Clinical Trial Protocol

Protocol Title: Expanding Xpert Ultra testing for TB diagnosis among HIV-positive patients admitted to hospital in Tanzania and Mozambique.

Short Title: EXULTANT

Protocol number: TB043-3/1-MOZ

Registration number: NCT04568967 (ClinicalTrials.gov)

Trial Sponsor: Foundation for Innovative Diagnostics (FIND)

Trial Coordinator: Barcelona Institute for Global Health (ISGlobal)

Protocol Version: 5.0

Date: 13 September 2023

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1. Clinical Trial Partners

SPONSOR REPRESENTATIVE:

Foundation for Innovative Diagnostics (FIND)
Dr Vinzeigh Nicholas Leukes
Email: Adam.Penn-Nicholson@finddx.org
Address: Chemin des Mines 9, Campus Biotech, 1202 Geneva, Switzerland

CLINICAL TRIAL COORDINATOR:

Barcelona Institute for Global Health (ISGlobal)

Dr Alberto L. García-Basteiro
Email: alberto.garcia-basteiro@isglobal.org
Address: Rosselló 132 4/2 08036 Barcelona, Spain

CLINICAL TRIAL SITES:

Mozambique

Instituto Nacional de Saude (INS)
Address: Vila De Marracuene. Estrada Nacional 1, Parcela 3934, Provincia de Maputo, Mozambique

Centro de Investigação em Saúde de Manhiça (CISM)
Address: Rua 12, Bairro Cambeve, Vila da Manhiça, Provincia de Maputo, Mozambique

Tanzania

National Institute for Medical Research (NIMR) – Mbeya Medical Research Centre (MMRC)
Address: P.O. Box 2410 Mbeya, Tanzania

Ifakara Health Institute Trust (IHI)
Address: Plot 463, Kiko Avenue, Mikocheni, Dar es Salaam, Tanzania,

OTHER CLINICAL TRIAL PARTNERS

Ospedale San Raffaele SRL (OSR)

Address: Via Olgettina 60, 20132 Milano, Italy,

Ludwig – Maximilians – Universitaet Muenchen (LMU)

Address: Geschwister Scholl Platz 1, 80539 Munich, Germany,

Swiss Tropical and Public Health Institute (Swiss TPH)

Address: Socinstrasse 57, 4051 Basel, Switzerland

African Society for Laboratory Medicine (ASLM)

Address: Nega City Mall, 8th Floor, Joseph Tito Street, Kirkos Subcity, Kebele 08, Addis Ababa,Ethiopia

University of Heidelberg (UoH)

Address: Im Neuenheimer Feld 324, 69120 Heidelberg, Germany

2. Signatures page

Protocol Title: Expanding Xpert Ultra testing for TB diagnosis among HIV-positive patients admitted to hospital in Tanzania and Mozambique.

Protocol number: TB043-3/1-MOZ

The signatures below confirm that the signatories have reviewed and approved this documentwhich shall govern the conduct of the specified research study.

SPONSOR:

Foundation for Innovative New Diagnostics (FIND)

Name	Signature	Date
Vinzeigh Leukes	DocuSigned by: Vinzeigh Leukes 6FFAC2EB4CF44F2...	25-Nov-2023

CLINICAL TRIAL COORDINATOR

Barcelona Institute for Global Health (ISGlobal)

Name	Signature	Date
Alberto Garcia-Basteiro	DocuSigned by: Alberto Garcia-Basteiro 1205713894D84FD...	19-Mar-2024

OTHER CLINICAL TRIAL PARTNERS

Ludwig-Maximilians-Universitaet Muenchen (LMU) (optional)

Name	Signature	Date

Ospedale San Raffaele SRL (OSR) (optional)

Name	Signature	Date

Swiss Tropical and Public Health Institute (Swiss TPH) (optional)

Name	Signature	Date

African Society for Laboratory Medicine (ASLM) (optional)

Name	Signature	Date

Statement of Principal Investigator

Protocol Title: Expanding Xpert Ultra testing for TB diagnosis among HIV-positive patients admitted to hospital in Africa.
Trial Number: *TB043 -3/1 - MOZ*

In signing this page, I, the undersigned, agree to conduct the trial according to the protocol and ICH-GCP E6 (R2) guidelines and in compliance with applicable regulations.

I will ensure that the requirements relating to obtaining Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) review and approval are met. I will promptly report to the IRB/IEC any and all changes in the research activities covered by this protocol.

I have sufficient time to properly conduct and complete the trial within the agreed trial period and I have adequate resources (staff and facilities) for the foreseen duration of the trial.

I am responsible for supervising any individual or party to whom I delegate trial related duties and functions conducted at the trial site. Further, I will ensure this individual or party is qualified to perform those trial-related duties and functions.

I certify that key individuals involved with the conduct of this trial, including myself, have completed GCP training and, if applicable, Human Subjects Protection Training.

I understand that all information obtained during the conduct of the trial with regard to the subjects' state of health will be regarded as confidential. No participant's names or personal identifying information may be disclosed. All participant data will be anonymized and identified by assigned numbers on all Case Report Forms, laboratory samples and other trial related information (such as essential documents) forwarded to FIND. Monitoring and auditing by FIND, and inspection by the appropriate regulatory authority(ies), will be permitted.

I will maintain confidentiality of this protocol and all other related investigational materials. Information taken from the trial protocol may not be disseminated or discussed with a third party without the express consent of FIND.

Clinical Trial Site: Centro de Investigação em Saúde de Manhiça (CISM)

Name of Principal Investigator: Dinis Nguenha

Signature: DocuSigned by: Dinis Nguenha D5A05D1EED15490...

Date: 27-Nov-2023
DD/MMM/YYYY

Statement of Principal Investigator

Protocol Title: Expanding Xpert Ultra testing for TB diagnosis among HIV-positive patients admitted to hospital in Africa.
Trial Number: *TB043 -3/1 - MOZ*

In signing this page, I, the undersigned, agree to conduct the trial according to the protocol and ICH-GCP E6 (R2) guidelines and in compliance with applicable regulations.

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Clinical Trial Site: Instituto Nacional de Saúde (INS)

Name of Principal Investigator: Bindiya Meggi

Signature: DocuSigned by: 3500E36440D3447... Date: 25-Nov-2023
DD/MMM/YYYY

3. Protocol History/Amendment Summary*

Version number	Release date	Comments
3.0	28 NOV 2021	Initial version (subsequent to TB-CAPT EXUBERANT revised study for new protocol)
3.1	01 APR 2022	Revisions after IRB/IEC review
4.0	16 JAN 2023	Minor changes and clarifications included
5.0	13 SEP 2023	Minor changes

*Refer to Appendix Document for Protocol Amendment History

3. List of Abbreviations and Acronyms

AHD:	Advanced HIV disease
ASLM:	African Society for Laboratory Medicine
ARV:	Antiretroviral Therapy
AlereLAM:	Alere Determine TB LAM Ag
CFU:	Colony Forming Units
CISM:	Centro de Investigação em Saude de Manhiça (Mozambique)
CNBS:	Centro Nacional Bioética para a Saude (National Bioethics Committee)
CRF:	Case Report Form
DST:	Drug susceptibility testing
FIND:	Foundation for Innovative New Diagnostics
FujiLAM:	Fujifilm SILVAMP TB LAM
HDSS:	Health and Demographic Surveillance System
HIV:	Human immunodeficiency virus
INS:	Instituto Nacional de Saúde (Mozambique)
IHI:	Ifakara Health Institute Trust (Tanzania)
LF-LAM:	Lateral flow urine lipoarabinomannan assay
ISGlobal:	Barcelona Institute for Global Health
LJ:	Lowenstein Jensen (solid culture)
LMU:	Ludwig-Maximilians-Universitaet Muenchen
LPA:	Line probe assays
Mtb:	*Mycobacterium tuberculosis*
MDR-TB:	Multi drug resistant tuberculosis
MHC	Macia Health Centre
MHC	Magude Health Centre
MDH	Manhiça District Hospital
MHC	Maracuene Health Centre
NIMR:	National Institute for Medical Research (Tanzania)
NTP:	National Tuberculosis Programme
OSR:	Ospedale San Raffaele SRL
PLHIV:	People living with HIV
RIF:	Rifampicin
RR:	Rifampicin resistance
RS:	Rifampicin susceptible.
Swiss TPH:	Swiss Tropical and Public Health Institute
TB:	Tuberculosis
Ultra:	Xpert® MTB/RIF Ultra
XDR-TB:	Extensive drug resistant tuberculosis
Xpert:	Xpert® MTB/RIF
XRH	Xinavane Rural Hospital

4. Protocol Synopsis

Protocol Title	Expanding Xpert Ultra testing for TB diagnosis among HIV-positive patients admitted to hospital in Africa.
Protocol number	TB043-3/1-MOZ
Short Title	EXULTANT
Sponsor	Foundation for Innovative New Diagnostics (FIND)
Study Centres	This will be a multi-centre study involving 4 sites in 2 African countries (Mozambique and Tanzania)
Name of investigational products	Xpert MTB/RIF Ultra (Ultra), AlereLAM, Fujifilm SILVAMP TB LAM (FujiLAM), LumiraDx
Indication	TB diagnosis in hospitalized adults living with HIV (PLHIV)
Background	Among people living with HIV, microbiological confirmation of TB is suboptimal due to sputum collection challenges and paucibacillary sputum resulting in poor diagnostic yield. The limitations of existing sputum-based tests for *M. tuberculosis (Mtb)* diagnosis among PLHIV admitted to hospital create imperatives to explore the usefulness of easy to collect, non-respiratory specimens which could improve TB diagnosis, while supporting and accelerating treatment guidance.
Objectives	Primary Objective(s): • To investigate the effect of an expanded TB screening strategy among HIV-positive patients admitted to hospital (including Ultra on sputum, stool and urine, and AlereLAM on urine, performed regardless of presence of TB symptoms) on the proportion of bacteriologically confirmed TB cases starting treatment within 72 hours of enrolment, compared to Ultra testing (on sputum/any tissue) and AlereLAM (on urine) in only those patients who are symptomatic for TB or fulfill WHO testing recommendations. Secondary Objectives: • to assess the impact of this screening strategy on 2-month all-cause mortality. • to assess the feasibility of multiple specimen collection for TB diagnosis within 72 hours of enrolment. *Exploratory objectives* • To assess the diagnostic accuracy of CRP on blood compared to TB symptoms screening in those with microbiologically confirmed TB. • To compare the bacteriological TB confirmation rate in the control arm (Ultra on sputum/any tissue) and urine AlereLAM in only those patients who are symptomatic for TB or fulfill WHO testing recommendations, to local

	standard of care (current testing algorithms implemented at the participating sites before study initiation).
Study Design	This is a multicentre, parallel, individually randomized, controlled diagnostic trial in consecutively enrolled HIV positive adults admitted to hospitalin selected sites in Mozambique and Tanzania.
Intervention arm	The **intervention arm** for this trial consists of HIV patients with TB testing performed regardless of presence of TB symptoms. Testing will be done on expectorated sputum, stool and concentrated urine with Ultra, and urine with AlereLAM. To fulfil exploratory objectives, we will also collect and store 2x tongue swabs for molecular TB diagnostic assay (Xpert Ultra and/or LumiraDx) testing, blood for testing with CRP, and urine samples which will be stored for retrospective FujiLAM testing and analysis.
Control arm	The **control arm** for this trial will consist of patients managed according to the current WHO recommended TB testing practices for HIV positive inpatients (as of Q1 2020). TB testing will be done as follows: ***Sputum Ultra*** performed whenever the patient has cough, fever, weight loss over night sweats and/or ***Ultra performed on any tissue*** (including lymph nodes) from patients with clinical suspicion of extrapulmonary TB. **and/or:** ***Urine Alere TB-LAM*** performed if patients have signs and symptoms of TB (pulmonary and/or extrapulmonary), or with advanced HIV disease, or who are seriously ill, or else irrespective of signs and symptoms of TB, but combined with a CD4 cell count of less than 200 cells/mm^3.
Study Population and Sample Size	Our *study population* will be adult HIV-positive patients admitted to hospital and living in the catchment areas within the 4 participating district hospitals in Tanzania and Mozambique, regardless of presence of TB attributable symptoms. Sample size was chosen to be sufficient to detect a 36.8% relative increase in the primary endpoint in the intervention arm (as compared to the control arm). Calculations were based on following assumptions: - Alpha: 0.05 - Beta: 0.2 (power=80%)

