Culture free diagnosis and follow-up of multidrug resistant tuberculosis patients (DIAMA)

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Statement of Compliance & Confidentiality

The information contained in this study protocol is privileged and confidential. As such, it may not be disclosed unless specific permission is given in writing by the principal investigator or when such disclosure is required by federal or other laws or regulations. These restrictions on disclosure will apply equally to all future information supplied which is privileged or confidential.

Once the final protocol has been issued and signed by the Investigator(s) and the authorized signatories, it cannot be informally altered. Protocol amendments have the same legal status and must pass through the mandatory steps of review and approval before being implemented.

By signing this document, the Investigator commits to carry out the study in compliance with the protocol, the applicable ethical guidelines like the Declaration of Helsinki and consistent with international scientific standards as well as all applicable regulatory requirements. The Investigator will also make every reasonable effort to complete the study within the timelines designated.

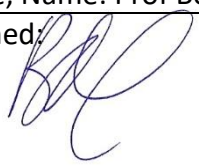
PROJECT COORDINATOR:

Title, Name: Prof Dissou Affolabi Date: 24 Nov 2016

Signed: 

PRINCIPAL INVESTIGATOR OF THE PARTNER INSTITUTION:

Title, Name: Prof Bouke de Jong Date: 24 Nov 2016

Signed: 

Synopsis

The proposed DIAMA (DIAgnostics for Multidrug resistant tuberculosis in Africa) study aims to address current gaps in the diagnosis and management of patients with Multi-Drug-Resistant (MDR) tuberculosis (TB). Building on existing networks and research collaborations, the project aims to evaluate and implement rapid and accurate molecular tests for resistance to anti-TB drugs, to replace the current dependency on phenotypic drug resistance testing (DST), which takes up to 4 months and is technically so demanding that few laboratories can perform these tests correctly.

The project builds on the World Health Organization (WHO) recommended continuous surveillance of patients for rifampicin resistance. Two African partners with advanced molecular laboratories will establish reference laboratories for the 'Deeplex' assay, a novel multiplex deep sequencing-based drug resistance diagnostic platform that simultaneously provides sequence information of genes that confer resistance to key TB drugs. Partners will recruit all patients with RR TB, and a subset of those with rifampicin sensitive TB. In a first phase, sputum will be shipped for the Deeplex assay, for comparison against phenotypic DST to 1st and 2nd line drugs. In a second phase, InSilixa and Cepheid 2nd line Xpert, two 'lower tech' tests at the last stages of laboratory validations, will be implemented in all countries that have established recruitment of retreatment patients. The InSilixa lab-on-chip assay, powered by a smartphone, is able to query 117 drug resistance conferring mutations at around $20 per test. The Cepheid Xpert 2nd line cartridge can be implemented in existing Xpert machines used for the Xpert MTB/Rif assays. These two tests will be compared versus the Deeplex assay.

Using the latest advances in e-Health software 'DataToCare', molecular results would be communicated in real time to the National TB Programs, so that MDR patients can swiftly start appropriate treatment.

Lastly, once patients have initiated MDR treatment, they will be monitored for treatment success by faster alternative approaches to the WHO recommended monthly cultures: serial sputum samples will have Fluorescein DiAcetate (FDA) vital stain microscopy and measurement of the bacterial load using the Xpert MTB/Rif.

Together, these advances are expected to dramatically improve the currently dismal prognosis of MDR-TB in health systems in resource-poor settings.

Introduction

1. Background

Background on MDR-TB diagnosis

The majority of MDR-TB patients worldwide today are neither detected nor accessing appropriate treatment (1). Recent advances in molecular diagnostics, especially the Xpert MTB/Rif test (2), have reduced the time to diagnose MDR-TB, and improved treatment regimens are rolled-out concurrently (3-5). Despite these advances, many questions remain. Only rifampicin resistance is diagnosed by the Xpert MTB/Rif test, leading to presumptive diagnosis of resistance to isoniazid and maybe other drugs. Resistance to other anti-TB drugs, linked to a range of mutations in other genes, affects the patients' prognosis and optimal treatment composition (6). Unpublished results from Institute of Tropical Medicine (ITM, Antwerp, Belgium) suggest that certain specific mutations in the gyrase gene confer high-level resistance to fluoroquinolones, and are associated with poor MDR TB treatment outcome. Patients with RR TB therefore need treatment adjusted towards other potent drugs. However, assessing resistance to other drugs currently takes weeks to months (7). In the present study, we aim to swiftly and accurately diagnose resistance to rifampicin and other drugs at country and regional levels by:

- Validating a higher complexity assay (Deeplex) that would be available in two regional reference laboratories for the benefit of the whole regions
- Validating lower complexity assays that, albeit less informative, could be available country-wide at district level (Xpert 2nd line) and/or as point of care tests (InSilixa)
- Identifying the thresholds of proportions of drug resistant mutants directly in sputum that predict clinical resistance and poor outcome.
- Informing the potential addition of phenotypically and/or clinically validated mutations identified by Deeplex to the InSilixa point-of-care assay

Technical background – Deeplex assay

The Deeplex test, developed by Genoscreen (8), consists of a targeted Next Generation Sequencing (NGS) assay, coupling multiplex PCR to deep sequencing. This approach is designed to be directly applicable on clinical samples, providing species- and *M. tuberculosis* lineage identification, and detection of drug resistance. The Deeplex test can simultaneously detect mutations in numerous genes that confer resistance to multiple anti-TB drug classes: The amplicons are deep sequenced on a MiniSeq platform, This molecular assay is coupled to a dedicated secured, cloud-based bioinformatics application, including analysis of deep sequencing data and final reporting of identification of resistance associated mutations. The MiniSeq based Deeplex test however requires specific infrastructure, equipment and technical skills and will only be deployed in well-established reference laboratories such as Laboratoire de Référence des Mycobactéries (LRM, Cotonou), Rwanda Biomedical Center (RBC, Kigali), and ITM in Antwerp. A sample referral system will be set up between the different countries to ensure shipment to the two African laboratories; ITM will serve as a back-up and external referent. Once concurrent phenotypic assays will not be needed any longer in the context of this project, sputum referral systems can include inactivation, such as in ethanol, to facilitate sample shipment (non-infectious, and no cold chain required).. The Deeplex assay will in a second phase also serve for validation of the point of care tests,

as it will detect all mutations in the respective genes. Moreover, it may identify novel mutations, including those unique to strains circulating in Sub-Saharan Africa.

