RIA Diagnostics

2nd European & Developing Countries
Clinical Trials Partnership Programme
(EDCTP2)

Grant Agreement Number DRIA2014-311

Evaluation of host biomarker-based point-of-care tests for targeted screening for active TB

Deliverable Nr. 2.1

Study protocol

Contractual delivery date:

Month 3 (30/06/2016)

Actual delivery date:

Month 3 (30/06/2016)

Responsible partner:

P1: Stellenbosch University (SUN)

Deliverable number	D2.1
Deliverable title	**Study Protocol**
Nature	R
Dissemination level	Co
Work package number	WP 2
Work package leader	SUN
Author(s)	Dr Elizna Maasdorp
Keywords	Study Protocol

The research leading to these results has received funding from the EDCTP2 programme supported by the European Union under grant agreement No DRIA2014-311.

Deliverable 2.1, the study protocol has been achieved.

A generic protocol was developed by the Stellenbosch University team for distribution to other sites. Other sites are to adapt this protocol according to local circumstances and requirements, for submission to their various regulatory bodies.

The protocol has been circulated to all the sites on 5 May 2016.

Please find attached the generic draft circulated.

Title:

Evaluation of host biomarker-based point-of-care tests for targeted screening for active TB

Acronym:

Screen TB

Protocol Number: to be allocated

Sponsored by:

The European & Developing Countries Clinical Trials Partnership (EDCTP)

Principal Investigator:

Prof Gerhard Walzl

Version Number:

0.3

Date:

05/05/2016

Protocol Summary

Title: Evaluation of host biomarker-based point-of-care tests for targeted screening for active TB (Screen TB)

Population: A total of 800 people presenting at primary health care clinics with presumed active pulmonary tuberculosis, aged 18 to 70 years, male or female gender, will be recruited. They should be willing to give informed, written consent, including consent for HIV testing. They should have symptoms that could be compatible with active TB (cough > two weeks, plus at least one of the following: fever, weight loss, hemoptysis and night sweats). Participants should not have been on TB treatment for the past 90 days and should not have received immune suppressive therapy, be known with alcohol of drug abuse or have a haemoglobin level <10g/dl. HIV co-infection is not an exclusion criterion. Participants will be recruited from primary health care clinics in Cape Town, South Africa, Windhoek in Namibia, Addis Ababa in Ethiopia, Banjul in The Gambia and Kampala in Uganda.

Number of Sites: See Section 1

Study Duration: 3 years

Subject Duration: 6 months for TB cases, 2 months for non-TB cases

Objectives:

The overall objective of the study is to incorporate a six-marker serum signature into a multiplex UCP-LFA format, referred to as TransDot, for finger-prick blood testing. The end point of the study is the accuracy (sensitivity and specificity) of the UCP-LFA TransDot test on finger-prick blood for active TB and will be prospectively compared against gold standard composite diagnostic criteria (GeneXpert, MGIT culture, TB sputum smear, CXR, TB symptom screen and response to TB treatment).

Primary:

• The primary outcome of interest will be accuracy, sensitivity and specificity of the TransDot finger-prick test when compared with the composite gold standard tests.

Secondary:

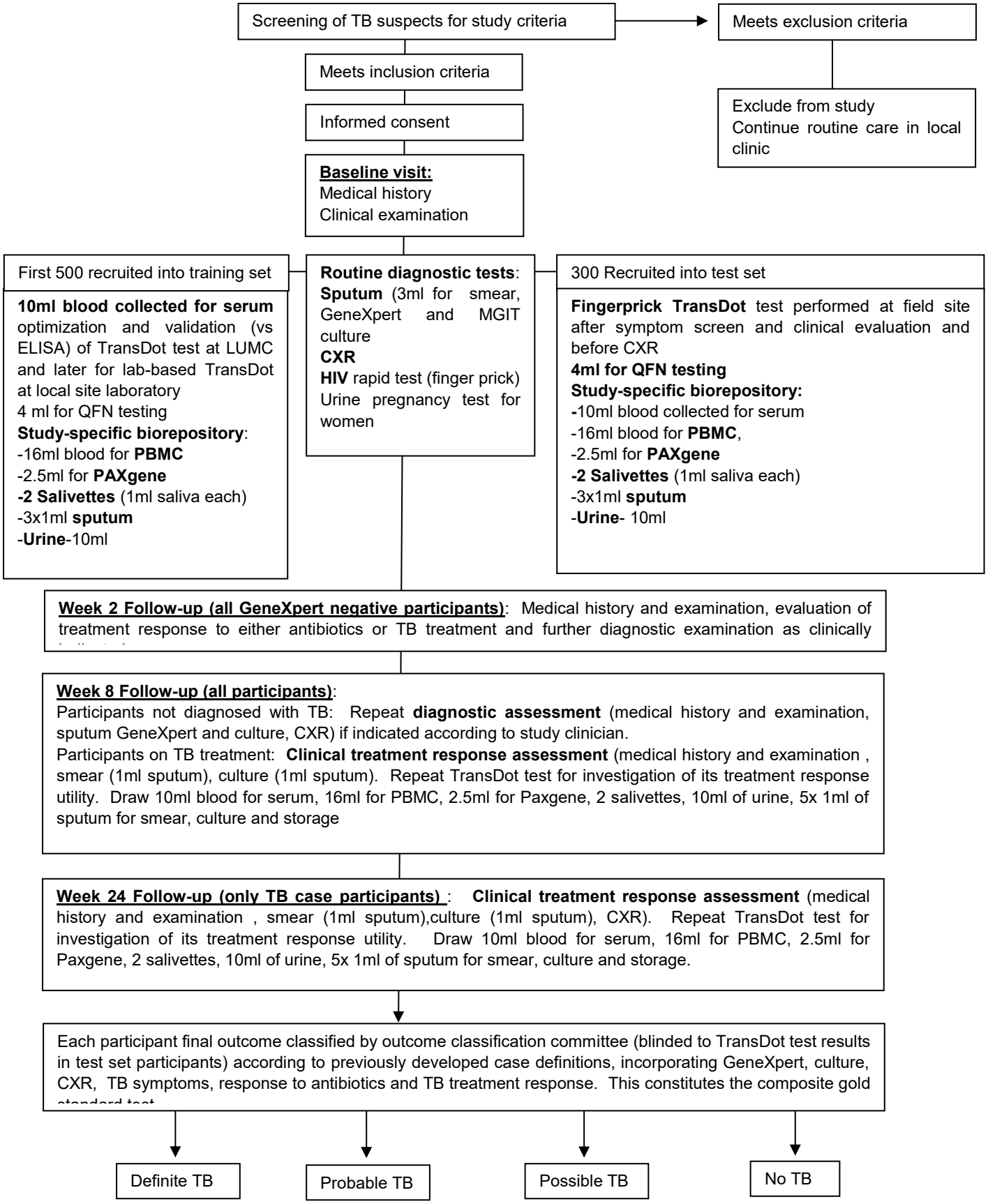
• To evaluate the agreement between the POC TransDot test and laboratory based ELISAs first (both on serum), and subsequently between POC TransDot (on fingerprick blood) and laboratory based TransDot (on serum).