	- Estimated proportion of participants with microbiologically confirmed TB in the control arm: 19% - Estimated proportion of participants with microbiologically confirmed TB in the intervention arm: 26% - Lost to follow up rate for primary endpoint: 5% of recruited participants. Based on these estimates, the study will require a total of 586 HIVparticipants to be enrolled per trial arm. For both arms together, this amounts to 1172 patients in total (293 per site).
Inclusion/Exclusion Criteria	Inclusion Criteria: 1. Adults (18 years old and above) 2. Confirmed HIV infection (including both antiretroviral (ART)- naïve and experienced) 3. Being admitted to the hospital Exclusion criteria: 1. Unable to provide informed consent (if no authorized relatives are in the position to provide the consent) 2. Living outside the catchment area of the participating hospital(s) 3. With plans to migrate outside the catchment area within 2 months after recruitment. 4. Currently receiving anti-TB therapy or having received anti-TB therapy (or a TB diagnosis) in the last 6 months prior to enrolment 5. Currently receiving or having received preventive TB treatment in the preceding 6 months 6. Patients admitted for traumatic reasons, acute abdomen, delivery or pregnancy (maternal conditions) or for planned/scheduled surgery. 7. Referred from other reference hospital 8. Positive result to a SARS-CoV-2 test (RT-PCR, antigen test, or other similar test that might define COVID-19 case in the study site)* *AND patients under investigation for COVID-19 if this situation hinders the execution of study activities (as judged by the site PI)
Evaluation Criteria	Primary Endpoint(s): The proportion of participants diagnosed with microbiologically confirmed TB and started on TB treatment within 72 hours of enrolment* in whom TB is microbiologically confirmed*/**, separately for control and intervention arms.

	*The numerator of the primary endpoint includes participants per study arm who are diagnosed with microbiologically confirmed TB and initiated TB treatment within 72 hrs of enrolment. The denominator is the number of enrolled patients in each study arm. **Patients are defined to have microbiologically confirmed TB if at least oneconfirmatory TB test (Ultra, AlereLAM) gives a positive result. Secondary endpoints: • Eight-week all-cause mortality (main secondary endpoint) among all participants enrolled. The numerator will be number of deaths during eight weeks after enrolment, the denominator is the number of participants enrolled. • Time to TB treatment initiation among microbiologically confirmed cases and among all TB cases since enrolment. We will estimate Kaplan Meier curves and hazard ratios at day 7, 14, 28 and 56. Additionally we will compare the time to related estimates (mean and standard deviation) of intervention and control group. • Proportion of cases re-admitted to hospital. The numerator will be the number of admissions after initial discharge and within 8 weeks from enrolment. The denominator will be the number of participants enrolled. • Proportion of participants who are diagnosed with TB (irrespective of bacteriological confirmation) and are started on TB treatment within 72 hours of enrolment. • Proportion of participants who are diagnosed with microbiologically confirmed TB and are started on treatment at 7 days, 14 days, 4 and 8 weeks of enrolment. • Proportion of study participants who are able to provide the different specimens (sputum, stool and urine samples) within 24, 48 and 72 hours of enrolment. • Time to laboratory confirmed TB diagnosis and any TB diagnosis since enrolment. We will estimate Kaplan Meier curves and hazard ratios at days 7, 14, 28 and 56. Additionally we will compare the time related estimates (mean and standard deviation) of intervention and control group. • In-hospital and 4-week all-cause mortality among all participants enrolled (assessed as the eight-week all-cause mortality). • Proportion of participants started on treatment without Rif resistance information (only AlereLAM or FujiLAM positive) Exploratory endpoints • Diagnostic performance of a molecular TB diagnostic assay (Xpert Ultra and/or LumiraDx) on tongue swabs and FujiLAM on urine stool against a microbiological reference standard (defined as any test positive in the

	intervention arm) or against a composite reference standard that includes bacteriologically confirmation or/and clinical evidence resulting in therapy initiation. • Diagnostic accuracy of CRP in blood compared to TB symptom's screening in those with microbiologically confirmed TB. Comparison of primary and secondary endpoints of the intervention arm with the local standard of care at the sites before the study initiation (a retrospective data collection from consecutive PLHIV admitted at participating hospitals). Sub-analysis • Primary endpoint will be calculated at different intermediatetimepoints (48 hours, 7 days and 14 days from enrolment). • Primary and selected secondary endpoints will be calculated for participants' subgroups including CD4 cells/mm^3 ≥200, <200 and <100, among participants with any TB symptoms, severely ill participants or with AHD, and among participants providing at least one sputum and (or) one urine sample for testing.
Study Timeline	Estimated period for ethics clearance May / 2022 Import permits and importation Jan-May / 2022 Kick off / training meeting Nov / 2021 Estimated date of first participant enrolled: Sep/ 2022 Estimated date of last participant enrolled: Jan/ 2024 Estimated date of last participant completed: Mar/ 2024 Estimated date for end of data cleaning: May / 2024 Estimated date for end of data analysis: July / 2024 Estimated date for first manuscript draft: Sep / 2024 Total duration of study: 47 months

5. Introduction

5.1 Background and statement of the problem

Tuberculosis (TB) is a leading cause of death worldwide and ranks above HIV/AIDS as the leading cause of mortality from a single infectious agent[1,2]. The World Health Organization(WHO) estimated that there were around 10 million new cases and 1.5 million deaths in 2018.[2] Nine and 17% of the total number of TB cases and deaths respectively occurred in people living with HIV (PLHIV), with 70% of these occurring in the African region.[2] PLHIV are at increased risk of acquiring *Mycobacterium tuberculosis* (*Mtb)* infection and have a >20-fold increased risk of TB disease progression compared to HIV-uninfected peers.[3–5] Inaddition, mortality during TB treatment remains as high as 11% globally, although fatal outcomes of PLHIV under TB treatment are even higher in Sub-Saharan African countries.[2]

One of the main barriers for ensuring timely TB treatment in PLHIV is the lack of an optimal diagnostic work up.[6] In fact, TB case detection among people with HIV remains disappointingly low and almost half (45%) of all HIV associated TB cases are estimated to be undiagnosed or unreported.[2] Mortality associated with undiagnosed TB among people with HIV is assumed to be higher than 70%.[7] Although the lack of a proper diagnosis is a multifactorial problem associated with quality of health care, social, economic and anthropological factors, the unavailability of rapid, sensitive and cheap diagnostic tools hasbeen deemed as an important factor contributing to under- and misdiagnosis. In addition, poor awareness of TB by health care workers and lack of established diagnostic algorithms further hinder adequate TB diagnosis in many high burden settings.[8,9] Autopsy studies conducted in sub-Saharan Africa show high rates of clinico-pathological discrepancies and missed TB which lead to high mortality [8,10–12]

Among PLHIV, microbiological confirmation of TB is suboptimal due to sputum collection challenges and paucibacillary sputum resulting in poor diagnostic yield.[13–15] Despite increasing availability, sputum Xpert® MTB/RIF (Cepheid Inc., Sunnyvale, CA, USA, hereinafter Xpert) confirms TB in only 77% of adult cases with HIV-infection[16] and only 66% of culture-positive pediatric cases.[17] Sensitivity of the next-generation Xpert MTB/RIF Ultra (hereinafter Ultra) is estimated to increase among adult TB patients with documented HIV-infection (90%)[16]. Yet, performance in immunosuppressed HIV positive patients (in whom sample collection is often unavailable) is probably suboptimal. Thus, estimates of Xpert diagnostic performance in general HIV positive patients may not reflect test performance within the majority of TB cases in hospitalized PLHIV. In addition, although Xpert results could, in theory, be available within a few hours, results are often available within several days after sample collection,[18,19] delaying TB treatment if not empirically

provided. The most sensitive test for TB diagnosis, liquid culture, is further handicapped as it is slow, expensive and contaminated 5-8% of the times.[20]

Options that have been recommended by WHO to ensure earlier diagnosis and reduce mortality include strategic placement of rapid molecular TB diagnostics such as Xpert[21] or Ultra[22] within HIV care settings (which could enable availability of results within 24 hours), and uptake of the lateral flow urine lipoarabinomannan assay (LF-LAM)[23] strip-test – the Alere Determine™ TB LAM Ag (AlereLAM) for seriously ill PLHIV. However, despite AlereLAM being a cheap and fast test, only 9 of the 30 high TB and HIV burden countries have a national policy and algorithm indicating the routine use of urine LF-LAM (with various levels of implementation). [2] A recent study showed that urine-based TB screening of PLHIV admitted to hospital might reduce 56-day mortality in those with low CD4 counts, severe anaemia or clinically suspected tuberculosis.[24] A recently published meta-analysis showed that, as an screening test among PLHIV, AlereLAM has limited sensitivity of 45% (95% CI: 29-63) and specificity of 92% (80-97) against a composite microbiological reference standard.[25] However, screening with AlereLAM improves the likelihood of being diagnosed with TB during admission. The WHO recently expanded the recommendations for the use of AlereLAM to include adults, adolescents and children living with HIV with signs and symptoms of TB (inpatient and outpatient setting) or irrespective of signs and symptoms of TB with CD4 cell count under 200 cells/mm^3 in inpatient settings irrespective of the CD4 cell count in outpatient settings.[26] The unavailability of CD4 counts upon admission or the assessment of the criteria for severely-ill or advanced HIV disease might delay quick testing of PLHIV in whom AlereLAM is recommended. In addition, LAM tests do not provide any information on potential resistance to anti TB drugs, which might hinder its implementation in high TB drug resistance settings.

The limitations of existing sputum-based tests for *M. tuberculosis (Mtb)* diagnosis create imperatives to explore the usefulness of easy to collect, non-respiratory specimens which could improve performance of all tests, while supporting treatment guidance and monitoring. In fact, the WHO has specified the need for rapid non-sputum based TB diagnostics among their high priority target product profiles for new TB diagnostics.[27]

5.2 Promising specimens for TB diagnosis using Xpert Ultra

Urine

Although there is no recommendation on the use of urine as a sample *for* Xpert TB testing, several diagnostic accuracy studies have shown an important added yield when used in hospitalized patients. Studies by Lawn in South Africa[28,29], showed that Xpert testing in urine among newly admitted unselected HIV-positive adults improved bacteriological confirmation relative to sputum Ultra,

sputum culture or urine LF-LAM. Xpert in urine provided the highest incremental diagnostic yield, with high specificity when used combined with sputum testing.[28,29] A recent clinical trial by Gupta-Wright and colleagues showed a lower diagnostic yield of urine-Xpert compared to TB LAM[30], although it was theonly test positive in 6% of microbiologically confirmed patients in the intervention arm.

