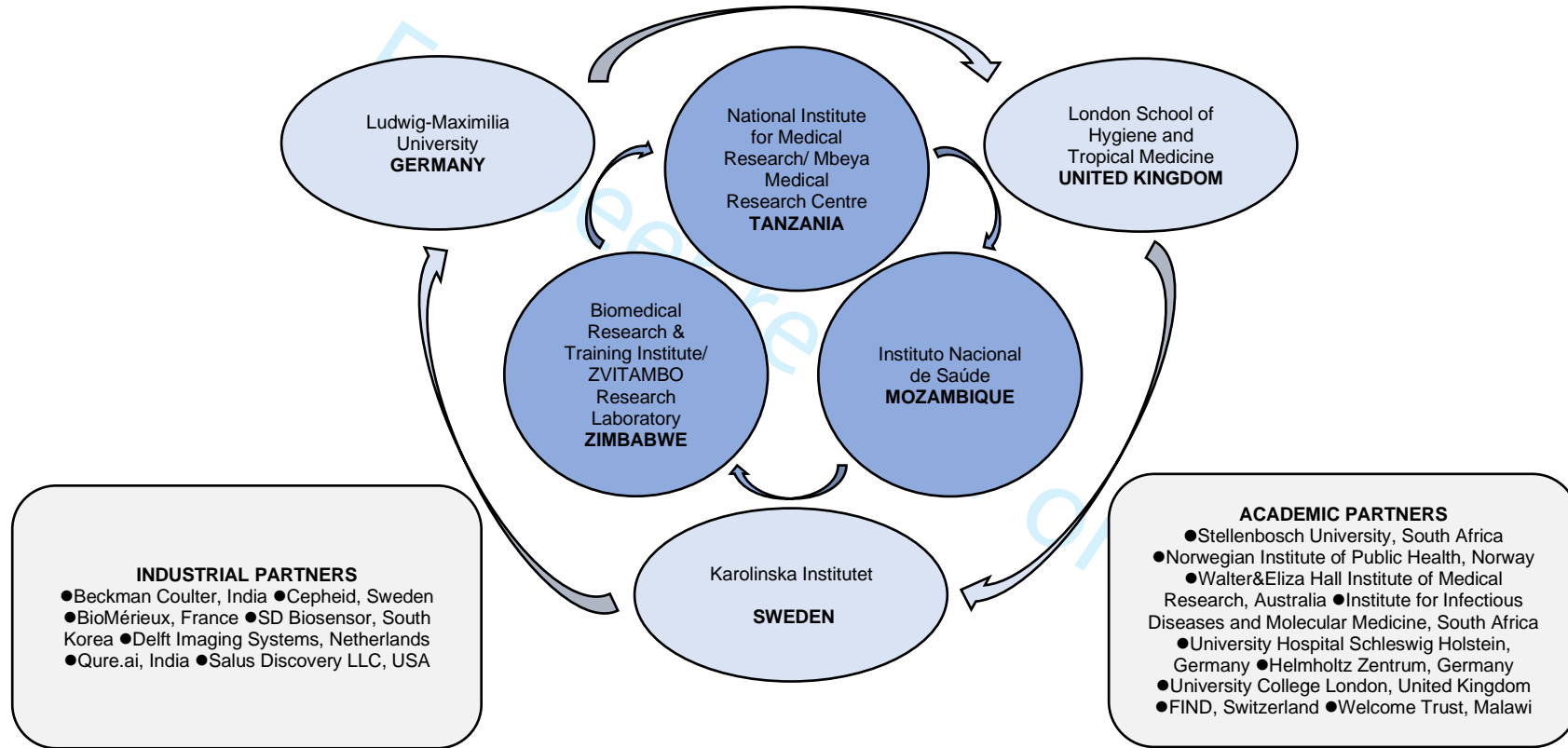


Figure 1. The ERASE-TB consortium

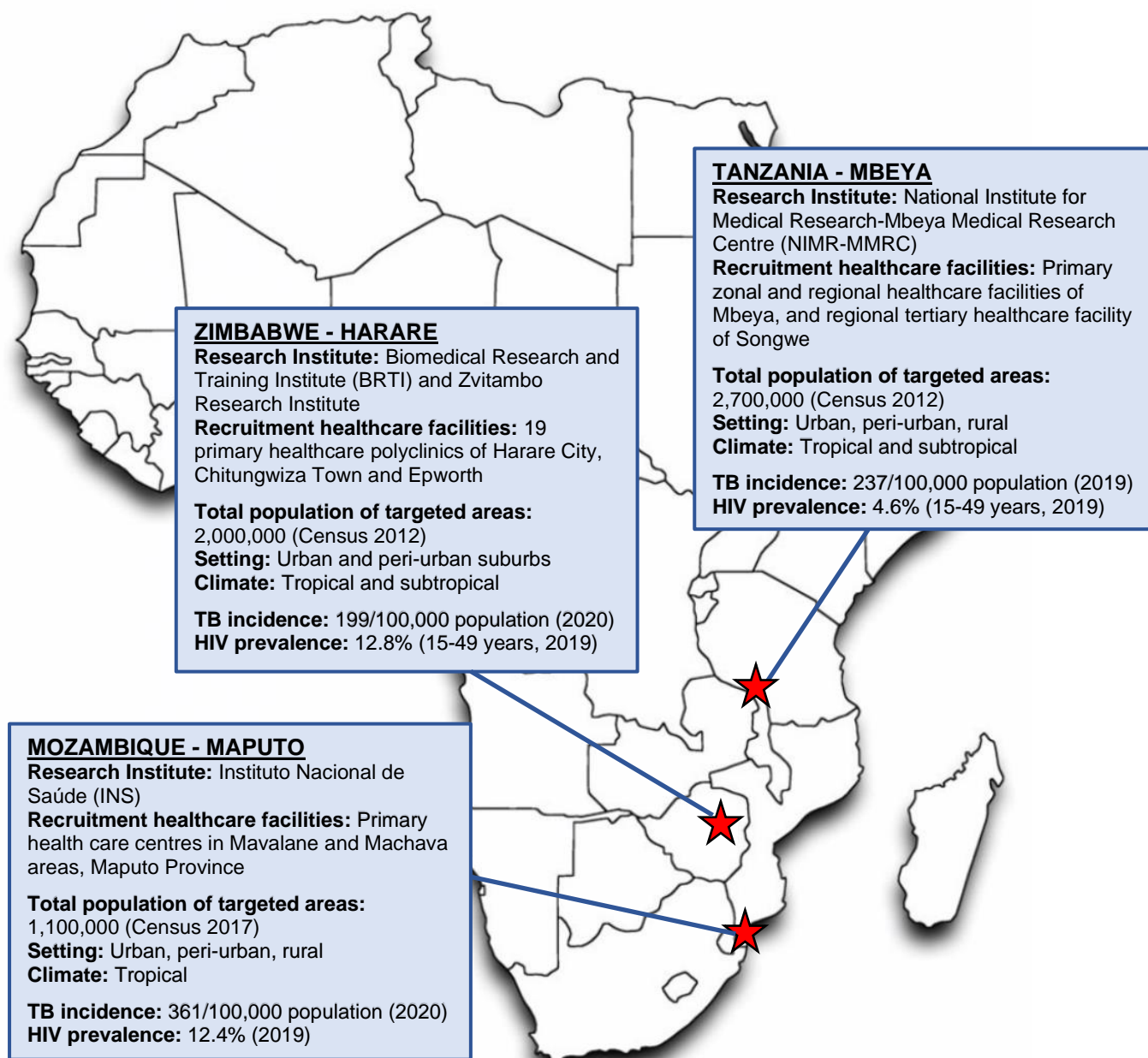


Figure 2. Location and characteristics of ERASE-TB study sites

INDEX CASE

HOUSEHOLD CONTACT

INCLUSION CRITERIA

- Aged ≥18 years
- Living with at least another person aged ≥10 years in the same household with a firm unchanged address for 6 months
- Pulmonary TB diagnosis within 4 weeks prior to enrolment
- Spontaneous sputum production
- Positive sputum smear, i.e. >+2 positivity by Ziehl-Neelsen stain or Auramine-O stain OR ≥+1 positivity on WHO symptom scale OR ≥medium positivity by GeneXpert (if no microscopy performed)
- <7 daily doses of anti-TB treatment since diagnosis
- Voluntary written informed consent obtained