• To investigate the utility of a TransDot test at month 2 and month 6 as a marker of treatment response.

• To identify additional host marker signatures that can be utilized for future improvement of diagnostic tests in the TransDot format or other point-of care tests that might become available in the future

• To collect appropriate additional host samples for future biomarker research.

Schematic of Study Design:



1. KEY ROLES

Individuals Stellenbosch University	Roles	Name	Qualification
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	Research nurses	Shirley McAnda	Dip Nursing
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Collaborating Institutions			
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	MRC, The Gambia		
	University of Namibia, Windhoek, Namibia		
	Makerere University School of Medicine, Kampala, Uganda		
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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION

Tuberculosis (TB) is a leading cause of death and places severe pressure on health care services of the developing world. Despite the introduction of the highly sensitive and specific GeneXpert MTB/RIF (GeneXpert) test [1] with a potential turn-around time of two hours, many people in high TB prevalence areas still do not have access to efficient TB diagnostic services due to logistical constraints that plague these settings.

Centralized laboratory placement of GeneXpert instruments necessitate transportation of sputum samples, which means additional clinic visits before initiation of treatment, often leading to loss to follow-up. Twenty-six percent of patients with positive sputum microbiology are not started on treatment, partly due to the need for multiple health care visits [2]. The relatively high cost of both instrument and cartridges are also problematic. Liquid culture is even less accessible and takes up to 42 days to deliver a result while sputum smear microscopy has a low sensitivity of 60% [3]. Microbiological tests are less sensitive in human immune-deficiency virus (HIV) co-infection [4] and are less suitable in young children, in whom it can be difficult to obtain sputum. The high burden of undiagnosed TB fuels ongoing transmission and poor treatment outcomes [5].

Although the policy of indiscriminate TB case finding by mobile mass chest X-rays (CXR) has not been successful and was abandoned decades ago, a recent World Health Organisation (WHO) document has revisited the concept of targeted CXR screening for active pulmonary TB, with strong screening recommendations for several high risk groups, including close contacts of TB cases, people living with HIV, residents of high TB prevalence areas or remote areas with poor access to health care [6]. CXR has high sensitivity (98%; 95% CI 95-100%) but lower specificity (75%, 95% CI 72-79%) [6]. Availability of X-rays in resource-limited settings is even less than for GeneXpert, requires skilled personnel, is relatively expensive (> 10 Euros/CXR) and is therefore not an optimal screening tool.

A cost effective, rapid, inexpensive, point-of-care screening test with high sensitivity and high negative predictive value would identify people with a high likelihood for active TB and would prioritize them for testing with more expensive and technically or logistically demanding assays including GeneXpert or liquid culture, and thus facilitate cost-effective diagnostic work-up in resource-limited settings.

2.2 SCIENTIFIC RATIONALE

Host serum protein biomarkers that indicate a high likelihood for active TB disease represent attractive targets for integration into screening tests. The EDCTP 1-funded African European Tuberculosis Consortium (AE-TBC) has previously investigated host biosignatures in whole blood culture supernatants after overnight stimulation with *Mycobacterium tuberculosis* (MTB) specific proteins. During this project >1,300 people from high TB prevalence areas (South Africa, Namibia, Malawi, The Gambia, Uganda and Ethiopia) with symptoms suggestive of active TB were recruited and worked up diagnostically with CXR, sputum culture, smear and GeneXpert. One third of the participants had confirmed active TB and 26% were HIV co-infected. A no-go decision was taken regarding the whole blood MTB stimulated culture signature as low sensitivity (75 - 80%) with specificity of 86% was deemed insufficient for further development and as an overnight assay is not suitable for rapid point of care (POC) testing.