Another analysis in a South African cohort of unselected HIV-positive hospitalized patients,showed a sensitivity of Urine Xpert of 41.8% (59/141) against a microbiological reference standard, very similar to that of AlereLAM (43.3% 61/141).[31] Interestingly, 15 cases detected with Urine Xpert were not detected with Alere LAM and 17 cases were detected with AlereLAM, but not with Urine Xpert.

Stool

Similarly to urine, no recommendation has been made about the use of Xpert or Ultra in stool so far. However, recent studies on stool Xpert in pediatric pulmonary TB suggest that it could be helpful in paucibacillary TB patients. A metanalysis showed the sensitivity and specificity of stool Xpert in 67% and 99%, respectively, being higher in HIV positive children[32], and other groups have reported a greater sensitivity (73%) compared to traditional tools[33]. Moreover, Xpert Ultra showed higher sensitivity in stool compared to Xpert[34]. While results of stool Xpert in adults are promising, showing a sensitivity of 90.2%-100% and specificity of 100% [35] and 81% in smear-negative patients[35], data is limited. Results of alternative in-house PCR show promising value in stool support of this specimen for PTB diagnosis (sensitivity 95-97% in smear positive and 77% in smear negative[33,36] and 69% for EPTB[33]). Although there are no published reports on HIV positive admitted patients, the abovementioned data suggest that the use of stool as a specimen for TB diagnosis could provide additional diagnostic yield in this vulnerable population.

5.3 Other promising tests and specimens

The Fujifilm SILVAMP TB LAM (FujiLAM)

A urine-based test which also detects LAM in urine, has shown promising results in frozen samples and is currently undergoing prospective evaluations in several settings. Recently published results using biobanked samples from studies conducted in South Africa showed higher sensitivity of FujiLAM compared to AlereLAM (70.4% vs 42.3%, using a microbiological reference standard), while maintaining high specificity.[31] FujiLAM performance includes a 5-step procedure according to manufacturer's instructions and takes around 50-60 minutes from sample collection to results availability. Thus, it is also considered a true point of care test, since results could easily be handed out the same day the patient provides the sample.

Given the substantially improved sensitivity of FujiLAM compared to AlereLAM, it could easilybe the best test for immunosuppressed HIV positive patients, and has the potential for being an important tool for TB diagnosis in this population subgroup. In addition, it might have a potential to impact on mortality. A recent study in South Africa showed that FujiLAM conducted retrospectively predicted TB mortality among hospitalized patients.[37] However further studies in different populations and settings, with endpoints including patient important outcomes are needed to further assess its impact where it is most needed. The FujiLAM assay is undergoing a version change for large scale manufacturing. The new version will not be available until the end of the planned trial, so analysis for FujiLAM will be done retrospectively on banked urine samples collected as part of this study.

Oral swabs

As sputum can be difficult to collect in some patient groups, alternative specimens for TB diagnosis have been evaluated in recent years. In 2015, a study conducted in South Africa[38] demonstrated the presence of *M.tb* in oral swabs. The diagnostic performance of this sample collected in tongue dorsum that two samples per subject showed a combined sensitivity of 92.8% and specificity of 91.5%.[39] A recent study[40] showed lower sensitivity and specificity when collecting tongue swabs on different days (88%-94.4% and 79.2%, compared to sputum Xpert). However, despite these promising results, further validation studies are needed to assess the potential utility of these innovative specimens.

CRP

C-reactive protein (CRP) indicates inflammation and can be detected on capillary blood. WHO has recently recommended CRP as a method for TB screening among ambulant people living with HIV who are newly in care and not yet on antiretroviral treatment, based on similar sensitivity and specificity to symptom screening in HIV-positive populations[41].

LumiraDx

LumiraDx is a recently developed point-of-care instrument that is currently available for SARS-CoV-2 testing in anterior nasal specimen, Dimer-D and CRP quantification in venous blood (https://www.lumiradx.com/us-en/what-we-do/diagnostics/test-technology/). While its use has not yet been tested for TB, the company is developing a *Mtb* kit to be tested in tongue swabs. LumiraDx is a fast (10min) and easy-to-use device, with potential for adding innovative POC solutions to TB diagnosis.

6. Aim and hypothesis

The overall aim of this study is to assess the potential of an expanded TB testing strategy toincrease the number of HIV-positive patients with microbiologically diagnosed TB who are started on treatment in adult wards of sub-Saharan Africa.

Previous studies have shown that Xpert testing of urine and stool are capable of confirming Mtb in patients who would otherwise test negative with traditional sputum-based methods. Evidence shows that these diagnostics may detect additional cases that are not detected by AlereLAM. Thus, an algorithm using the different tests (sputum Ultra, urine Ultra and stool Ultra) offered as a screening intervention to admitted HIV positive patients should increase the *Mtb* bacteriological yield (compared to current WHO recommended screening and testing strategies**) and contribute to earlier initiation of TB treatment. Given that some detected cases may not present with classical TB symptoms at the time of hospitalization, we believe that this strategy will increase the overall number of patients with diagnosed TB and started on treatment and ultimately reducing mortality.

*** Current WHO recommended strategies include sputum Xpert Ultra MTB/RIF whenever the patient has TB attributable symptoms, and urine Alere TB-LAM, if patients have signs and symptoms of TB, or with advanced HIV disease (1) or who are seriously ill (2) or else irrespective of signs and symptoms of TB and with a CD4 cell count of less than 200 cells/mm^3. These interventions will constitute the "control arm" across all study sites. Note that the current WHO recommended strategies might not be currently applied in a standardized manner as part of the local standard of care.*

6.1 Benefit/Risk Assessment

The study poses participants with minimal or no risk, given that no experimental intervention is provided to participants, but rather to samples from participants. The potential risks are associated to those associated to blood drawn, or discomfort produced while providing the sputum, urine or stool samples. The benefits of the study include probability of earlier confirmatory diagnosis of tuberculosis, which would allow immediate treatment initiation. This might have an impact on mortality in the first two months after enrolment. These benefits needto be confirmed as they are endpoints of the study. The study would surely allow a close clinical follow-up by study staff during the participants' involvement in the clinical trial.

7. Objectives

Primary Objective(s)

- To investigate the effect of an expanded TB screening strategy among HIV-positive patients admitted to hospital (including sputum Ultra, urine AlereLAM and urine and stool Ultra assay) on the proportion of microbiologically-confirmed TB cases starting treatment within 72 hours of enrolment, compared to Ultra testing (on sputum/any tissue) and AlereLAM (on urine) in only those patients who are symptomatic for TB or fulfill WHO testing recommendations

Secondary Objectives

- to assess the impact of this screening strategy on 2-month all-cause mortality.
- to assess the feasibility of multiple specimens' collection for TB diagnosis within 72hours of enrolment.

Exploratory Objectives

- To assess the added yield of a molecular TB assays (Xpert Ultra and/or LumiraDx) tongue swabs, and urine FujiLAM testing in microbiological TBconfirmation
- To assess the diagnostic accuracy of CRP in blood compared to TB symptoms screening in those with microbiologically confirmed TB.
- To compare the bacteriological confirmation rate of our control arm (Ultra on sputum/any tissue and AlereLAM in only those patients who are symptomatic for TB or fulfill WHO testing recommendations)to local standard of care (current testing algorithms implemented at the participating sites before study initiation).

8. Endpoints

8.1 Primary Endpoint(s)

The primary endpoint is:

- The proportion of participants diagnosed with microbiologically confirmed TB and started on TB treatment within 72 hours of enrolment, separately for intervention andcontrol arm. The numerator is the number of participants per study arm who are diagnosed with microbiologically confirmed TB and start on treatment within 72 hours of enrolment. The denominator is the number of participants enrolled per studyarm. We will compare this indicator between both study arms.
  Patients are defined to have microbiologically confirmed TB if at least one confirmatory TB test (Ultra, AlereLAM) gives a positive result.

Hospitalized PLHIV experience high mortality and TB underdiagnosis and misdiagnoses arefrequent. TB diagnosis is important when you can quickly start appropriate therapy. This endpoint will capture both diagnostic dimensions of the intervention (higher rates of *Mtb* laboratory confirmation) as well as the relevant patient outcome (TB treatment initiated).

Diagnosic procedures and treatment initiation (if appropriate) should be completed within 72 hours of enrolment given the intervention is aimed at rapid collection of samples for all patients upon admission and that Ultra testing can provide results within a few hours from specimen collection at all participating sites.

The numerator of the primary endpoint also includes patients whose bacteriological confirmation might arrive after 72 hours of enrolment. There might be cases where liquid or solid culture might be requested and results arrive after 72 hours. If the patient had been put on treatment empirically (before a positive culture results has arrived), that case will contributeto the numerator, given that treatment would have been started in a “true” TB case.

8.2 Secondary endpoints

- Eight-week all-cause mortality (main secondary endpoint) among all participants enrolled. The numerator will be number of deaths during eight weeks after enrolment, the denominator is the number of participants enrolled. We will compare this indicator between both study arms. In addition, this endpoint will also be calculated considering right censored observation when a participant is lost to follow up during the observation period or a

participant experiences a different event that makes further follow up impossible.

- Time to TB treatment initiation among microbiologically confirmed cases and among all TB cases since enrolment. We will estimate Kaplan Meier curves (event= TB treatment initiation among laboratory confirmed TB patients and among all TB cases) and hazard ratios at day 7, 14, 28 and 56. Additionally we will compare the time to related estimates (mean and standard deviation) of intervention and control group.
- Proportion of cases re-admitted to hospital. The numerator will be the number of admissions after initial discharge and within 8 weeks from enrolment. The denominatorwill be the number of participants enrolled.
- The proportion of participants who are diagnosed with TB (irrespective of bacteriological confirmation) and started TB treatment within 72 hours of enrolment. The numerator is the number of participants diagnosed with TB (with or without bacteriological confirmation) who started treatment within 72 hours of enrolment; the denominator is the number of participants enrolled.
- The proportion of participants who are diagnosed with microbiologically confirmed TBand are started on treatment at 7 days, 14 days, 4 weeks and 8 weeks of enrolment. Inaddition, this endpoint will also be calculated considering right censored observation when a participant is lost to follow up or dead during the observation period or a participant experiences a different event that makes further follow up impossible.
- Proportion of participants who are able to provide sputum, stool and urine samples for Ultra/AlereLAM testing within 24, 48 and 72 hours of enrolment (the numerator is the number of urine samples or (and) sputum samples provided within 72 hours of admission and the denominator is the number of patients enrolled).
- Time to laboratory confirmed TB diagnosis and any TB diagnosis since enrolment. We will estimate Kaplan Meier curves (events = laboratory confirmed diagnosis and any TB diagnosis) and hazard ratios at day 7, 14, 28 and 56. Additionally we will compare the time related estimates (mean and standard deviation) of intervention and control group.
- In-hospital and 4-week all-cause mortality as assessed by estimating a proportion (numerator: number of deaths occurring at the hospital over the number of enrolled participants and number of deaths by week 4 (irrespective of where it occurs) over the number of enrolled participants)
- Proportion of participants started on treatment without RIF resistance information (only AlereLAM positive)

The study has been powered to detect a statistically significant difference in the primary endpoint. Nonetheless, given that some patients might not present typical TB symptoms and TB misdiagnosis

is frequent among admitted HIV-positive patients, we believe the intervention could be associated with a higher proportion of patients diagnosed with TB (irrespective of bacteriological confirmation) and thus, overall survival. In consequence, several secondary endpoints are related to survival at different time points.

