EVALUATION OF XPERT MTB/RIF ULTRA IN STOOLS AND URINE TO IMPROVE TB DIAGNOSIS IN CHILDREN

STUDY PROTOCOL – VERSION 1.13
Date: 1st November 2021

MÉDECINS SANS FRONTIÈRES & MINISTRY OF HEALTH

Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral treatment
ATT	Antituberculosis treatment
CHW	Community health worker
CSF	Cerebral spinal fluid
CRF	Clinical report form
DST	Drug susceptibility testing
EPTB	Extrapulmonary tuberculosis
IDP	Internal displaced population
IPD	In-patient department
ITFC	Inpatient Therapeutic Feeding Centre
HIV	Human immunodeficiency virus
MSF	Médecins Sans Frontières
MoH	Ministry of Health
MTB/RIF	Mycobacterium tuberculosis / Rifampicin resistance
OCBA	Operational Centre Barcelona-Athens
OPD	Out-patient department
NICU	Neonatal intensive care unit
PICU	Paediatric Intensive Care Unit
POCUS	Point-of-care ultrasound
SAM	Severe acute malnutrition
SOP	Standard operating procedures
TB	Tuberculosis
US	Ultrasound

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1. Summary

Title	**EVALUATING XPERT MTB/RIF ULTRA IN STOOLS AND URINE TO IMPROVE TB DIAGNOSIS IN CHILDREN**
Design	Multicentric study (South Sudan and Guinea Bissau). Cross-sectional study.
Rationale	TB diagnosis in children is challenging in low-resource and conflict-affected settings, and a high number of children remain undiagnosed and untreated. A delay in TB diagnosis can lead to an increase in preventable morbidity and mortality. This study aims to provide more evidence of the utility of Xpert MTB/RIF Ultra in stools and urine for TB diagnosis in children, assuming a higher sensitivity than conventional microscopy and acknowledging that TB culture is rarely available in the contexts where MSF works.
Main objectives	To determine the sensitivity and specificity of Xpert MTB/RIF Ultra in stool and urine samples in suspected paediatric TB cases in comparison with the gold standard (Xpert MTB/RIF Ultra).
Secondary objectives	1. To determine the sensitivity and specificity of Xpert MTF/RIF Ultra rifampicin resistance using stools and urine in pulmonary and extra pulmonary TB confirmed and unconfirmed TB cases as composite. 2. To disaggregate according to HIV status (positive, negative) and to nutritional status (SAM or not). 3. Describe the prevalence of positive TB LAM in HIV-infected, HIV-uninfected and SAM patients with and without TB.
Population	Children with presumptive TB in Malakal (South Sudan) and in Bissau (Guinea Bissau).
Timeframe	January 2019 to July 2021.
Expected results	Show that stool and/or urine Xpert MTB/RIF Ultra can be a sensitive and specific tool for TB diagnosis in children. This evidence can lead MSF, WHO and National TB programs to recommend stool and urine Xpert MTB/RIF Ultra as routine tests for TB diagnosis in children.
Partnership	Ministry of Health in South Sudan Ministry of Health in Guinea Bissau

2. General Information

Title and date	EVALUATING XPERT MTB/RIF ULTRA IN STOOLS AND URINE TO IMPROVE TB DIAGNOSIS IN CHILDREN, July 2018
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3. Background

a. Tuberculosis in children

Despite the increased availability of diagnostic tests as well as a curative and preventive therapy, tuberculosis (TB) remains an important cause of morbidity and mortality. TB is the main infectious disease killer in the world and remains an important burden in resource-limited settings. WHO estimates that 10.4 million people suffered from TB in 2016, of which 1.7 million people died (1). Developing countries account for more than 90% of these cases and deaths.

Tuberculosis is also a major cause of morbidity and mortality among children: in 2016, 1,000,000 children became ill from TB, of which 250,000 died -80% of them were under 5 years old (2). Childhood TB has been a largely neglected subject for organizations dedicated to TB or child health.

Active TB in children is also a serious and frequent co-infection of HIV or a co-morbidity of malnutrition in endemic environments (3-6). Malnutrition is an independent risk factor for both development of TB and mortality due to TB (7-9), and is associated with more severe forms of TB (8). It is essential to better integrate the diagnosis of tuberculosis into vertical programs for non-infectious diseases, especially for malnutrition services (5,6,10,11).

b. TB diagnosis in children

According to WHO, the TB diagnostic gap world-wide is still enormous: 39% of all patients suffering from TB remained undiagnosed in 2016 (1). This gap is even higher when it comes to paediatric TB. In some African settings, only 20% of estimated cases were diagnosed (12).

The diagnosis of tuberculosis in children is particularly difficult and is essentially based on clinical symptoms. TB scoring systems have not proven to be effective and young children have difficulty expectorating adequate samples for microbiologic investigation. Even if samples are obtained by partly invasive procedures such as naso-pharyngeal aspiration or gastric lavage, the positivity rate in smear or culture is low (13,14). Similarly, radiographs and skin tests of tuberculin give only very limited information in children and are rarely available in contexts where Médecins sans Frontières (MSF) works.

In addition, TB cultures and Xpert MTB / RIF® (Cepheid, Inc.) are often not available in endemic areas. Traditionally, TB culture has been considered as the gold standard for TB diagnosis. However, access to culture and time till final result make culture not feasible as a regular test in most of the contexts where MSF works. This is the reason why an international expert panel established that the case definition of “confirmed tuberculosis” includes a positive WHO-endorsed nucleic acid amplification test (Xpert MTB/RIF) in addition to culture confirmation using a valid respiratory specimen for microbiologic examination, irrespective of the culture result (13).

Even when there is access to TB culture or Xpert MTB/RIF, we are confronted with the challenge of getting an appropriate sample from children. Clinical signs and symptoms alone are often variable and therefore considered too inaccurate for TB screening. This is particularly true for

children under 5 years old, children with HIV and for children with severe acute malnutrition. TB suggestive signs and symptoms such as cough, fever and low weight gain are also common in other childhood diseases, further complicating diagnosis (15).

It is therefore essential to continue research that may improve the diagnosis of tuberculosis in children. Timely initiation of appropriate treatment reduces morbidity and mortality. As such, there is an urgent need for appropriate tools which allow reliable and timely diagnosis of childhood tuberculosis. Improving childhood diagnosis will also reduce over-diagnosis and over-treatment, which exposes children to unnecessary toxicity and wastes resources.

c. Use of Xpert MTB / RIF® for TB diagnosis

Xpert MTB/RIF is widely used for TB diagnosis in pulmonary samples. A recent meta-analysis (16) showed that, compared with microscopy, Xpert offers better sensitivity (increased by 36-44%) for the diagnosis of pulmonary tuberculosis in children. However, its sensitivity remains suboptimal compared with culture tests (pooled sensitivity of 62-66% in respiratory samples, specificity 98%). Xpert scale-up can improve access to tuberculosis diagnostics for children. Culture remains the gold standard but, in the mentioned meta-analysis, among all samples analysed, only 12% had a culture positive.

The use of Xpert MTB/RIF has also been extended to extra-pulmonary TB forms. Recent meta-analysis and literature review (17,18,19) on the diagnostic accuracy of Xpert MTB/RIF in extra pulmonary samples, showed that Xpert sensitivity differed substantially between sample types, with higher sensitivity for lymph nodes (pooled sensitivity ranged between 87 and 96%) and CSF (pooled sensitivity ranged between 80.5 and 85%), and had a high specificity (pooled specificity between 97 and 98.7%). These findings led the WHO recommend Xpert MTB/RIF as the primary diagnosis test for lymph node and meningitis TB (20).

Xpert MTB/RIF for stools has also been considered in several studies (21-26) but its use is not yet recommended by the WHO. The sensitivity of the stool Xpert ranged between 45 and 83%, which suggests that it is a potential alternative screening test for children with suspected TB when sputum is unavailable. In one of the studies (21), stool Xpert could rapidly confirm TB in children who present with radiologic findings suggestive of severe TB.

The main challenge is confirming TB diagnosis with the gold standard – TB culture. Thus, many of the studies had very few patients (all of them < 75 children). However, the evidence suggests that stools, which are easily obtainable, are an appropriate alternative sample for the diagnosis of pulmonary TB, especially for children unable to give respiratory samples. This is true for both HIV positive and negative individuals. “Valid specimens” for the detection of DNA of M. tuberculosis with Xpert include respiratory specimens from sputum sampled by expectoration, sputum induction, gastric aspirates, or nasopharyngeal aspirates and can include the use of a stool sample (19,20).

Urine Xpert has produced less interest among clinicians. Of the few studies (27,28,29), all of them focus on adult HIV+ patients. As main conclusions, urine-based MTB/RIF, alone or in combination with LAM antigen detection and/or sputum Xpert MTB/RIF, may potentially aid the

diagnosis of TB in HIV-infected patients with advanced immunosuppression when sputum-based diagnosis is not possible. Further studies are necessary to assess the utility of urine Xpert MTB/RIF in the diagnosis of TB in children.

Cepheid developed Xpert MTB/RIF Ultra to improve the sensitivity of TB detection. Clinical studies have shown improvements in sensitivity from 77% to 90% amongst culture positive HIV co-infected patients (30) and from 45% to 95% amongst HIV co-infected patients suspected of having tuberculous meningitis (31). A mini-review on Xpert MTB/RIF Ultra (32) included two studies for children which showed an increased sensitivity of 11% (22% when HIV co-infected) and a specificity of 97% in comparison to culture; four studies for pauciballiray disease in adults should an increased sensitivity of 5 to 17% and similar specificity when compared to conventional Xpert MTB/RIF; a study showed 50% increase of sensitivity in CSF in comparison to Xpert.The increase of sensitivity has been detrimental to specificity, especially amongst patients with previous treatment history. Xpert Ultra provides a new "trace" result for potential false positive patients. This is the reason why, in 2017, the Global Laboratory Initiative recommended that "among persons with HIV, children and extra-pulmonary specimens 'trace calls' should be considered to be true positive results" (33). WHO recommends Xpert Ultra as a replacement of conventional Xpert as an acknowledgement of the improvement in sensitivity.

4. Rationale for this study

As described above, TB diagnosis in children is challenging in low-resource and conflict-affected settings, and a high number of children remain undiagnosed and untreated. A delay in TB diagnosis can lead to an increase in preventable morbidity and mortality.

This study aims to provide more evidence of the utility of Xpert MTB/RIF Ultra in stools and urine for TB diagnosis in children, assuming a higher sensitivity than conventional microscopy and acknowledging that TB culture is rarely available in the contexts where MSF works. This evidence can lead MSF, WHO and National TB programs to recommend stool and urine Xpert MTB/RIF Ultra as routine tests for TB diagnosis in children.

Our future ambition is to incorporate Xpert MTB/RIF Ultra in stools and urine in diagnostic algorithms for TB diagnosis in childhood.

5. Context

In 2016, 2.5 million people fell ill with TB in the African region, accounting for a quarter of new TB cases worldwide. An estimated 417,000 people died from the disease in the African region (1.7 million globally) in 2016. Over 25% of TB deaths occur in the African Region (34,35).

a. Malakal (South Sudan)

In South Sudan, TB remains a major public health problem. According to 2016 WHO data, the estimated TB incidence (all forms of TB) in South Sudan was 146 per 100.000 population (11% affecting children) in 2016, with an overall treatment coverage of 54%. HIV prevalence in the

country and in Upper Nile region is 2.7%. Estimated rate of MDR TB in new and already treated cases is estimated to be 3 and 13% respectively (36). People living in IDP camps, have an increased risk to develop TB (37), and the estimated incidence in Malakal is 3.5 times higher than in the country, with high HIV co-infection (11%).

Médecins sans Frontières (MSF) started the intervention in Malakal (figure 1) by the end of 2013 due to a conflict crisis in the area which required big humanitarian needs. MSF is currently supporting primary and secondary health care in Malakal Protection of Civilians (POC) and Town, and supporting the rural area through a decentralised model of care. MSF is currently working without MoH staff at the facilities. HIV/TB support started in 2015 and is currently supporting a cohort of around 100 HIV patients and is currently providing TB-DRTB integrated care. All components of the program (diagnostic, medical care, counselling and health promotion-community engagement) are supported by MSF, even if all data is reported to National TB Program according to MoH requirements.

Figure 1:

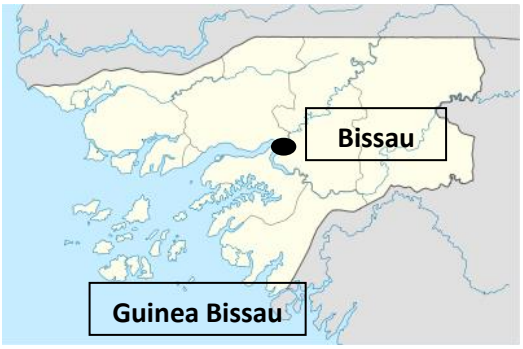
b. *In 2017, a total number of 236 patients were diagnosed of TB and started on treatment. Among them, 34 (14%) were children, 14 among them under 5 years old. In 2018, the MoH placed Xpert MTB/RIF machine in MSF supported laboratory in Malakal POC. Improved diagnostic skills and community strategy with active case finding have increased the TB diagnosis by 25%, with 28 cases in the first 8 months of the year.* ***Bissau (Guinea Bissau)***

In Guinea Bissau, TB remains a major public health problem. According to 2017 WHO data (38), the estimated TB incidence (all forms of TB) in Guinea Bissau was 374 per 100.000 population (15% affecting children) in 2017, with an overall treatment coverage of 32%. HIV prevalence in the country is 3.4% according to UNAIDS (39) and HIV coinfection in TB patients is as high as 32%. Estimated rate of MDR TB in new and already treated cases is estimated to be 2.5 and 14% respectively. The treatment success for new and relapse TB cases is 76 and 43% respectively.

