Version 17 230524

Increased Tuberculosis Case Detection - a cluster-randomized trial combining available resources and novel strategies for high endemic areas

DiOpTB

Protocol for a multi-centre, cluster-randomized clinical trial and PhD-project

Department of Infectious Diseases, Aarhus University Hospital, Aarhus University, Linköping University, Gondar University and Bandim Health Project

Table of Contents

1 Summary

As estimated by the WHO 10.6 million new Tuberculosis (TB) cases were identified in 2022– while more than three million went undetected and untreated. The low detection rate illustrates the failure to recognise and diagnose TB in the current cascade of healthcare and is a major obstacle to effective TB control programs. This multi-centre cluster-randomised clinical trial will evaluate the effect (i.e., diagnostic yield) of improving the point-of-care diagnostics already in place in most primary health-care centres in low-resource settings. The present study will be conducted in two different geographical settings in the Western and Eastern African countries of Guinea Bissau and Ethiopia. This improved clinical diagnostic pathway may improve case detection rates at primary healthcare level, ensuring prompt commencement of treatment, thereby diminishing transmission risk in the community and improving treatment outcomes. The Optimized Diagnostic Procedure (ODP) will utilize instructed sputum sampling and pooling as well as computer-aided detection (CAD) chest X-ray (CXR) and additional pooled sputum sample as well as non-sputum sampling (faecal and a buccal/tongue swab and saliva) for GeneXpert Ultra PCR (Xpert) as state-of-the-art add-ons to the routine diagnostic pathway for TB. This adds to the key components of the WHO "End TB" strategy – early diagnosis – and if successful, may be rapidly approved by the WHO and implemented by governments globally with potentially major public health benefits.

The study will be conducted in close liaison with the national Ministries of Health and TB programs in Guinea-Bissau and Ethiopia. This will facilitate any relevant findings to be taken forward for implementation into policy and practice. Capacity development, training and educational activities will be closely aligned to this study.

2 Objectives

2.1 Primary objective

1. Diagnostic yield of active TB within ten days, comparing Enhanced Usual Diagnostic Procedure (EUDP) to Optimized Diagnostic Procedure (ODP).

2.2 Secondary objectives

1. Number of patients treated for TB within two weeks comparing EUDP to ODP.
2. The additional diagnostic yield of CAD CXR compared to Xpert and culture.
3. Improved follow-up (FU) rates in the cascade of care (i.e., one week and six months FU for all included and treatment start and outcome for all TB diagnosed).

4. Differences in diagnostic yield of active TB between routine sputum samples, instructed sputum samples and non-sputum samples (faecal and saliva combined with buccal/tongue swabs).
5. Feasibility of including Oxford Nanopore sequencing for detection and molecular resistance patterns measured as rate of analysed samples within two weeks.

3 Background

In 2022, the WHO estimated that of a total 10.6 million new TB cases, more than three million went undiagnosed and of the remaining seven million only 57% were bacteriologically confirmed (1). In sub-Saharan African settings such as Ethiopia and Guinea Bissau, smear microscopy remains the major diagnostic tool in most areas despite the rollout of rapid diagnostic tests such as Xpert. In a multi-centre trial by Theron et al, the implementation of Xpert in African settings did indeed reduce diagnostic delay but unfortunately without any effect on the numbers who were initiated on TB treatment nor on mortality (2). The trial showed that Xpert rollout was not superior to enhanced, well-equipped, microscopy-based diagnostic facilities. In an editorial to the Lancet written by our group, it was concluded that TB elimination could be better advanced by improving currently available tools than by expanding Xpert testing to peripheral health facilities (2, 3). Nevertheless, when TB is diagnosed with a point of care test, there is a need to ensure that correct treatment is provided. However, culture-based drug susceptibility testing is very scarce in high endemic areas and often takes several weeks or months to perform (4). Recently, the Cryptic study (5) has shown that genotypic drug susceptibility testing (gDST) may guide treatment with a sensitivity and specificity well above 90% for key drugs. New techniques such as MinION (Oxford Nanopore Technologies) have also made it possible to perform sequencing and gDST directly from sputum samples. Such point-of-care-based sequencing technology is comparable in size to a USB flash drive (6) and may be attached to a laptop computer at a health centre in a high endemic area.

A cluster-randomised trial implementing the TBscore recently showed a fourfold increase in case detection rate in Ethiopia but not in Guinea-Bissau (7). It identified that factors such as laboratory capacity and routines in collecting and examining sputum smear samples may have a high impact on case detection rate and could be optimized based on the available resources. Surprisingly, the sensitivity of sputum smear microscopy ranges from 20-80% with an average of about 50% (8), which may partly be due to patient selection but also depend on considerable variability in sputum collection strategies and/or laboratory procedures. In a comparison of sputum collection methods

by Datta et al (9), pooling of sputum and structured instructions before sampling on average led to a twofold higher diagnostic yield whereas a spot versus morning sample showed no difference. A multi-centre study including Ethiopia, comparing fluorescence microscopy to conventional light microscopy showed a small but significant increase in sensitivity (72.8 vs 65.8%)(10). Further, recent research has shown that buccal and tongue swabs, that are easily obtained, can hold valuable diagnostic potential. (11) In smear-negative patients with presumed TB, the available diagnostic tools in high endemic countries include CXR but standardized procedures for evaluation of CXR have been scarce. Recently, CAD software based on artificial intelligence algorithms such as qXR (Qure.ai, India) have improved detection of microbiologically confirmed TB from 50-60% by experienced radiologists to 70-84% with a specificity of 80% by CAD (12). However, a recent systematic review concluded as did the WHO that there are too few high-quality studies to fully assess its diagnostic accuracy (13). We now propose to conduct a multi-centre cluster-randomised clinical trial to evaluate whether improvements on available diagnostic resources can increase the diagnostic yield of active TB and decrease mortality for patients diagnosed with TB.