8.3 Exploratory endpoints

- Diagnostic performance of a molecular TB diagnostic assay (Xpert Ultra and/or LumiraDx) on tongue swabs, and FujiLAM on urine against a microbiological reference standard (as defined in intervention arm) or against a composite reference standard that includes bacteriologically confirmation or/and clinical evidence resulting in therapy initiation. This endpoint will only be studied in participants of the intervention arm
- Diagnostic accuracy of CRP in blood in order to evaluate compared to TB symptom's screening in those with microbiologically confirmed TB.
- Comparison of primary and secondary endpoints of the intervention arm with the local standard of care at the sites before the study initiation (a retrospective data collection from consecutive HIV-positive patients admitted at participating hospitals).

9. Trial Design and study arms

This is a multicentre, parallel, individually randomized controlled diagnostic trial in consecutively enrolled HIV positive adults to evaluate the impact of the extended use of Xpert Ultra (3 specimens) for TB diagnosis, using sputum, stool and urine, AlereLAM among consecutive PLHIV admitted to hospitals in 4 sites of 2 high burden African countries (Tanzania and Mozambique). Thus, the **intervention arm** for this trial will include TB testing performed irrespective of the presence of TB symptoms. Testing will be done on expectorated sputum, stool and urine with Ultra and urine with AlereLAM.

To fulfil exploratory objectives, we will also collect 2x tongue swabs for molecular TB diagnostic assay (Xpert Ultra and/or LumiraDx) testing, blood for CRP, and urine samples which will be stored for retrospective FujiLAM testing and analysis.

The **control arm** for this trial consist of patients managed according current WHO testing recommendations (as ofQ1 2020) for HIV-positive inpatients. TB testing will be done as follows:

a) ***Sputum Ultra*** whenever the patient has cough, fever, weight loss or night sweats and/or Ultra performed on any tissue (including lymph nodes) in patients with clinical suspicion ofextrapulmonary TB.

and/or

b) ***Urine Alere TB-LAM***, if patients have signs and symptoms of TB (pulmonary and/or extrapulmonary), or with advanced HIV disease or who are seriously ill or else irrespective of signs and symptoms of TB and with a CD4 cell count of less than 200cells/mm^3.

As part of a secondary objective, we will retrospectively collect data of a cohort of consecutively hospitalized HIV-positive patients that were admitted prior to the trial initiation (**third arm**), that will be used to compare the difference between the intervention arm or control arm with the real standard of care practiced at the sites.

9.1 Study population and trial setting

Our *study population* will be adult (≥18 years) PLHIV admitted to hospital and living in thecatchment areas of 4 district hospitals in Mozambique and Tanzania.

These two countries are included in the World Health Organization's high TB and HIV burden country list. In 2020 Mozambique and Tanzania had estimated TB incidence ratesof 368 and 222 per 100 000 population respectively. The prevalence of HIV among new TB patients was 27 and 21% in 2020, respectively. The following table shows basic epidemiological indicators from the World Tuberculosis Report 2021 released by the World Health Organization.

	Mozambique	Tanzania
TB incidence Rate (per 100 000 population)	368	222
HIV-positive TB incidence (per 100 000 population)	101	47
HIV-positive TB mortality (per 100 000 population)	20	16
Proportion of pulmonary TB who are bac confirmed (%)	35%	44%
TB treatment coverage (%)	84%	64%
TB case fatality ratio (%)	11%	22%
TB patients with known HIV status who are HIV-positive (%)	27%	21%
TB treatment success in HIV positive patients (2019)(%)	88%	90%

Table 1. Basic World Health Organization TB burden of disease indicators (year 2020)[42]

Four research institutions from 2 different countries will be recruiting participants for the trial at associated district hospitals (non-referral hospitals). All the research sites have:

- Capacity to enroll at least 270 eligible patients in a year.
- Continuous power supply
- Access to laboratory testing which can provide Ultra results and other relevant TB tests (AlereLAM) within 24 hours.
- Access to CD4 cells count testing and blood chemistry services.
- Adequate space for the research team to work in or to erect a temporary structure if the existing facility is not adequate to accommodate the research team.
- At least one physician on the research team with experience in management of patientswith TB and HIV in order to make the relevant clinical decisions.
- Storage capacity for samples that will be analyzed at a later stage.

9.1.1 Mozambique

9.1.1.1 Manhiça District Hospital (MDH) and Xinavane Rural Hopsital (XRH) are two health facilities located in the district of Manhica, 80 and 120 km north from Maputo (respectively). MDH is a 110-bed rural hospital (60 beds in the adult ward),receiving referrals from 11 health care centers of the district. It is adjacent to Centro de Investigação em Saude de Manhiça (CISM), which provides support with clinical staff and performing some of the laboratory procedures for the hospital. It offers obstetric services including obstetric emergency care, operation room and, in collaboration with CISM, a morbidity surveillance 24h 7 days per week, hospital-based morbidity surveillance system for children under 15 years of age attending the MDH. XRH is a 50-bed rural hospital, 50km from MDH with 40 beds in the adult ward, receiving referrals from 3 health care centers of the northern part of Manhiça district. Both hospitals have acess to smear microscopy, GeneXpert, X-Ray facilities.

XRH is a 50-bed rural hospital (40 beds in the adult ward), receiving referrals from 3 health care centers of the northern part of Manhiça district. XPH is a provincial 40 – bed provincial hospital in the adult ward and MHC a relatively small health centre with a hospitalization capacity of 10 beds in the adult ward.

Additional sites such as the Maracuene, Magude and Macia Health Centres could be considered during the study if the recruitment rate in the MDH and XRH is inadequate. These 3 Health Centres are relatively small health centres surrounding the Manhiça District Hospital, located at 50, 150 and 160 km from Maputo (respectively), with a hospitalization capacity of 10, 54 and 31 beds (respectively) in the adult wards.

9.1.1.2 Mavalane General Hospital (MGH) and José Macamo General Hospital (JMGH) are hospitals located in Maputo city, with 72 and 70 adult beds respectively. Both hospitals collaborate with the Instituto Nacional de Saúde (INS), the national state funded health research institution in Mozambique. Both hospitals have access to smear microscopy, GeneXpert®, X-ray and CD4 cell count and HIV testing. Both hospitals serve some of the poorest regions of the city, have integrated Emergency Department (for children and adults), with those requiring intensive caretransferred to Maputo Central Hospital.

9.1.2 Tanzania

9,1,2,1 NIMR Mbeya Medical Research Center is located on the same ground of the Mbeya Zonal Referral Hospital (MZRH), a public tertiary healthcare facility serving a population of about 8 million people in Southwestern regions of Tanzania. MZRH has the average number of hospitaladmissions

of 30,000 per year and total of 400 bed capacity. MZRH admit over 200 HIV positive patients per month. NIMR work very closely with MZRH which provide a pool for recruitment of participants into various research studies carried out by NIMR. For this study recruitment of study participants will be conducted within the inpatient ward of MZRH and Mbeya Regional Referral hospital (MRRH). MRRH is a 150-bed regional hospital located in Mbeya city, Tanzania. It receives around 100 to 150 admissions of HIV infected patients per month. It works closely with NIMR Mbeya Center. It has microscopy, GeneXpert and X-ray facilities. Sputum, urine and other samples will be processed and tested at the NIMR laboratory.

9.1.2.2 St Francis referral hospital (SFRH) is located in the south-east of Morogoro region in Kilombero district. SFRH is a non-profit making organization belonging to the Roman Catholic Dioceses of Ifakara. It is a 371 beds Hospital and was declared a District (Council) Designated Hospital for Kilombero and Ulanga Districts in 1976 with catchment population of about 600,000 habitants and in 2010, the hospital was upgraded to the level of Referral Hospital. SFRH offers preventive, curative, rehabilitative and teaching services. SFRH is divided into departments for Surgery, Internal Medicine, Gynecology and Obstetrics, Pediatrics, Chronic Diseases and Intensive Care. Additionally, there are outpatients' departments and wards for Tuberculosis, Dental Medicine, Physiotherapy, Occupational Therapy, Psychiatry, Leprosy, Rehabilitation, an X-ray department, laboratory and a special laboratory for the production of infusions. SFRH incollaboration with the Ifakara Health Institute runs a HIV cohort with over 11,000 HIV positive patients ever enrolled. The cohort has been running since 2005. The average numberof inpatients treated per year is 16,480.

9.2 Participant eligibility criteria

9.2.1 Pre-eligibility screening criteria

Adult HIV-positive patients admitted in the hospitalization wards of the participating hospitals.

9.2.2 Inclusion Criteria:

1. Adults (18 years old and above)
2. Confirmed HIV infection (including both antiretroviral (ART)- naïve and experienced)
3. Admitted to the hospital (adult medical wards) at the time of enrolment.

9.2.3 Exclusion criteria:

1. Unable to provide informed consent (if no authorized relatives are in the position to

provide the consent)

2. Living outside the catchment area of the participating hospital(s)
3. With plans to migrate outside the catchment area within 2 months after recruitment.
4. Currently receiving anti-TB therapy or having received anti-TB therapy (or a TB diagnosis) in the last 6 monthsprior to enrolment
5. Currently receiving or having received preventive TB treatment in the preceding 6 months
6. Patients admitted for traumatic reasons, acute abdomen, delivery or pregnancy (maternal conditions), or for planned/scheduled surgery.
7. Referred from other reference hospital.
8. Positive result to a SARS-CoV-2 test (RT-PCR, antigen test, or other similar test that might define COVID-19 case in the study site)*

*AND patients under investigation for COVID-19 if this situation hinders the execution of study activities (as judged by the site PI)

9.3 Sample size

In order to detect a 36.8% relative difference (26% vs. 19%) in the proportion of admitted PLHIV who are bacteriologically confirmed TB positive between the intervention and control arm, thestudy will require a total of 586 participants to be enrolled per trial arm, whereby drop-out rates are considered (details see below). For both arms together, this yields 1172 patients in total. Given that 4 sites will be recruiting patients, this represents a mean of 293 participants per site (147 per arm at each site). Further details on sample size estimation can be found in (statistical considerations).

10. Study procedures

10.1 Pre-eligibility screening

Initial pre-eligibility based on HIV status will be assessed by study staff, who will visit the adultmedical wards every morning to identify any newly admitted patients. HIV documentation can be written proof of a positive HIV test, CD4 count records or ART prescription. From the information available in-patient registers and/or patient files, only those with unknown or documented HIV-positive status will be approached to participate in the study. Routine health personnel need to be aware of the study and contact study personnel whenever a new admission occurs.

For those with unknown HIV status, a rapid antibody-based test will be performed (further details under laboratory procedures) by authorized personnel (after ICF is obtained).

Basic anonymized information on participants who are admitted in the adult ward but not enrolled in the study will be recorded in a pre-screening log which should include: age, gender and the reason not eligible for trial participation, or if they are eligible but declined.