However, a promising serum host inflammatory signature was identified during this project after investigation of more than 70 serum host inflammation markers, including acute phase proteins, T helper cell 1, T helper cell 2 and regulatory cytokines, soluble cytokine receptors and growth factors. The original set of markers for screening were chosen according to the availability of multiplexed assays (Luminex platform) and their known roles in inflammation and represented a wide spectrum of markers with diagnostic potential. The most promising host serum protein signature was subsequently validated on 687 people from five African countries with suspected TB, regardless of HIV infection status or ethnicity, providing the basis for the follow-up work suggested herein. The six-analyte signature of C-reactive protein (CRP), Interferon gamma (IFN-γ), pre-albumin, complement factor H (CFH), apolipoprotein A1 and inducible protein 10 (IP-10) ascertained TB disease with a sensitivity of 89% (CI 78 – 95%) and specificity of 76% (CI 68 – 83%), (positive predictive value of 61%; negative predictive value of 94%) ([7], [8]). Measurement of multiple markers and evaluation of their ratios offset a lack of disease specificity of individual markers. These performance characteristics would constitute a valuable screening test if converted into an inexpensive POC test and would prioritize individuals with a high likelihood of active TB for confirmatory testing by GeneXpert.

During the AE-TBC project, partner Leiden University Medical Centre (LUMC) developed and validated a user-friendly lateral flow assay (LFA) for simultaneous detection of multiple host TB biomarkers (cytokines and antibodies) in blood or other body fluids [9, 10]. These LFAs are likely to assist in rapid TB diagnosis in low-resource settings and were tested in the AE-TBC field sites. Importantly, our test formats utilize novel, nano-sized upconverting phosphor (UCP) reporter particles as read-out [11, 12, 13]. The UCP-technology delivers flexibility with respect to sensitivity and robustness as the ultrasensitive fluorescent label is not hampered by common auto-

fluorescence background. Moreover, the label does not fade, allowing UCP-test strips to be stored indefinitely for reanalysis. The LFA can also be modified for measurement of markers in fingerprick blood, which will further enhance its POC utility.

The overall objective of the study is to incorporate the six-marker signature into an UCP-LFA format, the TransDot assay, enabling fingerprick blood testing. The end point of the study is the accuracy (sensitivity and specificity) of the UCP-LFA TransDot test on fingerprick blood for active TB and will be prospectively compared against composite gold standard diagnostic criteria of GeneXpert, mycobacterial growth indicator tube (MGIT) culture, TB sputum smear, CXR, TB symptom screen, response to antibiotics and response to TB treatment).

2.3 POTENTIAL RISKS AND BENEFITS

2.3.1 POTENTIAL RISKS

Potential risks are limited to the minor risks associated with phlebotomy and the small risk of subject confidentiality being compromised. As this study does not involve any intervention, we do not expect to add any additional risks other than the ones inherent to standard of care, to participants.

2.3.2 KNOWN POTENTIAL BENEFITS

Participants will be investigated for tuberculosis more thoroughly than during standard of care and will therefore benefit from a more certain diagnosis. They will also be followed up for 6 months and have additional investigations during follow up and at end of treatment, which will guide their further care. They will be referred to the appropriate level of care for any incidental findings during investigation, like malignancy, which would most likely not have been found at that stage in other circumstances.

3 OBJECTIVES

Primary objective:

To prospectively evaluate the accuracy (sensitivity and specificity) of the UCP-LFA TransDot fingerprick test (hereafter called TransDot) in a laboratory-free manner, based on the six marker serum biosignature as a screening test for active TB, as compared to a composite gold standard (GeneXpert, MGIT culture, chest X-ray, TB symptom screen, response to antibiotics and response to TB treatment) in a population of TB suspects from resource-poor, high TB incidence settings in South Africa, Uganda, The Gambia, Namibia and Ethiopia.

Secondary objectives:

To evaluate the agreement between the TransDot test and laboratory based ELISAs first (both on serum), and subsequently between the TransDot (on fingerprick blood) and laboratory based TransDot (on serum).

To investigate the utility of a TransDot test at month 2 and month 6 as a marker of treatment response.

To identify additional host marker signatures that can be utilized for future improvement of diagnostic tests in the TransDot format or other point-of care tests that might become available in the future.

To collect appropriate additional host samples for future biomarker research.

4. STUDY DESIGN

A non-interventional, diagnostic, prospective cohort design will be utilized, incorporating two separate sets of participants – overall a training set of 500 and a test set of 300 participants. The purpose of the training set will be to optimize the TransDot test and Reader to excellent sensitivity (90% or above) and acceptable specificity (80%). The test set will then be utilized to evaluate the TransDot test accuracy on fingerprick blood at the point-of-care site in a laboratory-free manner on a new prospective cohort of participants.

Training set participants will be recruited and receive investigations for TB. Blood samples will also be collected from them for performance of ELISAs and laboratory-based TransDot tests. These blood samples will be drawn at baseline, week 8 and week 24 at end of treatment for confirmed TB cases and at baseline and for non-TB cases.

Test set participants will be recruited and receive investigations for TB. A POC TransDot test will be performed on fingerprick blood at baseline, and at week 8 and week 24 in participants on TB treatment, as well as a laboratory based TransDot test on serum at baseline. The week 8 and week 24 TransDot tests will be used to investigate the test's utility as an indicator of treatment response.

Follow up of both sets of participants will be to week 24 for those found to have active TB, and week 8 for those found to be non-TB cases. There will be study visits at baseline, week 2 (GeneXpert negative participants only), week 8 and week 24 (participants on TB treatment only).

