# Proficiency testing programme – guidelines and SOPs

## 8.1. Xpert MTB/RIF PT Preparation Readiness Assessment Checklist

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Section 1: Quality Management Systems** | | | | | | | | | | | | | | | | | | | | | | |
| This section of the checklist will determine if the laboratory has defined and documented policies, procedures and instructions necessary to assure the quality of an Xpert MTB/RIF DTS proficiency-testing program. This portion of the checklist can be completed in an office or conference room. **Request documentation necessary for accurately answering questions below and have it brought to the assessment room.** | | | | | | | | | | | | | | | | | | | | | | |
|  | |  | | | | | **Yes** | | | | | **No** | | | | | **Partial** | | | | **Comments** | |
| 1 | | Does the laboratory have a document control procedure to prevent the use of invalid or obsolete documents? | | | | |  | | | | |  | | | | |  | | | |  | |
| 2 | | Does the procedure ensure that: | | | | |  | | | | |  | | | | |  | | | |  | |
| 2a | | Documents are periodically reviewed and updated, as necessary; | | | | |  | | | | |  | | | | |  | | | |  | |
| 2b | | Invalid or obsolete documents are promptly removed from all points of issue or use; | | | | |  | | | | |  | | | | |  | | | |  | |
| 2c | | All documents are uniquely identifiable; | | | | |  | | | | |  | | | | |  | | | |  | |
| 2d | | Changes to documents are reviewed and approved. | | | | |  | | | | |  | | | | |  | | | |  | |
| 3 | | Does the laboratory have managerial and technical personnel with the necessary authority, resources and technical competence required to implement the Xpert MTB/RIF PT program? | | | | |  | | | | |  | | | | |  | | | |  | |
| 4 | | Does the laboratory provide adequate supervision of technical staff, including trainees, by persons familiar with procedures for each activity? | | | | |  | | | | |  | | | | |  | | | |  | |
| 5 | | Does the laboratory define the minimum levels of qualification and experience necessary for positions within its organization and ensure those qualifications are met? | | | | |  | | | | |  | | | | |  | | | |  | |
| 6 | | Are test results legible, technically verified by an authorized person, and confirmed against patient identity? | | | | |  | | | | |  | | | | |  | | | |  | |
| 7 | | Are there documented procedures to prevent the loss of test result data in the event of hardware/software failure, fire or theft? | | | | |  | | | | |  | | | | |  | | | |  | |
| 8 | | Does the laboratory maintain up-to-date records of the following: | | | | |  | | | | |  | | | | |  | | | |  | |
| 8a | | Competency test results? | | | | |  | | | | |  | | | | |  | | | |  | |
| 8b | | Educational and professional qualifications? | | | | |  | | | | |  | | | | |  | | | |  | |
| 8c | | Records of trainings? | | | | |  | | | | |  | | | | |  | | | |  | |
| 8d | | Skills and experience of all technical personnel? | | | | |  | | | | |  | | | | |  | | | |  | |
| 9 | | Does the laboratory have a policy and procedures for identifying relevant training needs and providing training of personnel? | | | | |  | | | | |  | | | | |  | | | |  | |
| 10 | | Does the laboratory monitor quality (performance) indicators (e.g. testing statistics)? | | | | |  | | | | |  | | | | |  | | | |  | |
| 11 | | Are the outcomes of quality indicators, PT, customer feedback and all other information derived from the tracking of quality indicators used to improve lab performance? | | | | |  | | | | |  | | | | |  | | | |  | |
| **Section 2: Proficiency Test Management** | | | | | | | | | | | | | | | | | | | | | | |
| This section covers questions related to procurement, supply chain management, storage, shipping, data management, data analysis, and corrective action follow-up. **Request documentation necessary for accurately answering questions below and have it brought to an office or conference room.** | | | | | | | | | | | | | | | | | | | | | | |
|  | | |  | | | **Yes** | | | | | **No** | | | | | **Partial** | | | | | **Comments** | |
| 1 | | | Are supply quantities correctly projected and procured to ensure no/limited stock outs? | | |  | | | | |  | | | | |  | | | | |  | |
| 2 | | | Are Xpert MTB/RIF supplies stored appropriately and is the temperature of the storage area monitored and documented? | | |  | | | | |  | | | | |  | | | | |  | |
| 3 | | | Do all storage areas comply with the safety requirements related to the supplies stored in them? | | |  | | | | |  | | | | |  | | | | |  | |
| 4 | | | Are all supplies stored according to the ﬁrst-in-ﬁrst-out (FIFO) and first expiry first out (FEFO) system? | | |  | | | | |  | | | | |  | | | | |  | |
| 5 | | | Does the laboratory have a documented procedure on maintenance of its inventory? | | |  | | | | |  | | | | |  | | | | |  | |
| 6 | | | Does the laboratory ensure that purchased supplies, equipment and consumable materials are not used until they have been inspected and verified to comply with specifications or requirements? (Perform new lot testing) | | |  | | | | |  | | | | |  | | | | |  | |