Table 1. Antibiotics, and their gene targets, to which resistance is detected in Deeplex

Gene target	Antibiotic	Deeplex
aphC	Isoniazid	+
eis	kanamycin	+
embB	ethambutol	+
ethA	ethionamide	+
fabG1	isoniazid	+
gidB	streptomycin	+
gyrA	Fluoroquinolones*	+
gyrB	Fluoroquinolones*	+
inhA	isoniazid	+
katG	isoniazid	+
pncA	pyrazinamide	+
rplC	linezolid	+
rpoB	rifampicin	+
rpsL	streptomycin	+
rrl	linezolid	+
rrs	Injectable agents	+
tlyA	capreomycin	+

*including ofloxacin, levofloxacin, moxifloxacin and gatifloxacin

Technical background – InSilixa 'HYDRA-1K' chip

The InSilixa 'HYDRA-1K' chip (9) can detect 117 resistance conferring single nucleotide polymorphisms (SNPs), in addition to the wild-type sequence, all in triplicate, in a disposable chip and a hand held reader that can be operated on a smart phone (10). This test, rapid and cheap, including integrated sample processing, is at the last stage of laboratory validation and is expected to be ready for field testing in the second phase of the project. The platform is flexible, like Deeplex, and can have novel SNPs added.

Preliminary results – InSilixa 'HYDRA-1K' chip

InSilixa's chip has been validated on the WHO Tropical Disease Research (TDR) TB strain collection of 229 strains and showed good results.

Sputum processing will be done in an integrated platform and has been completed successfully on artificial sputum spiked with different concentrations of Mycobacterium tuberculosis complex bacteria.

Technical background- Xpert 2nd line

The Xpert MTB/Rif (developed by Cepheid) has already revolutionized the rapid detection of resistance to rifampicin (2). As this platform is rapidly expanded, and is based on a closed system that limits contamination, a cartridge for 2nd line drugs will be easily implementable and provide rapid indications on resistance to 2nd line drugs. Based on communications by Cepheid representatives, the Cepheid 2nd line cartridge is expected to be released in early

2017. It will be implemented for patients who test RR in the Cepheid Xpert MTB/RIF and will be compared against the Deeplex and InSilixa tests.

Background on continuous surveillance

WHO recommends to perform continuous surveillance for rifampicin resistance among retreatment patients, in whom such resistance occurs in around 15-20% in most Sub Saharan African settings (1). This tends to be done using the Xpert MTB/Rif, which in most settings is highly sensitive for rifampicin resistance, yet does not detect additional resistance to other antibiotics. The Xpert moreover misses the *rpoB* mutations outside of the test region, of which mainly the *rpoB*572 mutation is clinically relevant and is included in the novel assays tested in this proposal.

Background on MDR notification rates at partner institutes

The partners in the proposal expect to recruit at least 80% of the eligible retreatment patients (those with rifampicin resistance and the next rifampicin sensitive patient) presenting for diagnosis and care. The expected numbers of rifampicin-resistant TB are as follows:

Table 2. Annual new Rifamipicin-resistant (RR) TB diagnoses among patients at the African partner institutions

	Estimated RR-TB patients diagnosed /year
Benin, LRM	30
Cameroon, TBRL (*)	80
DRC, INRB	150
Ethiopia, JU	55
Guinea, GNRL	100
Mali, SEREFO	20
Nigeria, DF	55
Rwanda, RBC	90
Senegal, CHUD	10
Total	**590**

Background on MDR treatment monitoring

Currently, culture on monthly sputum samples is recommended by WHO for follow-up of MDR-TB patients under treatment (11), who are monitored through the end of their treatment. Unfortunately, culture is often not locally available and samples need to be transported from field to culture laboratories. The associated transport delays lead to high rates of contamination and false negative culture, thus leading to missing and unreliable data, particularly in laboratories in low resource settings. Even when locally available, negative cultures require 42 days of incubation, with the associated delay in knowing whether a patient is responding to therapy (5).

Technical background – FDA

Fluorescein DiAcetate (FDA) microscopy is a low cost diagnostic based on the FDA ester that needs to be cleaved by enzymes in live bacteria before it fluoresces, and slides can be read in the same fluorescent microscopes used for auramine microscopy (6). Preliminary results suggest that FDA vital stain microscopy correlates strongly with culture in MDR patients on follow-up.

Technical background – Xpert MTB/Rif

Similarly, we hypothesize that the now widely available Xpert MTB/Rif assay can be used to follow the decline in bacterial load, akin to the measurement of viral loads for the monitoring of HIV patients on therapy (12). We expect that the slope of the decline will be predictive of successful outcome.

Technical background on the connectivity solutions for GeneXpert

The Xpert has revolutionized the rapid diagnosis of RIF resistance in endemic settings, yet the logistics of data reporting can be challenging.