10.2 Informed consent

Informed consent will be sought from potential participants using information sheets and consent forms available in relevant languages. Written informed consent will be sought, with the assistance of a translator or a witness where necessary, using standard consent forms. In occasions where the participant is not able to undergo the informed consent process (mental retardation, altered level of consciousness, somnolence, obtundation, stupor or certain psychiatric conditions) an authorized relative might provide the written consent as a witness. Inthe case these conditions are temporal, patients would be reconsented when they are in full capacity. Participants unable to read or write will be asked to make a mark or thumbprint in thepresence of a witness. Details on standard informed consent procedures will be specified in sitestandard operating guidelines. The ICF will include a section to access to medical records and collection of relevant data from them.

10.3 Assessment for eligibility

All inclusion/exclusion criteria must be assessed among those HIV-positive patients admitted inthe ward and who have signed the informed consent.

For those patients who are not eligible, study staff will inform them that they will not be able to participate in the study and will receive the local standard of care.

10.4 Recruitment

A participant is considered recruited for the study when the participant has signed the informed consent, meets all the inclusion criteria and does not fulfill any of the exclusion criteria.

For those patients enrolled in the study, all the screening procedures will be documented in the screening logs and appropriate case report forms (CRFs) will be completed before starting the procedures specific to the control or intervention arm, including:

a) Basic socio-demographic information
b) Relevant medical history
c) Physical exam

Basic sociodemographic information and relevant medical history will be collected from the

participant/caregiver or, if available, from existing medical records. A locator form (as a source document) will include contact information of the participant and/or caregiver or relative (with at least one mobile phone number to reach out the participant after discharge).

Time from admission (as recorded in medical history) to study enrolment will be recorded.However, this time should be as short as possible, preferably less than 12-24 hours.

10.5 Randomization

This is an individually randomized clinical trial. Eligible participants will be randomized to eitherthe intervention or control arm, with an expected 50% of participants allocated to each of the arms. Randomization will be conducted using a web-based application (Enketo software) that can be used in any smartphone device or computer with internet connection. The day of randomization for each participant will be the enrolment day (study Day 0). At the time a participant is randomized, the study staff enters in the application several key data (patient study number and date of birth) and in reply, the randomization arm is provided a few seconds later in the same application interface. Thus, internet connection is needed for randomizing participants. Pre-registered users of the study team will be allowed to use the randomization application.

Developing a reliable training plan by which study teams (at the clinic and at the lab) are fully aware of the diagnostic allocation is critical so that arm-specific testing can be implemented without mistakes in a timely manner. This includes careful strategic measures (posters, signs, weekly meetings, etc) as part of the site internal planning, which also need to consider staff shifts, holidays (both in the hospital and at the laboratory). A screening and randomization log need to be daily updated. Day 0 CRF needs to be data entered in a timely manner to allow the central monitoring of number of recruited subjects per arm, site and week and overall mapping of participants included in the study.

10.6 Blinding

This is an unblinded trial. Neither participants, nor study staff will be blinded to tests practicedor test results.

Enrolment and testing will be done by study staff. AlereLAM will be done at the hospital or at a short distance from hospital (for example, same building hospital laboratory). Results of all tests will be provided to routine clinical staff who will make decisions on treatment initiation.

10.7 Minimisation of Error and Bias

Selection bias will be minimized by a) the randomization process and b) consecutive enrolmentof participants meeting inclusion criteria (and not meeting exclusion criteria). Study staff from all sites will be trained prior to study initiation in order to guarantee that inclusion and exclusion criteria and

well understood and applied homogenously across sites.

Other potential sources of bias (ascertainment, information, interpretation), will be also addressed. Laboratory procedures will be standardized across sites, using the same laboratory SOPs and specific lab training, in order to minimize differences by site. Given that most tests are different by arm, and that AlereLAM will be preferably done at the health facility (by the bed side), no blinding of attending physician or lab technologist will be possible. In order to explore the differences between WHO recommended procedures and real standard of care at the sites, there is an exploratory objective aiming at assessing the current yield of TB investigations under routine conditions prior to the study initiation at all sites. This will be consecutive collection of retrospective data from PLHIV admitted at participating hospitals prior the start of the study. The quality and completeness of this data might differ from participating sites.

Decision to start TB treatment will be based on any test results and the criteria of thephysician responsible for the patient's care.

10.8 Allocation to study arm

Enrolled patients will be randomly assigned to receive the diagnostic procedures specified forthe intervention or control arm. Date and time of patient admission and recruitment need to be clearly recorded in the patient file (hour and when possible, minutes). Once the patient hasprovided the relevant baseline information specified above (recruitment section), the researcher will proceed to randomization and then, will open a patient file where the patient trial identification number will be clearly visible. A specific study card with study team contact information and patient study number will be handed to the participant.

The allocation of the study arm will be recorded in the participant study file under the appropriate CRF. Different coloured study files (to identify allocation) should be used. Patientscould be recruited any day of the week although teams are encouraged to plan the relevant shifts over the weekend periods.

10.9 Arm-specific procedures (See table 2)

10.9.1 Intervention arm

The intervention arm for this trial will consist of testing sputum, stool and urine with Ultra and urine with AlereLAM in all recruited patients regardless of symptoms. Thus, all patients allocated to this arm will be asked to provide urine, stool and/or sputum.

If urine or sputum cannot be provided spontaneously at the time of recruitment, participants will be asked to do so within 72 hours (aim: within 24 hours from enrolment). Patients will be encouraged to drink water to stimulate diuresis (unless contraindicated). Urine will be collected in specific containers and stored for future testing. Ultra on Stool will be performed in fresh specimen according to the KNCV SOS method[33].
Additionally, we will ask for stool and oral swabs specimen for Xpert Ultra/ LumiraDx testing.

We aim to collect all samples specified for each intervention (including those for exploratory studies) within 72 hours from enrolment. Efforts will be made to collect all samples within the first 24 hours. Time of sample collection will be specified in the sample collection log and CRF.

10.9.2 Control Arm

The control arm screening and testing strategy will consist of:

Sputum Ultra whenever the patient has cough, fever, weight loss or night sweats and/orUltra on any tissue (including lymph nodes) from patients with suspected extrapulmonary TB.
Urine Alere TB-LAM, if patients 1) have signs and symptoms of TB (pulmonary and/or extrapulmonary), or 2) have advanced HIV disease* or 3) are seriously ill**. The latter two are all irrespective of signs and symptoms of TB.

*Adult patients with advanced HIV disease are those with CD4 cell count <200 cells/mm^3 or aWHO clinical stage 3 or 4 event at presentation to the clinic.

**A seriously ill patient is any individual with HIV or of unknown HIV status presenting with oneor more of the following danger signs:

- Unable to walk unaided
- A respiratory rate over 30/min
- A fever of more than 39ºC, and/or
- A pulse rate of over 120/min

Thus, sputum and urine collection will only be conducted for Ultra and AlereLAM testing if the above-mentioned symptoms/criteria are present. Otherwise, urine alone will be collected in specific containers and stored for future testing. Patients will be encouraged to drink water to stimulate diuresis (unless contraindicated).

The control arm for this trial follows WHO recommendations (as of January 2020) forbacteriological testing among hospitalized HIV-positive individuals.

Study staff will collect the samples for both arms.

In case any sample cannot be obtained within the first 72 hours from enrolment, reasons should be recorded in the appropriate CRF. In case the participant can provide the required sample(s) after 72 hours from enrolment, samples should still be collected and processed withclear indication of date and time. Special measures need to be implemented locally so the laboratory is aware of the tests to be performed despite some potential delay in specimen testing. After 72 hours from enrolment have passed, patient will be managed as per national guidelines.

Other than TB, patient clinical management will follow establishednational guidelines and protocols for hospitalized HIV-positive patients.

10.10 Follow up procedures (table 2)

Hospitalization stay (daily)

During hospitalization (daily) and at discharge, study team will verify and record whether a TB diagnosis has been made and/or anti TB treatment started. The study team will be working jointly with the routine health care staff so clinical decisions are made jointly. Study team will also record any ad-hoc TB investigation (additional Ultra, LAM testing, X-ray, etc) and their result. HIV care will follow national guidelines. .

Scheduled visits

After enrolment, there are two scheduled visits that need to take place unless the patient remains hospitalized (week 4 and week 8). These visits can occur either in the outpatient clinic, as home visits or by call or video-call depending on the patient and team preferences and COVID-19 epidemiologic situation. However, outpatient visits are recommended. A member of the study team will verify whether a TB diagnosis has been made on the relevant medical records (hospital or NTP office) and/or anti TB treatment started after discharge. Study staff will also record any ad-hoc TB investigation that was initiated by the care provider for clinical reasons (additional Ultra, LAM testing, X ray, etcetera) and their result (both by checking patient records and by asking the patient). HIV care will follow national guidelines. Vital status will also be recorded at these two visits. These visits will have a window period of -/+ 3 days (at week 4) and -3/+7 days at week 8.

In case a discharged participant does not attend the week 4 or week 8 visit, three phone call attempts will be made and, if unsuccessful, at least one home visit will be performed in order to collect relevant data to that visit. These attempts should be recorded in a patient tracking log. Week 4 visit should be sought until week 8 window visit opens, although will be considered a protocol deviation if done outside the week 4 window visit.

10.11 Participant study completion (week 8)

Patients might terminate their participation in the study at any point. Reason, if available, should be recorded in the patient file. Once participants have completed their week 8 visit (at the hospital/outpatient clinic or as home visit), they will be considered having completed their participation in the study. For those not attending week 8 visit, they will be considered lost to follow up after the three phone call attempts and home visit have occurred. Deaths occurring during the study at patient's household will be documented and relatives will be asked about signs and symptoms before death. In settings where verbal autopsies are routinely conducted (CISM), this data will be considered a study source document and information captured in the off-study-form.

10.12 Retention

Based on previous studies conducted at the sites, we expect a 5% lost to follow up rate at month 2. Sites will implement several strategies to maximize the retention rate, such as recording of several phone numbers in the locator form at recruitment, careful recording of physical address, additional contact information from relatives and friends, travel reimbursement to compensate them for the cost of transport to the clinic for study visits, etcetera, always according to local practice.

10.13 Participant termination and criteria

The study physician responsible and the PI may withdraw a patient from the trial (or certain procedures of the trial) for appropriate medical reasons, because of individual adverse events or new information gained about a diagnostic or a treatment. The physician should contact the Trial Management Team to inform of this decision. Likewise, any patient can withdraw from the study any time without explaining the reasons.

10.14 Trial discontinuation

The study may be discontinued at any time by the funder, sponsor, investigators, or by any ofthe relevant regulatory bodies and reasons for this decision will be appropriately communicated to the sites and enrolled patients.

10.15 End of trial

The sponsor will notify the authorities of the end of the clinical trial field activities within 90 days of its completion (the date of the last visit of the last patient undergoing the trial).

10.16 Safety Assessments

Refer to Section 10.17

Laboratory safety assessments will be done during study site assessment and/or study initiation visits to ensure that potentially infectious specimens are processed according to national and/or international guidelines.

Medical device incidents will be recorded and reported according to the MEDDEV 2.12/1 Rev 8 (see summary in 10.17.1 Medical Device Incidents (including Malfunctions)).

10.17 Safety and Incident Reporting

The probability of an AE or SAE occurring to a trial participant to be associated with the investigational products, or with the additional procedures used (such as sputum and urine collection, venipuncture for those HIV patients and fingerstick), is extremely low.