4 Methods

4.1 Location and nature of sites

The present study will be conducted in two African countries: Guinea-Bissau and Ethiopia. In Bissau, the capital of Guinea-Bissau, The Bandim Health Project has been a Health and Demographic Surveillance Site (HDSS) for 45 years and has a well-defined study population of approximately 100,000 under continued surveillance. Within the study area there are two health centres (HCs) from where patients with presumed TB will be enrolled (Bandim HC, Belem HC).

In Ethiopia the study will be conducted in collaboration with the University of Gondar in the region of North-Gondar, which has a population of more than two million. Two health centres located in North Gondar Zone, namely Azezo HC and Gondar HC will participate. The Gondar University Hospital is a teaching and referral hospital and will be used for further management of severe TB cases during this study.

4.2 Epidemiology and study population

4.2.1 Guinea-Bissau

The epidemiology of TB in the Bissau study population has been extensively described (14-18). The overall incidence of TB has declined only slightly since 2004 and was estimated at 273/100,000

population in 2020, while TB/HIV co-infection declined from 108 per 100.000 to 14 per 100,000 over the period (19). Smear negative cases and case fatality rate likewise declined over the period. The incidence of smear positive TB remained stable at 188 per 100,000 between 2004 and 2011 (20). All HCs have basic laboratory facilities to carry out sputum smear microscopy and all provide TB treatment. The national referral hospital for TB, Hospital Raoul Follereau, is located adjacent to the study area and is a close collaborating partner. The incidence rate of TB in Guinea-Bissau as a whole is 361 per 100,000 with a case detection rate estimated at 35% (21, 22).

4.2.2 Ethiopia

TB continues to be a major public health concern in Ethiopia fuelled by the expansion of the HIV epidemic since the 1990s. According to the 2022 WHO TB report (1), Ethiopia is among high-burden countries for both TB and TB/HIV and has an estimated incidence rate of 119 per 100,000, and a TB mortality of 17.7 per 100,000 (21). HIV-positive TB incidence is 6.2 per 100,000 and case detection rate is currently estimated at 73% (21, 22). These figures are high considering that Ethiopia is the second most populous country in Africa with an estimated total population size of more than 100 million. HIV screening is carried out as a routine.

4.3 Design

The present study is designed as an open-label, stepped-wedge cluster-randomised controlled trial (23) to investigate an optimized diagnostic procedure for active TB in healthcare centres in Guinea-Bissau and Ethiopia. Applying the stepped-wedge design ensures that all participating HCs will implement the intervention during the study period. This design is particularly useful for evaluating the population-level impact of an intervention, which is of interest in this study. All clusters (i.e., HCs) start with Enhanced Usual Diagnostic Procedure (EUDP) and are then randomized to switch to the intervention phase at predefined time points (see table 1).

See below for detailed description of sample size calculations.

Table 1 Intervention implementation by cluster

Date Commencing		03/06/2024 – 03/11/2024	04/11/2024 – 06/04/2025	07/04/2025 – 07/09/2025
Week		1-22	23-45	46-68
Cluster/HC	1	0	1	1
	2	0	1	1
	3	0	0	1
	4	0	0	1
Week		1-22	23-45	46-68

4.4 Bandim TBscore

The Bandim TBscore (TBscore) (Table 2) consists of five symptoms (cough, haemoptysis, dyspnoea, chest pain, and night sweats) and six signs (pale inferior conjunctivae, pulse >100 per minute, positive finding at lung auscultation, temperature >37°C (axillary), body mass index (BMI) <18/<16, and mid-upper-arm circumference (MUAC) <220 mm/<200 mm) (24). Each variable contributes one point while BMI and MUAC contribute an additional point if BMI<16/MUAC<200 mm; hence, the maximum score is 13. The score divides patients into three severity classes (SC): SC-I, TBscore 0-5; SC-II, TBscore 6-7, and SC-III, TBscore≥8. A simplified version of the score – TBscoreII – with a maximum score of 8 points has also been developed (25). The advantage of the latter score is that it can be performed without a physician present. The TBscore has been assessed in both Gondar and Bissau and found to be a useful add on in the diagnostic cascade of care.(7)

Table 2 Bandim TBscore – symptoms and signs.

Variables included	TBscore	TBscoreII
Symptoms		
Cough	1	1
Haemoptysis	1	-
Dyspnoea	1	1
Chest pain	1	1
Night sweats	1	-
Signs		
Anemia	1	1
Pulse >100 beats/min	1	-
Positive finding at lung auscultation	1	-
Temperature >37°C	1	-
BMI<18	1	1
BMI<16	1	1
MUAC<220 mm	1	1
MUAC<200 mm	1	1
Total number of points possible	13	8

BMI=body mass index; MUAC=mid upper arm circumference.