| 7 | | | Does the laboratory evaluate suppliers of critical supplies and services, and does it maintain records of these evaluations, and list those suppliers that are approved? | | |  | | | | |  | | | | |  | | | | |  | |
| 8 | | | Does the laboratory have the capacity to provide secure storage areas or stock rooms, or both, which prevent damage or deterioration of any proficiency test item between preparation and distribution? | | |  | | | | |  | | | | |  | | | | |  | |
| 9 | | | Does the laboratory participate in Xpert MTB/RIF test external proficiency testing? | | |  | | | | |  | | | | |  | | | | |  | |
| 10 | | | Does the laboratory have access to well characterized mycobacterial strains (e.g., ATCC / culture EQA programme strains)? | | |  | | | | |  | | | | |  | | | | |  | |
| 11 | | | Does the laboratory have biological specimen storage policies documented? | | |  | | | | |  | | | | |  | | | | |  | |
| 12 | | | Are necessary staff trained on biological specimen storage policies and is training documented? | | |  | | | | |  | | | | |  | | | | |  | |
| 13 | | | Are specimens or cultures promptly stored at -70 to -80 °C to prevent death and promote good recovery of organism? | | |  | | | | |  | | | | |  | | | | |  | |
| 14 | | | Does the laboratory currently manufacture, distribute, analyse and report proficiency test items for other tests (e.g., smear microscopy / HIV)? | | |  | | | | |  | | | | |  | | | | |  | |
| 15 | | | Does the laboratory have the capacity to ensure that the environmental conditions (biological sterility, dust, humidity, electrical supply and temperature) do not compromise the PT scheme or the required quality of operations? | | |  | | | | |  | | | | |  | | | | |  | |
| 16 | | | Does the laboratory have access to analytical software and such as Microsoft Office (Excel and Word) to create labels, analyse data and generate reports? | | |  | | | | |  | | | | |  | | | | |  | |
| 17 | | | Does the laboratory have sufficient managerial buy-in to support the Xpert MTB/RIF proficiency-testing program? | | |  | | | | |  | | | | |  | | | | |  | |
| 18 | | | In respect of the proficiency testing scheme; does the laboratory have the technical capacity to: | | |  | | | | |  | | | | |  | | | | |  | |
| 18a | | | Manufacturer and produce quality DTS PT panels? | | |  | | | | |  | | | | |  | | | | |  | |
| 18b | | | Validate PT panels to ensure accuracy and precision? | | |  | | | | |  | | | | |  | | | | |  | |
| 18c | | | Label, pack, and distribute proficiency test panels? | | |  | | | | |  | | | | |  | | | | |  | |
| 18d | | | Collect and compile results and analyse results to determine aggregate results? | | |  | | | | |  | | | | |  | | | | |  | |
| 18e | | | Produce, verify and distribute timely PT reports? | | |  | | | | |  | | | | |  | | | | |  | |
| 18f | | | Follow up with testing sites performing unsatisfactory on PT to investigate and implement corrective actions? | | |  | | | | |  | | | | |  | | | | |  | |
| 19 | | | Is capacity available for appropriately-authorized personnel to validate and interpret PT results before release to participating sites? | | |  | | | | |  | | | | |  | | | | |  | |
| 20 | | | Does the laboratory have the capacity to inform participants in the PT scheme of nonconforming dispatched items, and does it have the capacity to recall items or reports already sent to participants? | | |  | | | | |  | | | | |  | | | | |  | |
| 21 | | | When a participating site scores an unsatisfactory result, does the procedure for corrective action start with an investigation to determine the root cause(s) of the problem? | | |  | | | | |  | | | | |  | | | | |  | |
| 22 | | | Where corrective action is needed, does the laboratory identify potential actions that are most likely to eliminate the problem and to prevent recurrence? | | |  | | | | |  | | | | |  | | | | |  | |
| 23 | | | Are corrective actions appropriate to the magnitude and risk of the problem? | | |  | | | | |  | | | | |  | | | | |  | |
| 24 | | | Are any required changes resulting from corrective action investigations documented and implemented? | | |  | | | | |  | | | | |  | | | | |  | |
| 25 | | | Are the results monitored to ensure that corrective actions have been effective? | | |  | | | | |  | | | | |  | | | | |  | |
| **Section 3: Biosafety** | | | | | | | | | | | | | | | | | | | | | | |
| This section of the checklist with aid in determining if the facility and safety procedures employed are adequate to conduct DTS preparation for Xpert MTB/RIF while ensuring risk to laboratory personnel is low. | | | | | | | | | | | | | | | | | | | | | | |
| **Conduct the first part of this assessment in the conference room or office, move to the laboratory space when indicated below.** | | | | | | | | | | | | | | | | | | | | | | |