The GeneXchange™ system, now improved as DataToCare is an information technology solution for capturing and analyzing data linked to Xpert, developed for low resource countries. This software permits to capture data directly from the laptop of the Xpert machine, such as the test results, including precise information such as cycle threshold (Ct) and individual probe results, basic epidemiological information and basic clinical data. This information is then sent by SMS or internet to a central server, which allows consultation, analysis and exportation of data using a web interface. The connectivity solutions will target the Xpert results and Specific systems will be developed to allow data capture and transmission of the novel tests.

2. *Rationale*

We anticipate that the impact of the DIAMA project, if the results are as promising as expected, will be potentially paradigm-shifting. We foresee that MDR-TB will move from an often terminal illness with grave prognosis to a readily recognizable and treatable disease, in which treatment success can be predicted early, so treatment can be modified in 'real' time. The novel tests that identify specific resistance to particular drugs used for the treatment of resistant TB allow for a way of 'personalized medicine', and by monitoring the impact of specific mutations on the outcome of MDR treatment, we anticipate learning which mutations should lead to treatment modifications in order to enhance the chance of treatment success. In addition, we will learn whether heteroresistance, which is the simultaneous occurrence of bacterial populations with and without resistance, is of clinical relevance. Lastly, the fact that the Deeplex assay gives full sequence information on a large number of genes allows us to detect, for the first time, whether different mutations are common in circulating drug resistant strains in the participating countries in Sub-Saharan Africa. Such results will thus not only inform the treatment algorithms used in clinical trials for MDR-TB, yet also provide fundamental insights into the pathogenesis underlying the development of TB resistance.

Once the validation of the novel diagnostic tests for TB resistance mutations will be completed in the DIAMA proposal, the companies that have developed these tests will

include these results in their respective applications for approval by regulatory authorities, and for endorsement by WHO. The monitoring of MDR patients on treatment with FDA microscopy is readily implementable in most peripheral microscopy centers.

Study Objectives

1. Overall Aim:

To improve diagnosis and management of MDR-TB patients with culture-free approaches

2. Primary objectives:

1. To diagnose TB resistance to 1st and 2nd line drugs through novel molecular multiplex assays (Study 1)
   a. To validate the Deeplex assay and establish a network for shipment of sputum samples in ethanol to regional reference laboratories (Study 1 – phase 1)
   b. To validate the InSilixa chip-based detection of resistance as a point of care test (Study 1 – phase 2)
   c. To validate the Cepheid Xpert 2nd line cartridge at the district level
2. To setup alternative culture-free approaches for the monitoring of patients' response to MDR treatment (Study 2), with:
   a. FDA microscopy
   b. Measurement of bacterial load by following Cycle threshold (C_t) values in Xpert MTB/Rif

3. Secondary objectives:

1. To estimate the proportion of additional resistance in patients resistant to RIF (isoniazid, and other drugs) in the countries involved using the novel validated tests and identify associations of programmatic outcome in MDR patients.
2. To measure the association of specific mutations against INH and PZA with programmatic treatment outcome
3. To pilot whether implementation of DataToCare for real-time monitoring of molecular test results can reduce delay between diagnosis and treatment of RR-TB patients

Methods

The study is expected to start in the first quarter of 2017, and will end May 31st, 2021.

1. Study design:

Two multi center observational studies will be conducted:

- *Study 1:* Cross sectional study for TB cases aged ≥ 15 (all RIF resistant (RR) ones (new cases or retreatment patients) and equal number of RIF sensitive retreatment

patients from the same country) with comparison of TB resistance diagnostic test performance at baseline. There are 2 phases in this project; phase 1 is the comparison of Deeplex results against the phenotypic results (Gold standard), phase 2 is the comparison of Insilixa and Xpert 2nd generation results against Deeplex (gold standard).

- *Study 2:* Cohort study of RR-TB patients (recruited in Study 1) under treatment with comparison of the performance of FDA or Xpert compared to solid culture

The study flow chart is placed in annex 1 of this protocol

2. Study settings

The study takes place in nine African countries (Benin, Cameroon, DRC, Ethiopia, Guinea, Mali, Nigeria, Rwanda and Senegal) with patients recruited at a national or regional level (depending on country size), and the samples tested in the respective reference laboratory (see partners). All partners involved in this study have or will implement(ed) a routine system of screening all TB patients at risk for resistant TB aged ≥ 15 with Xpert/MTbRif.

3. Study population

In order to be eligible for study 1 and 2, study participants **must meet the following criteria:**

- Being ≥ 15 year old
- Having a positive test on GeneXpert (*M. tuberculosis*) with or without resistance detected to rifampicin
- Willing and able to provide written informed consent, or for minors: assent from these patients and consent from a legal representative

In addition, for the study 2 (cohort study), only patients for whom RIF resistance was detected with GeneXpert at baseline and who started MDR-TB treatment will be eligible.

4. Sample size

The partners in the proposal expect to recruit at least 80% of the eligible retreatment patients (those with RIF resistance -considered as MDR- and equal numbers of RIF sensitive patients) presenting for diagnosis and care. Based on previous years data, over 4700 patients at risk of RR TB are on average collectively being screened annually out of which 590 (15%) are diagnosed as being RR-TB (Table 2, in Background.
For the validation of the novel tests (Study 1), we will recruit all RR patients and equal numbers of RIF susceptible patients, for a total of 1420 RR- and 1420 RIF susceptible patients. In the table below, we summarize the power we expect to have to detect resistance to the key drugs:

Table 3. Sample size considerations against **phenotypic** gold standard (Phase 1), and the **genotypic** gold standard (Phase 2, see study flow chart in Annex 1), with each phase including 1420 patients, half of whom will have RR TB, for a total of 2840 patients.