EXCLUSION CRITERIA

- Treatment for TB within the past 30 days OR on current TB treatment OR on current preventive TB therapy (if HIV negative) at the time of enrolment



INCLUSION CRITERIA

- Aged ≥10 years
- Substantial exposure, i.e. spending ≥3 nights/week over the past 4 weeks in the same household as the index case,
- Voluntary written informed consent or parent/guardian consent & assent in case of minors obtained



EVENTS AT BASELINE/SCREENING VISIT

- Administration of socio-demographic questionnaire
 - Assessment of vital signs, medical history
 - Digital CXR (if available)^I
- Specimen investigations**
- Sputum: culture, storage, WGS, MBLA

EVENTS AT BASELINE/ ±14days of SCREENING VISIT

- Administration of socio-demographic questionnaire
 - Assessment vital signs, medical history
 - Screening for diabetes (HbA1c)^N, high blood pressure^O, anaemia (Hb)^N, WHO TB symptoms
 - Digital CXR^I, spirometry^N
- Specimen investigations**
- Sputum: Xpert MTB/RIF Ultra^E, culture^F, storage, WGS^P
 - Blood: HIV/CD4 counts^G, Cepheid host response, TAM-TB, IGRA SD Biosensor, PBMC^L, storage^J
 - Urine: dipstick/LAM, storage



EVENTS AT UNSCHEDULED UNWELL VISIT(S)
(phone/on-site)^{C,K,M}

- Assessment of vital signs, medical history
 - Screening for high blood pressure, WHO TB symptoms,
 - Digital CRX^{H,I}
- Specimen investigations**
- Sputum: Xpert MTB/RIF Ultra, culture, storage, WGS^P
 - Blood: Cepheid host response, TAM-TB, storage^J
 - Urine: dipstick/LAM, storage

EVENTS AT UN-SCHEDULED FOLLOW-UP VISIT(S)^B (phone)

- Assessment of medical history
 - Screening for WHO TB symptoms
- Specimen investigations**
- None

EVENTS AT 6months ±30days/ 12months ±30days/ 18months ±30days/ 24months ±30days^A FOLLOW-UP VISITS

- Assessment of vital signs, medical history
 - Screening for high blood pressure, WHO TB symptoms
 - Digital CXR^I, spirometry^N
- Specimen investigations**
- Sputum: Xpert MTB/RIF Ultra^E, culture^F, storage^D, WGS^P
 - Blood: Cepheid host response, TAM-TB, PBMC^L, storage
 - Urine: dipstick/LAM, storage^J



Optional

Optional

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Figure 3. Eligibility criteria and schedules of events for index cases and household contacts