The TransDot test will be evaluated against a composite gold standard in the form of previously developed case definitions, consisting of GeneXpert, MTB culture, CXR, TB symptoms, response to antibiotics and TB treatment response. The case definitions will be applied to participant data by an outcome classification committee (see 4.2) who will remain blinded to the TransDot results of the test set, until after completion of all participants' outcome classification.

The primary outcome of interest will be performance of the TransDot test as compared to the gold standard, measured by sensitivity and specificity.

4.1 STUDY PHASES

The study will be conducted in three phases. During the first phase a blood POC test using UCP-LFA TransDot [14] technology will be developed by LUMC, incorporating six serum markers, CRP, IFN-γ, pre-albumin, complement factor H, apolipoprotein A1 and IP-10. During this phase optimal antibody pairs for the lateral flow strips will be evaluated. In the event of a lack of optimal test performance for individual markers, replacement markers will be selected according to ongoing marker evaluation at the respective sites.

The TransDot test device technology will be optimized using serum from a subset of the first 500 recruited participants. These samples will be shipped to LUMC.

During a second phase of the study, TransDot devices will be available in the African laboratories, to test the remaining training set participants' serum locally. The first two phases will aim to optimise the sensitivity and specificity of the TransDot test.

The third phase will be the evaluation of the TransDot test in the field setting, by research nurses testing test set participants' fingerprick blood.

4.2 EVALUATION OF TB DIAGNOSIS

An outcome classification committee will classify each participant according to the standard case definitions below. The committee will consist of clinician investigators from the participating sites. They will assign a classification of Definite TB, Probable TB, Possible TB or non-TB (see table below) to each participant, as soon as possible after the week 8 follow up time point. Participants started on TB treatment at clinics will be followed up for 6 months to evaluate treatment response. During phase 3 of the study, the committee will remain blind to the point of care TransDot test results on the test cohort, until outcome classification is complete.

The case definitions will be applied as shown in the table below:

Pos = positive; neg = negative; con = contaminated; GR = good response; PR = poor response; NG = none given									
Category	**Culture**	**GeneXpert**	**AFB smear**	**CXR**	**Symptoms**	**TB Treatment started by clinic**	**TB Treatment response**	**Response to antibiotic (AB), if prescribed**	**Class**
A	pos				pos				Definite
B	neg or con	pos (no TB in past year)			pos				Definite
C	neg or con	neg or pos (TB in past year)	pos	pos	pos	yes	GR		Definite
D	neg or con	neg or pos (TB in past year)	pos	neg	pos	yes	GR		Probable
E	neg or con	neg or pos (TB in past year)	neg	pos	pos	yes	GR		Probable
F	neg or con	neg or pos (TB in the past year)	neg	pos	pos	No		PR	Possible
G	neg or con	pos (TB in past year)	neg	neg	pos	yes	GR		Possible
H	neg	neg	neg	pos	pos	no		GR	No TB
I	neg	neg	neg	neg	pos	no		NG	No TB
J	Not done or con	Not done	Not done						Exclude
K	Not done or con	pos (TB in past year)	pos or neg	neg or not done	pos	yes	PR		Exclude
L	Not done or contam	Pos TB in past year	pos or neg	neg or not done	pos	no	NG	PR	Exclude

Definitions:

TB symptoms or signs: symptoms volunteered or specifically elicited by interviewer and signs objectively observed by examiner that include

Cough ≥ 2 weeks; *Fever* (subjective, present ≥ 2 weeks); *Weight loss* (significant, unintentional loss of ≥ 10% of body weight in last 6 months. If no previous weight available, the patient's judgement of weight loss will be used); *Haemoptysis* (blood stained to blood clots); *Wasting* (clinical finding of examining health care professional) ; *Night sweats* (drenching of sleeping clothes or bedding, not related to environment); *Adenopathy* (clinical finding of significantly enlarged lymph nodes by examining health care professional), of particular significance in HIV co-infected individuals; *Other:* may be considered in exceptional cases and on individual basis by case review team and include shortness of breath and chest pain

Sputum culture positive: liquid or solid agar positive with confirmed speciation for *M. tuberculosis* complex

CXR compatible with active TB: CXR pattern regarded by research clinician as typical for adult TB (any of the following: cavitation, diffuse upper lobe or mid-zone infiltrates, including air-space nodules or clustered nodules, consolidation in upper or mid zones, unilateral or bilateral lymph node enlargement, particularly in HIV co-infected participants, pleural effusion). CXR pattern not typical for adult TB (diffuse infiltrates in lower lobe, miliary type infiltrate) may be included after consultation of the outcome classification committee.

TB treatment response: baseline symptom improvement of ≥50%, weight gain, sputum smear or culture conversion, CXR improvement after at least two months of TB treatment.

- **Failed antibiotic (AB) treatment**: No response of symptoms to a 7 day, broad spectrum oral or intravenous antibiotic

5. STUDY POPULATION

5.1 SELECTION OF THE STUDY POPULATION

Adult participants with presumed active TB disease will be recruited in South Africa, the Gambia, Uganda, Namibia and Ethiopia. Each site will recruit approximately 160 participants with suspected TB, but faster recruiting sites may recruit more and slower recruiting sites fewer, until the desired overall total of about 800 participants is reached. In South Africa, up to 300 participants will be recruited from primary health care clinics in Cape Town.

Patients presenting to the health care facility with symptomatic pulmonary disease and a high likelihood of having tuberculosis will be enrolled and followed up for outcome classification.