According to National TB Program data, in 2018 2200 persons were started on TB treatment and 45% among them were ≤ 15 years old.

MSF OCBA launched the permanent mission in Guinea Bissau (figure 2) in 2016 as a strategic choice with the objective to test and develop innovative medical strategies in paediatric care to decrease childhood mortality and which will ultimately help to overcome operational challenges, by being capitalized and exported to more unstable settings. MSF supports several departments in the Simão Mendes National Hospital in collaboration with MoH: paediatric intensive care unit (PICU), neonatal intensive care (NICU), in-patient therapeutic feeding centre (ITFC) and few beds in the paediatric ward. MSF is currently diagnosing TB in children and referring them to MoH / National TB program to initiate and continue treatment.

Figure 2:



6. Objectives

a. Main objective

To determine the sensitivity and specificity of Xpert MTB/RIF Ultra in stool and urine samples in suspected paediatric TB cases in comparison with the WHO recommended test (Xpert MTB/RIF Ultra in sputum sample or validated extrapulmonary sample).

b. Secondary objective

1) To determine the sensitivity and specificity of Xpert MTF/RIF Ultra rifampicin resistance using stools and urine in pulmonary and extra pulmonary TB confirmed and unconfirmed TB cases as a composite.
2) To disaggregate according to HIV status (positive, negative) and to nutritional status (SAM or not).
3) Describe the prevalence of positive TB LAM in HIV-infected, HIV-uninfected and SAM patients with and without TB.

7. Patients and Methodology

a. Study design

We plan to conduct a multicentric study in 2 different centres: Malakal (South Sudan) and Bissau (Guinea Bissau). The methodology for the evaluation of Xpert MTB/RIF Ultra in stools and urine will be a cross-sectional study.

b. Study sites

This study will be conducted in South Sudan in two different sites, the Malakal POC and the Malakal town, and in Bissau in one site, the Simão Mendes National Hospital.

c. Partnership and study coordination

In South Sudan, this study will be conducted in partnership between Médecins sans Frontières Operational Centre Barcelona-Athens (OCBA) and the Ministry of Health of South Sudan, in collaboration with the National TB Program. The MoH is willing to provide administrative and political support. The Xpert MTB/RIF Ultra machine was procured by MoH as well. No academic institutions have been involved in the study.

In Guinea Bissau, this study will be conducted in partnership between MSF OCBA and the MoH of Guinea Bissau, in close collaboration with the National TB Program. MSF works closely with MoH in Simão Mendes hospital, and the General Director of the TB hospital (Raoul Follereau Hospital), as well as the Responsible of the TB ambulatory care at Simão Mendes, are co-authors of the study and willing to provide technical support.

d. Study participants and sample size

1. Research population

The research population are children with presumptive TB in (Malakal town and POC hospitals) or in Bissau (Simão Mendes National Hospital).

Note that the same population will be offered to participate in the study "Evaluation of point of care ultrasound (POCUS) for the diagnosis of TB in children" (ref: MSF ERB 18116) which will be submitted in a separate protocol. Note that all participants will be asked to sign 2 separate consent forms.

2. Participants: inclusion criteria

- Any child from 6 months of life to 15 years old
- TB suspected according to diagnostic criteria
- Informed consent provided by legal responsible or companion

3. Participants: exclusion criteria

- Patients who have received TB treatment for > 1 day in the last 3 months
- Patients not producing stools during period of hospitalization

4. Sample size

The primary analysis will to describe the performance of Xpert MTB/RIF Ultra from stools and urine in patients with confirmed TB. The estimated total sample size is **327 suspected TB patients: 143 in Malakal and 184 in Bissau.** The sample size difference is justified by the number of suspected patients currently consulted in both settings. Note that this is the same sample size

that will be used for the study "Evaluation of point of care ultrasound (POCUS) for the diagnosis of TB in children".

The sample size has been estimated with a variety of assumptions: we assume that sensitivity of Xpert MTB/RR in stools and urine will be 60% and specificity 95%.

Expected sensitivity	Precision			
	0.05	0.07	0.1	0.12
0.5	385	196	97	67
0.6	369	189	93	**64**
0.7	323	165	81	56

With a precision of 12%, total number of 64 patients will be required to have a diagnosis of confirmed TB. If we assume that positivity of Xpert MTB/RR in sputum or validated extra pulmonary sample will be 20% (rate non-sick/sick = 4), we will need a total of 320 suspected TB patients will be needed (160 per project) to achieve 64 patients with confirmed TB (32 per project). We assume that 2% of the children won't be capable of producing stools, therefore the final sample size will be 327.

e. Definitions

1. SAM (severe acute malnutrition) in children:

- Children < 5 years old: Z-score weight / height <-3 and / or MUAC <11.5 cm, and / or bilateral oedema.
- Children 5-15 years old: weight for height < 70% (see table in Appendix 16) or bilateral oedema

2. HIV infection:

All patients with suspected TB will be tested for HIV, after consent of the legal responsible and pre-test counselling. The algorithm test will be adjusted to the age of the child (see Appendix 12). All children < 18 months will need PCR DNA for HIV diagnosis. All children ≥ 18 months and > 6 weeks of breastfeeding cessation will follow same diagnostic algorithm as for adults, which requires 3 positive rapid tests.

3. Suspected case of tuberculosis (40,41)**:**

Any child with at least one of the following signs, symptoms or features is considered a **suspected case of tuberculosis** (Appendix 4):

- Persistent cough for more than 2 weeks
- Unexplained fever for more than a week
- Suspected extra-pulmonary tuberculosis (TBEP), e.g.:
  - Angular deformation of the spine (gibbous)
  - Lymphadenopathy
  - Subacute meningitis
  - Abdomen distended with ascites

- > 2 weeks of diarrhoea
- Painless enlarged joints
- Pleural effusion
- Other

All **HIV positive** and **SAM children,** as well as all **TB contacts**, will systematically and actively be screened for TB.

Tuberculosis screening in children is done on admission and during hospitalization. If children are not identified as suspected TB cases at admission, a new assessment will be done after 1 week of admission. In addition to the above criteria, tuberculosis is suspected based on the following signs, symptoms and characteristics during hospitalization:

- Low weight gain despite correct nutritional treatment
- Persistent pneumonia after adequate and well-followed antibiotic therapy
- Persistence of cough
- Persistent fever (>38ºC) beyond one week after exclusion from classical causes such as malaria or pneumonia
- Persistence or aggravation of fatigue
- Chest radiographs imaging suggestive of tuberculosis

4. **Case with diagnosis of tuberculosis** (40,41)

- **Confirmed TB:** patients with Xpert MTB/RIF Ultra positive (or TB culture positive when available) in a respiratory sample (pulmonary TB) or in other sample, such as CSF, lymph node or other tissue/liquid (extra pulmonary TB); TB LAM positive in HIV-infected patients.

- **Unconfirmed TB:** no microbiological confirmation but clinical diagnosis of TB and started on anti TB treatment (as established in Appendix 5).

- **Unlikely TB:** good response to other treatments (antibiotic, nutritional support, etc.) and no suggestive symptoms after 1 week of admission and until the 2 month follow-up.

8. Study procedures

a. Identification of participants

Eligible children will be identified upon admission to paediatric ward, ITFC, PICU/NICU (in Bissau) or as suggestive cases in OPD. The identification of suspected TB cases will be mainly based on clinical criteria. A diagnostic algorithm (Appendix 4) will be applied to all suspected cases. The populations of interest for this study are hospitalized children, severely malnourished (SAM) and HIV positive or exposed children.

b. Laboratory

1. Sample collection

All patients included in the study will have requested one stool and one urine sample. The team will follow a specific SOP for respiratory sample collection (naso-pharyngeal aspirate, gastric lavage or spontaneous sputum), stools, urine and lymph nodes (or other validated extra pulmonary samples) as described in the appendixes. In hospitalized children, all samples will be collected during hospitalisation and patients not capable of producing the required sample (stools/urine) during this period will be excluded from the study. For ambulatory patients, samples will be collected within 2 hours and, if child not capable of producing stools/urine, samples will be requested to be brought by parents or guardians the day after.

2. Laboratory procedures

Xpert MTB/RIF Ultra will we performed to respiratory or extra pulmonary validated samples, stools and urine for all patients at baseline, and for those with negative results and persistent symptoms, will be repeated after 1 to 2 weeks.

TB LAM in urine will be tested in all Malakal patients (starting from November 2021) and the result (negative/positive and grade 1-4) recorded in the CRF Lab form. The result will be shared with Research Manager who will only communicate to clinicians in case of HIV-infected patient; in these patients, TB LAM will be used as per clinical protocol.

3. Sample handling and storage

Respiratory specimens will be collected at the clinic and samples will be kept between 2 and 8ºC until processed. Processing will be done within the 24 hours and not more than 72 hours after collection. A sterile stool container and instructions will be provided to the parent or guardian for specimen collection and handling. Following delivery to the laboratory, the stool specimen will be kept in a cool dry place and processed within 2 hours. Samples obtained in this study will adhere to all local laboratory procedures governing the storage of biological specimens.

c. Linkage to care for HIV/TB patients

All patients HIV positive will be enrolled into **HIV care** either in MSF program (Malakal) or MoH programs (Bissau). MSF will guarantee that all children will be started on ART after 2 weeks of TB treatment (or as soon as possible if TB has been excluded) or differed to 4-6 weeks in case of TB meningitis.

All TB cases (confirmed or unconfirmed) will be enrolled in **TB care** in MSF program (Malakal) or MoH program (Bissau). MSF will ensure all patients to be started on adequate weight-based dose fix combinations and followed up during the length of treatment. All children diagnosed of DRTB will be initiated on DRTB treatment regimen according to national recommendation.

In patients where stool samples yield positive results and sputum tests are negative, stool results will be communicated to Study Coordinator on site for interpretation and appropriate clinical management. Where stools results are concordant with sputum results, no additional information will be provided.

As a transversal study, it is not foreseen to follow-up patients for study purposes. The follow-up will be required though for the study “Evaluating POCUS to improve TB diagnosis in children”.

d. Data collection

The following information will be noted in an individual case study report form (CRF) for each participant:

1. **DEMOGRAPHIC INFORMATION AND INFORMED CONSENT OF PATIENTS:**

- Study code
- Date of birth / age
- Sex
- Parent or guardian's informed consent: Yes / No
- Consent to be traced in case of defaulting

2. **CLINICAL AND DIAGNOSTIC INFORMATION OF STUDY PARTICIPANTS AT BASELINE:**

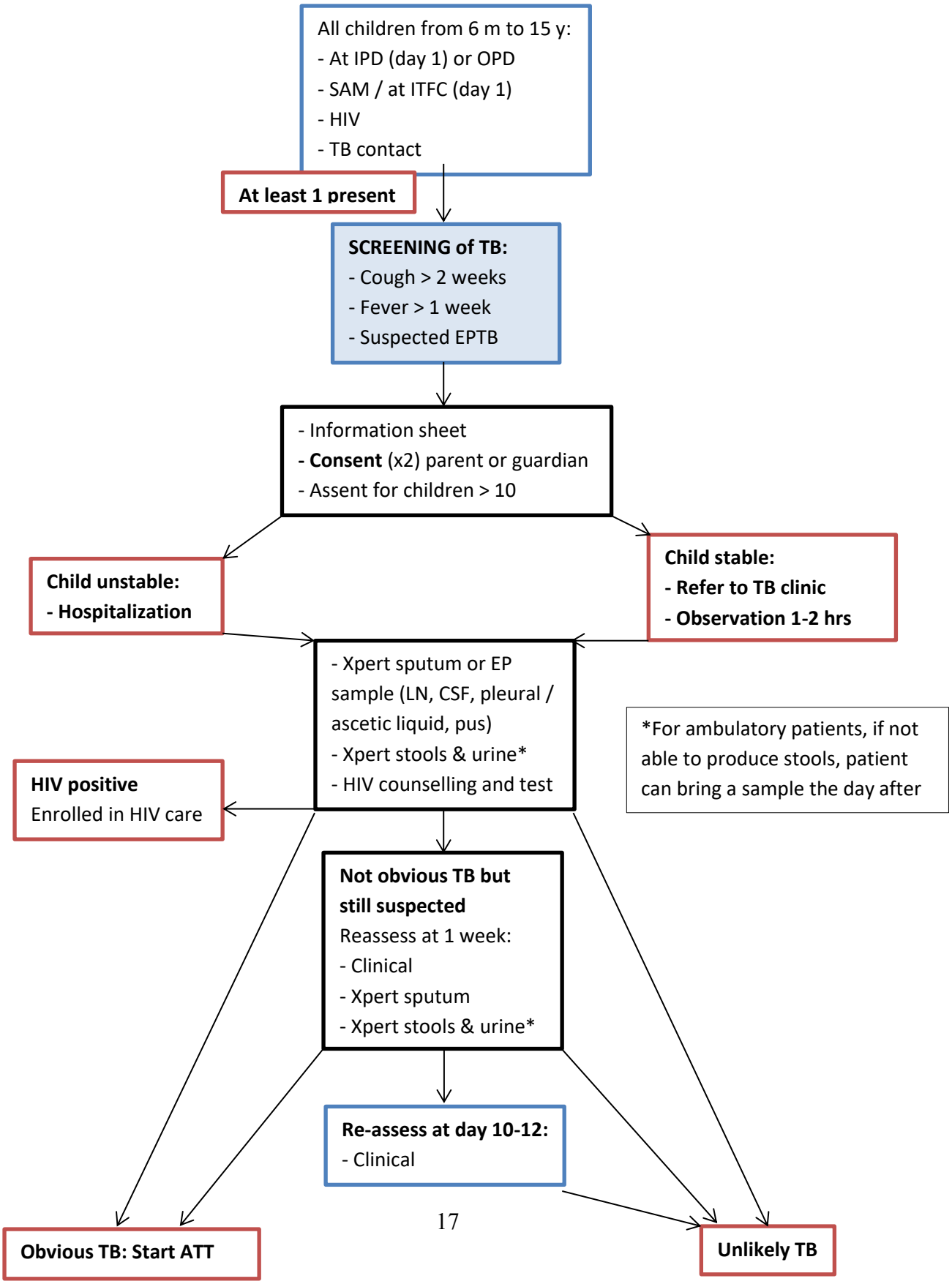
- Entry point (OPD, TB or HIV or Nutrition program, IPD)
- History of TB contact, HIV or malnutrition
- Temperature (axillary) (◦C)
- Weight and size
- Nutritional status
- Co-morbidities: malaria, respiratory infection, HIV status
- Signs and clinical symptoms suggestive of tuberculosis
- Final diagnosis
- TB treatment started: Yes / No
- Date of TB treatment started