Drug	Expected prevalence of resistance in RIF R (n=710 each phase)	Expected total prevalence of resistance (n=1420 each phase)	Expected sensitivity and precision against phenotypic test	Expected specificity and precision against phenotypic test	Expected sensitivity and precision against genotypic test	Expected specificity and precision against genotypic test
RIF	710 (100%)	710 (50%)	98% +/- 1%	98% +/-1%	98% +/- 1%	98% +/-1%
INH	675 (95%)	923 (65%)	90% +/- 1%	98% +/-1%	98 +/- 1%	98% +/-1%
FQ	43 (6%)	43 (3%)	85% +/- 8%	98% +/-1%	98%, range 88%-100%	98% +/-1%

RIF= rifampicin, INH=isoniazid, FQ=fluoroquinolones

The estimated prevalence of resistance is based on the WHO report (1) and personal communications. This sample size will be ample to validate these tests for resistance to RIF, isoniazid, and fluoroquinolones, according to established guidelines (9).

5. Study outcomes

- For the study 1 (validation of deeplex test, Insilixa and Xpert 2nd line):

Drug resistance to INH, RIF, ofloxacin, kanamycin as measured by phenotypic methods and Deeplex method.
Secondary outcome: for both RR- and RS patients programmatic treatment outcome will be recorded.

- For the study 2 (cohort study, on RR patients only):

Three types of unfavorable treatment outcomes will be considered:

- Early treatment failure defined as a culture positive at month 6 of the TB treatment (+/- 2 weeks) not followed by negative consecutive cultures and with molecular confirmation of the similarity of the strain with the baseline one
- End of treatment failure defined as a positive culture at the end of the TB treatment (+/- 2 weeks) not followed by negative consecutive cultures and with molecular confirmation of the similarity of the strain with the baseline one
- Relapse defined as a positive culture after the end of the TB treatment not followed by negative consecutive cultures and with molecular confirmation of the similarity of the relapse strain with the baseline one.

6. Data management & Data Collection

Data collected:

For each patient enrolled in study 1 & 2, an expanded laboratory request form will be completed.

For all patients the following data will be collected:

- Baseline characteristics (mostly those collected from routine monitoring, including demographic- and contact information, detailed treatment history including details such as failure vs relapse prior MDR treatment, return after loss to follow-up), risk factors for (resistant) TB
- Phenotypic drug sensitivity tests (phase I of Study 1 only)
- Deeplex results (phase I and II of the Study 1)
- Programmatic treatment outcomes

In addition:

- for the phase 2 of the study 1 (validation of InSilixa and 2nd line Xpert test) the results of these 2 tests (InSilixa and 2nd line Xpert) will also be recorded

- for all patients enrolled as RR, the following additional information will be recorded:
  - monthly FDA results,
  - monthly GeneXpert and culture results,
  - treatment outcomes

Database:

A Master database will be developed by Savics company together with the central study Data Manager (DM) of Benin. A unified data entry system will be used, with a unique identifying number for each patient who is recruited in the study.

Data entry and control:

Each partner will be responsible for regular data entry with built-in validation checks, internal quality control and regular back-up. All data collected will be kept in a secure place with access restricted to study staff.

The Study Data Manager of Benin will remotely follow data entry. Quality control checks will be performed. After review of the files, necessary queries, if arising, will be immediately addressed to the country-site data manager for clarification using patient query forms. After resolution of the queries, corrected data will be entered in the database. All databases will be secured with password-protected access systems.

7. Patient management

An informed consent form will be required to be signed by all patients enrolled in the studies.

For minors, an assent will be sought for participating in the studies and a legal representative will provide consent.

If patients or legal representative are illiterate a literate witness will check that the consent process is done as per scheduled and will sign the consent form.

Patients found to have RR-TB will be referred to initiate MDR-TB treatment and will be eligible to participate in both Study 1 and Study 2, while the RIF sensitive patients will participate in Study 1 only.

RR TB patients will be followed during the whole course of the MDR-TB treatment, as per routine recommendations (study flow-chart in annex 1).

8. Laboratory procedures

Sampling schedule

The DIAMA project is based on routine diagnosis and patient management.
While country TB programs recommend collecting sputum samples, a maximum of two additional samples will be asked at the same visit.

All patient interventions and associate laboratory tests are summarized in Table 4 below.

Table 4. Schedule of assessment

	Screening [1]	***Enrollment*** [2]	***Follow up***[3]
Medical/demographic history	X		
Informed Consent		X	
Sputum collection	X	X	X
Programmatic outcome follow-up[1]			X

[1] *Includes routine programmatic interventions*

[2] *May be combined with screening*

[3] *RR patients only*

As no additional visit is required, and intervention is minimal, no compensation fee will be given to the participants.

Specific tests

In all settings, smear microscopy with Ziehl-Neelsen (ZN) or auramine/rhodamine (A/R), culture, 1st line drug susceptibility testing (DST) and Xpert MTB/Rif are routinely used. Where 2nd line DST on solid culture is not yet available, the project will help implementing the recommended tests on a routine basis. If implementation is not possible in a reasonable framework, respective samples will be tested in one the study reference labs (LRM Cotonou, RBC Kigali or ITM Antwerp), together with the Deeplex.

Samples will undergo direct auramine microscopy and direct FDA microscopy. All samples (including AFB negative) will undergo Xpert MTB/Rif testing and culture.

As all partners already conduct phenotypic DST, the culture and first-line phenotypic DST will be conducted on-site, following training for second line DST by the Lowenstein Jensen proportion method as necessary. Participating laboratories will receive periodic External Quality Assessment (EQA) panels from the ITM in Antwerp. Surplus sputum will be stored at

-20C and serves for testing additional bacterial diagnostic tests, such as the InSilixa and Cepheid 2nd line tests available in the second phase of study 1.