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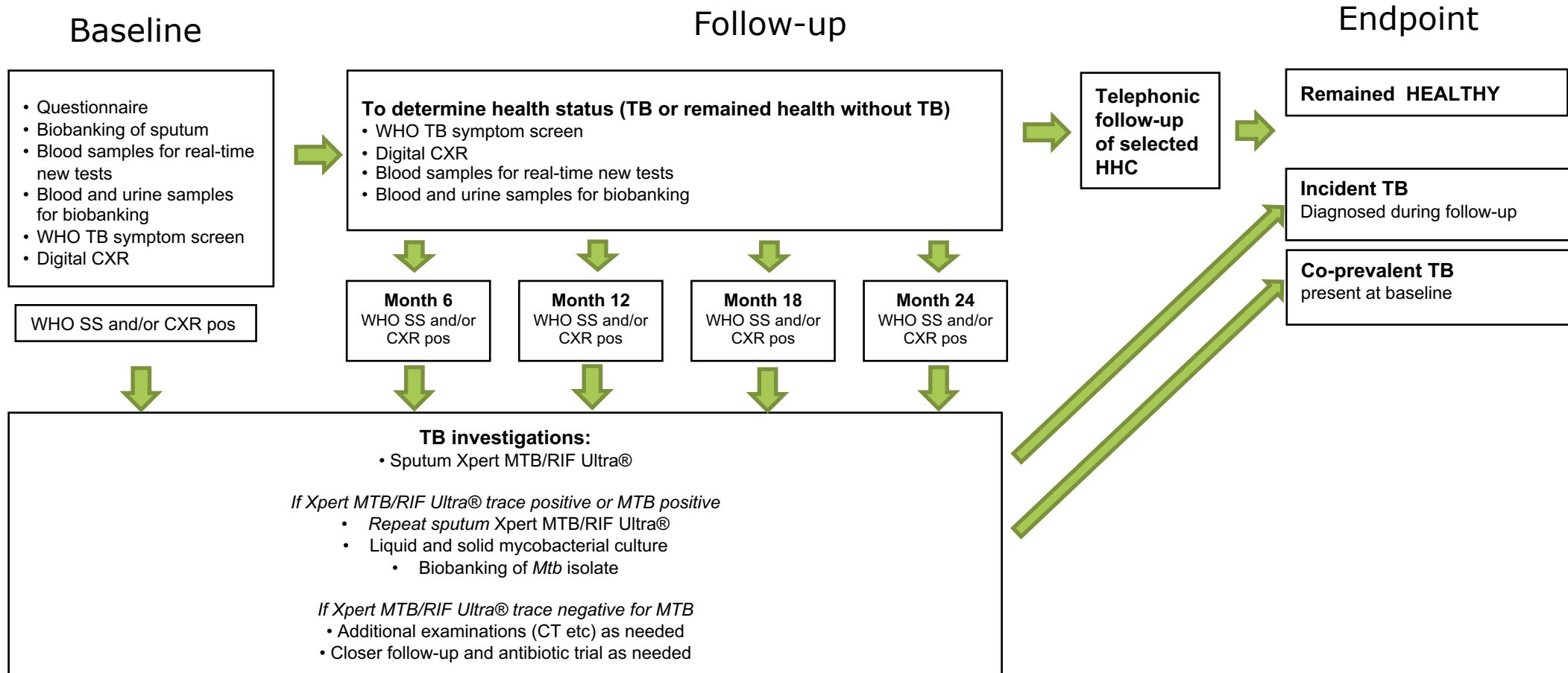


Figure 4. Study design.

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2-3
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	5-6
	4	Study objectives and hypotheses	6
METHODS			
<i>Study design</i>	5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	6-7
<i>Participants</i>	6	Eligibility criteria	7 (Figure 3)
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	7 (Figure 3)
	8	Where and when potentially eligible participants were identified (setting, location and dates)	7
	9	Whether participants formed a consecutive, random or convenience series	7, 11
<i>Test methods</i>	10a	Index test, in sufficient detail to allow replication	8-10, 11-13
	10b	Reference standard, in sufficient detail to allow replication	8-10, 11-13
	11	Rationale for choosing the reference standard (if alternatives exist)	8-10, 11-13
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	13-14
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	13-14
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	13-14
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	13-14
<i>Analysis</i>	14	Methods for estimating or comparing measures of diagnostic accuracy	13-14
	15	How indeterminate index test or reference standard results were handled	13-14
	16	How missing data on the index test and reference standard were handled	14
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	13-14
	18	Intended sample size and how it was determined	11
RESULTS			
<i>Participants</i>	19	Flow of participants, using a diagram	8-10 (Figure 3)
	20	Baseline demographic and clinical characteristics of participants	8-10 (Figure 3)
	21a	Distribution of severity of disease in those with the target condition	
	21b	Distribution of alternative diagnoses in those without the target condition	
	22	Time interval and any clinical interventions between index test and reference standard	
<i>Test results</i>	23	Cross-tabulation of the index test results (or their distribution) by the results of the reference standard	
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	13-14
	25	Any adverse events from performing the index test or the reference standard	
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	3
	27	Implications for practice, including the intended use and clinical role of the index test	5-6
OTHER INFORMATION			
	28	Registration number and name of registry	2
	29	Where the full study protocol can be accessed	
	30	Sources of funding and other support; role of funders	16

STARD 2015

AIM

STARD stands for “Standards for Reporting Diagnostic accuracy studies”. This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition**. This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test**. A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross-tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross-tabulation (sometimes referred to as the contingency or “2x2” table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on <http://www.equator-network.org/reporting-guidelines/stard>.

