

Stool GeneXpert MTB/Rif Assay

Standard Operating Procedure

1.0. Purpose

The purpose of this standard operating procedure (SOP) is to detail the steps for correctly performing, interpreting, and documenting valid results from GeneXpert MTB/Rif using stool samples. GeneXpert MTB/Rif test (for use with the Cepheid GeneXpert systems) is a semi quantitative real time PCR in-vitro diagnostic test validated for the detection of *Mycobacterium tuberculosis* complex DNA in sputum samples and for the detection of rifampicin resistance associated mutations of the *rpoB* gene.

2.0. Scope

This procedure is not validated by Cepheid nor is it yet endorsed by World Health Organization (WHO). One of the main challenges to timely diagnosis of childhood Tuberculosis is the difficulty in obtaining sputum, thereby necessitating induction (which requires skill and infrastructure). In addition, sputum from children is often paucibacillary as children are less likely to form cavity lesions in the lungs to contain bacilli [1-4]. Children tend to swallow sputum when they cough and *M. tuberculosis* DNA has been shown to survive the harsh acidic and digestive environment of the gastro-intestinal tract [5]. Studies have shown that use of stool samples increases the yield and speed of TB diagnosis, especially among children [1-6].

3.0. Responsibility and Authorization

The persons responsible for performing this test are Laboratory Technologist and trained non-laboratory personnel.

4.0. Materials

4.1. GeneXpert DX System

- 4.1.1. GeneXpert Instrument, computer, barcode reader
- 4.1.2. GeneXpert MTB/Rif cartridge and sample reagent bottle

4.2. Materials required but not provided in the kit

- 4.2.1. Centrifuge
- 4.2.2. Gloves
- 4.2.3. Pipette (capable of dispensing 2mL)
- 4.2.4. Biohazard disposable bags

5.0. 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.

6.0. Principle

The GeneXpert 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.

8.1 Reagent storage and preparation

GeneXpert MTB/Rif cartridges must be stored at 2-28°C. 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).

8.2 Test Procedure

- 8.2.1 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
- 8.2.2 Add 5 ml of Phosphate Buffered Saline (PBS) and vortex to homogenize the mixture
- 8.2.3 Centrifuge the mixture at 3200x g for 15 min
- 8.2.4 Add 2 ml of the Xpert reagent into 1ml of the re-suspended pellet mixture and mix thoroughly.
- 8.2.5 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).

9.0 Interpretation of test results

- Interpret the results as you normally do with results from sputum samples processed in GeneXpert 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

8.3 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).

9 REFERENCES

- 9.1 Marais BJ. Childhood tuberculosis: epidemiology and natural history of disease. *Indian J Pediatr.* 2011;78:321–327.
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- 9.5 Cordova J, Shiloh R, Gilman R H, et al. Evaluation of molecular tools for detection and drug susceptibility testing of Mycobacterium tuberculosis in stool specimens from patients with pulmonary tuberculosis. *J Clin Microbiol* 2010; 48: 1820–1826.
- 9.6 Chipinduro M, Mateveke K, Makamure B, Ferrand RA, et al. Stool Xpert MTB/Rif test for the diagnosis of childhood pulmonary tuberculosis at primary clinics in Zimbabwe. *Int Journal of of Tubercu Lung.* 2017; 21 (2): 161-166