10.17.1 Serious adverse events (SAE) reporting

An SAE is any unfavourable and unintended sign (including abnormal laboratory finding), symptom or disease temporally associated with the use of an investigational product, whether or not related to the investigational product with a) results in death b) is life-threatening; c) requires inpatient hospitalization or prolongation of existing hospitalization; d; results in persistent disability/incapacity; e) is a congenital anomaly/birth defect or f: requires intervetnion to prevent permanent impairment or damage to the human subject.

Given the nature of this study (in which no investigational product is administered to the participant), SAE reporting is limited in scope to:

a) SAEs that may be associated with sputum, urine and blood collection
b) SAEs which results in death.

SAE reports must be sent to the FIND on a six monthly basis (line listing format). Contacts for SAE reporting can be found on the front of the protocol. SAE reports should be sent to national IRB and regulatory authorities with a similar frequency or as per national regulations for diagnostic clinical trials.

10.17.2 Medical Device Incidents (including Malfunctions)

Medical devices are being provided for use in this Trial for TB diagnosis in PLHIV. In order to fulfil regulatory reporting obligations worldwide, the investigator is responsible for the detection and documentation of events meeting the definitions of incident or malfunction that occur during the

trial with such devices. The definition of a Medical Device Incident can be found in Appendix 1.

NOTE: Incidents fulfilling the definition of an SAE related to a medical device will also follow the processes outlined above and in Appendix 1 of the protocol.

10.17.3 Time Period for Detecting Medical Device Incidents

Medical device incidents or malfunctions of the device that result in an incident will be detected, documented, and reported during all periods of the trial in which the medical device is used.
If the investigator learns of any incident at any time after a participant has been discharged fromthe trial, and such incident is considered reasonably related to a medical device provided for thetrial, the investigator will promptly notify FIND.
The method of documenting Medical Device Incidents is provided in Appendix 1.

10.17.4 Follow-up of Medical Device Incidents

The investigator is responsible for ensuring that follow-up includes any supplemental investigations as indicated to elucidate the nature and/or causality of the incident. New or updated information will be recorded on the originally completed form with all changes signed and dated by the investigator.

10.17.5 Reporting of Medical Device Incidents to FIND

Medical device incidents will be reported to FIND within 24 hours after the investigator determines that the event meets the protocol definition of a medical device incident.
The Medical Device Incident Report Form will be sent to the sponsor scanned by e-mail. If e-mailis unavailable, then the investigator should phone the sponsor and send an e-mail as soon as possible. Contact details will be provided in the Trial Manual.
The same individual will be the contact for the receipt of medical device reports. Medical device incidents will then be communicated by the sponsor to the manufacturer within 24 hours after the sponsor has received the Medical Device Incident Report Form.

10.17.6 Regulatory Reporting Requirements for Medical Device Incidents

The investigator will promptly report all incidents occurring with any medical device provided for use in the Trial for FIND to fulfil the legal responsibility to notify appropriate regulatory authorities and other entities about certain safety information relating to medical devices being

used in clinical studies.

The investigator, or responsible person according to local requirements (e.g., the head of themedical institution), will comply with the applicable local regulatory requirements relating tothe reporting of incidents to the IRB/IEC.

10.18 Retrospective data collection

Given that our control arm may not represent the local standard of care across sites, we will compare the main study endpoints of both study arms with a third cohort of patients, which follows the real standard of care at the different sites. We will retrospectively collect data of a cohort of consecutively hospitalized HIV-positive patients that were admitted prior to the trial initiation. Data to allow the evaluation of primary objectives will be collected from medical records (source documents) in an anonymized fashion. We aim to collect data from patients who were admitted to the site two months or more before the day of the trial first study patient recruitment. Patient records will be selected from consecutive admissions from that day backwards until completing the sample size (around 147 cases per site). Data collection will be obtained retrospectively in order to capture the real practice at the different sites, including potential phone calls to confirm the life status of the patients after two months of hospitalization, minimizing the effect of a potential Hawthorne effect. Given the complexity and often impossibility of obtaining informed consent from patients that have been discharged, no informed consent will be sought for these patients. This IC exemption is an exception contemplated in the International Ethical Guidelines for Health-related Research Involving Humans of the Council for International Organizations of Medical Sciences (CIOMS) (Geneva, 2016). The study has not been powered to detect differences in the primary endpoint between intervention or control arm and the retrospective cohort (which may vary between sites). This is a secondary objective that will be analyzed at site level.

11. Schedule of visits (table 2)

	Day 0 (recruitment) *Control arm*	Day 0 (recruitment) *Intervention arm*	Day 0-3 *(<72h)*	Daily	At discharge *If dischargedbefore day 28/56*	Week 4 *+/- 3 days*	Week 8 *+/- 3 days*
Baseline Evaluation							
Informed consent	X	X					
Eligibility criteria verified	X	X					
CD4 count / Viral Load[&]	X	X					
Locator information	X	X					
Brief medical history	X	X					
Proof of HIV status stored	X	X					
WHO Symptom screening	X	X			X		
TB Investigations							
Samples collected and tests	**Urine AlereLAM#** **Sputum Ultra*** Blood CD4	**Sputum Ultra** **Stool Ultra** **Urine Ultra** **Urine AlereLAM** 2x Oral Swabs Urine FujiLAM Blood CD4 Blood for CRP					
Results issued			X				
Further contacts							
Verification TB diagnosis / ad hoc TB investigations				X	X	X	X
Verification TB treatment initiation				X	X	X	X
Verification vital status				X	X	X	X
HIV care / ART				X	X	X	X

*Sputum Ultra whenever the patient has cough, fever, weight loss or night sweats and/or Ultra on any tissue (including lymph nodes) from patients with suspected extrapulmonary TB

#Urine LAM if patients have currently signs and symptoms of TB (pulmonary and/or extrapulmonary), or with advanced HIV disease (1) or who are seriously ill (2) or else irrespective of signs and symptoms of TB and with a CD4 cell count of less than 200 cells/mm^3.

[&]CD4 count testing will be done if not available within the last 3 months. Most recent viral load will be captured (if available)

**urine should be collected in all participants in the control arm, irrespective of whether LAM test is done.

12. Laboratory procedures and storage of samples

Samples will be processed and results issued back to attending physicians as soon as possible. AlereLAM results should be available within two hours from specimen collection. Ultra results should be provided within 24 hours from being collected at the participating hospital laboratory associated facilities.

12.1 Xpert Ultra for TB

This WHO recommended assay, which is currently substituting the previous version (Xpert), uses a cartridge-based polymerase chain reaction platform.

12.1.1 Sputum: Ultra testing will be performed in a sample of raw sputum (without any prior decontamination) and it requires mixing sputum with a buffer (proportion 2:1) for 15 minutes. Following incubation, 2ml of the sputum-buffer mix are transferred into the Ultra cartridge, which is placed in the Xpert instrument. Results are ready within 100 minutes. It detects *Mtb* DNA in a sputum sample and is able to detect a mutation in the resistance-determining region (RRDR) of the *rpoB* gene which is the responsible of 95% of drug resistance to rifampicin (Rif).[33] Rifampicin resistance is generally considered to be predictive of MDR since most Rif resistant strains are also resistant to isoniazid.

12.1.2 Stool: Ultra testing on stool will be done in a sterile loop from bulk stool following the KNCV SOS method (https://www.kncvtbc.org/en/sos-stoolbox/).

12.1.3 Urine: Following the removal of 200 µL of urine for AlereLAM testing (see below) and 12 ml for storage for future testing the remaining urine (around 40-50mls) will be cooled centrifuged at 3000g for 15mins in a bucket centrifuge. The supernatant will be discarded, and the urine pellet resuspended in approximately 2ml of PBS. 0.75ml of the resuspended pellet will be added to 1.25ml of Ultra sample reagent and incubated for 15 minutes, then added to the cartridge and processed as per the manufacturer's instructions.

12.1.4 Tongue swab: After the sample is collected (from brushing the swab along the length/breadth of the anterior 2/3 of the tongue dorsum for about 10 seconds) the head of theswab will be broken off and the shaft of the swab discarded. The tubes will be stored dry and later Ultra and/or LumiraDx (see 12.3) testing will be performed following instructions in SOPs developed.

12.2 Other urine-based tests procedures:

12.2.1 AlereLAM testing:

Recommended by WHO for TB diagnosis in severely immunocompromised HIV-positive patients, will be performed under the specified criteria in the control arm and in all participants in the intervention arm. A urine sample (at least 1-3 ml) will be taken at the hospital facilities (see below). The test is performed by applying 60 µL of unconcentrated urine to the Alere Determine™ TB *LAM* Ag assay strip and incubating at room temperature for 25 minutes (time-set). The strip is then inspected by eye.

The intensity of any visible band on the test strip is graded by comparing it with the intensities of the bands on a manufacturer-supplied card (LAM positive will be deemed using the grade 1 cut-off on the manufacturer's post 2014 reference card). AlereLAM tests will be interpreted by two independent readers at the clinic, and results will be recorded. In case of discrepancy, a third reader will be used as a tie-breaker. Invalid tests will be repeated using the same urine. Indeterminate results will be repeated with a different urine sample.

Alere-LAM test strips will be stored until the end of the study for data monitoring purposes. Alere-LAM tests will be read prior to the results of the sputum Ultra (if performed) and without knowing any clinical details of the patient to reduce bias.

12.2.2 FujiLAM testing (Appendix 2): Briefly, urine is added to the reagent tube up to the indicator line (approximately 200 µL), mixed, and incubated for 40 min (set timer) at ambient temperature. Aftermixing again, two drops of urine are added to the test strip. Following this, button two is immediately pressed to release a reducing agent for silver amplification. After the Go Next colour indicator mark turns orange (within 3–10 min), button 3 is pressed to release a silver-ion solutionto activate the silver amplification reaction. The result has to be read within 10 min (set timer). TheFujiLAM assay does not use a reference scale card and any line identified on the test line is deemedpositive. This test will be done retrospectively in banked samples.

12.3 LumiraDx: The test strip is introduced into the LumiraDx device through opening the instrument's door. A drop of the prepared oral tongue swab / blood specimen is applied to the test strip and a confirmation of the sample detection appears in the instrument's screen. After this, the door is closed immediately. The results are provided with in 10 minutes.

12.4 HIV testing: Rapid point-of-care antibody-based HIV testing will be performed in those patientswith unknown or undocumented HIV status using fingerprick blood. Testing will be done by trained health care workers. If the result of the first "rapid" test (Determine®, Abbott Laboratories, USA) is positive, a second confirmatory test will be performed. CD4 counts will be obtained fromaTB cases coinfected with HIV (if no recent documented CD4 counts are available-within last 3 months)

as well as all newly identified HIV-positive cases. CD4 testing will be done at each site using point of care PIMA equipment or FACSCalibur flow cytometers. Newly diagnosed HIV- positive patients will be referred to appropriate counselling services at each of the sites.

12.5 Sample Storage details:

Storage of all specimens will follow detailed SOP which will be similar at all participating sites.

Specimen storage is not a mandatory component of the protocol, but will be encouraged to accomplish exploratory objectives and retrospective testing. Storage should be done the same day of sample collection.

12.5.1 Urine samples. After removing necessary volumes for Ultra and AlereLAM testing, transfer raw urine (12 ml) to a sterile 50ml tube. Aliquot the supernatant in two 3.5 ml aliquots. Aliquots should be stored at -80 or -70ºC. In case no criteria for AlereLAM testing is in place in the control arm, urine would still be collected for storage.

12.5.3 Tongue swabs samples: Two tongue swabs will be collected. After sample collection, the head of both swabs will be broken off and stored dry in a 5ml tube at -80°C for later testing with Ultra and the LumiraDx platform.