4.5 Buccal, tongue swap and saliva sample

Buccal and tongue samples will be collected using the Omniswab (Whatman, catalogue #WB100035) and added to a container where patients leave a saliva sample. Samples will be collected by trained laboratory staff, who gently brush the inside of each cheek and then the tongue of the participant for 10 seconds with the OmniSwab. The OmniSwab has a breakpoint and the head will be ejected into 500 µl buffer containing 50 mM Tris pH 8.0, 50 mM EDTA, 50 mM sucrose, 100 mM NaCl, and 1% SDS, and transported to the laboratory at 4˚C. (11) There, the OmniSwab-collected samples will be vortexed in the saliva, and the swabs heads removed. One part of the sample will be analyzed using Xpert Ultrawhile the other part will be stored at – 80 °C until further processing.

4.6 Computer-aided detection chest X-ray (CAD CXR) applying artificial intelligence (AI)

A preliminary study using an AI based CAD CXR software (qXR, Qure) compared to two Ethiopian radiologists included 498 CXRs from a previously performed randomized controlled trial on the TBscore. Of those, the less experienced radiologist found 50, the more experienced radiologist found 100 and CAD CXR found 83 to be indicative of TB. Using Xpert PCR as the gold standard for TB diagnosis, the overall AUC for the CAD CXR was 0.84 while the less experienced radiologist performed at a sensitivity of 41.4% and a specificity of 94.1% and the experienced radiologist's assessments were 55.2% sensitive and 85.0% specific. The agreement between the radiologists was moderate (κ=0.45), as was the agreement between each radiologist and the software (κ=0.36, κ=0.59).

In the present study we will include a mobile phone app to guide photographing analog X-ray films. These photographs will then be uploaded to a locally placed box (qbox) and analyzed on site.

4.7 Enrolment

At all sites adult patients will be screened during consultations carried out at primary healthcare centres. All patients presenting with cough of any duration, sputum production, or weight loss will have their TBscore assessed. All participating health centres have previous experience collecting the symptoms and signs necessary for the TBscore and completing a score chart from which the TBscore can be calculated. All patients with a TBscore≥4 will be referred for TB diagnostics. Patients with 4≤TBscore<6 will be referred to fluorescence microscopy while patients with TBscore≥6 will be referred to Xpert PCR. The staff at the sites will receive general training in TB diagnosis and then the healthcare facilities will, following a random sequence, switch from Enhanced Usual Diagnostic Procedure (EUDP), consisting of standard TB program diagnostics but ensuring availability of all reagents, to intervention (i.e., OPD).

4.7.1 Enhanced Usual Diagnostic Procedure (EUDP)

The standard TB diagnostics in both settings consist of performing the sputum smear analysis by the clinical routine. Smear-negative cases will be followed as per standard routine (Figure 1A).

4.7.2 The Optimized Diagnostic Procedure (ODP) intervention

A three-step package which involves (Figure 1B):

1. **Oral and mobile phone-guided instructions by study staff**

Patients will be instructed to take several deep breaths, hold their breath for a moment, and repeat this several times until coughing is induced including instructions to cough deeply and vigorously whilst breathing out (26). Instructions will be presented to the participating patients on a mobile phone to ensure consistent instructions to all participants.

2. **Pooling of two spot sputum samples (9)**

Two instructed pooled spot samples will be split into two parts (27) and investigated by fluorescence microscopy (28). The other part of the pooled sputum sample will be frozen for later confirmation with batch-wise BACTEC 960 MGIT as a gold standard for microbiological diagnosis.
As an add on, we will analyze a subgroup of samples with the MinION to assess applicability in a low resource setting.

3. **Smear-negative cases at the first visit will be assessed for persisting symptoms and referred to a CXR unit**

Those with a CXR CAD result suggestive of active TB will be treated for TB. The smear negative cases will also leave an additional instructed spot sputum sample which will be pooled and one part analyzed by Xpert Ultra and the other part by BACTEC 960 MGIT culture. Additionally, non-sputum sampling will be performed and analyzed by Xpert Ultra including saliva combined with buccal and tongue sample using the same swab as well as a faecal sample Xpert Ultra using the WHO-recommended direct procedure. (29)
In a feasibility study, on the basis of intention to treat (either by smear microscopy or CXR/clinical grounds) the participants will be asked for an additional instructed sputum sample which will extracted using EZ1 and sequenced using nanopore sequencing and the EPI2ME bioinformatic platform as previously described. (6)

4.7.3 Implementation of the ODP and EUDP

Upon commencement of the intervention arm, the staff will be trained in applying optimized diagnostic procedures.
For all included patients (both in the EUDP and the ODP) a follow-up visit one week from first encounter will take place, to ensure initiation of treatment (for smear positive cases) or to screen for persisting symptoms and carry out a second clinical evaluation (smear negative cases). Diagnosis will be according to local standards and based on smear microscopy, CXR, and WHO clinical criteria including for extrapulmonary cases (30, 31). During the intervention, the physician can overrule the TBscore if needed. At all clinics, both in the EUDP and ODP (i.e. intervention) phase, all included

patients will be referred to HIV testing at adjacent HIV-treatment clinics, where pre- and post-testing counselling will be carried out.