|  | |  | | **Yes** | | | | | | **No** | | | | | **Partial** | | | | | **Comments** | | |
| 1 | | Does the laboratory have a policy in place for conducting Risk Assessments? | |  | | | | | |  | | | | |  | | | | |  | | |
| 2 | | Are policies in place for the safe handling of sharps? | |  | | | | | |  | | | | |  | | | | |  | | |
| 3 | | Is there a chemical storage and handling policy? | |  | | | | | |  | | | | |  | | | | |  | | |
| 4 | | Does the laboratory management provide regular biosafety training? | |  | | | | | |  | | | | |  | | | | |  | | |
| 5 | | Is biosafety training provided to axillary staff, such as cleaners and maintenance staff if they have access to BSL3 laboratory? | |  | | | | | |  | | | | |  | | | | |  | | |
| 6 | | Is there an occupational health program? | |  | | | | | |  | | | | |  | | | | |  | | |
| 7 | | Are policies in place for use of PPE within the Laboratory? | |  | | | | | |  | | | | |  | | | | |  | | |
| **Move to the laboratory to observe work in specimen processing, culture work up, DST, and molecular testing areas.** | | | | | | | | | | | | | | | | | | | | | | |
| 8 | | Are all PPE stored appropriately when not in use? | |  | | | | | |  | | | | |  | | | | |  | | |
| 9 | | Is there a visual monitoring device so that staff can ensure proper directional airflow? | |  | | | | | |  | | | | |  | | | | |  | | |
| 10 | | Is the laboratory separated form areas that are open to unrestricted foot traffic? | |  | | | | | |  | | | | |  | | | | |  | | |
| 11 | | Is there a controlled ventilation system that maintains directional airflow within the laboratory? | |  | | | | | |  | | | | |  | | | | |  | | |
| 12 | | Is there an autoclave in the laboratory? | |  | | | | | |  | | | | |  | | | | |  | | |
| 13 | | Are spore strips or other comparable method used to verify autoclave function at least monthly? | |  | | | | | |  | | | | |  | | | | |  | | |
| 14 | | Are procedures involving infectious materials conducted within a BSC? | |  | | | | | |  | | | | |  | | | | |  | | |
| 15 | | Are N-95 respirators worn when manipulating culture (liquid or inoculating suspensions) due to the high concentration of organisms in easily aerosolized liquid? | |  | | | | | |  | | | | |  | | | | |  | | |
| 16 | | Are procedures and practices performed in such a way that minimizes the formation of aerosols? | |  | | | | | |  | | | | |  | | | | |  | | |
| 17 | | Is a hand washing policy in place and are handwashing stations available near laboratory exits and stocked with needed supplies? | |  | | | | | |  | | | | |  | | | | |  | | |
| **Section 4: Equipment for Xpert DTS** | | | | | | | | | | | | | | | | | | | | | | |
| This section will determine if the necessary equipment and supplies are on site, calibrated and properly maintained, and in appropriate condition. **This portion will need to be completed in the laboratory.** | | | | | | | | | | | | | | | | | | | | | | |
|  | | |  | | | | | **Yes** | | | | | **No** | | | | | **Partial** | | | | **Comments** |
| 1 | | | Does the laboratory contain the following essential equipment necessary for preparation of DTS for Xpert MTB/RIF: | | | | |  | | | | |  | | | | |  | | | |  |
| 2 | | | BACTEC MGIT 960/320 instrument | | | | |  | | | | |  | | | | |  | | | |  |
| 3 | | | Safety centrifuge | | | | |  | | | | |  | | | | |  | | | |  |
| 4 | | | Biological Safety cabinet (BSC), Class II | | | | |  | | | | |  | | | | |  | | | |  |
| 5 | | | Vortex | | | | |  | | | | |  | | | | |  | | | |  |
| 6 | | | GeneXpert Dx System equipped with 6-color modules and GX2.1 software or higher, including: | | | | |  | | | | |  | | | | |  | | | |  |
| 6a | | | GeneXpert instrument | | | | |  | | | | |  | | | | |  | | | |  |
| 6b | | | Computer | | | | |  | | | | |  | | | | |  | | | |  |
| 6c | | | Barcode wand reader | | | | |  | | | | |  | | | | |  | | | |  |
| 6d | | | Printer | | | | |  | | | | |  | | | | |  | | | |  |
| 6e | | | UPS back-up battery (sufficient to finish a run when power goes down) | | | | |  | | | | |  | | | | |  | | | |  |
| 7 | | | Refrigerators for reagents and isolate storage set to 2-8°C | | | | |  | | | | |  | | | | |  | | | |  |
| 8 | | | Freezer, -80C° | | | | |  | | | | |  | | | | |  | | | |  |
| 9 | | | Axillary incubator set for 35-37°C | | | | |  | | | | |  | | | | |  | | | |  |
| 10 | | | Automatic pipettes (p20, p200, p1000) | | | | |  | | | | |  | | | | |  | | | |  |
| 11 | | | Repeat pipettors (recommended) | | | | |  | | | | |  | | | | |  | | | |  |
| 12 | | | Hot air oven 80-85°C | | | | |  | | | | |  | | | | |  | | | |  |
| 13 | | | Is annual calibration/certification service information readily available for the: | | | | |  | | | | |  | | | | |  | | | |  |
| 13a | | | GeneXpert system | | | | |  | | | | |  | | | | |  | | | |  |
| 13b | | | Safety centrifuge | | | | |  | | | | |  | | | | |  | | | |  |
| 13c | | | BSC | | | | |  | | | | |  | | | | |  | | | |  |
| 13d | | | Pipettes | | | | |  | | | | |  | | | | |  | | | |  |