Partners are moreover encouraged to follow WHO guidelines regarding the use of Line Probe Assays (LPA) for FQ and injectable resistance for RR patients, which is not part of the DIAMA study protocol. Given the intrinsic delays of shipments in phase I, and the fact that the Deeplex is not yet validated in phase I, the use of such LPAs will support the appropriate choice of treatment regimen for RR patients. The partner laboratories will however be able to request results from Deeplex testing to weigh these 'unvalidated' results in the choice of a salvage regimen in case patients fail their RR treatment.

Shipment samples

The shipment of samples to the reference laboratories in Cotonou and Kigali will be organized, based on parallel sputum aliquots in validated sputum preservative, to avoid contamination, for phenotypic testing if not completed on-site; as IATA Category A shipment), and for Deeplex testing. Quality control and troubleshooting will be provided by shipping samples to ITM, where the same Deeplex tests will be set-up, as well as to Genoscreen, which will offer support to all three laboratories using the test.
Sample shipping has been reinforced through organizing a 2-day IATA shipping course in Cotonou, followed by an examination for IATA certification for all partners.

DataToCare

To help increase the GeneXpert connectivity, we will assess the landscape and their eventual existing connectivity solutions in all countries at the start of the project. This will allow us to develop a plan for implementation and upgrade of connectivity solutions in selected sites adapted to the local context. In the selected sites, we will provide ongoing support after installation, and monitor the Xpert results, diagnostic delay, and users' satisfaction.

9. Data Analysis

For the phase I and II of the study 1 (validation of Deeplex against phenotypic DST & validation of InSilixa and Xpert 2nd generation against Deeplex results), we will validate the molecular assays for their performance (Se and Sp) on the diagnosis of RIF resistance, against phenotypic DST on solid medium as gold standard, with resolution of discordances by classic target sequencing of the *rpoB* gene. Similar analyses will be conducted to determine the performance for the diagnosis of isoniazid and fluoroquinolone resistance.

An interim analysis will be performed after 350 RR-TB patients have been recruited in order to test our assumptions regarding resistance proportions and consider adjustment of sample size or recruitment strategy.

For the study 2, the results will be analyzed with time dependent Cox regression to identify the optimal time point to identify patients who are at risk of failing treatment.

Ethical considerations

This study is mainly based on routine procedures, already in place or to be implemented as capacity building. All patients will receive the diagnostic testing as per recommendations. The only intervention concerns the request of two additional sputum samples after inclusion in the study. Minors from 15 years old will be included, as they produce sputum samples like adults. During follow-up, MDR patients will have to give two samples, i.e. an additional one to the one requested for routine management. The production of an additional sputum sample is very unlikely to cause any major harm to the patient. Personal data will be protected, with a unique study identifier number used in the password protected database.

1. Potential risks

The risk to the participants from the standard collection of additional sputum specimens is minimal. There is a small risk of disclosure of personal information obtained from the clinical interviews.

2. Protection against potential risks

Confidentiality among participants will be protected by assigning each patient a unique identifying number, and no patient identifying information will be included in study database. Care will be taken to ensure that patients understand that study documents will be kept in strict confidence by the investigator, though absolute confidentiality cannot be guaranteed.

3. Risks in relation to benefits

Patients will benefit from intensive screening for resistance to the standard TB/MDR-TB drug regimens; drug resistance is a significant cause of mortality for patients with TB. These benefits significantly outweigh the minimal risks involved from specimen collection and the low risk of disclosure of patient information.

4. Ethical (and Regulatory) Review

This study will be submitted for formal review and approval to the Benin ethics committee (i.e. the central ethics committee), and the Ethics Committee(s) and/or Competent Authorities of the nine countries where the study is carried out and of selected collaborating partners.
The study will be carried out according to the principles stated in the Declaration of Helsinki, all applicable regulations and according to established international scientific standards.

Each partner will submit the protocol of the studies, including the information sheet and consent form as well as a Material Transfer Agreement on the shipment of samples to the competent body of their country for independent examination of the scientific merits and ethical acceptability. No participants will be enrolled or subject related activities performed in a site, before written approval from the local- as well as the central ethics committees is

obtained. The studies will moreover not start at each site until a copy of a written and dated approval has been received by the project coordinator.

The choice of treatment for the patient will not depend on the results of the test under validation but on locally conducted tests. In case study results are suggestive of discordant results with the local site laboratory, the interpretation of all available results will be discussed with experts in the field to determine whether the patient's standardized MDR regimen would best be adjusted.

5. Informed Consent

An informed consent form will be required to be signed by all patients enrolled in the studies 1 through 3. For participants aged 15-17 years of age, assent will be sought, as well as consent from their parent or legal representative. Appropriate information as to the purpose and the nature of the studies will be available in French, English and other local languages when appropriate. When patients are unable to read, an independent witness will be present to ensure that the patients receive complete information. Patients may be enrolled in other studies and still participate in this low risk diagnostic study.

6. Confidentiality

All study procedures will be conducted in private, and every effort will be made to protect participant privacy and confidentiality. A standard operating procedure for confidentiality protection that reflects the study implementation plan will be established. In addition, the procedures described below will be implemented:

- All study-related information will be stored securely at the study site.
- All participant information will be stored in locked filing cabinets in areas with access limited to study staff.
- Data collection, administrative forms, laboratory and other reports, will be anonymised to maintain participant confidentiality.
- All records that contain names or other personal identifiers, such as locator forms and informed consent forms, will be stored separately from study records identified by the randomisation number.
- All databases will be secured with password-protected access systems.