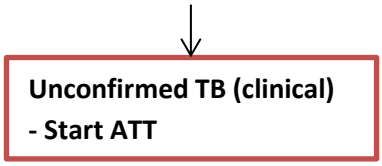
3. **LAB EXAMS INFORMATION:**

- Program identification number
- Inclusion number in the study
- Date of respiratory sample collection
- Type of procedure: spontaneous sputum, naso-pharyngeal aspirate, gastric aspiration
- Date of Xpert MTB/RIF Ultra in respiratory sample performed (1 sample)
- Result of Xpert MTB/RIF Ultra (negative, positive RIF resistance negative, positive RIF resistance positive, indeterminate)
- Date of stools collection
- Date of Xpert MTB/RIF Ultra in stools performed (1 or 2 samples)
- Result of Xpert MTB/RIF Ultra (negative, positive RIF resistance negative, positive RIF resistance positive, indeterminate)
- Date of urine collection
- Date of Xpert MTB/RIF Ultra in urine performed (1 or 2 samples)
- Result of Xpert MTB/RIF Ultra (negative, positive RIF resistance negative, positive RIF resistance positive, indeterminate)

e. Flow of patients

OPD patients who don't need hospitalization will be requested to stay in observation for 2 hours to perform all exams after obtaining the informed consent. If the participant is not able to produce stools within this time, s/he will be requested to bring the sample once produced at home (all information in Appendix 3). Please, find the flow in the following page.





9. Safety Considerations

There are safety considerations related to infection control of TB, even if children are only considered contagious after they acquire the capacity of expectorating (> 8 years old). For older children, isolation procedures for coughing patients, as well as protection measures for medical staff, need to be implemented. Lab staff needs to wear respirator and gloves when manipulating samples for Xpert MTB/RIF Ultra. Safety procedures related to Xpert MTB/RIF Ultra need to be in place.

Diagnostic procedures are routinely done in all children with suspected TB (not specifically for the study). Gastric lavage and naso-pharyngeal aspirate can be uncomfortable. For gastric lavage, there's a small risk of spasms of vocal cords, tube entering in the airway instead of the oesophagus, minor bleeding or aspiration pneumonia. Regarding naso-pharyngeal aspirate, the actual risks for participants are still smaller; it can cause nausea or local annoyance. Lymph node puncture is a routine procedure as well and is only performed in palpable lymph nodes (≥ 2 cm). The risks are small and can comprise blood vessel punction or accidental exposure to blood for the staff. All three are common procedures which will only be performed by trained staff. Any possible complication will be treated by MSF.

Collection of stool and urine specimens poses no risk. Since individual risks to children are small, reporting of adverse events and setting up of an external data safety monitoring board are not considered necessary for this study.

10. Data Management and Analysis

The number of children screened by the program for suspected tuberculosis, the number of eligible children and the number of cases included in the study during the recruitment period will be noted. The number of parents or guardians of eligible children who refuse their participation will also be registered. Demographic, clinical and diagnostic characteristics at baseline and during patient follow-up under the program will also be recorded.

This information will be used to describe the characteristics of the patient population included, and will allow comparison of the characteristics of patients with positive Xpert in stools and urine in comparison with those who test negative. The main outcome of interest for the Xpert MTB/RIF Ultra study is establishing the sensitivity and specificity for Xpert in stools and urine (compared with gold standard).

Data will be collected in the files by the study team members. Data will be coded and entered into RedCap database, and analysed with SPSS statistical software. Continuous variables (e.g.

age, distance) will be summarized using mean and standard deviation or median and interquartile range as appropriate and may also be expressed as ordinal categories with frequencies; frequencies will be reported with corresponding 95% confidence intervals.

11. Ethical Considerations

The study will be conducted in accordance with the Declaration of Helsinki on Ethical Principles of Medical Research on Human Subjects, and in accordance with the rules of the National Ethics Committee of South Sudan and Guinea Bissau. The protocol will be submitted for approval to the National Committee for Ethics of South Sudan and Guinea Bissau, and the MSF Ethics Committee.

a. Informed consent

Participation in the study is on a voluntary basis and requires prior informed consent. Trained staff will provide eligible parents or guardians with detailed information on the study's objectives and procedures, as well as a clear explanation of the risks and benefits of participation. A written information sheet will be given to the parent or guardian, in English or in local language (annex 1). The study staff will assist the parent or guardian on all the points indicated in the form and answer all the questions. If the parent or guardian is not able to read the information sheet, staff will read it aloud. All children will be briefed on the study and the information will be adjusted to the age range and an assent process will take place for children older than 10 years old, but following national legislature, the consent has to be signed by an adult. All questions will be answered by medical staff responsible for the study.

Parents or guardians agreeing to have their child participating in the study will need to sign an informed consent, which will also be signed by the study staff (annex 2). For illiterate parents or guardians, a witness who can read and write, designated by the parent or guardian himself and who will not be a staff member of the study, will be present throughout the information process and will co-sign the form to confirm that the parent or guardian has understood and willingly accepts participation in the study.

The process of informed consent will be done in an area of the clinic that offers enough privacy to make a voluntary decision without constraint. Parents or guardians will be explicitly informed that they have the right to refuse participation or withdraw from the study at any time during the research. Copies of both forms (the study information sheet and informed consent, in English or in the local language) will be given to the parent or guardian.

b. Confidentiality

All information collected during the study will be recorded and identified by a specifically assigned number for the study and the number assigned routinely for the program. Patient or parent or guardian names will not be recorded in the study form or electronic databases. Access to the study information (in paper or electronic format) is restricted to authorized staff.

Informed consent forms will be kept in a secure and confidential place in a locked cabinet by the study coordinator throughout the study. The consent forms will be archived for a minimum of 5 years after the end of the research, in a secure and locked archive by the investigator at MSF OCBA, in Barcelona. After this period, all documents will be destroyed. If the Ministry of Health requests to archive copies of the informed consents, they will be given at the end of the research study.

c. Benefits

We expect that the study will validate the use of Xpert MTB/RIF Ultra in stools and urine for pediatric TB diagnosis. Improved tools for paediatric TB diagnostic will ultimately have an impact in reducing mortality in children with TB disease. We expect that the results of the study will build the base of an improved diagnostic strategy in which diagnostic tools are more adapted to a particular operational/epidemiological context.

At an individual level, a potential benefit would be a most efficient diagnosis of TB in the child, which will lead to an earlier treatment and improved prognosis. All patients will be included in the TB (and HIV when required) program. The community will benefit of the improved TB detection in children.

d. Risks

The potential risk for the implementation related to the study is eventual challenges in availability and implementation of new tools and consumables (Xpert MTB/RIF Ultra). The risks linked to the field might be instability of the context and lower recruitment rate than expected which would lead to a longer duration of the study.

Regarding the potential individual risks for the subjects in the study could be a longer stay at the hospital. The risks related to sample collection are described in section 9 and can be related to gastric lavage, naso-pharyngeal aspirate or lymph node punction; these procedures are used routinely for the diagnosis and not specifically for this study. No harms are expected in stools or urine collection.

Being diagnosed of TB can be associated to stigma. MSF has been supporting the TB program in Malakal for the last 3 years and a health promotion and community engagement strategy to increase knowledge and reduce stigma is already in place; an assessment on TB related stigma showed that stigma on TB is relatively low in the area. In Bissau, the National TB Program is responsible for TB treatment initiation and follow-up. TB stigma in the capital was evaluated as low. Moreover, all children in both settings will be treated with strict confidentiality.

12. Timeline

The estimated overall duration will be 36 months, starting from January 2019, 28 of them in the field:

- Ethical review: 2 months
- Training to staff: 1 month
- Inclusion and data collection: 24 months
- Data analysis and report: 3 months

- Dissemination: 6 months

13. Dissemination of Results and Publication Policy

Programme results will be presented to MoH authorities, as well as to WHO, to advocate for the inclusion of both Xpert MTB/RIFUltra in stools and/or urine in international guidelines if the study show promising results and added value in the TB diagnosis.

The results of the study will be presented in conferences and published in a peer reviewed journal in order to share results with the TB public health professionals and other medical-humanitarian actors.

14. Budget

Costs related to the study are guaranteed by MSF operational budget, including necessary human resources and all consumables and diagnostic tests, which will be available through standard MSF procurement and supply mechanisms.

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16. Appendixes

Please, note that many of the following appendixes will be used for the study "Evaluating POCUS to improve diagnosis in children". Only the appendixes marked with an asterisk are specific for this study:

Appendix 1: Study Information Sheet: Xpert MTB/RIF*

Appendix 2: Informed Consent Form Xpert MTB/RIF*

Appendix 3: Information for caretaker on stool and urine sample collection*

Appendix 4: Clinical form (CRF)

Appendix 5: TB diagnostic algorithm (SOP of TB diagnosis in children)

Appendix 6: SOP for naso-pharyngeal aspirate

Appendix 7: SOP for gastric lavage

Appendix 8: SOP for pulmonary sample collection, storage and shipment

Appendix 9: SOP for lymph node punction and Xpert MTB/RIF assay

Appendix 10: SOP for stools Xpert MTB/RIF assay*

Appendix 11: SOP for urine Xpert MTB/RIF assay*

Appendix 12: SOP for Xpert MTB/RIF assay performance

Appendix 13: SOP for the diagnosis of HIV in children

Appendix 14: Checklist for adherence counselling (HIV and TB)

Appendix 15: Anti-TB dosages for children

Appendix 16a: Weight for height from 5 to 10 year old

Appendix 16b: Wight for height in adolescents (10 to 18 year old)

Appendix 17: CRF Lab*

Appendix 18: Interpretation of Trace results (based on GLI)

Appendix 1. Study Information Sheet Xpert MTB/RIF

Title of the study: EVALUATING XPERT MTB/RIF IN STOOLS AND URINE TO IMPROVE TB DIAGNOSIS IN CHILDREN

Principal investigator: Laura Moretó (MSF OCBA)

Co-authors South Sudan: Endashaw Mengistu (MSF OCBA), Maria José Sagrado (MSF OCBA), Laurence Flevaud (MSF OCBA), Godwin Ohisa (MoH), Mohamed Eltom (MSF OCBA)

Co-authors Guinea Bissau: Jonathan Gallo (MSF OCBA), Pablo Nuozzi (MSF OCBA), Maria José Sagrado (MSF OCBA), Laurence Flevaud (MSF OCBA), Jamila Vieira Fonseca (MoH), Cándida Redina Rodrigues (MoH), Armando Sifna (MoH), Miguel Camará (MoH), Mercè Rocaspana (MSF OCBA)

Partnership: Ministry of Health of South Sudan and Ministry of Health of Guinea Bissau

ERB South Sudan: Dr. Richard Lako Lino Loro, Director Generral for Policy Planning, Budgeting and Research, MoH SS, HQ Ministerial Complex, Juba, SS – PO Box 88, Juba, Tel: +2110177800281.

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Version: version 1.11, March 2019

Dear Sir or Madam,

Médecins sans Frontières (Operational Centre Barcelona-Athens - OCBA) and the Ministry of Health, invite you to participate with your child in a study to evaluate Xpert MTB/RIF in stools and urine to improve the diagnostic of TB in children. Before making your decision and accepting or refusing to participate, it is important that you understand this information sheet which explains various aspects of the study. You can read it, or we can read it and explain it to you, and we will answer any questions you wish to ask.

Please, note that we will also offer your child to participate to the research "Evaluating POCUS to improve diagnosis in children" and a separate information sheet and consent form will be shared with you as well. Please note that it is your decision whether to participate only in one of the studies, both studies or not at all, without any consequences.

All the relevant information will be shared also with your child adjusted to age range and all questions will be answered, but for ethical reasons we ask you to sign the consent form.

Background and purpose of the study: Tuberculosis (TB) is a very common disease but its diagnosis is difficult, especially in children. Xpert MTB/RIF for sputum samples is already validated as a standard test, but using it in both stools and urine can help us diagnose TB in children. This test requires collecting a small amount of urine and stool, and the results are available within 72 hours. We hope that this research will help improve the diagnosis of tuberculosis in children in the area as well as in other places where resources are limited.