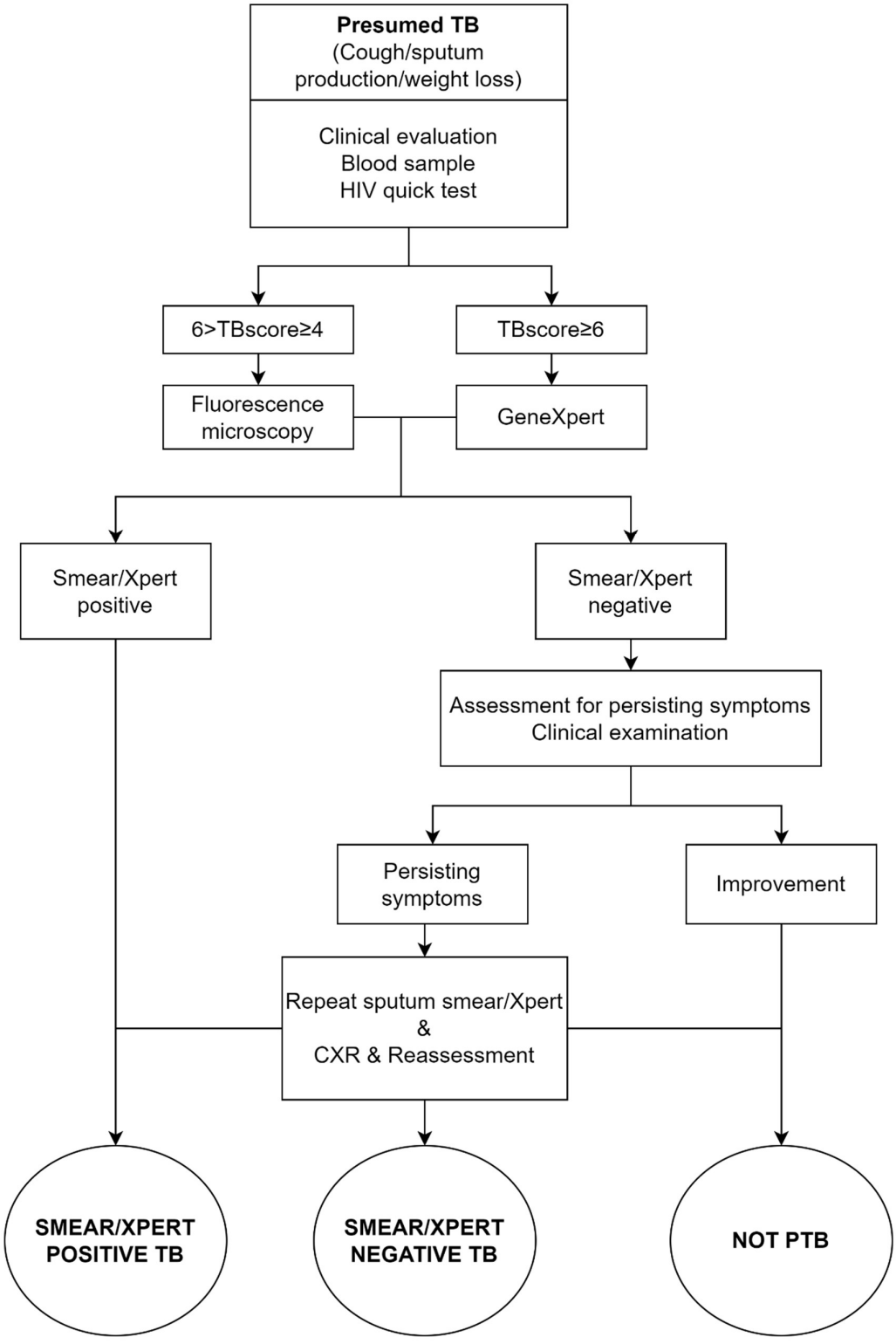

Figure 1A Diagnostic flow of the Enhanced Usual Diagnostic Procedure (EUDP), serving as control.