| 14 | | | Please list the most recent calibration/certification date for each instrument listed in the comments section here: | | | | |  | | | | |  | | | | |  | | | | GeneXpert: \_\_\_\_\_\_\_  Centrifuge: \_\_\_\_\_\_\_  BSC: \_\_\_\_\_\_\_\_\_\_\_\_  Pipettes: \_\_\_\_\_\_\_\_\_ MGIT: \_\_\_\_\_\_\_\_\_\_\_ |
| 15 | | | Is any of the following equipment under an annual service contract? | | | | |  | | | | |  | | | | |  | | | |  |
| 15a | | | GeneXpert system | | | | |  | | | | |  | | | | |  | | | |  |
| 15b | | | BACTEC MGIT 960 | | | | |  | | | | |  | | | | |  | | | |  |
| 15d | | | BSC | | | | |  | | | | |  | | | | |  | | | |  |
| 16 | | | Has daily, monthly, and annual maintenance and/or certification been routinely performed and documented | | | | |  | | | | |  | | | | |  | | | |  |
| 16a | | | GeneXpert system | | | | |  | | | | |  | | | | |  | | | |  |
| 16c | | | BACTEC MGIT 960 | | | | |  | | | | |  | | | | |  | | | |  |
| 16d | | | BSC | | | | |  | | | | |  | | | | |  | | | |  |
| 16e | | | Incubators | | | | |  | | | | |  | | | | |  | | | |  |
| 16f | | | Thermometers | | | | |  | | | | |  | | | | |  | | | |  |
| 16g | | | Refrigerator | | | | |  | | | | |  | | | | |  | | | |  |
| 16h | | | Automatic and repeat pipettors | | | | |  | | | | |  | | | | |  | | | |  |
| 17 | | | Is all equipment mentioned above validated in accordance with the documented procedures before being placed in service? | | | | |  | | | | |  | | | | |  | | | |  |
| 18 | | | Does the GeneXpert computer system maintenance include a back-up process with routine archiving of testing data and a system recovery plan? | | | | |  | | | | |  | | | | |  | | | |  |
| 19 | | | Is a dedicated BSC available for DTS preparation and can it be left running through the drying process? | | | | |  | | | | |  | | | | |  | | | |  |
| 20 | | | Is all the required equipment maintained, serviced, and in good working condition? | | | | |  | | | | |  | | | | |  | | | |  |
| 21 | | | Are other axillary equipment listed below readily available? | | | | |  | | | | |  | | | | |  | | | |  |
| 21a | | | Pipette aid | | | | |  | | | | |  | | | | |  | | | |  |
| 21b | | | Microcentrifuge/cryovial tube racks | | | | |  | | | | |  | | | | |  | | | |  |
| 21c | | | Racks for 50 ml conical tubes | | | | |  | | | | |  | | | | |  | | | |  |
| 21d | | | Rack for 16 mm glass tubes | | | | |  | | | | |  | | | | |  | | | |  |
| 21e | | | Trolley cart | | | | |  | | | | |  | | | | |  | | | |  |
| 21f | | | Timer | | | | |  | | | | |  | | | | |  | | | |  |
| **Section 5: Supplies for Xpert DTS** | | | | | | | | | | | | | | | | | | | | | | |
| This section will determine if necessary supplies are on site and of appropriate specifications and procurement needs before DTS training begins. **This portion will need to be completed in the laboratory or the store room.** | | | | | | | | | | | | | | | | | | | | | | |
|  |  | | | | **Yes** | | | | **No** | | | | | **Partial** | | | | | **Comments** | | | |
| 1 | 4 ml cryovials, sterile, skirted, screw cap with O-ring or other appropriate tube for DTS | | | |  | | | |  | | | | |  | | | | |  | | | |
| 2 | 2 ml cryovials, sterile, skirted, screw cap with O-ring, appropriate for storage at -70-80°C | | | |  | | | |  | | | | |  | | | | |  | | | |
| 3 | 1 ml graduated sterile plastic transfer pipettes | | | |  | | | |  | | | | |  | | | | |  | | | |
| 4 | 3 ml graduated sterile plastic transfer pipettes | | | |  | | | |  | | | | |  | | | | |  | | | |
| 5 | Large, plastic, sealable, specimen transport bags | | | |  | | | |  | | | | |  | | | | |  | | | |
| 6 | 3 mm glass beads | | | |  | | | |  | | | | |  | | | | |  | | | |
| 7 | 16 x 100 mm glass tubes with screw cap, sterile | | | |  | | | |  | | | | |  | | | | |  | | | |
| 8 | 50 ml conical tubes | | | |  | | | |  | | | | |  | | | | |  | | | |
| 9 | Sterile, disposable 10 μl loops | | | |  | | | |  | | | | |  | | | | |  | | | |
| 10 | Pipette tips for repeat pipettor (Eppendorf Biopur Combitips 5 ml) | | | |  | | | |  | | | | |  | | | | |  | | | |
| 11 | Pipette tips: p20, p200, p1000 | | | |  | | | |  | | | | |  | | | | |  | | | |
| 12 | 10 ml and 25 ml serological pipettes | | | |  | | | |  | | | | |  | | | | |  | | | |
| 13 | Tuberculocidal disinfectants, prepared according to specifications | | | |  | | | |  | | | | |  | | | | |  | | | |
| 14 | Paper towel or cotton wool | | | |  | | | |  | | | | |  | | | | |  | | | |
| 15 | Beakers for preparing disinfectant | | | |  | | | |  | | | | |  | | | | |  | | | |
| 16 | Absorbent bench liners | | | |  | | | |  | | | | |  | | | | |  | | | |
| 17 | Food grade dye | | | |  | | | |  | | | | |  | | | | |  | | | |
| 18 | Verify answers to questions 2, 3, and 4 in Section 2 | | | |  | | | |  | | | | |  | | | | |  | | | |
| 19 | In Summary, the above supplies are needed to prepare DTS. Are all currently supplies available in the laboratory? If not are they reasonable to procure? | | | |  | | | |  | | | | |  | | | | |  | | | |