7. Insurance

Given the observational nature of the study with minimal risks no insurance will be obtained by the sponsor.

Study Committee and Study management

The project will be coordinated by LRM, which has close collaborative links with all research teams. Overall coordination will be the responsibility of the Project Coordinator (Prof Dissou Affolabi), who will be assisted in this task by an LRM based Study manager, an Administrator and the Project Steering Committee (PSC). The PSC will include the Principal investigator

from each of the collaborating partners, a representative of Savics company, and the financial administrator of the project (LRM).
The project manager under the supervision of the project coordinator will monitor the project on a daily basis. PSC meetings will occur at regular intervals (at the start of the project, and every 6 months). All salient issues will be discussed within the PSC in order to take full advantage of the expertise of the whole team, and to find the most appropriate solution to deal with the issue.

Dr Corinne Merle (WHO/TDR) and Bouke de Jong (ITM) will assist the project Coordinator (Prof Dissou Affolabi) for the overall coordination of the project. The coordination mechanism between the partners is shown through the organogram in Annex 2.

Monitoring visits will be conducted at the sites by the study manager, assisted by other partners as needed, to review the recruitment procedures and numbers and the laboratory activities and sample shipments. Depending on the findings and recommendations made at the monitoring visit, the schedule of follow-up visits will be determined for each site.

Dissemination of Results

Data and results will be made available to the consortium partners as early as the specific study requirements will allow. The rules/guidelines regarding authorship will be discussed and decided jointly by the members of the PSC prior to the start of the project.

Both the design and the results of the studies will also be discussed with representatives of the national TB programs of the respective countries in order to favor change in practices (in case of positive findings).

The involvement of WHO TDR in the design and execution of the project will facilitate links with WHO Global TB and the ultimate translation of the findings into policy.

At the start of the project, an agreement will be prepared and signed between all partners, which will balance the access to scientific results and the protection of Intellectual Property for the Deeplex- and InSilixa diagnostics, based on the premise that drug resistance mutations, and their prevalence, as identified in this project, were the results of public funding and should be exploited for scientific advancement as widely as possible. Any such data sharing will be done without patient identifiers and will be included in the informed consent procedure and proposals for ethics approvals.

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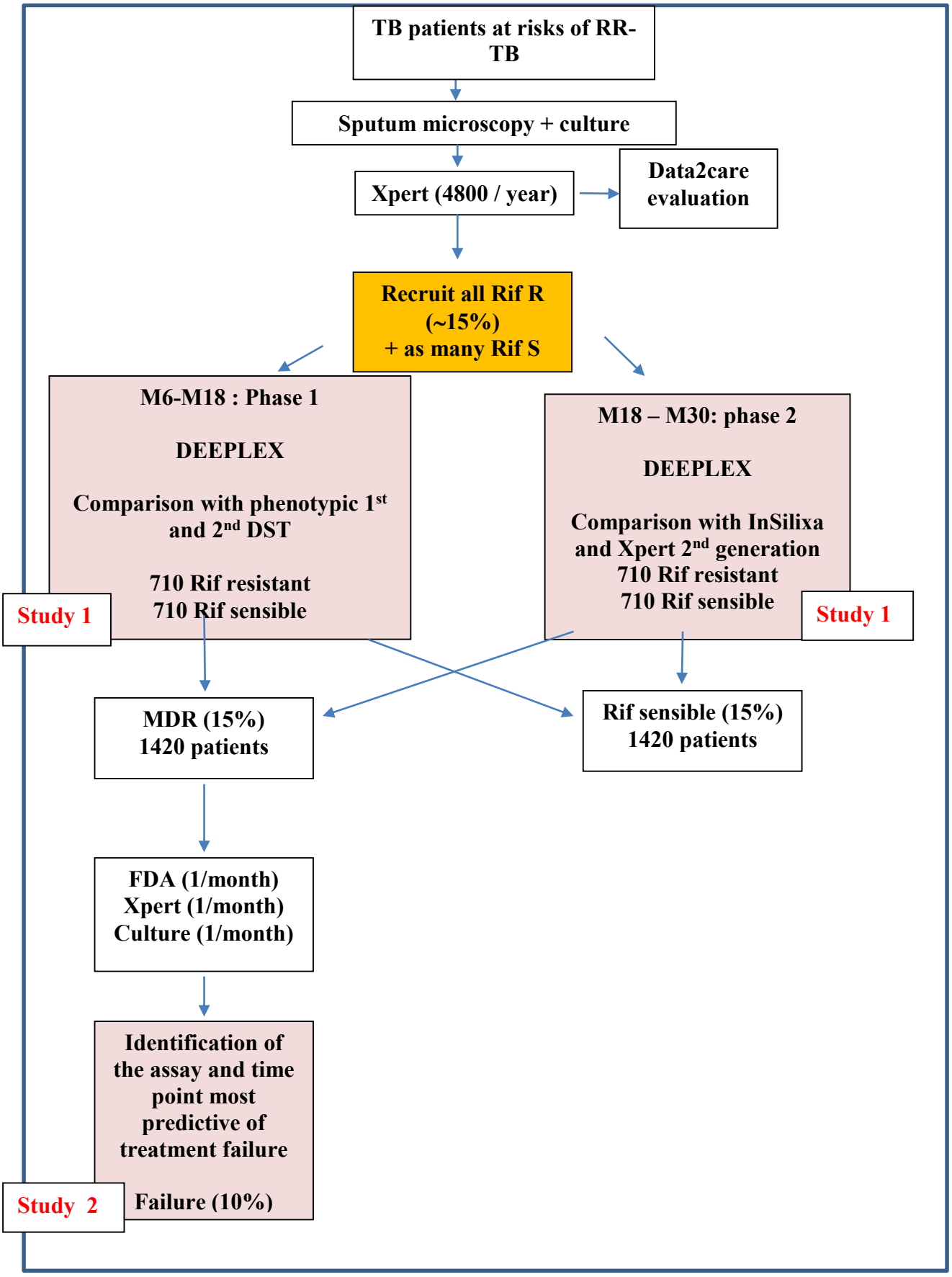
List of Abbreviations