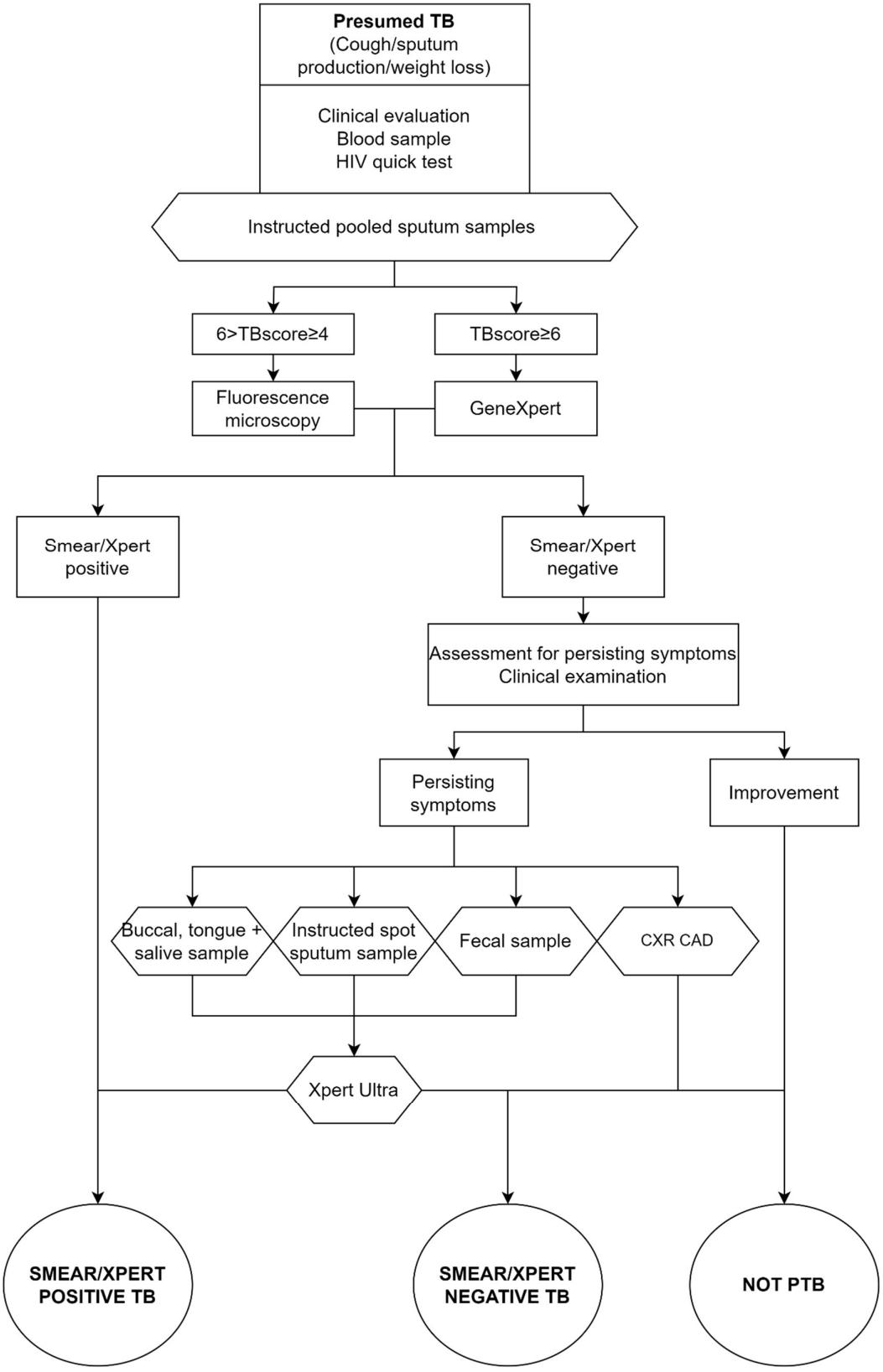

Figure 1B Diagnostic flow of the Optimized Diagnostic Procedure (ODP), serving as the intervention. Hexagons indicate deviations from the EUDP.

4.7.4 Inclusion criteria

At the participating healthcare facilities, all patients ≥15 years old with presumed TB with cough, sputum production, and/or weight loss of any duration are eligible to participate. Healthcare facilities (n=4, two in each of the countries) will switch from Enhanced Usual Diagnostic Procedure (EUDP) to Optimized Diagnostic Procedure (ODP) following a random sequence.

4.7.5 Exclusion criteria

1. TB treatment within the past year.
2. Cerebral disturbances impairing the ability to give informed consent or follow the treatment regime.

5 Outcomes

5.1 Primary outcomes

1. Number of smear positive, Xpert PCR positive, or CXR positive patients comparing EUDP to ODP.

5.2 Secondary outcomes

1. Number of patients on active TB treatment comparing EUDP clinics to ODP clinics.
2. Diagnostic yield of CAD CXR compared to smear microscopy, Xpert PCR, and culture.
3. Follow-up rates in the cascade of care (i.e. one-week and six-months follow-up for all included and treatment start and outcome for all diagnosed with TB)
4. Differences in diagnostic yield between instructed sampling, buccal samples, fecal samples and routine sputum sample.

6 Sample size and statistical analyses

Based on a previous study in the same setting, we found that among patients with a TBscore≥3 there were a total of 5% smear positive cases both in Guinea-Bissau and Ethiopia. Using a higher cut-off value at TBscore≥4 increases specificity and is estimated to increase the case detection yield to 6% (based on previous data) using routine sputum collection and sputum smear analysis. Increasing the TBscore cut-off value thus leads to a lower referral rate of 54% of all screened (instead of 73%) while increasing the number of smear positive cases in the sample (from 5% to 6%). As both settings now use Xpert MTB/RIF Ultra as the primary diagnostic method, initial case rate is estimated at 8% (32). Based on systematic reviews, it is estimated that the diagnostic yield of an instructed

sputum where two spot-samples are pooled and processed using LED microscopy will lead to an at least twofold increase in sensitivity (9, 10, 27, 33). Adding CAD CXR onto those that are smear negative by the optimized sputum smear strategy is estimated to increase the number of patients diagnosed with TB 2.5-fold. To show an increase in diagnostic yield using ODP from a conservative estimate of 7% to 14% with a power of 80% and a significance level of 0.05, two clusters in each country are needed, including 132 patients per time interval (of 22 weeks). The estimated inclusion rate per cluster per week is 6 patients, which means that it should take 66 weeks to reach a target of 1584 inclusions.

Sample size was calculated using the "steppedwedge" function in Stata (34).

The diagnostic yield, time to diagnosis, and treatment outcomes will be calculated.

Detection rates will be compared between control (EUDP) and intervention (ODP) in a generalized linear mixed effects model taking into account time and center effect and allowing for intra-cluster correlation, i.e., a mixed effect logistic regression for longitudinal data. A similar repeated measurements model will be used to analyze and compare groups on patient characteristics.

7 Timeframe

Study preparations will start August 2023 with establishment of enrolment procedures and training of staff. Enrolment will take place for 68 weeks (from June 2024 to September 2025). End of follow-up will be April 2026. For details, please see Table 3. Overall responsible body for activities planned is the PI in collaboration with local VIP and TAP personnel.