Why is your child asked to participate? We want your child to participate because he/she meets all the criteria of the study: he/she is between 6 months and 15 year old, he/she presents the symptoms of tuberculosis and he/she has not been treated for TB in the last 3 months.

What happens if your child participates in the study? If you agree to participate in the study, your child will be examined as normally done and will be given all the usual tests for the diagnosis of tuberculosis that are used in the hospital. In addition, he/she will be requested to provide a sample for stools and urine and will be given the Xpert MTB/RIF test from these samples The result of the Xpert MTB/RIF samples will only be used for the study, because it is not yet known whether this test works well in children. If needed, your child will be kept in hospitalization. Otherwise, s/he will be kept in Observation for a maximum of 2 hours and, if the child can't be produce stools / urine during this period, you will be asked to bring the samples the day after. The usual treatment for tuberculosis, like any other necessary treatment, will normally be given to your child, according to the recommendations in force in the country. Your child will be followed during the whole duration of the treatment as part of the TB program and regardless of the participation in the study.

Do you have the right not to participate? Participation in the study is left to your decision alone. You are free to decide whether your child will participate in the study or not. You are also free to withdraw your child from the study at any time. Your decision will not affect the care your child will receive at this hospital.

Are there disadvantages and risks if you participate in the study? Participation in the study entails no risk. However, the collection of urine and stool can be felt as a bit unpleasant for the child. The rest of procedures are routinely done (not specifically for the study).

What are the advantages if you participate? There are no direct benefits for you or your child if they participate in the study. But your child's participation in the study will improve the diagnosis of TB in the future, and we hope to be able to better treat and diagnose TB in children. You will not receive any direct monetary benefit such as food or payment as a reward for participating in the study.

Is confidentiality assured? All information we collect about your child during the course of the study will remain strictly confidential. Your child's name will not appear on the study documents or on the reports. All documents will be kept in a locked cupboard and only staff working for the study will have access to the information that will be collected.

How will the results of the study be used? The results of this research will be shared within MSF and with experts at the national and international levels in both national and international meetings, and will be published in specialized medical journals. All available results will be shared with you as well.

Who did verify and approve the study? This study was verified by the National Ethics Committee of South Sudan and by the MSF Ethics Committee.

If you agree to your child's participation in the study, please sign with your name below indicating that you have read and understood the nature of the study, your responsibilities as a

participant, the risks and benefits associated with your free and voluntary participation, and that all your questions about the study have been answered satisfactorily.

You will receive a copy of this consent form once it is signed, which you can keep at home.

Assent process for children older than 10 years old:

There's a disease called Tuberculosis which is quite common in the area where you live but unfortunately diagnostic is difficult and many children remain without diagnosis. This is the reason why we are proposing a study to analyse your stools and urine using a machine called Xpert MTB/RIF.

Your parent or guardian said it would be all right for you to take part of this study but this is your decision. If you don't want to participate, you don't have to and if you want to stop it at any time, just let us know and we will proceed accordingly. We will ask you to participate to a second study that wants to evaluate point of care ultrasound and you can accept to participate in both, in only one or in none of them.

If you agree to help us, we will ask you to collect stools and urine in the hospital (if you are hospitalized) or at OPD (if not) within 2 hours; if you can't produce today, we will ask your parent or guardian to bring the samples tomorrow morning.

If you sign or mark this document it means that you understand the information I just explained and that you agree to be part of the study. If you don't want, don't sign the document.

The above statement has been read to the child and he agrees to participate in the study.

The child accepts participating in the study: ☐ Yes ☐ No

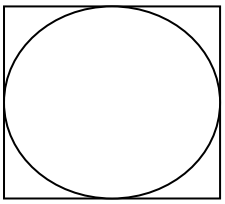
Appendix 2: Informed consent form Xpert MTB/RIF stools / urine

Declaration of the parent or guardian of the participating child

I confirm that I received orally and in writing all the information concerning this study, and that I understood what is asked if I participate. I know who to contact if I need more information about this study. I understood that the personal information will remain confidential throughout the study and that I have the freedom to stop the participation of my child at any time without this modifying the care s/he receives / will normally receive in the clinic.

I accept that my child under my care is participating in this study. I will receive a copy of this informed consent to keep.

Name of child participating in study: _____



| --- | --- | --- | --- |
| Date | Name parent/guardian | Signature | or thumbprint
If caretaker can't read or write |

| --- | --- | --- |
| Date | Name of staff | Signature |

| --- | --- | --- |
| Date | Name of the witness
If parent/guardian **can't read or write** | Signature |

Contact persons for further information

If you have a problem or have questions about this study, you can contact the staff collaborating in the study directly, and also contact:

Laura Moretó, Principal Investigator in the Study
MSF OCBA, Medical Department
Tel: +34 93 304 6168
Email: laura.moreto@barcelona.msf.org

Appendix 3: Information for caretaker on stool & urine sample collection

1) Stools

You will receive one (or more) special containers for stools. Do not bring stool in any other container. Keep the containers out of children's reach.

Preparing to collect the stool:

- For children in diapers, get 6-spoonful from the diaper, using the spoon from the stool container.
- For older children, put plastic wrap, newspaper, or a special collection container under the toilet seat or latrine to catch the stool. Place the plastic container or potty in the toilet bowl. Alternatively place clean newspaper or plastic wrap across the toilet seat opening.

Collecting the stool sample:

- Allow the child to the stool into the potty, plastic container, or onto the newspaper or plastic wrap. Make sure it does not touch the inside of the toilet.
- Take the caps off of the containers. Transfer 6 small spoonful of the stool into the blue specimen container using the spoon built into the lid of the specimen container. Never take the sample out of the water in the toilet bowl.
    - Do not overfill the specimen container. A walnut-sized amount, or a third of the container, is enough for testing.
    - Put on the specimen container lid and screw on tightly. If the outside of the container has got dirty, clean the outside with soap and warm water.

- Wash your hands thoroughly with soap and water.
- Dispose of the stool left in the potty, plastic container, newspaper, or plastic wrap into the toilet.

Storage (ambulatory patients):

- Fresh samples: Keep in the refrigerator 4^0C and bring to Laboratory within 12 hours.
- If no fridge, collect the sample in the morning on the day you are visiting the clinic. Store in a cool dry place and bring to Lab within 2 hours.

2) Urine

You will receive one (or more) special containers for **urine**. Keep the containers out of children's reach.

Collecting the urine sample:

- The urine samples of the participants of the study will be collected by the parent or guardian in the clinic. For infants, urinary bags will be used. For children who can self-emit urine on demand, sterile containers will be used. The minimum volume required is 20 ml.
- Wash your hands thoroughly with soap and water.
- Give the sample to medical staff or bring it to the laboratory as soon as possible (within 2 hours).

Questions?

This sheet is not specific to your child but provides general information. If you have any questions, please discuss with medical staff responsible for the study at the hospital.

Appendix 4: CRF clinical form

Note that this form will be shared between the 2 studies (Xpert MTB/RIF stools/urine & POCUS) and only filled once

Background information of study participant **Date:** / /

Study code patient:
Date of birth: Age:
Sex: Female □ Male □
Parent or guardian informed consent: Yes □ No □
Consent to be traced in case of defaulting: Yes / No

Baseline clinical information at entry of study **Date:** / /

Entry point to study:

OPD	□	TB program (ie. TB contact)	□	
IPD	□	HIV program	□	
Nutrition program	□	Other	□	Specify:

Screening in all children, HIV, SAM or TB contacts:

Fever > 1 week	□	
Cough > 2 weeks	□	
Suspected extra pulmonary TB	□	Site:

Screening in hospitalized children after 1 week hospitalisation if suggestive symptoms:

Loss weight despite correct nutritional treatment	□
Persistent cough or pneumonia despite antibiotic	□
Persistent fever once classical causes excluded	□
Persistent or aggravated fatigue	□
Chest radiography suggestive of TB	□

History of:

TB or chronic coughing contact:	Yes □	No □	
Unexplained weight loss in the last 3 months	□	MAM □	SAM □
Known HIV status: Negative □	Positive □	Unknown □	

Clinical condition:
Temperature (axillary) (◦C): Weight (kg): Size (cm):
Nutritional status: Not significantly malnourished □ MAM □ SAM □
Co-morbidities:
Malaria Respiratory infection:
HIV: Negative □ Positive □ Unknown □
First PCR DNA pending □ Confirmation PCR DNA pending (first positive) □
ART regimen: Date ART started: / /
Signs of pulmonary TB:
Cough □
Tachypnea □
Hypoxemia (sat O2 < 92%) □
Fever (axillary temperature > 38ºC) □
Signs of extra pulmonary TB:
Angular deformation of the spine (gibbous) □

Lymphadenopathy	□	
Subacute meningitis	□	
Abdomen distended with ascites	□	
> 2 weeks of diarrhoea	□	
Painless enlarged joints	□	
Pleural effusion	□	
Other	□	Specify:
POCUS done: Yes □ No □	Date: / /	

Information at discharge: final diagnosis **Date**: / /

Confirmed pulmonary TB	□	
Suspected pulmonary TB	□	
Confirmed extra pulmonary TB	□	
Suspected extra pulmonary TB	□	
Unlikely TB	□	
Other	□	Specify:

TB treatment
Started: Yes □ No □ Date ATT started: / /
Regimen:

Follow-up after 2 months of discharge (POCUS participants) Date: / /

Nutritional status:	Not significantly malnourished □		MAM □	SAM □
TB treatment prescribed:	Yes □	No □		
Under TB treatment:	Yes □	No □		
If interruption, specify reason:				
Smear at month 2:	Done □	Positive □	Negative □	
	Not done □			
POCUS done: Yes □	No □	Date: / /		

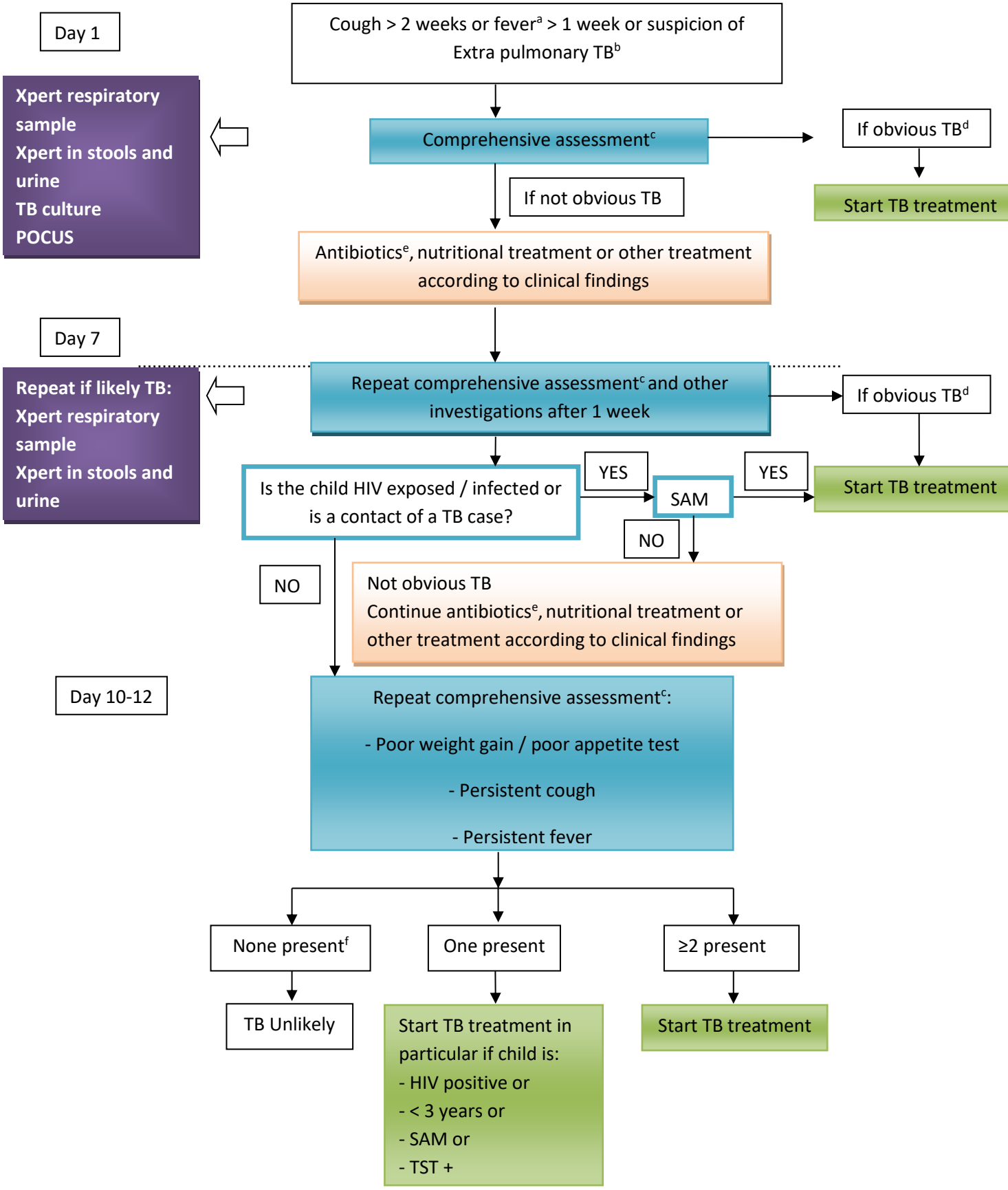
TB patients: follow-up after 6 months of discharge (POCUS participants) Date: / /