**2018 – A**

**Kit instructions**

## 

## 8.2. Xpert® MTB/RIF Proficiency Testing

**KIT CONTENTS**

1. Check kit contents upon receipt.

* 1 plastic bag containing 5 labeled Dried Tube Specimen (DTS) sample tubes
* 5 individually wrapped transfer pipettes
* 1 **Xpert® MTB/RIF Result Form**

1. Inspect each of the 5 sample tubes to ensure a small button of dried blue material is present at the bottom of each tube. Note the absence of the blue material from any of the sample tubes in the comments section of the **Results Form** but test the sample as instructed.
2. For replacement materials (DTS samples), notify within 10 calendar days of the receipt date. Please contact:

**STORAGE AND STABILITY INFORMATION**

1. Specimens are intentionally shipped ambient.
2. Upon receipt, store specimens in the dark at 2-8°C until testing.
3. Only rehydrate the number of DTS samples that can be tested immediately.
4. Time between DTS sample rehydration and testing should not exceed 30 minutes.

**DETAILED TESTING INSTRUCTIONS**

**Caution:** **These samples contain nonviable strains of mycobacteria. They however should be treated as infectious material.** These test samples should only be opened in a laboratory where the Xpert® MTB/RIF assay is performed, using the same precautions and safety measures employed when testing patient specimens.

1. Place DTS sample tubes in a rack.
2. Remove cap of DTS sample tube. Using the pipette provided in the PT sample kit, aspirate 2.5 ml of sample reagent (SR) from the Xpert® MTB/RIF assay kit. Add SR into the DTS tube to rehydrate the sample, then recap the tube.

**Note**: When processing more than one DTS sample at a time, open only one sample tube, add 2.5 ml SR then recap tube before moving to the next sample. Use separate pipette for each sample. Use one bottle of SR per sample.

1. Tighten cap and shake each tube vigorously 10 -20 times.
2. Incubate tubes for 10 minutes at room temperature.
3. After the 10-minute incubation period, shake each sample vigorously 10-20 times.
4. Incubate tubes for an additional 5 minutes at room temperature.
5. Open one Xpert® MTB/RIF assay cartridge lid, and then open the DTS sample tube. Using the transfer pipette provided with the Xpert® MTB/RIF assay kit, aspirate the rehydrated DTS sample up to the 2ml mark. Transfer the sample into the sample chamber of the cartridge. Close the cartridge lid firmly.