AFB	Acid Fast Bacillus
CNHPP	CENTRE NATIONAL HOSPITALIER DE PNEUMO-PHTISIOLOGIE
DST	Drug Susceptibility Testing
EDCTP	European & Developing Countries Clinical Trials Partnership
FDA	Fluorescein Di-Acetate
FM	Auramine Fluorescent Microscopy
HNID	Hôpital National Ignace Deen, Conakry, Guinea
INRB	Institut National de Recherche Biomédicale du Zaire
ITM	Institute of Tropical Medicine
LED	Light emitting Diode
LBV	Laboratoire de Bacteriologie Virologie, Dantec, Dakar
LRM	Laboratoire de Référence de Mycobactéries
MDR	Multi Drug Resistant
MTB	Mycobacterium tuberculosis
NTP	National TB Programme
RBC	Rwanda Biomedical Center
REG	Register
RIF	Rifampicin
SEREFO	Centre de Recherche et de Formation, Mali
SOP	Standard Operating Procedure
TB	Tuberculosis
TBD	To be determined
TBRL	Tuberculosis Reference Laboratory Bamenda
USTTB	Université des Sciences, des Techniques et des Technologies de Bamako

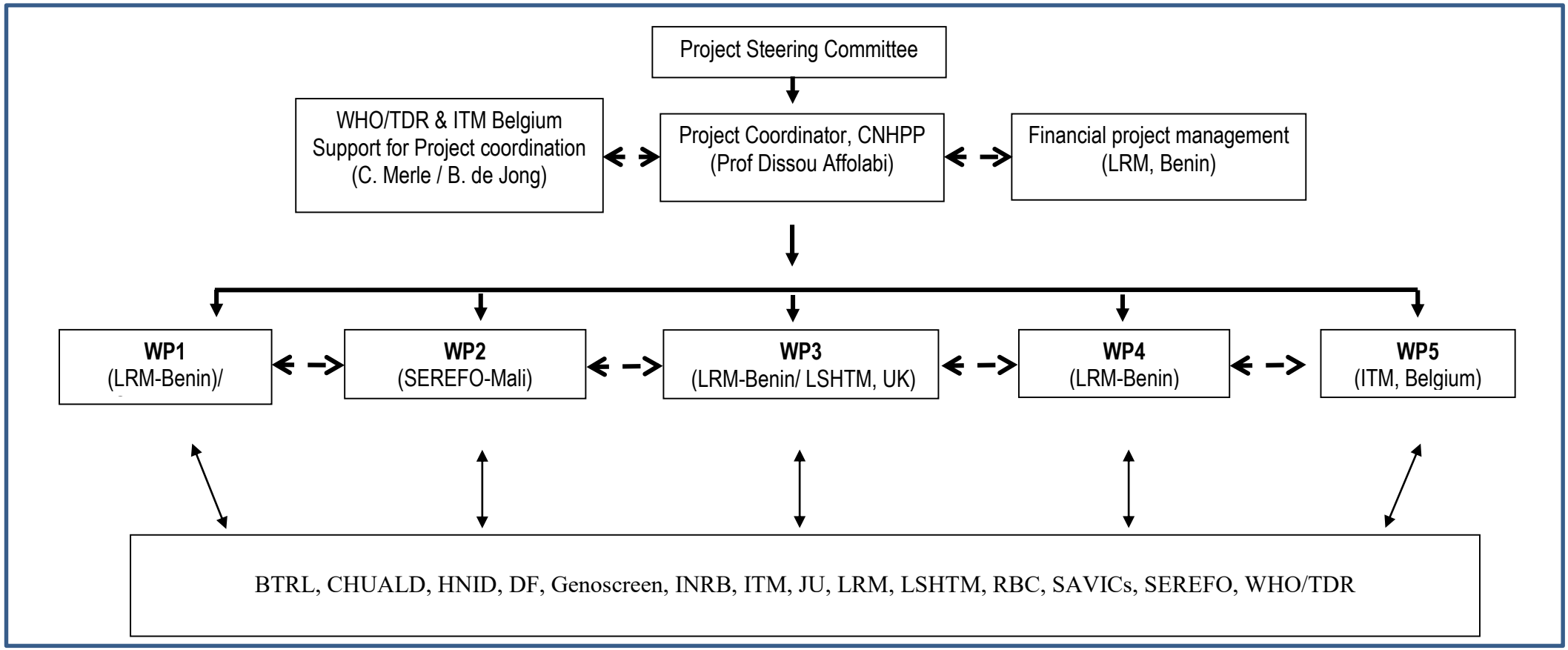
12. ANNEXES

1. Study Flow chart
2. Organogram
3. Patient information sheet and ICF

ANNEX 1: Study flow chart



Annex 2> DIAMA Organigram



TBRL: Tuberculosis Reference Laboratory Bamenda, Cameroon
CHUALD: Centre Hospitalier et Universitaire Aristide Le Dantec, Dakar, Senegal
HNID: Hôpital National Ignace Deen, Conakry, Guinea
DF : Damien Fondation, Ibadan, Nigeria
Genoscreen, Lille, France
INRB : Institut National Biomédicale, Kinshasa, Democratic Republic of Congo
ITM: Institute of Tropical Medicine, Antwerp, Belgium
JU : Jimma University, Jimma, Ethiopia
LRM :Laboratoire de Reference des Mycobatéries, Cotonou, Benin
LSHTM: London School of Hygiene & Tropical Medicine, London, UK
RBC : Rwanda Biomedical Center, Kigali, Rwanda
SAVICs company, Belgium
SEREFO: Bamako, Mali
WHO/TDR : Special programme of Tropical Disease Research at WHO, Geneve