13. Statistical considerations and data analysis

13.1 Sample Size calculations

Recent studies using Alere LAM and sputum Xpert to screen unselected admitted HIV positive patients in Southern Africa[24,29,31] show that around 17% of these patients are laboratory confirmed for tuberculosis. The added yield of using Xpert Ultra instead of Xpert would involve an 11-17%[43–45] relative increase in bacteriological confirmation. Thus, universal AlereLAM testing and sputum Ultra would likely confirm around 20% of unselected admitted HIV participants in our study. We estimate that the bacteriological confirmation in our control arm would be slightly lower than 20%, around 19%, given that we will not use a universal screening strategy but the WHO recommendations (sputum Ultra if TB compatible symptoms and AlereLAM testing if TB compatible symptoms or in those with advanced HIV disease, or seriously ill, or with a CD4 cell count of less than 200 cells/mm3). Based on existing literature, we estimated that up to 10% of participants in our control arm would not receive either Xpert Ultra or LAM testing.

We estimated that the intervention arm would yield around 26% of bacteriologically confirmed cases by universal testing (irrespective of symptoms) with AlereLAM and Xpert Ultra on sputum, urine, and stool. As mentioned before, universal AlereLAM and sputum Xpert Ultra would yield around 20% of bacteriological confirmation. Studies show that Xpert urine could increase bacteriological confirmation by 4%. Although there is limited evidence on the potential benefits of stool Xpert Ultra in adults, there are promising results observed in children (who, as HIV positive patients, often have extrapulmonary TB). Thus, we assume that adding stool Xpert could increase our bacteriological confirmation about 1%. If we apply a similar gain (observed in sputum) in urine and stool by substituting Xpert by Ultra, the combined added yield would be of around 6%. Thus, we expect to have a 26% bacteriological confirmation in our intervention arm by using universal LAM testing and Xpert Ultra on sputum, stool and urine. This would translate into a 7% difference (19-26%) between our control and intervention arm.

The estimation of the bacteriologically confirmed cases in both the control and intervention arm could have the following limitations:

- We estimate the increased yield of Ultra in urine and stool as if this additionality were independent, considering that this yield would come from potentially different phenotypes of TB patients. Otherwise, some of the gain observed by Urine Xpert Ultra or Stool Xpert Ultra might overlap and thus we could be overestimating the expected additionality in bacteriological confirmation in these samples.
- We obtained the Xpert Ultra added yield from studies performed in TB presumptive HIV positive inpatients and outpatients. Estimates of added yield vary widely in the literature. However, given that major gain of Xpert Ultra has been observed in HIV positive patients and smear negative patients, we think that the added yield of Ultra in our population (strictly admitted PLHIV) could be higher, and potentially greater in extrapulmonary samples such as stool or urine (as a recent study -Kabir et al, 2021 -of Stool Ultra in children

has shown). In this regard, we have taken a conservative approach by using the reference of Dorman et al, 2018.

- The added yield of Ultra over Xpert does not consider that some of that additional yield might be picked up by Urine AlereLAM (optimistic approach).
- Some of these limitations may impact both the control and the intervention arm and thus would probably have a limited effect on our expected difference in bacteriologically confirmed cases that we aim to detect through this study.

Assuming that we will find 19% of bacteriologically confirmed cases in the control arm following the WHO indications for sputum Xpert® Ultra and AlereLAM in HIV hospitalized patients (based on previously mentioned data), and a two-sided type I error of 5%, the inclusion of patients per arm would provide an 80% power to detect a 7% increase in the percentage of bacteriologically confirmed TB diagnosis allowing for a loss to follow-up of 5%.

Based on these estimates, the study will require a total of 586 PLHIV participants to be enrolled per trial arm. For both arms together, this yields 1172 patients in total.

Given that 4 sites will be recruiting patients, this means approximately 293 participants per site (around 147 per arm at each site).

13.2 Data analysis

A separate statistical analysis plan will be developed containing all details of the analysis strategy.

13.2.1 Analysis set

We will analyze the data using primarily an intention to treat analysis approach. This dataset is composed for all randomized patients by their assigned intervention. It also includes patients who might not complete week-4 and week-8 visit or who died or who did not receive the full assigned intervention. Lost to follow up patients and deaths will be considered for specific secondary endpoints.

13.2.2 Statistical methods

Variables will be summarized using descriptive statistical methods. Continuous data will be described using the mean and standard deviation statistics when these values follow the normal distribution. Otherwise we will use the median and interquartile range when we will not assume this distribution. Categorical values will be summarized using frequency and/or percentages.

For categorical comparisons we will use a Chi-square test or Fisher exact test according to the

data characteristics. Likewise, the continuous comparisons will be tested by Student t-test, Anova test or Kruskal Wallis test.

When necessary, we will transform the continuous data, especially laboratory data, in non-linear scale. For these cases we will describe these data with geometric mean. Heterogeneity between sites will be analyzed.

Assessment of loss to follow-up as random across arms

We will analyze the baseline characteristics between lost and non-lost follow up patients and across arms to ensure that loss to follow up occurs randomly. If we find any significant attribute we will adjust the analysis by this/these factors.

Time-to-event data

Time-to-event data (such as time to mortality, time to TB diagnosis or time to treatment initiation (among lab confirmed or all TB)) will be presented using Kaplan-Meier curves with a log rank test (two-sided 0.05 significance level). The analysis of covariates on time-to-event data will be performed using a Hazard Ratios estimated through Cox regression models.
Comparisons between control and intervention arm will be established at prespecified time points (day 7, day 14, day 28 and day 56). Mean time (and SD) to event per study arm will also be estimated.

Significance levels

The significance level for most comparisons is p=0.05. For selected secondary endpoints measured at several timepoints and subgroup analysis, the significance level will be p=0.01

Study discontinuations & patient disposition

Patient disposition and reasons for premature discontinuations and their frequencies will be tabulated.

Datasets analyzed

Number of patients in each analysis set will be tabulated and listings of any patients excluded from any analysis set will be provided.

Efficacy analysis

The primary efficacy endpoint has a composite numerator 'microbiologically confirmed TB and therapy initiated within 72 hours from admission' (success), 'non lab-confirmed TB or therapy

not initiated within 72 hours' (failure).

Microbiological confirmation may arrive later than 72 hours after enrolment (to account for scenarios in which culture is requested or there is any delay on sample processing).

The primary efficacy analysis will be compared between the proportion of microbiologically confirmed TB and therapy initiated within 72 hours of enrolment in each study arm.

Adjustment for other variables (country, hospital, seasonality or other possible confounders) may be considered and will be described in detail in the analysis plan.

Missing values will be examined in detail. We may consider a multiple imputation procedure if necessary (variables with more than 10% of missing values).

We will accept as significant difference the p-values less than 0.05.

13.2.3 Pre-specified sub-analysis

- Primary and selected secondary endpoints among PLHIV with ≥200 CD4, <200 CD4 and <100 CD4 cells/mm3 and
- Primary and selected secondary endpoints among admitted HIV positive patients providing at least one sputum, stool and (or) one urine sample for testing.
- The primary and selected secondary endpoints will also be calculated among those who had their test result available and were started on treatment within 72h of enrolment in both arms (as in a classical per-protocol (PP) analysis).

Both the main clinical trial analyses (related to primary and secondary objectives) and that related to the exploratory objectives (oral swab Ultra and LumiraDx and urine FujiLAM sub-studies) will be expanded in separate statistical analysis plans.

13.2.4 Interim analysis

For this study no formal interim analysis is planned.

13.2.5 Software documentation

The statistical analysis will be performed with the Stata software. We will use the Stata 17 version or the last most up to date available version when analysis takes place. Scripts used in the analysis can be published as supplementary material of the main publication or uploaded into a public repository

14. Data management

14.1 Type of data that will be collected

a) Participants' personal data

This trial will collect pseudonymized demographic and clinical data. This includes variables such as TB symptoms,weight, height, medical history (history of diabetes, renal failure, others), current and past medication, HIV status, ART use, tobacco and alcohol use, previous TB episodes, existence of TB contact at home, among others.

b) Laboratory parameters (biological data) derived from laboratory analyses of participants' specimens

Results from all TB microbiological assays performed to patients including CD4 counts (if not available within the last 3 months) and HIV test (if not documented status available), will be linked to study participant's information through a unique study identifier number. All samples and databases will not contain patient's name but only laboratory barcodes and anonymized patient study numbers.

14.2 CRFs

We have developed 10 main CRFs for all participants:

- Consent & Eligibility CRF
- Baseline Characteristics CRF
- Specimen Collection CRF
- AlereLAM & CRP CRF
- FujiLAM Results CRF
- Xpert Ultra Tongue Swab Results CRF
- Lumira Dx Test Results CRF
- Hospitalization assessment CRF
- 4 Week visit CRF
- 8 Week visit CRF
- Lab results CRF (including TB specific and other lab results (CD4 counts, viral load)
- Study Completion CRF(whenever a patient stops being part of the study (completed all procedures -at week 8), death before week 8, or LTFU at any point within the first 8 weeks).

In addition, a retrospective local standard of care CRF will be used (Retrospective CRF).

The Lab result CRF might be used at

any point within the study period if additional TB tests are performed on the patient.

14.3 Data entry

Data will be collected on paper based CRFs and entered to a secured encrypted and password protected electronic CRF system using OpenClinica® Software (Enterprise version 4), a secure web application for building and managing online surveys and databases. While OpenClinica can be used to collect virtually any type of data, it is specifically geared to support online data capture for research studies and operations.

Data collection and entry will be performed by study specific personnel trained in data collection and human subjects data protection. Data managers will design the electronic CRF, ensure all data quality and provide support whenever a problem occurs. Each completed CRF should be data entered within a week from its completion.

Although main data capture will be based on paper CRFs replicating electronic CRFs, there will be handwritten data source documentation (additional patient medical history, HIV proofs, X ray data, printouts of Ultra results, clinical follow up information and other), which will be kept with patient study files locally and that might be used for data quality checks. All personnel with direct access to data will be trained on human subjects' data protection and maintain training records.

Different data access privileges to OpenClinica will be set for different user types (local data clerks, local data managers, local study coordinators, site PIs, and project overall data manager from LMU). The Site PI and site project manager/study coordinator are responsible for assuring that the data entered into OpenClinica is complete, accurate, and recorded in a timely manner.

In addition to data capture, sites will maintain logbooks to record the dates of completed and upcoming study visits, specimen collection, transport and receipt at the respective laboratories, and time of receipt of test results. Laboratory tests will be requested on study laboratory test requisition forms. All logbooks and laboratory requisition forms will be kept in locked filing cabinets when not in use and will be accessible only by designated clinic personnel.

14.4 Data monitoring and quality assurance

Electronic CRFs will be designed in order to minimize data capture errors. Drop-down menus, data range limits/edit checks and data type protection will be included in open data collection fields. Data will be curated by local data clerks and routine data checks performed by local data managers. The project main data manager at LMU will periodically generate a list of data queries

derived from the reconciliation of different clinical and lab project data. Automated syntaxes will be designed in order to improve the efficiency of the quality assurance process.

No official onsite data monitoring visits will be done in this study. Central/remote monitoring will happen remotely through LMU/FIND.