Nutritional status:	Not significantly malnourished □		MAM □	SAM □
TB treatment finalised:	Yes □	No □		
Smear at month 6:	Done □	Positive □	Negative □	
	Not done □			
POCUS done: Yes □	No □	Date: / /		

TB treatment outcomes:

Cured:	□	Defaulted:	□	Date: / /
Completed:	□	Death:	□	
Failure:	□	Not evaluated:	□	

Appendix 5: TB diagnostic algorithm



a) xillar temperature > 38ºC

b) Enlarged lymph nodes, gibbous, > 2 weeks diarrhoea, meningitis

c) Comprehensive assessment includes:

   a. Clinical assessment

   b. Growth assessment

   c. Bacteriological test (sputum collection, naso-pharyngeal aspirate, gastric lavage, lymph node aspiration):

      - Xpert MTB/RIF testing (sputum, nasopharyngeal aspirate, gastric lavage, lymph node aspirate, CSF);
      - Xpert MTB/RIF in stools and urine;
      - TB culture and DST (only at baseline):

      For children:

      - < 5 years old: do nasopharyngeal aspirate (Appendix 5)
      - From 5 years old until child can expectorate: gastric lavage (Appendix 6)
      - For children who can expectorate: sputum

   d. POCUS (only at baseline)

   e. HIV testing if not yet performed (Appendix 16)

   f. When relevant and available: X-ray (CXR, spine)

d) Xpert MTB/RIF positive, CXR showing suggestive lesions (e.g. hilar lymphadenopathy, upper lobe infiltrates, and miliary picture), gibbous.

e) Broad spectrum antibiotics:

- **If no signs of severity:**
  - First line: amoxicillin PO for 7 days (NO fluoroquinolones)
  - If a second course of antibiotics is needed: azithromycin PO for 5 days
- **If signs of severity**:
  - Parenteral antibiotics (ceftriaxone ± cloxacillin if *S.aureus* is suspected)
  - If a second course of antibiotic is needed: azithromycin PO for 5 days
  - In addition: **PCP treatment** should be given presumptively to all HIV-exposed or infected children < 1 year of age and any older child with sever immune suppression and not on CTX prophylaxis. For all other HIV-exposed or infected children, it should be considered if there is poor response to broad spectrum antibiotics after 48 h

f) Clinical response to broad-spectrum antibiotic does not rule out TB. Continue follow up to see if symptoms re-occur

Appendix 6: SOP for naso-pharyngeal aspirate[2]

Equipment required:

- Disposable gloves and respirator masks (N95)
- Suction apparatus
- Sterile 6/7/8 G mucus extractor or nasogastric catheter
- Normal saline (0.9%) 5 ml
- Sterile syringe 50 ml (single use)

Performing nasopharyngeal aspiration:

1. Infection control measures: All personnel responsible for collecting respiratory specimens must wear personal protective equipment. The procedure will take place in a dedicated cough room or should be fitted with a door that can lock and windows that open to the outside. Alternatively, samples may be collected outside in the open air or in sputum collection booth/other appropriate structure housed outdoors.

2. The child's nose is cleaned with saline drops. If old enough, the child can be asked to blow the nose into a tissue. If the nasal mucus is too thick to be removed with the measures above, it can be suctioned prior to nasopharyngeal aspiration. A soft catheter size F6/7 is used for suctioning and is discarded immediately afterwards.

4. Two drops of sterile saline are instilled into each nostril.

5. The length of the cannula used for aspirating the NPA sample is measured as the distance from nostril to tragus of the ear; then the posterior nasopharynx is suctioned using a soft plastic cannula connected to a mucus trap.

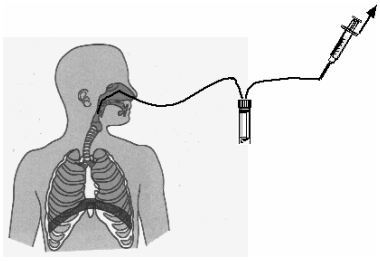
6. Suctioning is activated only when the tip of the cannula is in the posterior nasopharynx. When the cannula is passed through the nostrils (during introduction and extraction), the suction is de-activated.

7. Transfer full volume of sample into a sterile container (Falcon tube).

8. Clean Falcon tube with alcohol swabs.

9. Label sample: sample type and number, date, time, total sample volume.

10. Place specimen in sample bag, seal and put into a cold box for transport to lab.



[2] Based on SOP collection of respiratory specimens in children, Desmond Tutu TB Centre Paediatric Studies

Appendix 7: SOP for gastric lavage[3]

Equipment required:

- Gloves and respirator
- Suction catheter (6, 7, 8F)
- Sputum container
- 50 ml syringe
- Sterile water

Performing gastric lavage:

1. Infection control measures: All personnel responsible for collecting respiratory specimens must wear personal protective equipment. The procedure will take place in a dedicated cough room or should be fitted with a door that can lock and windows that open to the outside. Alternatively, samples may be collected outside in the open air or in sputum collection booth/other appropriate structure housed outdoors.

2. Child needs to be fasting for at least 4 hours and the procedure will take place early in the morning.

3. Place the child in a half-sitting or sitting position in the adult's arms.

4. Insert a nasogastric tube and check that it is correctly placed.

5. First suction to collect the gastric fluid and place it in the sputum container, then rinse the stomach with 30 ml of sterile water and suction again. Add the suctioned fluid to the first sample.

[3] Based on SOP of sputum collection in MSF TB guidelines 2014

Appendix 8: SOP for pulmonary sample collection, storage and shipment

Sputum collection techniques:

Regardless the collection technique used, staff member present during sputum collection should wear a respirator to prevent bacilli inhalation.

Sputum obtained spontaneously:

Two specimens are to be collected. When possible, specimens should be collected outside in the open air and far away from other people.

The first sample is collected on the spot, at the consultation, when the patient is identified as suspected TB case. If the patient has recently eaten, ask him/her to rinse his/her mouth with water in order to avoid the presence of food in the sample.

The second sample is collected the day after, in the early morning, right after the patient wakes up and before eating. The second sample may be collected at home then the patient brings it to the health facility.

Alternatively, two sputum specimens can be collected one hour apart (frontloaded microscopy). Collection technique:

- The patient must be given a labelled sputum container (or a Falcon® tube, if the sample is to be shipped by air).
- Have the patient take a deep breath, hold for a few seconds, exhale, repeat two or three times, then cough: sputum is material brought up from the lungs after a productive cough. One or two minutes of chest clapping are of benefit.
- Collect at least 3 ml and close the container hermetically.

The quality of sample determines the reliability of the result. Always check that the sample contains solid or purulent material and not only saliva. Take a new sample if unsatisfactory.

If the sample is collected at home, make sure that the patient has understood the technique, including closing the container hermetically after collecting the sputum.

Sputum specimen storage

When examinations are not performed on the site of collection:

- **Specimen for smear microscopy**

Smears should be performed within three-four days of collection and in the meanwhile stored refrigerated (2 to 8°C) and protected from light.

Contamination does not affect microscopy but heat make specimen liquefy, with selection of mucopurulent part of the sample more difficult.

- **Specimen for culture in liquid medium**

Keep the specimen refrigerated (2 to 8°C), protected from light. Do not use cethylpyrodinium chloride (CPC) as it is not compatible with MGIT.

The specimen should be processed as soon as possible.

- **Specimen for culture on Lowenstein-Jensen medium (LJ)**

- Specimens that can be cultured in less than 3 days after collection:

Keep refrigerated (2 to 8°C) and protected from light until transport OR immediately transport to the laboratory for processing.

- Specimens that will be cultured more than 3 days after collection:

Use Falcon tubes and add 1% CPC to preserve the specimen for up to 2 weeks. Specimens with CPC should not be refrigerated, as the CPC will crystallize and be ineffective. Samples with CPC

can be inoculated on LJ. For inoculation on agar, they require prior neutralization by neutralizing buffer (Difco).

CPC can be used for specimens tested by Xpert MTB/RIF.

Sputum specimen shipment

To a local laboratory

- Without CPC transport medium: between 2 and 8°C and protected from light;
- With CPC transport medium: should not be refrigerated because at low temperatures the CPC will crystallize and ruin the sample. Specimens should be kept at room temperature, protected from heat and light.

By air to a reference laboratory for culture

Samples are collected and shipped in 50 ml Falcon® conical tubes with screw caps. The tubes are labelled UN 3373, corresponding to Category B infectious substances. If transport times are less than 12 hours, even specimens without CPC can be transported at room temperature.

Samples are triple-packaged, in accordance with IATA packing instruction 650:

1. Primary container holding the sputum sample: tube tightly closed and placed into a latex glove;

2. Secondary container intended to protect the primary container: leak-proof box with enough absorbent material to absorb the entire sample, should the primary container break;

3. Outer packaging intended to protect the secondary container, with UN 3373 labelling.

Information to be provided:

- Primary container: label with the patient's name or identification number and the sample collection date and location;
- Outer package: indicate the name of the receiving laboratory, the complete address (name, street, postal code, locality, country), and telephone number.

All samples must be accompanied by the corresponding laboratory test request form (including clinical information).

Notes:

- Procedures for shipping bacterial strains obtained after culture are different, more complicated, and rarely feasible in practice. Cultures are classified as Category A infectious substances (UN 2814).
- For a detailed description of the shipment procedures, see MSF Medical catalogue, volume 4.

Appendix 9: SOP for lymph node puncture and Xpert MTB/RIF assay[4]

FNAC is used to obtain material from lymph nodes. The material is expressed onto slides and prepared for examination.

Two smears will be prepared with Giemsa staina2 to look for caseum, granuloma, giants cells, and epithelioid cells or histocytes and 1 or 2 will be prepared with Ziehl-Neelsen (ZN) stain to look for acid-fast bacilli (AFB).

Equipment

- Needle 23G (in very few cases, it would be possible to use 19G)
- 10 ml syringe
- 2 slides for Giemsa + one or 2 slides for ZN stain
- 10% polyvidone iodine, sterile gauze, gloves

Technique

- Disinfect the area.
- With the needle attached to the syringe, insert the needle deep into the lymph node.
- After the needle has entered the mass, pull back on the syringe plunger to create a vacuum.
- Rapidly move the needle in a to-and-fro fashion to allow material entering the needle.
- When blood or material appears in the needle hub the aspiration should be stopped. Try to aspirate as much as possible of materials, the amount of materials that has been aspirated would have effect on the specificity and sensitivity of diagnosis.
- Release the negative pressure before to take out the needle from the lymph node. Do not continue sucking while taking out the needle, this will avoid aspiration of materiel into the barrel of the syringe and avoid mixing the sample with the possible peripheral blood in the skin.

Appendix 10: SOP for stools Xpert MTB/RIF assay

Materials

- Xpert DX System
- Xpert Instrument, computer, barcode reader
- Xpert MTB/Rif cartridge and sample reagent bottle
- Materials required bud not provided in the kit
- Centrifuge
- Gloves
- Pipette (capable of dispensing 2mL)
- Biohazard disposable bags

Safety, Health & Environment

Treat all stool specimens as potentially infectious and follow basic universal precautions. Wear protective clothing (coat/apron and gloves) when handling the specimens.

Principle

The Xpert System integrates and automates sample processing, nucleic acid amplification, and detection of the target sequences using real-time PCR and reverse transcriptase PCR. The system requires the use of single-use disposable GeneXpert MTB/Rif cartridges that hold the PCR reagents and host the PCR process. Xpert MTB/RIF includes reagents for the detection of tuberculosis and RIF resistance as well as a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. The primers in the Xpert MTB/RIF assay amplify a portion of the rpoB gene containing the 81 base pair "core" region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with RIF resistance.

Specimen collection and storage

Collect stool samples from children in a clean wide open container. Stool samples must be processed within the day of collection. Fresh stool samples can be used within 3 hours if kept at room temperature.

Reagent storage and preparation

Xpert MTB/Rif cartridges must be stored at 2-28ºC. Do not use beyond expiration date and do not open the cartridge until you are ready to perform the test (use the cartridge within 30minutes after opening its lid).

Test Procedure

- Add an aliquot of 2-3 g (pea size) of stool sample into a centrifuge tube (with a lid) using a sterile disposable plastic loop
- Add 5 ml of Phosphate Buffered Saline (PBS) and vortex to homogenize the mixture
- Centrifuge the mixture at 3200x g for 15 min

- Add 2 ml of the Xpert reagent into 1ml of the re-suspended pellet mixture and mix thoroughly.
- Using the sterile pipette provided, aspirate the liquefied sample into the pipette until the meniscus is above the minimum mark. Add the mixture into the Xpert MTB/Rif cartridge and insert the cartridge into the GeneXpert instrument and conduct the assay according to the manufacturer's instructions (follow your laboratory Sop for operation of the GeneXpert Instrument).