1. RESEARCH PARTICIPANT INFORMATION SHEET

Diagnostics for Multidrug resistant tuberculosis in Africa

Investigator: <name of the Principal Investigator>
Organization: <name local hospital, organization,…>
Sponsor: Laboratoire National des Mycobactéries, Benin

You are invited to participate in a research study on drug resistant tuberculosis, which is difficult to diagnose. Before you decide to be part of this study, it is important that you understand the information in this form, because it explains your rights, as well as our responsibilities. In this information and consent form, the purpose, examinations, possible advantages, risks and inconveniences related to this study are explained to you. The right to withdraw your consent at any time to participate is also described below. You have the right to ask questions at any time, for example about the possible benefits and possible risks related to this study. Your participation is completely voluntary. You may talk to anyone you feel comfortable with about the research and you can take your time to think about whether you want to participate or not. If you consent to participate in the study, we suggest you keep this background information with you throughout the entire study period.

PURPOSE AND DESCRIPTION OF THE STUDY

This research study is being done to learn more about tuberculosis (TB), and especially about the detection of TB germs that do not respond to the drugs normally used to treat them, which is called "drug-resistant TB". We want to evaluate new tests, which will give more information about why some TB germs may not respond to treatment. Approximately 2800 people will participate in this study in nine different countries.

HOW THE STUDY IS DONE

If you accept to participate in this study and if you meet the conditions to participate, you will be asked to provide extra sputum samples. Also, we will record how well your treatment is working. If you have drug-resistant tuberculosis, you will also be asked to give one extra sputum sample at each of your follow-up visits. This sample will be used for extra testing to see how well your treatment is working.

RISKS AND INCONVENIENCES

The production of sputum is not dangerous to you. You can produce the extra sputum in the same way as you did for the first sample(s) you gave. It may be a minor inconvenience for you to spend extra time to produce more specimens.

BENEFITS

You will receive the results of the lab tests currently done in your health center as usual, and some additional lab tests will also be done. For a small number of people, the results of the additional tests may help to improve your treatment. You may also contribute to improving health care for others if the results of the tests under evaluation in this study help to improve treatment management for future tuberculosis patients.

COMPENSATION

You will not have to pay any fee for any test done for this study. You will also not be given any fee for participating in this study.

PROTECTION OF YOUR CONFIDENTIALITY

We will do everything we can to protect your confidentiality. The information collected about you will be stored in an electronic database. This information will not be shared with anybody except the study researchers and your doctor, and it may be written down in your medical record. This information may also be examined by the organizer of this study to check that the information is correct. Your identity will remain secret since your personal information will be given a unique code. Your name will not appear in any reports or publication resulting from this study.

ETHICS COMMITTEE

Before the start, this study was reviewed and approved by the regional/national Ethics Committee(s) in all the participating countries.

VOLUNTARY PARTICIPATION

Your participation in this study is entirely voluntary. It is your choice whether you want to take part in the study. Whether you choose to participate or not, all the services you receive at this clinic will remain the same.

You also have the right to stop your participation in the study at any time, even after you have signed this Informed Consent Form. You do not have to give a reason for wanting to stop being part of the study.

The study investigator can stop your participation in this study at any moment as well, even without having to request your permission, if he or she judges this in your best interest or if you do not follow the instructions for participation in the study.

CONTACT PERSON IN CASE OF QUESTIONS

If you have any questions concerning your participation in this study, your rights or think you have been harmed as a result of the study, you can contact, now, during, or after the study:

Principal investigator: Pr Dissou Affolabi, at Laboratoire de Référence des Mycobactéries, Cotonou, Benin. Phone : +229 21331533. Fax : +229 21337057. Email: affolabi_dissou@yahoo.fr.

Local investigator: [adapt for each country]

2. INFORMED CONSENT FORM

Diagnostics for Multidrug resistant tuberculosis in Africa

Part which is destined only to the participant (and his/her legal representative if applicable)

I confirm that I have been informed about the study and that I have received a copy of the research participant information sheet and the Informed Consent Form. I have understood the information. I have been given sufficient information concerning the conditions, the duration of the study and any possible inconvenience to me. In addition, I have received sufficient time to consider the information and to ask questions, to which I have received satisfying answers.
I freely consent to participate in this study and consent to cooperate in the examinations/activities requested. I am willing to give information concerning my medical history, use of medication and participation in other studies if any.
I agree that my doctor and other healthcare professionals involved in my treatment are informed about my participation in this study.
I agree that my samples may be used for additional scientific studies on TB or other germs.
I understand that I will give extra sputum samples.

☐ I agree that my samples may be stored after completion of this study.

Date: …… **Name: …….**

Signature (or thumbprint) of participant:

_____

Name of the witness: ……

Signature:

_____

Name of legal representative (for minors aged 15-17): ……

Signature (or thumbprint) of participant:

<u>Part only destined to the investigator's team</u>

I, the undersigned, …… confirm that I have informed the participant (and/or the legal representative) about all the relevant aspects of this study. I confirm that he/she has consented voluntarily to participate in the study.

Date: …… ***Signature:***