14.5 Record retention

Study records (source documents, signed informed consent forms, IRB/IEC correspondence and approval letters, and screening logs) will be kept in a secure location accessible only to authorized study staff, investigators, and monitors. Secure archives are available at the sites for preliminary storage after study closure, before moving them to an off-site, secure storage facility. All records will be archived in a secure storage facility for at least ten years after the completion of the study.

15. Ethical considerations

This study will be conducted according to the ethical principles set forth in the Declaration of Helsinki, ICH-GCP, and local regulatory requirements as applicable.

Written informed consent will be obtained from each participant prior to any protocol-specified procedures being conducted. An added IC for specimen storage (and future use) might be required at some sites. The protocol and informed consent form(s) will be reviewed and approved by the IRB or IEC of each participating clinical site prior to patient enrollment. Site investigators are responsible for ensuring that the protocol is reviewed by an IRB/IEC with the appropriate composition (per site guidelines). The investigator will inform the IRB/IEC as to the progress of the study at applicable intervals as defined by IRB/IEC policy. Depending on national policies, the protocol might be reviewed by national regulatory authorities.

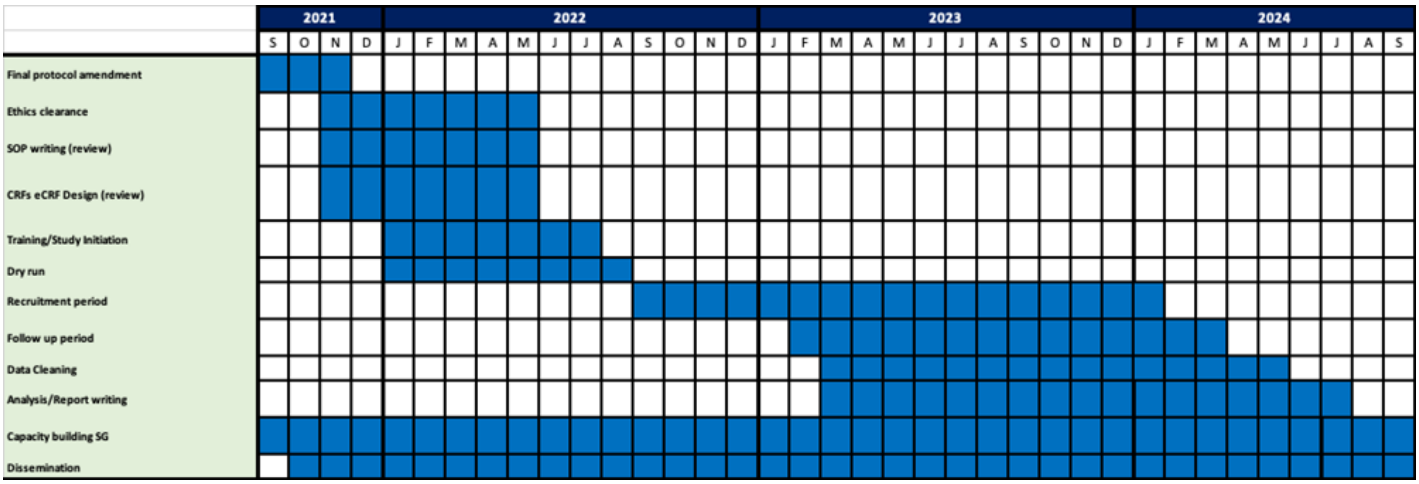
FIND will act as the main sponsor for this study. Delegated responsibilities will be assigned locally and documented. FIND holds Public Liability ("negligent harm") and Clinical Trial ("non-negligent harm") insurance policies which apply to this trial.

15.1 Confidentiality

Adequate measures to ensure personal data protection and confidentiality will be taken, according to the European General Data Protection Regulation (EU) 2016/679 (GDPR) on the protection of natural persons with regard to the processing of personal data and on the free movement of such data. National regulations on personal data protection will be implemented to safeguard patients' confidentiality throughout the duration of the clinical trial.

All study related data (study CRFs and clinical specimens) will be pseudonymized linked to an individual patient study number, and samples will be numbered/barcoded in accordance. Names or other obviousidentifiers will not be used on study CRFs, they only will appear at each site on source documents. Any hardcopy links between patient names and patient study numbers or data will be kept in a locked cabinet at each study site, under the responsibility of the PI/site coordinator. All informed consent forms will also be maintained in a locked cabinet at each of the sites. All study personnel and investigators will be trained prior to study initiation on all aspects related to patient's confidentiality. Study results will be reported and published in summary form without any patient-identifying information.

16. Timeline



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18. Appendices

Appendix 1: Incident Definition and Reporting

Incident: Any malfunction or deterioration in the characteristics and/or performance of a device, as well as any inadequacy in the labeling or the instructions for use which, directly or indirectly, might lead to or might have led to the death of a patient, or USER or of other persons or to a serious deterioration in their state of health.

The following criteria as defined in sections 1.1, 1.2 and 1.3 are to be considered to determine whether an adverse incident has occurred. Please note that all three criteria must be met to be deemed as reportable. Please also refer to MEDDEV 2.12-1, Guidelines on a Medical Devices Vigilance System for further guidance.

1.1 An Event has Occurred

This also includes situations where testing performed on the device, examination of the information supplied with the device or any scientific information indicates some factor that could lead or has led to an event.

Typical events include, but are not limited to:

1.1.1 A malfunction or deterioration in the characteristics or performance (should be for intended purpose and follow manufacturer's instructions).

1.1.2 For IVDs where there is a risk that an erroneous result would either (1) lead to a patient management decision resulting in an imminent life-threatening situation to the individual being tested, or to the individual's offspring, or (2) cause death or severe disability to the individual or foetus being tested, or to the individual's offspring, all false positive or false negative test results shall be considered as events.

For all other IVDs, false positive or false negative test result falling outside the declared performance of the test shall be considered as events.

1.1.3 Unanticipated adverse reaction or side effect.

1.1.4 Interactions with other substances or products.

1.1.5 Degradation / destruction of the device.

1.1.6 Inappropriate therapy.

1.1.7 Inaccurate labelling, Instructions for Use (IFU) and / or promotional materials (includes omissions and deficiencies but not those generally known by user).

1.2 The Manufacturer's Device is Suspected to be a Contributory Cause of the Incident

1.2.1 In assessing the link between the device and the Incident, the Manufacturer should take account of:

1.2.1.1 Results of the manufacturer's own preliminary assessment of the incident.

1.2.1.2 Evidence of previous, similar incidents.

1.2.1.3 Any other relevant evidence held by the manufacturer.

1.2.2 Where there are a number of devices involved, the manufacturer should always assume their device contributed to the incident until proven otherwise.

1.3 The Event Led, or Might Have Led, to One of the Following:

1.3.1 The death of a patient, user or other person.

1.3.2 Serious deterioration in the health of a patient, user or other person that can include:

1.3.3 Life-threatening illness.

1.3.4 Permanent impairment of a body function or permanent damage to a body structure.

1.3.5 A condition necessitating medical or surgical intervention to prevent the two points above; for example clinically relevant increase in surgical procedure, a condition requiring hospitalisation or prolongation of existing hospitalisation.

1.3.6 Any indirect harm as a consequence of incorrect diagnostic or IVD test result when used within the manufacturer's IFU (use errors reportable under section 5.1.5.1 of MEDDEV 2.12/1 must also be considered)

1.3.7 Foetal distress, foetal death or any congenital abnormality or birth defect.

Appendix 2: FujiLAM testing

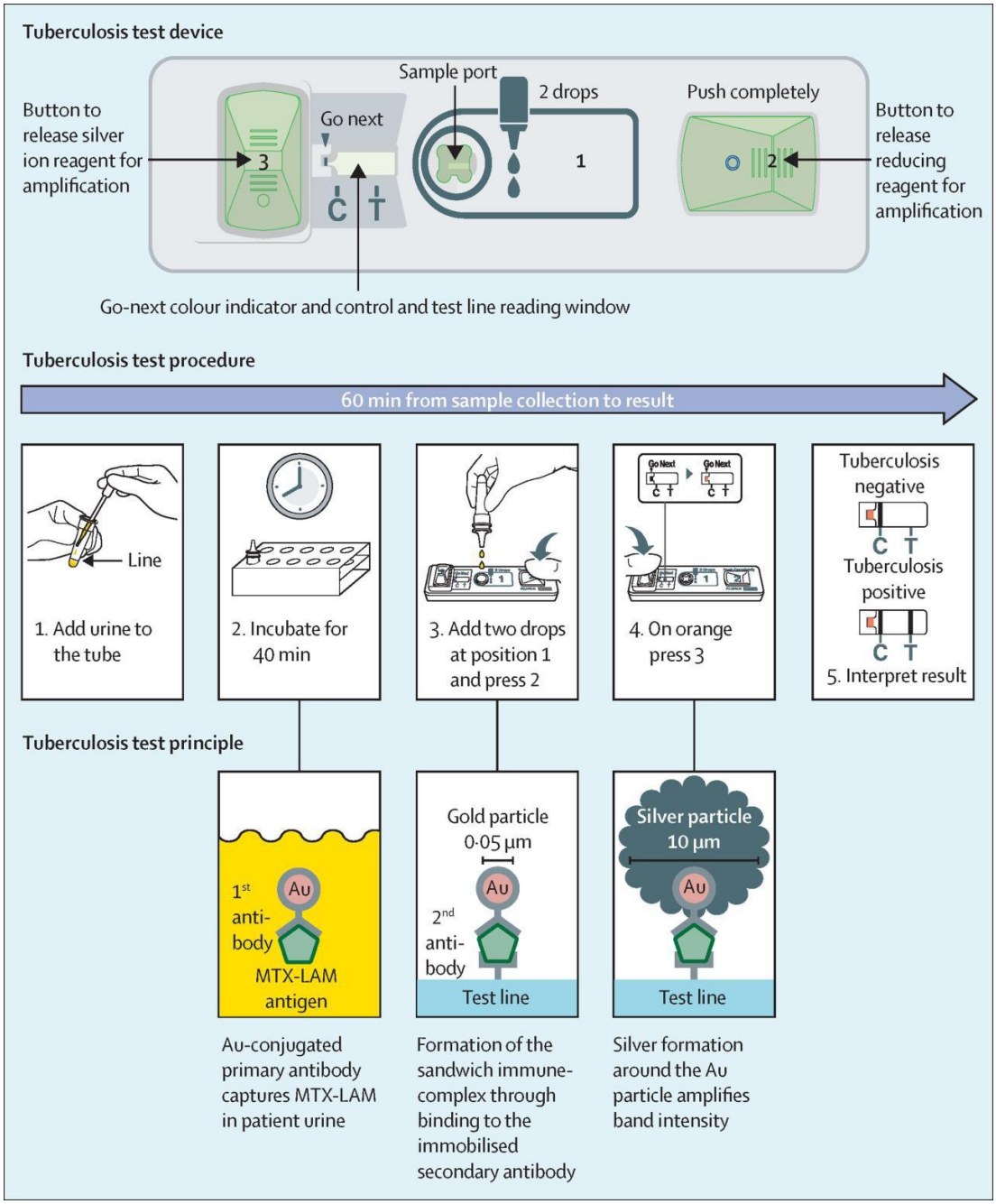

Figure 1: FujiLAM TB test device, test procedure and test principle. (MTX-Man refers to mannose caps further modified with a 5-methylthio-D-xylofuranose residue). Au=gold. C=control line. MTX-LAM=5-methylthio-D-xylofuranose-lipoarabinomannan. T=test line. Image obtained from Broger et al, 2019.[31]