5.2 INCLUSION/EXCLUSION CRITERIA

Inclusion criteria

1. Symptoms suggestive of TB disease: cough for more than two weeks, fever, malaise, weight loss, night sweats, haemoptysis, chest pain, loss of appetite.
2. Willingness to give consent to take part in the study.
3. Willingness to undergo HIV testing or be willing to have their HIV infection status disclosed to the study field workers.
4. Eighteen years or older and aged 70 years or younger.

Exclusion criteria

1. Permanent residence in study area for less than 3 months or with no permanent address.
2. Pregnancy or breastfeeding.
3. HB<9g/l
4. On TB treatment currently or in the last ninety days.
5. HIV positive patients currently on INH prophylaxis, or in the last ninety days.
6. Known quinolone or aminoglicozide antibiotic use reported in the past 60 days.

Patients who had previous TB and patients with extra-pulmonary TB in addition to pulmonary TB or other concomitant diseases will not be excluded. Patients with drug resistance detected on GeneXpert or culture will not be excluded from the primary analysis. Both HIV positive and HIV negative individuals will be enrolled.

6 STUDY PROCEDURES/EVALUATIONS

6.1 STUDY PROCEDURES

Patients who present to a primary health care clinic with pulmonary symptoms and who are presumed to have TB disease, will be approached to participate in the study by research recruiters or nurses. Sputum results from the National Health Laboratory System may also be accessed to identify patients who were investigated for TB.

Participants will be recruited into either the training or the test cohort, with the test cohort recruitment starting after the training cohort has completed the enrolment of 500 participants. Participants will be required to attend the baseline visit, a week 2 visit (to review results and antibiotic response in those not already diagnosed with TB at this point), a week 8 visit (all participants, to assess TB treatment response in TB cases and to assess alternative diagnosis or resolution of symptoms in non-TB cases) and a week 24 visit (TB participants only, to assure TB was treated successfully).

Willing and eligible patients will be informed about the study by a research nurse, guided by the informed consent document, and asked to sign the document, after being given the opportunity to ask questions. The participant will receive a copy of the informed consent form, while the original consent form will be filed in the regulatory file and another copy will be filed in the participant's file.
Each recruited participant will be assigned a unique study ID number by the recruiting nurse.
Personal and demographic information on each participant will be collected by the nurse using an electronic case report form on a tablet computer. Information such as name, address, telephone numbers, other contact person, age, sex, ethnicity, language and employment will be collected and uploaded to a secure, password protected and centralized database hosted at Stellenbosch University.

Standardized TB symptoms case report forms and medical history case report forms will be completed for all participants at each visit by the research nurse and recorded electronically via the tablet into the database. Information collected will include TB symptoms presence, severity and duration, previous TB episodes and treatment outcome, other medical conditions, medication use, HIV status (if known), previous surgery, family history, smoking history, alcohol consumption and recreational drug usage.

A directed physical examination will be performed at each visit and recorded in the physical examination case report form via the tablet into the database.

Sputum for smear, GeneXpert and TB culture will be collected at the baseline, week 8 and week 24 visits by asking the participant to cough and collect expectorated sputum in a sputum container, while seated at a sputum collection booth, which is fitted with a high-efficiency particulate arrestance (HEPA) filter for infection control purposes.

CXRs will be performed on all participants at baseline. At week 8 and 24 only those who were diagnosed on CXR, where the radiographic treatment response pattern is required and those in whom a follow-up CXR is ordered by a clinician for clinical reasons, will have follow-up x-rays.

Any clinical information or test results that could potentially impact a participant's clinical care, will be shared with the participant's local clinic.

Rapid HIV tests will be performed on all participants with previously negative or unknown HIV status, after pre-test counselling. Results will be given to participants, along with post-test counselling. A negative result will be considered negative, while a positive result will be confirmed by a second rapid test from a different manufacturer. Incongruent results will finally be confirmed by an HIV Elisa. Newly diagnosed HIV infected participants will receive a CD4 count and be referred to their local clinic for possible antiretroviral treatment.

Blood, sputum, urine and saliva samples will be collected at baseline, week 8 and 24, and additionally at week 2 if clinically indicated for TB diagnosis.
The table below outlines the sample types, volumes, frequency and other investigations performed.

Sample collection and Investigations performed						
			Participant group and Time Point			
			Baseline	Week 8		Week 24
Sample type	Processing and analysis destination	Sample volume	All	TB arm	Non-TB arm	TB arm
Whole blood in Serum Seperator Tube (SST)	Serum separation for ELISA, Luminex, TransDot optimisation and laboratory-based evaluation, storage in study specific bio-repository	10 ml	Yes	Yes	Yes	Yes
Whole blood in Sodium Heparin tube	Peripheral blood mononuclear cell isolation for future biomarker discovery work	2 x 8 ml = 16ml	Yes	Yes	No	Yes
Whole blood 4 QFN tubes	Quantiferon Gold test	4 x 1ml = 4ml	Yes	No	No	No
Whole blood in PAXgene tube	Future whole blood RNA signature discovery work	2.5ml	Yes	Yes	No	Yes
Fingerprick blood	HIV rapid test	1 to 2 drops	Yes	No	No	No
Whole blood in EDTA tube	CD4 count	2 ml	New HIV diagnosis only	No	No	No
Fingerprick blood	POC TransDot test in Test set participants	1 to 2 drops	Yes (Test set)	Yes (Test set)	No	Yes (Test set)
TOTAL BLOOD VOLUME	Total volume over 24 weeks for TB arm: 89.5 ml (+2 ml for CD4 in some) Total volume over 8 weeks for non-TB arm: 42.5 ml (+ 2ml for CD4 in some)		32.5ml (+ 2ml for CD4 in some)	28.5 ml	10 ml	28.5 ml
Sputum	AFB smear	1 ml	Yes	Yes	Only if clinically indicated	Yes
Sputum	GeneXpert	1 ml	Yes	No	Only if clinically indicated	No
Sputum	Culture	1 ml	Yes	Yes	Only if clinically indicated	Yes
Sputum	Future sputum-based diagnostic or treatment response investigations, including GeneXpert Ultra and new culture techniques	2 x 1ml for storage	Yes	Yes	No	Yes
Induced sputum (hypertonic saline nebulisation)	Diagnostic or Future sputum-based diagnostic or treatment response investigations, including GeneXpert Ultra and new culture techniques	Up to 5ml as necessary	If not enough sputum spontaneously	If not enough sputum spontaneously	No	If not enough sputum spontaneously
Saliva from two salivettes chewed	Stored for future biomarker discovery work	2 x 1ml	Yes	Yes	No	Yes
Urine	Stored for future biomarker work	10 ml	Yes	Yes	No	Yes
Urine	Urine pregnancy test in women	Dipstix	Yes, women	No	No	No
CXR	Diagnosis and treatment response evaluation		Yes	Only if indicated for treatment response	Only if clinically indicated	Only if indicated for treatment response