Interpretation of test results:

- Interpret the results as you normally do with results from sputum samples processed in Xpert Instrument.
- The results are produced by the GeneXpert DX System from measured fluorescent signals and embedded calculation algorithms and will be displayed in the "View Results" window. Lower Ct values represent a higher starting concentration of DNA template; higher Ct values represent a lower concentration of DNA template.
- MTB Detected=> MTB target DNA is detected. The MTB result will be displayed as High, Medium, Low or Very Low depending on the Ct value of the MTB target present in the sample.
- RIF Resistance Detected=> will be displayed if the mutation in the rpoB gene has been detected. This is only displayed in MTB detected results.
- MTB Not Detected=>MTB target DNA is not detected

Quality control testing

Each GeneXpert test cartridge is a self-contained test device with an in-built control for each sample. Normally, no external controls are required. The internal controls enable the system to detect specific failure modes within each for each sample

- Instrument system control: Check status-it checks the optics, temperature of the module and the mechanical integrity of each cartridge. If the system controls fail, an ERROR test result will be reported.
- Probe Check control (PCC): after sample preparation, bead reconstitution and tube filling (prior to thermal cycling), multiple fluorescent readings are taken at different temperatures and compared to default setting. PCC controls for:
    - Missing target specific reagent (TSR) and or enzyme reagent beads which contain all primers, probes and internal control template.
    - Incomplete reagent reconstitution
    - Incomplete reaction tube filling
    - Probe degradation
- If the PCC fails, an ERROR test result will be reported.
- Sample processing control (SPC) assesses the effectiveness of the sample processing steps, including and up-to reaction tube filling. SPC ensures that the sample was correctly added to the cartridge and detects degradation of the enzyme(s) or other components of the system. SPC does not compete with target DNA.
    - SPC must be Positive when target is Negative
    - SPC can be Positive or Negative when the target is Positive

- SPC passes if it meets the validated acceptance criteria. E2097: too less sample volume added (<1mL), E2096: no sample added

- Internal Quantitative Standard High and Low (IQS-H and IQS-L): IQS-H and IQS-L are two dry bead armored RNAs nonspecific to HIV in the form of a dry bead that goes through the whole GX process. The IQS-H and IQS-L are standards calibrated against the WHO 3rd International Standard. They are used for quantification by using lot specific parameters for the calculation of HIV-1 RNA concentration in the sample. The IQS-H and IQS-L pass if they meet the validated acceptance criteria. They run internally with every cartridge and they control for reagent performance due to improper storage and they confirm that reaction components are set up correctly.

- External Controls: not available in the kit, but they can be used (positive and negative controls).

[4] Based on OCB SOP Stools GeneXpert MTB/Rif Assay

Appendix 11: SOP for urine Xpert MTB/RIF assay[5]

Materials

- Xpert DX System
- Xpert Instrument, computer, barcode reader
- Xpert MTB/Rif cartridge and sample reagent bottle
- Materials required bud not provided in the kit
- Centrifuge
- Gloves
- Pipette (capable of dispensing 2mL)
- Biohazard disposable bags

Safety, Health & Environment

Treat all stool specimens as potentially infectious and follow basic universal precautions. Wear protective clothing (coat/apron and gloves) when handling the specimens.

Principle

The Xpert System integrates and automates sample processing, nucleic acid amplification, and detection of the target sequences using real-time PCR and reverse transcriptase PCR. The system requires the use of single-use disposable GeneXpert MTB/Rif cartridges that hold the PCR reagents and host the PCR process. Xpert MTB/RIF includes reagents for the detection of tuberculosis and RIF resistance as well as a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. The primers in the Xpert MTB/RIF assay amplify a portion of the rpoB gene containing the 81 base pair "core" region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with RIF resistance.

Specimen collection and storage

Collect midstream urine in a fresh standard urine collection container. Fresh urine samples can be used within 3 hours if kept at room temperature.

Urine samples should be stored at 2-8°C if the test is to be run within 3 days of collection.

- If testing is delayed more than 3 days, the samples should be frozen (-20°C or colder). For frozen or refrigerated urine bring all samples to room temperature one hour prior to use. Frozen samples may contain aggregates.
- All thawed samples must be centrifuged at 3000 g for 15 minutes at room temperature Specimens that have been frozen and thawed more than 3 times cannot be used.

Reagent storage and preparation

GeneXpert MTB/Rif cartridges must be stored at 2-28oC. Do not use beyond expiration date and do not open the cartridge until you are ready to perform the test (use the cartridge within 30minutes after opening its lid).

Test Procedure

- The sample of urine, 4ml, is centrifuged at 3000g for 5 minutes.
- Decant the supernatant and re-suspend the pellet/sediment in 2mL of the GeneXpert sample reagent.
- Thoroughly mix the re-suspension mixture, and add 2ml of the mixture into the GeneXpert MTB/Rif cartridge.
- Insert the cartridge into the GeneXpert instrument and proceed with testing (follow your laboratory Sop for operation of the GeneXpert Instrument).

Interpretation of test results:

- Interpret the results as you normally do with results from sputum samples processed in Xpert Instrument.
- The results are produced by the GeneXpert DX System from measured fluorescent signals and embedded calculation algorithms and will be displayed in the "View Results" window. Lower Ct values represent a higher starting concentration of DNA template; higher Ct values represent a lower concentration of DNA template.
- MTB Detected=> MTB target DNA is detected. The MTB result will be displayed as High, Medium, Low or Very Low depending on the Ct value of the MTB target present in the sample.
- RIF Resistance Detected=> will be displayed if the mutation in the rpoB gene has been detected. This is only displayed in MTB detected results.
- MTB Not Detected=>MTB target DNA is not detected

Quality control testing

Each GeneXpert test cartridge is a self-contained test device with an in-built control for each sample. Normally, no external controls are required. The internal controls enable the system to detect specific failure modes within each for each sample

- Instrument system control: Check status-it checks the optics, temperature of the module and the mechanical integrity of each cartridge. If the system controls fail, an ERROR test result will be reported.
- Probe Check control (PCC): after sample preparation, bead reconstitution and tube filling (prior to thermal cycling), multiple fluorescent readings are taken at different temperatures and compared to default setting. PCC controls for:
    - Missing target specific reagent (TSR) and or enzyme reagent beads which contain all primers, probes and internal control template.
    - Incomplete reagent reconstitution
    - Incomplete reaction tube filling
    - Probe degradation
- If the PCC fails, an ERROR test result will be reported.
- Sample processing control (SPC) assesses the effectiveness of the sample processing steps, including and up-to reaction tube filling. SPC ensures that the sample was correctly added to the cartridge and detects degradation of the enzyme(s) or other components of the system. SPC does not compete with target DNA.

- SPC must be Positive when target is Negative
- SPC can be Positive or Negative when the target is Positive
- SPC passes if it meets the validated acceptance criteria. E2097: too less sample volume added (<1mL), E2096: no sample added

- Internal Quantitative Standard High and Low (IQS-H and IQS-L): IQS-H and IQS-L are two dry bead armored RNAs nonspecific to HIV in the form of a dry bead that goes through the whole GX process. The IQS-H and IQS-L are standards calibrated against the WHO 3rd International Standard. They are used for quantification by using lot specific parameters for the calculation of HIV-1 RNA concentration in the sample. The IQS-H and IQS-L pass if they meet the validated acceptance criteria. They run internally with every cartridge and they control for reagent performance due to improper storage and they confirm that reaction components are set up correctly.

- External Controls: not available in the kit, but they can be used (positive and negative controls).

[5] Based on OCB SOP Urine GeneXpert MTB/Rif Assay

Appendix 12: SOP for Xpert MTB/RIF assay performance

Xpert MTB/RIF assay is based on hemi-nested real time PCR for simultaneous detection of M. tuberculosis (MTB) and rifampicin (RIF) resistance. The target is the rpoB gene, critical for detection of mutations associated to rifampicin resistance. Xpert MTB/RIF automates all aspects of real time PCR analysis, with results available in 2 hours.

Sample processing

The test can be performed using fresh sputum samples or decontaminated samples prior culture inoculation.

Procedure with fresh sputum samples:

- Ask the patient to rinse the mouth twice before collecting the sample.
- Collect a minimum of 1.5 ml good quality sputum.
- Follow the procedures outlined below:

Procedure for Xpert MTB/RIF*

Step 1	Step 2	Step 3
		
Add the reagent 2:1 (v/v) to the sample and shake 10-20 times. Incubate at room temperature for 15 min.; during incubation repeat once shaking for 10- 20 times.	With a pipette transfer the diluted sample into a cartridge.	Insert the cartridge in the machine and start the test.

* Source: National Health Laboratory Services, South Africa.

Procedure for sediment samples:

- Sediments can be prepared according to standard decontamination procedure (NALC-NaOH method) and re-suspend with phosphate buffer.
- Ensure 0.5 ml is available for the test; add 1.5 ml of reagent for 0.5 ml of re-suspended sediment.
- Follow the procedure described in the above figure.

Interpretation of the results:

Proper test performance is ensured by 2 internal controls:

- Sample processing control (SPC) ensures adequate processing and monitors presence of inhibition.
- Probe check control (PCC) verifies that the steps of the tests (rehydration, filling of the cartridge, etc.) take place correctly.

When the test is completed the display can show:

- "MTB detected" expressed by levels (the higher the level, the higher the amount of MTB detected in the sample) or "MTB not detected";
- RIF results expressed as "detected", "not detected" or "indeterminate" are available only if MTB is detected.

Other possible results:

- Invalid: MTB invalid and SPC failed due to one of several reasons, such as inhibition;
- Error: MTB no result, SPC no result, PCC failed; fail of system components;
- No result: e.g. tests stopped during processing.

Storage of samples and cartridges:

Samples:

- For a period ≤ 3 days: store at 35°C maximum. A cold chain is not required for up to 3 days after collection. During this period of time, overgrowth of normal flora does not have negative influence on the test. However, if a cold chain is available, samples should be stored at 2 to 8°C in order to help their preservation.
- For a period of 4 to 10 days: store refrigerated at 2 to 8°C.

If samples require other testing (i.e. smear microscopy and/or culture), sample storing conditions adequate for microscopy and culture have to be followed. CPC does not interfere with Xpert MTB/RIF testing.

Cartridges:

- To be stored at 2 to 28°C.
- The cartridge should be used within 30 minutes of opening the cartridge lid.
- Cartridges are stable for 7 days after opening the packaging.

Logistic requirements

Power supply

- The device requires stable and uninterrupted power supply.
- Each GeneXpert instrument will need a uninterruptible power supply (UPS).
- The minimum requirement for the functioning of the GeneXpert instrument is to have a 800VA UPS.

Operating temperature

- The operating temperature for GeneXpert instrument device is 15 to 30°C. According to climate conditions, the installation of air conditioning can be recommended to keep the area within the temperature ranges indicated by the manufacturer.

Calibration

- The GeneXpert modules require annual calibration, which must be performed by an authorised service provider or carried out by swapping out the modules. A detailed commercial sales contract and customer support plan should be negotiated with the supplier, guaranteeing regular maintenance, calibration, repair and replacement (when needed).
- Cartridges and reagents shelf-life
- 12 months from date of production.

Storage space

- Each kit contains 10 cartridges and all reagents necessary to run 10 tests. The dimensions of the kit are 27 x 20 x 17 cm and the weight is 800 g.

Lab space

- The GeneXpert IV instrument (4 modules allowing the processing of 4 specimens at the same time) has the following dimensions:
- 29.8 cm wide, 35.6 cm high, 31.1 cm deep; weight: 12 kg. It is designed for indoor use only.
- Provide at least 5 cm of clearance on each side of the instrument to ensure adequate ventilation.
- Do not place the instrument close to the vents of other instruments or air-handling units.

Safety

- The personal protection requirements for microscopy should be adopted, including use of gloves and respirators.

Waste disposal

- Same procedure as for sputum containers. To be noted is the large volume of additional waste generated by Xpert MTB/RIF compared to smear microscopy.

Appendix 13: SOP for the diagnosis of HIV in children

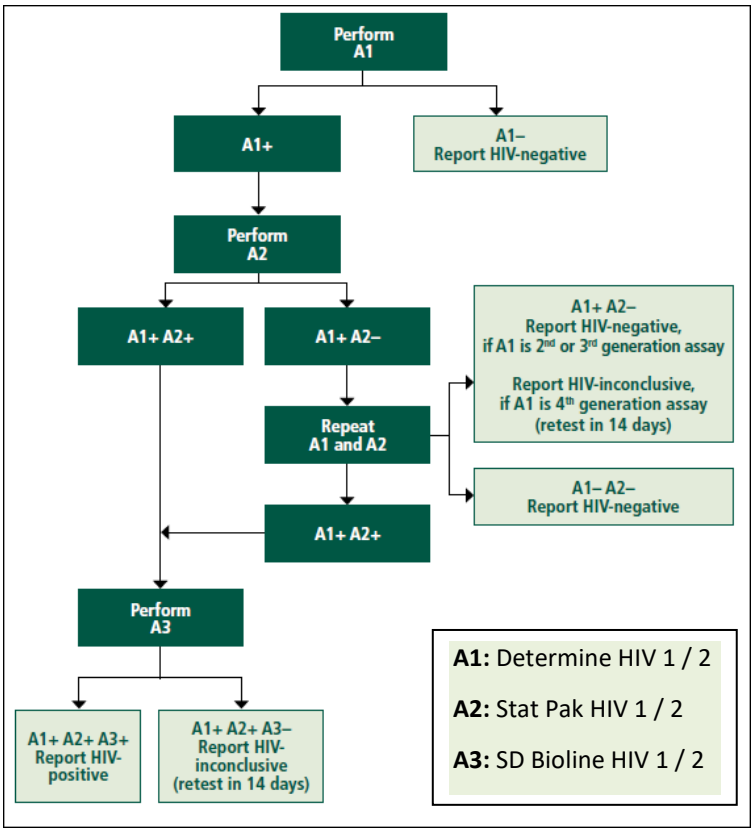
1.1. Indication:

HIV infection should be systematically assessed in all children admitted to the ITFC ward and in children admitted to the Paediatric ward who present with clinical suspicion of HIV infection.