Table 3 Gantt chart of proposed activities.

	2023					2024											2025											2026											2027
	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J
Consortium meetings	x					x							x					x							x					x							x					
Preparation																																										
Database and forms																																										
Material																																										
PhD students and Post Doc registration																																										
Site visit																																										
Ethics approval																																										
Workshop																																										
Enrollment																																										
Data entry																																										
Follow-up																																										
Communication																																										
Data analysis																																										
Manuscript writing																																										
Dissemination																																										
Data dissemination (local/regional)																																										
Information meetings (policy)																																										

8 Public health importance

8.1 Major advances

TB case-finding remains a challenge, particularly in overburdened healthcare facilities with limited access to diagnostics (35-37). A simple disease management strategy combined with improved utilization of available diagnostics may ensure that more patients with TB are treated and earlier and that those at high risk of dying are targeted appropriately with a rational use of limited resources. The major advance of adding a standardized approach to patients with presumed TB will be to decrease the substantial burden of undiagnosed TB with simple means, which makes it a sustainable, affordable, and practicable tool.

8.2. New approach

The strength of the Bandim TBscore strategy is that it guides the health care professional through a structured interview and uses the existing laboratory set-up, which is of great benefit compared to other more advanced diagnostic tools. The diagnostic algorithm simply utilizes what is already part of the primary healthcare setup, thereby increasing the chance of being applicable in similar settings worldwide. Similarly, the novel technologies employed in the ODP can easily be integrated into clinical settings worldwide.

8.3. Generalizability of trial results

This trial will test implementation of a cheap, readily available, practical point-of-care package which requires only minor additional training of staff. If shown to be effective in identifying additional TB cases, use of the Bandim TBscore and improved diagnostics may be expected to be endorsed with little delay by the WHO and national governments as a standard part of TB diagnosis and management.

8.4. Contribution to improved disease management and public health

In under-funded over-burdened healthcare facilities with a large patient load presenting with co-morbidities, many patients with TB remain undiagnosed and untreated (35-37). Simple clinical tools at points of care which can identify up patients with active TB may have great potential for diagnosing patients with TB early and thereby preventing TB-associated morbidity and mortality. Targeting delayed TB diagnosis and TB-related mortality will add to the agenda of reducing poverty-related diseases. The resources used to combat TB and the productive years lost to TB inflict a punishing toll on the economies of TB-endemic countries and helps perpetuate the cycle of poverty. The majority of TB is now concentrated in the World's poorest countries, thus effective and

practicable programs to detect and cure TB early is the most feasible method of controlling the disease.

8.5 Improvements in patient care

A major strength of the Bandim TBscore is its ability to continually assess disease severity during treatment as has previously been shown by our group (25, 38, 39). Thus, the score may be used both to enhance the number of confirmed TB cases among patients with presumed TB and serve as an easily adaptable monitoring tool during the treatment or re-evaluation of these.

9 Ethical considerations

Consultative approval is expected to be granted from the Regional Ethics Committee in the Central Denmark Region, Denmark and permission to carry out the study will likewise be sought from the national ethics committees in Guinea-Bissau and Ethiopia. Written information will be provided in the official language Portuguese/Amharic and oral information will be provided to all eligible patients in the widely spoken language Portuguese Creole/Amharic. Informed written consent or a fingerprint if illiterate will be kept together with case report forms.

10 Capacity Building and future implications

The present study will serve as a platform for relevant capacity building for good clinical practice and good clinical laboratory practice in the two African sites as well as promote a stronger linkage within Africa, building on the existing links with Institutions in the EU. In addition, the capacity building and networking activities planned for this project aim to integrate African partners into the rapidly developing global network of TB trial sites; in particular it will build a resource of patients with presumed TB with detailed clinical data which is rare among TB studies in Africa(1). We have built a well-functioning infrastructure to carry out trials at the primary healthcare level, which is seldom done in TB research. We now aim to utilize this solid basis and our experience to improve TB case detection further.

11 The Nordic collaboration and study group

The Nordic collaborators in this trial have been working together since 2016. The main aim of the collaboration is to improve case finding of TB using applicable interventions in high endemic settings. Capacity building is an essential part of the collaboration and all trials make efforts to

involve existing structures and political elements in the settings in which they are carried out. Workshops will be held throughout the trial period.

Christian Morberg Wejse (CW), MD, PhD is a consultant at the Department of Infectious Diseases, Aarhus University Hospital and Professor of Cross-Cultural Medicine and Global Health at the Department of Public Health, Aarhus University. He has supervised more than 20 research projects in Guinea-Bissau.

Thomas Schön (TS), MD, PhD is a consultant of clinical microbiology and infectious diseases and a professor at the Department of Biomedical and Clinical Sciences Linköping University. He has undertaken multiple research projects in Ethiopia and Sweden. Both CW and TS have been involved in the development of the present research project and act as supervisors during the trial.

Frauke Rudolf (FR), MD, PhD is senior registrar at the Department of Infectious Diseases, Aarhus University Hospital where she is responsible for TB patients, and clinical associate professor at Aarhus University. She has completed her PhD in Guinea-Bissau, evaluating a clinical score for TB. In recent years her research has focused on improving TB case detection and assessing gender differences in TB.