Sample types and investigations per timepoint

Fingerprick TransDot test

For test set participants, serum will be collected for local laboratory based TransDot tests. POC TransDot tests will also be performed on fingerprick blood by the research nurse in a standardized manner across sites, at the baseline visit and before any results from the composite gold standard tests are available. A drop of blood will be applied to the test strip and the test strip will be submitted to the reader. The reported result will be recorded in the case report form. Agreement between the laboratory based and point of care TransDot tests will be calculated. Study investigators will be blinded to the TransDot test results until the final outcome classification has been completed for each participant. TransDot test results will also not be shared with clinic staff and no clinical decisions will be made based on TransDot test results.

A TransDot test will also be done at weeks 8 and 24 to investigate the utility of the test for monitoring treatment response as a secondary outcome. It will be compared to week 8 and 24 treatment outcomes as defined by sputum results, radiology, where applicable and TB symptoms.

It is expected that approximately one third of the participants will have a final diagnosis of active TB, whereas the non-TB cases will have a range of conditions including viral and bacterial lower respiratory tract infections and non-infectious conditions like chronic obstructive pulmonary disease.

The decision to treat for TB and the administration of treatment will remain the responsibility of the national healthcare system, although all relevant results to aid the decision will be shared with clinic staff. Referrals to the appropriate level of care for any accidental discoveries of other conditions or positive HIV results will also be done by the research team.

Participants at Stellenbosch University will receive R150 for participation in the study to compensate for travel expenses and loss of hours at work.

6.2 STUDY SCHEDULE:

Baseline visit (all participants)

Obtain written informed consent.

Assign unique study participant ID.

Conduct TB symptoms questionnaire.

Obtain relevant medical history.

Perform directed physical examination.

Collect sputum samples for smear (except if results are obtainable from the government clinic), GeneXpert, MGIT culture and storage.

Perform chest x-ray.

Perform pre- and post-test counselling for HIV test, except in participants who disclose their known positive status to study nurses.

Collect 10ml blood for serum separation for laboratory based TransDot testing (Training and Test cohort).

Collect 4ml for Quantiferon (QFN) Gold test

Collect 16ml of blood for peripheral blood mononuclear cell (PBMC) isolation.

Collect 2.5ml of whole blood into PAXgene tubes for the study-specific biorepository.

Collect 2ml of saliva into the study-specific biorepository.

Collect 10ml urine into the study-specific biorepository.

Perform POC TransDot test and record the result (Test cohort).

Assure that all participants with symptoms of TB have attended the government clinic to obtain routine TB investigations.

Week 2 visit (only participants not diagnosed with TB by the baseline visit GeneXpert or sputum smear)

Conduct TB symptoms questionnaire and judge whether any symptoms improved or resolved on course of antibiotics for GeneXpert or smear negative patients.

Week 8 visit (all participants)

Conduct TB symptoms questionnaire.

Obtain relevant medical history.

Perform directed physical examination.

Perform CXR (only when indicated for treatment response).

Collect sputum samples for TB sputum smear (except if smear result is available from the government clinic), MGIT culture and storage (only participants on TB treatment or those for whom it is clinically indicated).

Collect 10 ml blood for serum separation for laboratory based TransDot testing (Training cohort).

Collect 16ml of blood for PBMC isolation (participants on TB treatment only).

Collect 10ml urine into the study-specific biorepository (participants on TB treatment only).

Perform POC TransDot test and record the result (Test cohort participants on TB treatment only).

Week 24 visit (only participants who started TB treatment)

Conduct TB symptoms questionnaire.

Obtain relevant medical history.

Perform directed physical examination.

Collect sputum samples for TB sputum smear (except if smear result is available from the government clinic), MGIT culture and storage.

Perform induced sputum if inadequate sputum volumes are available.

Perform CXR (only when indicated for treatment response).

Collect 10 ml blood for serum separation for laboratory based TransDot testing (Training and Test cohort).

Collect 16ml of blood for PBMC isolation.

Collect 2.5ml PAXgene tube for study-specific biorepository.

Collect 2ml saliva for study-specific biorepository.

Collect 10ml urine into the study-specific biorepository.

Perform TransDot test and record the result (Test cohort).