1.2. General principles:

- HIV testing should be performed by trained staff.
- HIV testing can only be performed after counselling and informed consent from the parents.
- In children under 5 years old, the mother will be tested first and the children will be tested only when:
    - Mother is HIV positive
    - Mother is negative but strong suspicion of HIV infection (ie. unsafe blood transfusion, traditional scarification or circumcision) or
    - In the absence of the mother
- HIV testing will be performed by using standard MSF (algorithm 1) or National testing algorithm.
- Children and/or mother with positive HIV diagnosis will be immediately linked to the HIV program, MSF supported or not.

1.3. Algorithm 1: MSF testing algorithm for mothers and children 18 months or more[1]



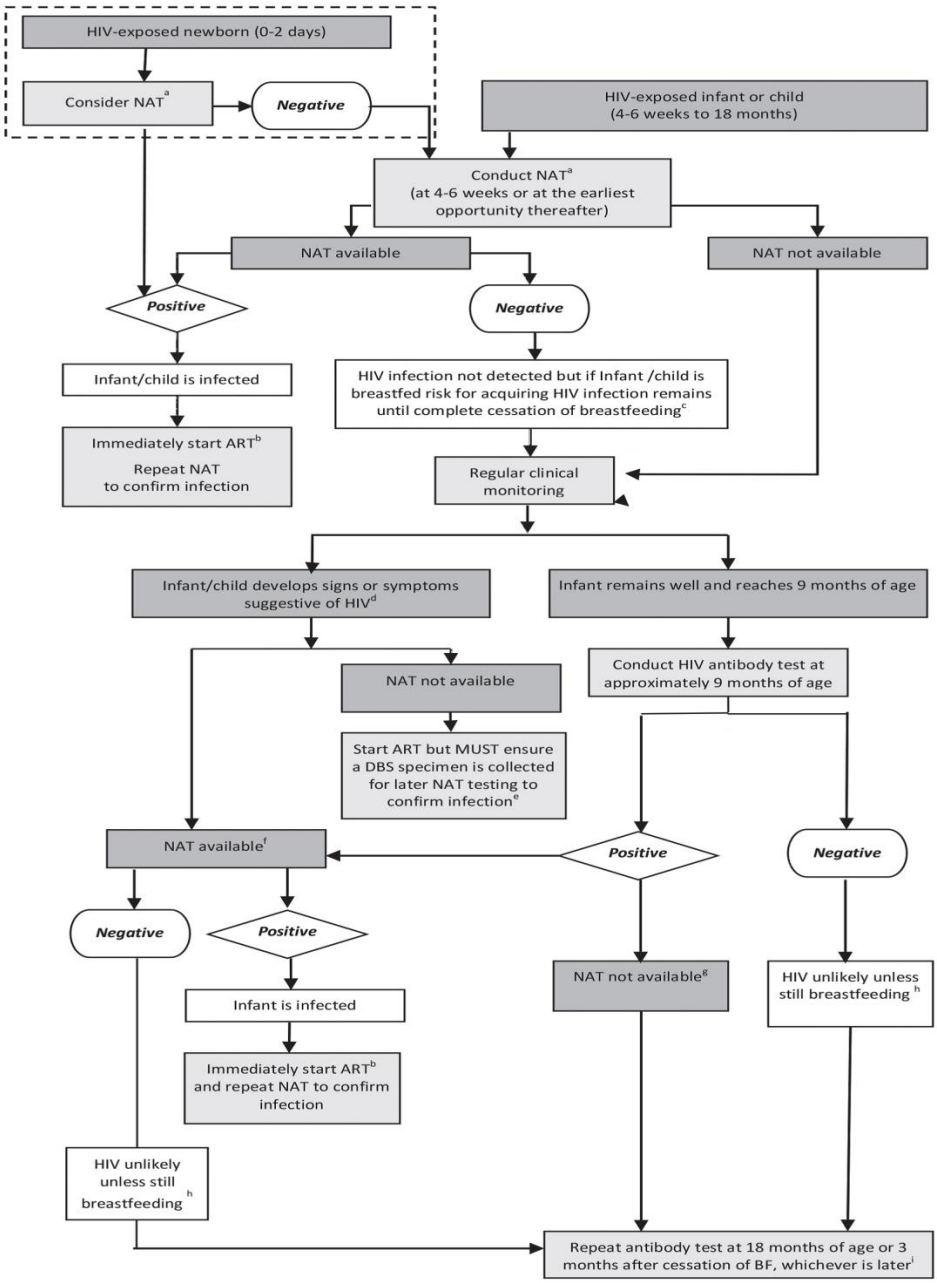
[1] New HIV testing algorithm for MSF setting, October 2017

1.4. MSF testing for children under 18 months:

- In Guinea Bissau, all children under 18 months will undergo a PCR DNA.

For South Sudan:

- If child < 9 months and mother HIV positive or unknown: perform PCR DNA.
- If child is 9 or more months but not yet 18 months, and mother positive or unknown: perform rapid test and confirm with PCR DNA if positive.
- If child is 18 months and not breastfed in the last 6 weeks: follow algorithm 1.



*Source WHO guidelines 2016 '*Consolidated guidelines on the use of Antiretroviral drugs for treating and preventing HIV infection'

Appendix 14: Checklist for adherence counselling (HIV and TB)

Pre HIV test counselling check list: individual or in group

Reasons and benefits of having an HIV test

What the patient knows about HIV?

What is HIV and its modes of transmission

A brief description of prevention options

Reinforce that results will remain confidential

Reinforce the fact that the patient can deny the test, but explain the importance of doing it

The HIV testing process (finger-prick, testing procedure, timing of results)

The meaning of an HIV-positive and an HIV-negative result

The potential for incorrect results if a person already on ART is given an HIV test

Information regarding the follow-up services that are available in the case of an HIV-positive test result

Assess personal risky behaviour an discuss risk reduction plan

Information about support mechanisms, who will the patient tell about the results? Where can he get support?

Individual testing procedure

Ask for consent for testing

Address any fears related to testing

Post-test individual HIV counselling check list

For NEGATIVE results

Ask the patient if he is ready to receive the results

Give the results simply and clear

Explain the meaning of negative HIV test and the window period and assess if there has been at risk behaviour in the preceding 3 months; recommend re-testing in case of recent exposure

Discuss the importance of remaining negative by educating on methods of prevention (provision of male and female condoms and explain how to use them)

Emphasize on the importance of knowing the HIV status of sexual partner(s) and give information about the availability of partner and couples testing services

Patient education and counselling at screening of TB

For POSITVE results

Ask the patient if he is ready to receive the results

Give the results simply and clear

Check with open-ended questions that the person understand the meaning of the result

Allow and encourage the patient to ask questions

Let the patient talk about his feelings about the results

Acknowledge the shock of the diagnosis ; offer support

Determine how the patient will get through the next few hours

Discuss eventual disclosure of the results and to whom

Discuss how to protect partner(s) from infection and explain use of condoms

Encourage and offer HIV testing for sexual partners, children and other family members of the client

Provide clear information on ART and its benefits for maintaining health and reducing the risk of HIV transmission, as well as where and how to obtain ART

Encourage and provide time for the client to ask additional questions

Topics

Give emotional support regarding the diagnosis

Assess previous knowledge and explain facts on TB disease

- What is TB (Card 1)
- What are the signs and symptoms of active TB (Card 2)
- What are the ways of transmission and prevention of TB (Card 3)
- What is the relationship between HIV and TB (Card 14)
- What is the difference between drug-sensitive and drug-resistant TB

Assess previous knowledge and explain the facts on TB treatment:

- What does the treatment consist of (Card 5)
- How to adhere (Card 15)
- Side effects of treatment (Card 6)
- Clinic follow-up for DSTB patients
- Clinic follow-up for DSTB patients

Explain risk for family members and ask the patient to bring any family member with TB symptoms and all children < 5 years old

Make a plan on how to adhere to TB treatment (getting ready to start treatment, support system, getting to the clinic, medication schedule, managing missed doses, reminder strategies, storing medication)

When Patients suspected to have active TB disease, including DR-TB, referred for sputum collection

Mode In group or individually

Duration 15 minutes

Topics

Explain basic facts on TB (what is TB, signs & symptoms, transmission, prevention, relationship with HIV) (Card 1-2-3)

Explain the procedure of TB diagnosis

Explain how to produce sputum (Card 4)

Patient education and counselling at the start of DSTB treatment

When Before or at the day of TB treatment initiation

Mode Individual

Duration 30-45 min

Patient education and counselling after starting DSTB treatment

When One week after treatment initiation or at the first visit after commencing treatment

Mode Individual

Duration 30 min

Topics

Give emotional support

Evaluate adherence to TB medication (annex 8) and work around any difficulties taking medication since starting

Review knowledge on TB disease and its treatment

Explain monitoring tests for TB treatment

Explain on use of traditional medication, alcohol and drugs in combination with TB treatment

Remind patient to bring any family member with TB symptoms and all children < 5 years old (if the patient didn't yet)

Patient education and counselling at the end of DSTB intensive phase

When At the end of DSTB intensive phase

Mode Individual or in group

Duration 15 min

Topics

Evaluate adherence

Solve any adherence issues

Explain the change in drug regimen for the continuation phase

Explain the need to continue to take treatment throughout the maintenance phase, even if the patient might feel better. Assess and reinforce the patient's motivation to continue treatment

Remind patient to bring any family member with TB symptoms and all children < 5 years old (if the patient didn't yet)

Additional PEC sessions throughout DSTB treatment

When At any moment of TB treatment if adherence problems are detected

Mode Individual

Duration 20 min

Topics

Evaluate adherence

Solve any adherence issues

Explain the need to continue to take treatment throughout the treatment, even if the patient might feel better. Assess and reinforce the patient's motivation to continue treatment

Remind patient to bring any family member with TB symptoms and all children < 5 years old (if the patient didn't yet)

Support group TB sessions

When At any moment of TB treatment if adherence problems are detected

Mode	Group
Duration	45 minutes
Methodology	Discuss challenges and share personal experience without being blamed, judged or stigmatised Can be facilitated by staff member or not Attention to infection control concerns

Appendix 15: Anti-TB dosages for children[6]

Intensive phase

Weight (kg)	Paediatric formulations		Adult formulations		
	HZR 50/150/75	E 100	E 400	H 100	EHZR 275/75/400/150
4	1 tab	1 tab			
5	1 tab	1 tab			
6	1 tab	1 tab			
7	1 tab	1 tab			
8	2 tab	2 tab			
9	2 tab	2 tab			
10	2 tab	2 tab			
11	2 tab	2 tab			
12	3 tab	3 tab			
13	3 tab	3 tab			
14	3 tab	3 tab			
15	3 tab	3 tab			
16	4 tab	–	1 tab		
17	4 tab	–	1 tab		
18	4 tab	–	1 tab		
19	4 tab	–	1 tab		
20	4 tab	–	1 tab		
21	4 tab	–	1 tab		
22	4 tab	–	1 tab		
23	–	–	–	1 tab	2 tab
24	–	–	–	1 tab	2 tab
25	–	–	–	1 tab	2 tab
26	–	–	–	1 tab	2 tab
27	–	–	–	1 tab	2 tab
28	–	–	–	1 tab	2 tab
29	–	–	–	1 tab	2 tab
30-34	–	–	–	–	2 tab
35-39	–	–	–	–	2 ½ tab
40-54	–	–	–	–	3 tab
55-70	–	–	–	–	4 tab
> 70	–	–	–	–	5 tab

[6] Based on the Appendix 8a of MSF TB Guideline 2014

Continuation phase

Weight (kg)	Paediatric formulation	Adult formulation
	HR 50/75	HR 75/150
4	1 tab	
5	1 tab	
6	1 tab	
7	1 tab	
8	2 tab	
9	2 tab	
10	2 tab	
11	2 tab	
12	3 tab	
13	3 tab	
14	3 tab	
15	–	2 tab
16	–	2 tab
17	–	2 tab
18	–	2 tab
19	–	2 tab
20	–	2 tab
21	–	2 tab
22	–	3 tab
23	–	3 tab
24	–	3 tab
25	–	3 tab
26	–	3 tab
27	–	3 tab
28	–	3 tab
29	–	3 tab
30-34	–	2 tab
35-39	–	2 ½ tab
40-54	–	3 tab
55-70	–	4 tab
> 70	–	5 tab

	Daily dosing in patients < 30 kg	Daily dosing in patients ≥ 30 kg
E	15 to 25 mg/kg once daily	15 to 25 mg/kg once daily
H	7 to 15 mg/kg once daily	4 to 6 mg/kg once daily
Z	30 to 40 mg/kg once daily	20 to 30 mg/kg once daily
R	10 to 20 mg/kg once daily	8 to 12 mg/kg once daily

Appendix 16a: Weight for height in children from 5 to 10 year old

RECONSTRUCTED FROM BMI charts: height between 120,5 and 140,0 cm

Reconstruction based on WHO 2007 references

Older children from 60 months to 10 years (110,0 - 140,0 cm)