Anita Zalisz, will be working on the project in the capacity of PhD-student. Anita will be responsible for supervising procedures in Bissau including data collection, data entry, as well as disseminating results.

Mulugeta Aemero (MA), professor, Head, Tropical & Infectious Diseases Research Centre, CMHSCSH, University of Gondar, Ethiopia, will be working on the project in the capacity of project collaborator in Gondar, Ethiopia.

Temesgen Tadesse (TT), MD, is a physician, who will be working on the project in the capacity of radiologist in Gondar, Ethiopia.

Segenet Bizuneh (SB), MD, is a physician, who will be working on the project in the capacity of internist in Gondar, Ethiopia.

Dessie Abebaw Angaw, Assistant Professor of Epidemiology and Biostatistics, will be working on the project in the capacity of project collaborator and supervisor in Gondar, Ethiopia.

Masresha Seyoum, BSc, MSc, Diagnostic Coordinator at UoGCSH, will be working on the project in the capacity of microbiologist in Gondar, Ethiopia.

Lilica Sanca (LS), BSc, and Ebba Abate (EA), PhD, are key personnel in Guinea-Bissau and Ethiopia respectively. LS is responsible for the national refence laboratory for TB in Guinea-Bissau while EA

has completed his PhD in Gondar, has formerly worked as Director General, Ethiopian Public Health Institute (EPHI), Ethiopia and is now Project Director for the North Africa Saving Lives and Livelihood Initiative, Project Hope Namibia, Namibia.

Armando Sifna (AS), MD, is a physician with extensive experience in TB and is part of the TB program under the ministry of health in Guinea-Bissau.

12 Exploiting and disseminating the project results

The standardised approach to TB management resulting from implementing the Bandim TBscore and improving available diagnostics is simple to communicate and translate into policy and WHO guidelines. The national partners in this project work within the national TB programs and may disseminate project results rapidly into policy changes at the national level. There are no intellectual property rights issues preventing a global dissemination of the Bandim TBscore which will be attempted through peer-reviewed publications and conference presentations.

13 References

1. WHO. GLOBAL TUBERCULOSIS REPORT 2022. 2022.
2. Theron G, Zijenah L, Chanda D, Clowes P, Rachow A, Lesosky M, et al. Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial. Lancet. 2014;383(9915):424-35.
3. Wejse C. Point-of-care diagnostics for tuberculosis elimination? Lancet. 2014;383(9915):388-90.
4. Harries AD, Kumar AMV. Challenges and Progress with Diagnosing Pulmonary Tuberculosis in Low- and Middle-Income Countries. Diagnostics (Basel). 2018;8(4).
5. Consortium CR, the GP, Allix-Beguec C, Arandjelovic I, Bi L, Beckert P, et al. Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing. N Engl J Med. 2018;379(15):1403-15.
6. Votintseva AA, Bradley P, Pankhurst L, Del Ojo Elias C, Loose M, Nilgiriwala K, et al. Same-Day Diagnostic and Surveillance Data for Tuberculosis via Whole-Genome Sequencing of Direct Respiratory Samples. J Clin Microbiol. 2017;55(5):1285-98.
7. Rudolf F, Abate E, Moges B, Mendes AM, Mengistu MY, Sifna A, et al. Increasing smear positive tuberculosis detection using a clinical score - A stepped wedge multicenter trial from Africa. Int J Infect Dis. 2021;113 Suppl 1:S55-S62.
8. Steingart KR, Ng V, Henry M, Hopewell PC, Ramsay A, Cunningham J, et al. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis. 2006;6(10):664-74.
9. Datta S, Shah L, Gilman RH, Evans CA. Comparison of sputum collection methods for tuberculosis diagnosis: a systematic review and pairwise and network meta-analysis. Lancet Glob Health. 2017;5(8):e760-e71.
10. Cuevas LE, Al-Sonboli N, Lawson L, Yassin MA, Arbide I, Al-Aghbari N, et al. LED fluorescence microscopy for the diagnosis of pulmonary tuberculosis: a multi-country cross-sectional evaluation. PLoS Med. 2011;8(7):e1001057.
11. Mesman AW, Calderon RI, Pollock NR, Soto M, Mendoza M, Coit J, et al. Molecular detection of Mycobacterium tuberculosis from buccal swabs among adult in Peru. Sci Rep. 2020;10(1):22231.
12. Nash M, Kadavigere R, Andrade J, Sukumar CA, Chawla K, Shenoy VP, et al. Deep learning, computer-aided radiography reading for tuberculosis: a diagnostic accuracy study from a tertiary hospital in India. Sci Rep. 2020;10(1):210.
13. Harris M, Qi A, Jeagal L, Torabi N, Menzies D, Korobitsyn A, et al. A systematic review of the diagnostic accuracy of artificial intelligence-based computer programs to analyze chest x-rays for pulmonary tuberculosis. PLoS One. 2019;14(9):e0221339.
14. Gustafson P, Gomes VF, Vieira CS, Jensen H, Seng R, Norberg R, et al. Tuberculosis mortality during a civil war in Guinea-Bissau. JAMA. 2001;286(5):599-603.
15. Gustafson P, Gomes VF, Vieira CS, Rabna P, Seng R, Johansson P, et al. Tuberculosis in Bissau: incidence and risk factors in an urban community in sub-Saharan Africa. Int J Epidemiol. 2004;33(1):163-72.
16. Gustafson P, Gomes VF, Vieira CS, Samb B, Naucler A, Aaby P, et al. Clinical predictors for death in HIV-positive and HIV-negative tuberculosis patients in Guinea-Bissau. Infection. 2007;35(2):69-80.