6.3 LABORATORY EVALUATIONS

6.3.1 LABORATORY EVALUATIONS/ASSAYS

Sputum will be collected according to standard protocols for GeneXpert (1ml), mycobacterial growth indicator tube (MGIT) (1ml) and performance of direct smears for acid-fast bacilli (1ml), if smear results cannot be accessed from department of health facilities. Any remaining sputum will be used either for cryostorage for the study-specific biorepository (we will aim for 2x1ml aliquots) or performance of new microbiological tests, including GeneXpert Ultra and the detection of extracellular mycobacterial deoxyribonucleic acid (DNA). We will share the findings of our routine diagnostic tests with the clinics, who will remain responsible for treatment decisions and management.

Any future use of sputum samples will be restricted to detection of mycobacterial or host molecules in the context of TB diagnostic or biomarker research.

6.3.2 SPECIMEN COLLECTION, PREPARATION, HANDLING AND SHIPPING

Serum (approximately 5ml) will be obtained through centrifugation of 10ml of whole blood. Serum aliquots of 500µl each will be stored at minus 80 °C until either shipment to Leiden University Medical Centre for direct comparison between TransDot tests and ELISA or Luminex for host markers or investigation by multiplex cytokine array technology or ELISA for host markers with diagnostic potential.

Peripheral blood mononuclear cells will be isolated through the Histopaque/Ficoll Density Media method and stored in liquid nitrogen until further use. The future experiments will be aimed at detection of surface markers or intracellular markers on different immune cells to investigate differences between active TB cases and non-TB cases and to track changes during treatment.

PAXgene tubes will be stored at minus 80 °C until use. RNA will subsequently be extracted to investigate the gene expression profile of immune cells during active TB and during treatment to compare this to non-TB cases. There have been recently described gene expression biosignatures with promise as diagnostic tests but such signatures will require validation in different cohorts.

Sputum aliquots for future use, will be stored raw and in Trizol at minus 80 °C.

Saliva will be stored at minus 80 °C until use. We have previously found promising diagnostic biosignatures in saliva that are made up of host inflammatory markers and these observations require validation in larger studies.

Urine (10ml) will be stored at minus 80 °C for future biomarker investigations.

6.3.2.1 INSTRUCTIONS FOR SPECIMEN PREPARATION, HANDLING AND STORAGE

Specimens will be prepared, handled and stored according to our established SOPs. Serum will be transported at room temperature, saliva, PAXgene tubes and sodium heparin tubes (for peripheral blood mononuclear cell isolation) on ice.

All sample tubes will only be labelled with study-specific participant identifiers (bar codes) and will not contain any personal identifiers. Similarly, tubes for storage will only be marked by unique study and participant-specific codes and not with personal identifiers. The identities of participants can only be linked back to the names of individuals through the consent form, which contains the link between personal identifiers and study ID. Informed consent documents and source documentation will be stored in the regulatory file and participant files in access controlled, locked cabinets at the study site.

6.3.2.2 SPECIMEN SHIPMENT

Shipment of a total of 374 serum samples from each site (a projected recruitment of 160 participants per site of which one third (53) have active TB with 3 time points for TB cases and 2 for non-TB cases) to LUMC will take place. Shipments will occur at approximately 6-monthly intervals and each shipment will contain the samples obtained during the preceding 6-month period. Shipments might also contain fewer samples if the aims to align TransDot assay performance with ELISA or Luminex have been achieved. Shipment will be done according to International Air Transport Association (IATA) approved procedures and through accredited shippers of biohazardous materials and all Material Transfer Agreements, export and import permits will be in place. Shipments will be temperature controlled. Appropriate contact details of senders and receivers of shipments will be noted on shipping documents.

7. DATA MANAGEMENT PLAN

7.1 DATA COLLECTION

Participant information will be collected directly into electronic case report forms by research nurses using tablet computers, equipped with the Research Electronic Data Capture (REDCap) application for this purpose. X-ray report forms will be captured in an electronic case report form in REDCap by the study clinician and laboratory test result reports will similarly be captured by laboratory personnel.

All reports form part of source documentation and will be filed in participant folders.

Outcome classification conducted by the clinical committee will be done using standard outcome classification forms, which will be captured into an electronic case report form in the database.

7.2 DATA MANAGEMENT

Information captured into electronic case report forms, is uploaded in real time to a secure, password protected database, hosted by Stellenbosch University. The database will be managed by the database manager of the study team, who will oversee regular data checks for missing data and data validation.

7.3 DATA STORAGE

Informed consent documents and source documentation, including clinical result reports, will be stored in the regulatory file and participant files in access controlled, locked cabinets at the study site. Records will be kept for a minimum of 15 years, in compliance with local and international policies.

A daily automated back-up of the database onto an off-site back-up server will done. In addition, a 2 weekly manual back-up of the data onto an external hard drive will be done by the database managers.

8. ASSESSMENT OF SAFETY

An adverse event(AE) is any untoward medical or psychological occurrence in a human research participant, including any abnormal laboratory finding, symptom or disease, and which does not necessarily have a causal relationship with the research or any risk associated with the research. It also includes any event that can affect research participants or data integrity negatively, or that has the potential to impact negatively on members of the research team, or on the project as a whole, and that is deemed significant by the investigator.

A serious adverse event (SAE) is an adverse event that, in the view of either the investigator or sponsor, is life-threatening or results in death, hospitalisation, persistent or significant disability, a birth defect or requires intensive intervention to prevent permanent impairment or any of the previously mentioned outcomes.