	MALE			Weight-for-Height (Standing up)				FEMALE	
	-3	-2	-1	Median	cm	Median	-1	-2	-3	
	14.4	15.6	17.0	18.5	110	18.6	17.0	15.5	14.2	
	14.5	15.8	17.1	18.7	110.5	18.8	17.1	15.7	14.4	
	14.6	15.9	17.3	18.9	111	19.0	17.3	15.8	14.5	
	14.8	16.0	17.5	19.1	111.5	19.2	17.5	16.0	14.7	
	14.9	16.2	17.6	19.2	112	19.4	17.7	16.2	14.8	
	15.0	16.3	17.8	19.4	112.5	19.6	17.9	16.3	15.0	
	15.2	16.5	18.0	19.6	113	19.8	18.0	16.5	15.1	
	15.3	16.6	18.1	19.8	113.5	20.0	18.2	16.7	15.3	
	15.4	16.8	18.3	20.0	114	20.2	18.4	16.8	15.4	
	15.6	16.9	18.5	20.2	114.5	20.5	18.6	17.0	15.6	
	15.7	17.1	18.6	20.4	115	20.7	18.8	17.2	15.7	
	15.8	17.2	18.8	20.6	115.5	20.9	19.0	17.3	15.9	
	16.0	17.4	19.0	20.8	116	21.1	19.2	17.5	16.0	
	16.1	17.5	19.2	21.0	116.5	21.3	19.4	17.7	16.2	
	16.2	17.7	19.3	21.2	117	21.5	19.6	17.8	16.3	
	16.4	17.9	19.5	21.4	117.5	21.7	19.8	18.0	16.5	
	16.5	18.0	19.7	21.6	118	22.0	19.9	18.2	16.6	
	16.7	18.2	19.9	21.8	118.5	22.2	20.1	18.4	16.8	
AGE	16.8	18.3	20.0	22.0	119	22.4	20.3	18.5	16.9	AGE
year:month	16.9	18.5	20.2	22.2	119.5	22.6	20.5	18.7	17.1	year:month
06:08	17.1	18.6	20.4	22.4	120	22.8	20.7	18.7	17.2	06:10
06:09	17.5	19.0	20.6	22.4	120.5	22.8	20.8	18.8	17.3	06:11
06:10	17.9	19.2	20.8	22.5	121	22.9	20.9	18.9	17.3	07:00
6:11 / 7:00	18.0	19.3	21.0	22.9	121.5	23.0	20.9	19.0	17.4	07:01
07:01	18.3	19.6	21.1	23.1	122	23.1	21.0	19.1	17.6	7:02 / 7:03
07:02	18.5	19.8	21.3	23.3	122.5	23.3	21.1	19.2	17.7	07:04
07:03	18.6	20.0	21.6	23.4	123	23.4	21.2	19.4	17.9	07:05
07:04	18.8	20.1	21.8	23.8	123.5	23.6	21.4	19.5	18.0	07:06
07:05	18.9	20.3	22.0	24.0	124	23.8	21.5	19.7	18.1	07:07
07:06	19.1	20.5	22.2	24.2	124.5	24.2	21.7	19.8	18.3	07:08
07:07	19.2	20.6	22.3	24.4	125	24.4	22.0	20.0	18.4	07:09
07:08	19.4	20.8	22.5	24.6	125.5	24.6	22.2	20.3	18.7	07:10
07:09	19.7	21.1	22.7	24.9	126	24.9	22.4	20.5	18.9	07:11
07:10	19.8	21.3	23.0	25.1	126.5	25.1	22.6	20.6	19.0	08:00
7:11 / 8:00	20.0	21.5	23.2	25.3	127	25.3	22.7	20.8	19.2	08:01
08:01	20.2	21.6	23.4	25.7	127.5	25.5	23.1	21.0	19.3	08:02
08:02	20.3	21.8	23.6	25.9	128	25.9	23.3	21.1	19.5	08:03
08:03	20.5	22.0	23.8	26.1	128.5	26.1	23.4	21.5	19.6	08:04
08:04	20.6	22.3	24.1	26.3	129	26.3	23.6	21.6	20.0	08:05
08:05	21.0	22.5	24.3	26.7	129.5	26.7	24.0	21.8	20.1	08:06
08:06	21.1	22.6	24.5	26.9	130	26.9	24.2	22.0	20.3	08:07
08:07	21.3	22.8	24.7	27.1	130.5	27.1	24.4	22.1	20.4	08:08
08:08	21.5	23.0	24.9	27.3	131	27.5	24.5	22.5	20.6	08:09
08:09	21.6	23.2	25.2	27.7	131.5	27.7	24.9	22.7	20.9	08:10
8:10 / 8:11	21.8	23.5	25.4	27.9	132	28.1	25.1	22.8	21.1	08:11
09:00	22.1	23.7	25.6	28.1	132.5	28.3	25.3	23.0	21.2	09:00
09:01	22.3	23.9	25.8	28.5	133	28.5	25.6	23.3	21.4	09:01
09:02	22.5	24.1	26.2	28.7	133.5	28.9	25.8	23.5	21.6	09:02
09:03	22.6	24.2	26.4	28.9	134	29.1	26.0	23.7	21.9	09:03
9:04 / 9:05	22.8	24.6	26.6	29.3	134.5	29.5	26.4	23.9	22.1	09:04
09:06	23.1	24.8	27.0	29.5	135	29.7	26.6	24.2	22.2	09:05
09:07	23.3	25.0	27.2	29.9	135.5	29.9	26.8	24.4	22.4	09:06
09:08	23.5	25.2	27.4	30.1	136	30.3	27.2	24.6	22.8	09:07
09:09	23.7	25.5	27.6	30.4	136.5	30.6	27.4	25.0	22.9	09:08
09:10	23.8	25.7	28.0	30.8	137	31.0	27.6	25.2	23.1	09:09
9:11 / 10:00	24.2	25.9	28.2	31.0	137.5	31.2	28.0	25.3	23.3	09:10
10:01	24.4	26.3	28.6	31.4	138	31.6	28.2	25.5	23.6	09:11
10:02	24.6	26.5	28.8	31.7	138.5	31.8	28.4	25.9	23.8	10:00
10:03	24.7	26.7	29.0	32.1	139	32.3	28.8	26.1	24.0	10:01
10:04	25.1	26.9	29.2	32.3	139.5	32.5	29.0	26.3	24.1	10:02
10:05	25.3	27.2	29.6	32.5	140	32.9	29.4	26.7	24.5	10:03

Appendix 16b: Weight for height in adolescents

Weight adaptations between 140,0 (BMI charts WHO 2007) to 145,0 cm (NCHS 198

Reconstruction based on NCHS-CDC-WHO 1982 references

Adolescents from 10 to approx 18 years (140,0 - 165,0 cm)

MALE		Weight-for-Height (Standing up)						FEMALE
70%	80%	85%	Median	cm	Median	85%	80%	70%
25.1	27.2	29.6	32.5	140	32.9	29.4	26.7	24.5
25.2	27.4	29.7	33.0	140.5	33.2	29.6	26.8	24.7
25.2	27.6	29.9	33.4	141	33.6	29.8	27.0	24.9
25.3	28.0	30.0	33.8	141.5	33.8	30.2	27.4	25.0
25.4	28.2	30.2	34.1	142	34.1	30.4	27.6	25.2
25.4	28.4	30.3	34.7	142.5	34.8	30.7	27.8	25.6
25.5	28.8	30.5	35.0	143	35.6	31.1	28.0	25.7
25.6	29.0	30.6	35.3	143.5	36.0	31.3	28.4	25.8
25.6	29.1	30.8	35.7	144	36.4	31.4	28.6	25.9
25.7	29.2	30.9	36.1	144.5	36.9	31.5	29.1	26.0
25.8	29.2	31.0	36.5	145	37.1	31.5	29.7	26.0
25.8	29.5	31.3	36.8	145.5	37.4	31.8	29.9	26.2
26.0	29.7	31.6	37.2	146	37.8	32.1	30.2	26.5
26.3	30.0	31.9	37.6	146.5	38.1	32.4	30.5	26.7
26.5	30.3	32.2	37.9	147	38.4	32.6	30.7	26.9
26.8	30.6	32.5	38.3	147.5	38.8	33.0	31.0	27.2
27.0	30.9	32.8	38.6	148	39.1	33.2	31.3	27.4
27.3	31.2	33.1	39.0	148.5	39.5	33.6	31.6	27.7
27.5	31.5	33.4	39.3	149	39.8	33.8	31.8	27.9
27.8	31.7	33.7	39.7	149.5	40.1	34.1	32.1	28.1
28.0	32.0	34.0	40.0	150	40.5	34.4	32.4	28.4
28.3	32.3	34.3	40.4	150.5	40.8	34.7	32.6	28.6
28.5	32.6	34.7	40.8	151	41.2	35.0	33.0	28.8
28.8	32.9	34.9	41.1	151.5	41.5	35.3	33.2	29.1
29.1	33.2	35.3	41.5	152	41.9	35.6	33.5	29.3
29.3	33.5	35.6	41.9	152.5	42.3	36.0	33.8	29.6
29.6	33.8	35.9	42.3	153	42.6	36.2	34.1	29.8
29.8	34.1	36.2	42.6	153.5	43.0	36.6	34.4	30.1
30.1	34.4	36.6	43.0	154	43.4	36.9	34.7	30.4
30.4	34.7	36.9	43.4	154.5	43.8	37.2	35.0	30.7
30.7	35.0	37.2	43.8	155	44.2	37.6	35.4	30.9
30.9	35.4	37.6	44.2	155.5	44.6	37.9	35.7	31.2
31.2	35.7	37.9	44.6	156	45.1	38.3	36.1	31.6
31.5	36.0	38.3	45.0	156.5	45.5	38.7	36.4	31.9
31.8	36.3	38.6	45.4	157	46.0	39.1	36.8	32.2
32.1	36.7	38.9	45.8	157.5	46.5	39.5	37.2	32.6
32.4	37.0	39.3	46.2	158	47.0	40.0	37.6	32.9
32.7	37.3	39.6	46.6	158.5	47.6	40.5	38.1	33.3
33.0	37.7	40.0	47.1	159	48.2	41.0	38.6	33.7
33.3	38.0	40.4	47.5	159.5	48.9	41.6	39.1	34.2
33.6	38.4	40.8	48.0	160	49.7	42.2	39.8	34.8
33.9	38.7	41.1	48.4	160.5	50.5	42.9	40.4	35.4
34.2	39.1	41.5	48.8	161	51.6	43.9	41.3	36.1
34.5	39.4	41.9	49.3	161.5	52.8	44.9	42.2	37.0
34.8	39.8	42.3	49.8	162	54.5	46.3	43.6	37.8
35.1	40.2	42.7	50.2	162.5	56.1	47.7	44.9	38.3
35.5	40.5	43.1	50.7	163	56.4	47.9	45.1	38.8
35.8	40.9	43.5	51.1	163.5	56.7	48.2	45.4	39.0
36.1	41.3	43.9	51.6	164	57.0	48.4	45.6	39.5
36.5	41.7	44.3	52.1	164.5	57.3	48.7	45.8	39.8
36.8	42.1	44.7	52.6	165	57.6	48.9	46.0	40.0

Appendix 17: CRF Lab

Patient study code:

Respiratory Xpert MTB/RIF Ultra
Date of sample collection: / /
Type of procedure:
□ Spontaneous sputum
□ Naso-pharyngeal aspirate
□ Gastric aspiration
Date of Xpert MTB/RIF performed: / /
Result of Xpert MTB/RIF:
□ Negative
□ Positive RIF resistance negative
□ Positive RIF resistance positive
□ Indeterminate

Respiratory Xpert MTB/RIF Ultra (second sample if done)
Date of respiratory sample collection: / /
Type of procedure:
□ Spontaneous sputum
□ Naso-pharyngeal aspirate
□ Gastric aspiration
Date of Xpert MTB/RIF in respiratory sample performed: / /
Result of Xpert MTB/RIF:
□ Negative
□ Positive RIF resistance negative
□ Positive RIF resistance positive
□ Indeterminate

Validated extra pulmonary Xpert MTB/RIF Ultra
Date of sample collection: / /
Type of sample:
□ Lymph node
□ Pus or tissue Specify location:
□ Pleural effusion
□ CSF
□ Ascetic fluid
□ Other Specify:
Date of Xpert MTB/RIF in respiratory sample performed: / /
Result of Xpert MTB/RIF:
□ Negative
□ Positive RIF resistance negative
□ Positive RIF resistance positive
□ Indeterminate

Validated extra pulmonary Xpert MTB/RIF Ultra
Date of sample collection: / /
Type of sample:
□ Lymph node
□ Pus or tissue Specify location:

□ Pleural effusion
□ CSF
□ Ascetic fluid
□ Other Specify:

Date of Xpert MTB/RIF in respiratory sample performed: / /

Result of Xpert MTB/RIF:

□ Negative
□ Positive RIF resistance negative
□ Positive RIF resistance positive
□ Indeterminate

Stools Xpert MTB/RIF Ultra

Date of stools collection: / /

Date of Xpert MTB/RIF in stools performed: / /

Result of Xpert MTB/RIF:

□ Negative
□ Positive RIF resistance negative
□ Positive RIF resistance positive
□ Indeterminate

Stools Xpert MTB/RIF Ultra (second sample if done)

Date of stools collection: / /

Date of Xpert MTB/RIF in stools performed: / /

Result of Xpert MTB/RIF:

□ Negative
□ Positive RIF resistance negative
□ Positive RIF resistance positive
□ Indeterminate

Urine Xpert MTB/RIF Ultra

Date of urine collection: / /

Date of Xpert MTB/RIF in urine performed:

Result of Xpert MTB/RIF:

□ Negative
□ Positive RIF resistance negative
□ Positive RIF resistance positive
□ Indeterminate

Urine Xpert MTB/RIF Ultra (second sample if done)

Date of urine collection: / /

Date of Xpert MTB/RIF in urine performed: / /

Result of Xpert MTB/RIF:

□ Negative
□ Positive RIF resistance negative
□ Positive RIF resistance positive
□ Indeterminate

Appendix 18: Interpretation of Trace results in Xpert MTB/RIF Ultra