**Note**: When processing more than one sample at a time, open only one cartridge, add the rehydrated sample and close the cartridge before moving to the next sample. Use separate pipette for each sample.

1. Load the cartridges into the GeneXpert instrument immediately.

**REPORTING YOUR RESULTS**

1. Complete the **Xpert® MTB/RIF PT Result Form** included in the kit. Completely fill the circles indicating your results: MTB, RIF Resistance, Uninterpretable, or Exception (Unable to test or analyse the sample).
2. If a test results in an error, document the error code on the space provided on the Result Form.
3. If you are unable to test a sample, record the Exception Code (E1, E2, etc.) on the space provided on the **Result Form**. The list of Exception Codes is on the **PT Result Form**.

**SUBMITTING RESULTS**

1. Submit the completed **PT Result Form** to your country’s Xpert proficiency testing coordinator at the email address on the bottom of the Result Form or submit results electronically (if available).
2. **A** **PT Participants Evaluation and Results Summary** will be sent to you later via your Xpert PT Country Coordinator or electronically (if available).

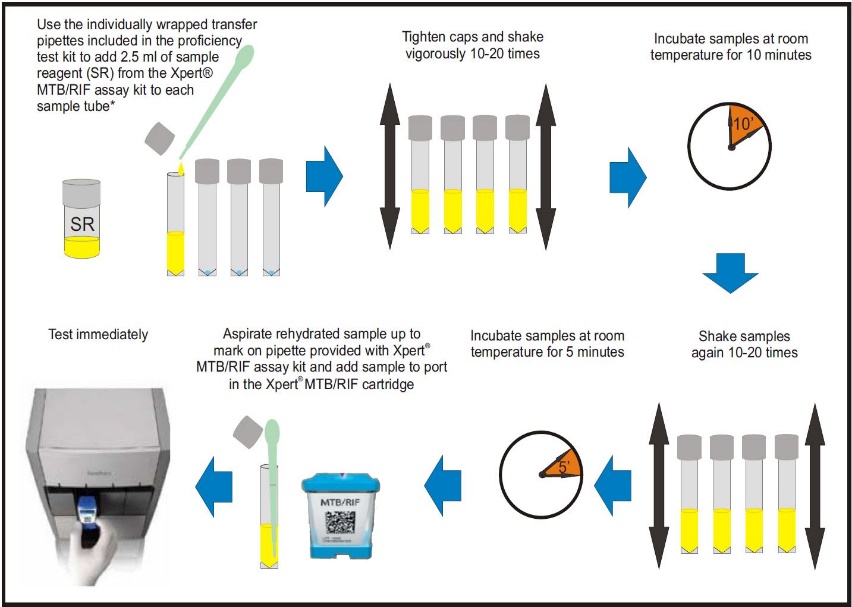
## 

## 8.3. Proficiency Testing Job Aid

Testing Xpert® MTB/RIF Dried Tube Specimen (DTS) Proficiency Testing Samples

Caution: Xpert MTB/RIF DTS samples contain nonviable strains of mycobacteria. They should be treated as infectious material. The DTS samples should only be opened in a laboratory where the Xpert MTB/RIF assay is performed, using the same precautions and safety measures employed when testing clinical samples.

* Performance evaluation testing should be incorporated into the flow of clinical testing
* Only re-hydrate the number of samples that can be tested immediately. Typically, four samples for a 4-module instrument, as shown in the diagram below
* Samples should not be shaken vigorously, as this may introduce bubbles which will interfere with the Xpert MTB/RIF assay
* Time between sample rehydration and testing should not exceed 30 minutes.
* Store samples at 2-8°C in the dark until testing



\*Avoid cross-contamination by using a separate transfer pipette for each sample, one bottle of sample reagent per sample and only having one sample open at a time