AEs/SAEs can be considered related or unrelated to study procedures. As this is not an interventional study, we do not expect clinical AEs or SAEs to be related to any study procedures. All SAEs will be reported in a standardized way.

Additional reporting of adverse events to the EDCTP will be done according to their reporting requirements.

All SAEs will be reported for the whole study period and even after the study has been completed, if the study team becomes aware of SAEs potentially related to study procedures. The principal investigator should be notified of the SAE immediately. SAEs should be captured on a SAE form, which will be filed in

the study regulatory file. Additionally, SAEs will be reported to the Stellenbosch University Human Research Ethics Committee within 7 calendar days.

9. CLINICAL MONITORING

As this study is not a clinical trial, no clinical regulatory organisation will be involved in monitoring. We will comply to any monitoring requirements from the EDCTP.

10. STATISTICAL CONSIDERATIONS

10.1 STUDY OUTCOME MEASURES AND ANALYSIS

The main outcomes of interest are sensitivity and specificity of the point of care TransDot test during phase 3 of this study. Sensitivity will be calculated as the proportion of true positives (as determined by the composite gold standard of TB case definitions), who test positive on the TransDot test. Specificity will be calculated as the proportion of true negatives who test negative on the TransDot test.

For the primary analysis, the true positives as determined by the composite gold standard of TB case definitions, will consist of the Definite and Probable TB classes. The Possible TB class will be excluded as their diagnosis may involve a significant amount of uncertainty. This group will be included in a secondary analysis to show the TransDot test's performance in this uncertain group of participants.

Secondary outcomes of interest are positive and negative predictive values of the TransDot test, which will be calculated as the proportion of participants with positive or negative tests who are positive or negative according to case definitions, respectively.

Sensitivity, specificity, positive and negative predictive values will be reported with 95% confidence intervals.

Reproducibility of the test will be determined between laboratory based tests and point of care tests, as well as between the point of care tests conducted in the different countries, using the Kappa statistic for agreement.

10.2 SAMPLE SIZE CONSIDERATIONS

95% Confidence intervals (CI) for sensitivity and specificity evaluate how precisely sensitivity and specificity have been estimated. The width of CI is dependent on the sample size. 100 TB cases in the final point of care test evaluation (test set), would result in a CI of 85 to 95% for a target sensitivity of 90% (as obtained in the AE-TBC study), based on the binomial distribution. As we expect that about one third of those screened for TB, will be diagnosed with TB, we will aim to recruit 300 participants into this test set. In order to provide for the training set that will be utilized during phase 1 and 2 of this study, we will recruit 500 participants into the training set, for a total of 800 participants recruited for this study.

11. ETHICAL CONSIDERATIONS

11.1 SUBJECT CONFIDENTIALITY

Results obtained from the analysis of data obtained in this study may be submitted for publication by the investigators. No personal identifying information will be included in publications. All study documentation will be identified by the unique study IDs assigned to participants with no personal identifiers of the participants. All records will be stored in locked cabinets. Electronic database access is password protected and any data exported and shared for analysis purposes from the database, will contain only study IDs and no personal identifiers.

No data will be shared with parties not involved in the study through data collection, monitoring, analysis or sponsoring of the study. New ethical approval will be obtained for any data sharing beyond the scope of this project.

11.2 FUTURE USE OF STORED SPECIMENS

Some of the participants' serum samples will be shipped to LUMC for testing in their laboratories, using techniques not available in the African laboratories. Up to 5ml of serum (from 10ml blood), 2.5ml blood in PAXgene tubes, peripheral blood mononuclear cells from 8ml of blood and 2ml saliva per participant per visit will also be stored for future use, not directly related to this project but concerning diagnostic or treatment response aspects of TB. The samples will be stored at the sites where they were collected and in the case of the South African component of the study at the Stellenbosch University Immunology Research Group laboratories. However, up to 5x1ml serum aliquots from up to 500 participants will be stored at LUMC for optimisation of TransDot assays. Participants will have the opportunity during the informed consent process to decline the storage of their samples for future use, or to specify whether they want to restrict future use to tuberculosis related research or are willing to make their samples available for any biomedical research. The samples from subjects who decline consent for storage and future use will be destroyed at the end of the study.

11.3 INFORMED CONSENT PROCESS

Obtaining informed consent is a process rather than an event and discussions to ensure participant understanding and continued willingness to participate should take place at every study visit.

No trial activities will take place before the informed consent form has been signed by a participant or before the respective ethical review boards have given permission for the study. A research nurse will discuss the purpose, duration, procedures, alternatives, risks and benefits of the study with potentially eligible participants. The potential participant will be given the opportunity to ask questions or discuss the

study with family members before signing the consent form. The nurse conducting the discussion will also ask the participant questions to assess the participant's understanding.

People who are not able to give informed consent for themselves, will not be included in this study. At Stellenbosch University the informed consent discussion will be conducted in English or Afrikaans, according to the preference of the participant. In the case of Xhosa speaking participants, an interpreter could be used to translate English or Afrikaans to Xhosa. Informed consent forms will be available in English, Afrikaans and Xhosa. The language used will be documented in the participant file. In the case of a participant who cannot read and write, an independent witness will be present during the discussion and will sign in addition to the participant's thumbprint. This will also be documented in the participant file.

The participant may withdraw consent at any stage and will be informed that withdrawal will not influence their routine clinical care.

A participant may also be withdrawn from the study at the discretion of the study investigators if their further participation is somehow deemed to be a risk to their safety or if lack of the participant's cooperation compromises the quality of the study.

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