17. Gustafson P, Lisse I, Gomes V, Vieira CS, Lienhardt C, Naucler A, et al. Risk factors for positive tuberculin skin test in Guinea-Bissau. Epidemiology. 2007;18(3):340-7.
18. Lienhardt C, Fielding K, Sillah JS, Bah B, Gustafson P, Warndorff D, et al. Investigation of the risk factors for tuberculosis: a case-control study in three countries in West Africa. Int J Epidemiol. 2005;34(4):914-23.
19. Bohlbro AS, Mendes AM, Sifna A, Gomes V, Rudolf F, Wejse C. Incidence of pulmonary tuberculosis in suburban Bissau, Guinea-Bissau between 2004 and 2020: a prospective cohort study. Infection. 2023;51(4):955-66.
20. Lemvik G, Rudolf F, Vieira F, Sodemann M, Ostergaard L, Rodrigues A, et al. Decline in overall, smear-negative and HIV-positive TB incidence while smear-positive incidence stays stable in Guinea-Bissau 2004-2011. Trop Med Int Health. 2014;19(11):1367-76.
21. TWB. Tuberculosis case detection rate (%, all forms) 2020. 2020.
22. WHO. The World Health Organization. TB country, regional and global profiles.
23. Hussey MA, Hughes JP. Design and analysis of stepped wedge cluster randomized trials. Contemp Clin Trials. 2007;28(2):182-91.
24. Wejse C, Gustafson P, Nielsen J, Gomes VF, Aaby P, Andersen PL, et al. TBscore: Signs and symptoms from tuberculosis patients in a low-resource setting have predictive value and may be used to assess clinical course. Scand J Infect Dis. 2008;40(2):111-20.
25. Rudolf F, Lemvik G, Abate E, Verkuilen J, Schon T, Gomes VF, et al. TBscore II: refining and validating a simple clinical score for treatment monitoring of patients with pulmonary tuberculosis. Scand J Infect Dis. 2013;45(11):825-36.
26. Monkongdee P, McCarthy KD, Cain KP, Tasaneeyapan T, Nguyen HD, Nguyen TN, et al. Yield of acid-fast smear and mycobacterial culture for tuberculosis diagnosis in people with human immunodeficiency virus. Am J Respir Crit Care Med. 2009;180(9):903-8.
27. Anagaw B, Mulu A, Abate E, Anagaw B, Belay T, Gelaw A, et al. Improved detection of acid-fast bacilli in sputum by the bleach-concentration techinique at Gondar University Teaching Hospital, northwest Ethiopia. Ethiop Med J. 2012;50(4):349-54.
28. Steingart KR, Henry M, Ng V, Hopewell PC, Ramsay A, Cunningham J, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. Lancet Infect Dis. 2006;6(9):570-81.
29. WHO. Practical manual of processing stool samples for diagnosis of childhood TB. 2022.
30. Wilkinson D, Newman W, Reid A, Squire SB, Sturm AW, Gilks CF. Trial-of-antibiotic algorithm for the diagnosis of tuberculosis in a district hospital in a developing country with high HIV prevalence. Int J Tuberc Lung Dis. 2000;4(6):513-8.
31. Harries A, Maher D, Graham S. TB/HIV: a clinical manual 2004 [updated 2004. 4th:[24-]. Available from: http://whqlibdoc.who.int/publications/2004/9241546344.pdf.
32. Zifodya JS, Kreniske JS, Schiller I, Kohli M, Dendukuri N, Schumacher SG, et al. Xpert Ultra versus Xpert MTB/RIF for pulmonary tuberculosis and rifampicin resistance in adults with presumptive pulmonary tuberculosis. Cochrane Database Syst Rev. 2021;2:CD009593.
33. Cattamanchi A, Huang L, Worodria W, den Boon S, Kalema N, Katagira W, et al. Integrated strategies to optimize sputum smear microscopy: a prospective observational study. Am J Respir Crit Care Med. 2011;183(4):547-51.
34. Hemming K, Girling A. A menu-driven facility for power and detectable-difference calculations in stepped-wedge cluster-randomized trials. The Stata Journal. 2014;14(2):363-80.

35. Hoa NB, Tiemersma EW, Sy DN, Nhung NV, Vree M, Borgdorff MW, et al. Health-seeking behaviour among adults with prolonged cough in Vietnam. Trop Med Int Health. 2011;16(10):1260-7.
36. Senkoro M, Hinderaker SG, Mfinanga SG, Range N, Kamara DV, Egwaga S, et al. Health care-seeking behaviour among people with cough in Tanzania: findings from a tuberculosis prevalence survey. Int J Tuberc Lung Dis. 2015;19(6):640-6.
37. Yimer S, Holm-Hansen C, Yimaldu T, Bjune G. Health care seeking among pulmonary tuberculosis suspects and patients in rural Ethiopia: a community-based study. BMC Public Health. 2009;9:454.
38. Rabna P, Andersen A, Wejse C, Oliveira I, Gomes VF, Haaland MB, et al. Utility of the plasma level of suPAR in monitoring risk of mortality during TB treatment. PLoS One. 2012;7(8):e43933.
39. Rudolf F. The Bandim TBscore--reliability, further development, and evaluation of potential uses. Glob Health Action. 2014;7:24303.