## 8.4. Xpert® MTB/RIF Proficiency Testing Result Form

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Name of Site:** | | | | | | | | **PT Panel ID: 2018-A** | | | | **Due Date:** \_\_\_\_\_\_\_\_\_\_ | | | | |
| **Site PT-ID Number:** | | | | | | | | **Date received:**\_\_\_\_\_\_\_\_\_\_ | | | | **Date results reported:** \_\_\_\_\_\_\_\_\_\_ | | | | |
| **Instructions:** Fill in the circles below corresponding to the results from testing each sample provided. Document the error code for tests resulting in error. Record the cycle thresholds (Ct) in the cells provided for all successful tests. \*Results cannot be accepted if received after the due date. | | | | | | | | | | | | | | | | |
| **Sample ID** | **Result** | | | | | | | | | **Cycle Threshold (Ct) Value** | | | | | | |
| **MTB** | | **RIF Resistance** | | **Uninterpretable** | | **Exception** | | | Probe D | Probe C | | Probe E | Probe B | SPC | Probe A |
| 2018 -A-1 | O | Detected, Very Low | O | Detected | O | Invalid | O | | Unable to Test |  |  | |  |  |  |  |
|  | O | Detected, Low | O | Not Detected | O | No Result | Exception Code: \_\_\_\_\_\_\_\_\_\_\_\_ | | | Date tested: \_\_\_\_\_\_\_\_\_\_\_ | | | | | | |
|  | O | Detected, Medium | O | Not Applicable | O | Error  Code: \_\_\_\_\_\_\_ |  | | | Tested by: \_\_\_\_\_\_\_\_\_\_\_ | | | | | | |
|  | O | Detected, High | O | Indeterminate |  | | | GeneXpert module number: \_\_\_\_\_\_\_\_\_\_\_ | | | | | | |
|  | O | Not Detected |  |  |  |  |  | | |  | | | | | | |
|  | | | | | | | | | | | | | | | | |
| 2018 -A-2 | O | Detected, Very Low | O | Detected | O | Invalid | O | | Unable to Test |  |  | |  |  |  |  |
|  | O | Detected, Low | O | Not Detected | O | No Result |  | |  | Date tested: \_\_\_\_\_\_\_\_\_\_\_ | | | | | | |
|  | O | Detected, Medium | O | Not Applicable | O | Error  Code: \_\_\_\_\_\_\_ | Exception Code: \_\_\_\_\_\_\_\_\_\_\_\_ | | | Tested by: \_\_\_\_\_\_\_\_\_\_\_ | | | | | | |
|  | O | Detected, High | O | Indeterminate |  | | | GeneXpert module number: \_\_\_\_\_\_\_\_\_\_\_ | | | | | | |
|  | O | Not Detected |  |  |  |  |  | | |  | | | | | | |
|  | | | | | | | | | | | | | | | | |
| 2018 -A-3 | O | Detected, Very Low | O | Detected | O | Invalid | O | | Unable to Test |  |  | |  |  |  |  |
|  | O | Detected, Low | O | Not Detected | O | No Result |  | |  | Date tested: \_\_\_\_\_\_\_\_\_\_\_ | | | | | | |
|  | O | Detected, Medium | O | Not Applicable | O | Error  Code: \_\_\_\_\_\_\_\_ | Exception Code: \_\_\_\_\_\_\_\_\_\_\_\_ | | | Tested by: \_\_\_\_\_\_\_\_\_\_\_ | | | | | | |
|  | O | Detected, High | O | Indeterminate |  | | | GeneXpert module number: \_\_\_\_\_\_\_\_\_\_\_ | | | | | | |
|  | O | Not Detected |  |  |  |  |  | | |  | | | | | | |
|  | | | | | | | | | | | | | | | | |
| 2018 -A-4 | O | Detected, Very Low | O | Detected | O | Invalid | O | | Unable to Test |  |  | |  |  |  |  |
|  | O | Detected, Low | O | Not Detected | O | No Result |  | |  | Date tested: \_\_\_\_\_\_\_\_\_\_\_ | | | | | | |
|  | O | Detected, Medium | O | Not Applicable | O | Error  Code: \_\_\_\_\_\_\_\_ | Exception Code: \_\_\_\_\_\_\_\_\_\_\_\_ | | | Tested by: \_\_\_\_\_\_\_\_\_\_\_ | | | | | | |
|  | O | Detected, High | O | Indeterminate |  | | | GeneXpert module number: \_\_\_\_\_\_\_\_\_\_\_ | | | | | | |
|  | O | Not Detected |  |  |  |  |  | | |  | | | | | | |
|  | | | | | | | | | | | | | | | | |
| 2018 -A-5 | O | Detected, Very Low | O | Detected | O | Invalid | O | | Unable to Test |  |  | |  |  |  |  |
|  | O | Detected, Low | O | Not Detected | O | No Result |  | |  | Date tested: \_\_\_\_\_\_\_\_\_\_\_ | | | | | | |
|  | O | Detected, Medium | O | Not Applicable | O | Error | Exception Code: \_\_\_\_\_\_\_\_\_\_\_\_ | | | Tested by: \_\_\_\_\_\_\_\_\_\_\_ | | | | | | |
|  | O | Detected, High | O | Indeterminate |  | Code: \_\_\_\_\_\_\_\_ |  | | | GeneXpert module number: \_\_\_\_\_\_\_\_\_\_\_ | | | | | | |
|  | O | Not Detected |  |  |  |  |  | | |  | | | | | | |

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**Xpert® MTB/RIF Proficiency Testing Result Form**

|  |  |  |
| --- | --- | --- |
|  | | |
| **Name of Site:** | **Site PT-ID Number:** | **PT Panel ID: 2018-A** |

|  |
| --- |
| Xpert MTB/RIF cartridge or pouch lot no.: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |
| Xpert MTB/RIF cartridge expiry date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |
| Date of last calibration of the GeneXpert instrument: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |
| **Exception codes:** E1: Power failure; E2: No reagents available; E3: Instrument not working; E4: GeneXpert computer not working; E5: Lack of testing personnel; E6: PT sample missing; E7: Others, (explain in the Comments section) |
| **Comments:** |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Attestation statement**: We, the undersigned, recognizing that some special handling may be required due to the nature of PT materials, have as closely as is practical, performed the analyses on these specimens in the same manner as regular patient specimens. We confirm that results were not shared or PT specimens referred or tested outside our testing site. | | | | | | |
| **Testing Site supervisor:** | | |  | | | |
| Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | Signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |  |  |
|  |  | | | | | |
| **Testing personnel:** | |  | | | | |
| Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | Signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |  |  |
| Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | Signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |  |  |
| Email results to: | | | | | | |

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## 8.5. Proficiency testing evaluation report

|  |  |  |
| --- | --- | --- |
| **Country** | **Testing Site** | **PT-ID Number** |
|  |  |  |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Summary of All Reporting Sites** | | | | | |
|  | **2018-A-1** | **2018-A-2** | **2018-A-3** | **2018-A-4** | **2018-A-5** |
| Total number of reporting sites |  |  |  |  |  |
| **TB Detection** |  |  |  |  |  |
| Sites detecting TB (%) |  |  |  |  |  |
| Sites not detecting TB (%) |  |  |  |  |  |
| Sites reporting uninterpretable TB result\*(%) |  |  |  |  |  |
| Sites not reporting TB detection result (%) |  |  |  |  |  |
| **RIF Detection** |  |  |  |  |  |
| Sites detecting Rif resistance (%) |  |  |  |  |  |
| Sites not detecting Rif resistance (%) |  |  |  |  |  |
| Sites reporting indeterminate Rif result (%) |  |  |  |  |  |
| Sites reporting uninterpretable Rif result\*(%) |  |  |  |  |  |
| Sites not reporting Rif detection result (%) |  |  |  |  |  |
| \* Uninterpretable result = invalid, error, or no result   |  |  |  |  | | --- | --- | --- | --- | | **Site Results** | | | | |  | **MTB Detected** | **Rif Resistance** | **Score** | | **Sample ID: 2018-A-1** |  |  |  | | Expected Results |  |  |  | | All Participating Sites’ Consensus Results |  |  |  | | **Testing Site’s Result** |  |  |  | | **Sample ID: 2018-A-2** |  |  |  | | Expected Results |  |  |  | | All Participating Sites’ Consensus Results |  |  |  | | **Testing Site’s Result** |  |  |  | | **Sample ID: 2018-A-3** |  |  |  | | Expected Results |  |  |  | | All Participating Sites’ Consensus Results |  |  |  | | **Testing Site’s Result** |  |  |  | | **Sample ID: 2018-A-4** |  |  |  | | Expected Results |  |  |  | | All Participating Sites’ Consensus Results |  |  |  | | **Testing Site’s Result** |  |  |  | | **Sample ID: 2018-A-5** |  |  |  | | Expected Results |  |  |  | | All Participating Sites’ Consensus Results |  |  |  | | **Testing Site’s Result** |  |  |  | |  | **Percentage** | **Satisfactory/Unsatisfactory** |  | | **FINAL SCORE for Testing Site** |  |  |  | | | | | | | |

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**Proficiency testing evaluation report**

|  |
| --- |
| **Comments** |

|  |  |
| --- | --- |
| **2018 Panel A** | |
| Name of Testing Site: | *PT provider will provide this information on this report* |
| Testing Site PT ID Number: | *PT provider will provide this information on this report* |
| Attention: | *(Testing site supervisor or Lab Director) PT provider will provide this information on this report* |
| Report Reviewed By (Name): |  |
| Report Reviewed By (Signature): |  |
| Review Date: |  |
| Reviewed with the staff on (date): |  |

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## 8.6. DTS Preparation Standard Operating Procedure and Worksheets

**STANDARD OPERATING PROCEDURE**

1. **TITLE**

Preparation of Dried Tube Specimens (DTS) for Xpert MTB/RIF Proficiency Testing

1. **PURPOSE**

Proficiency testing programs are used to monitor and improve the quality of laboratory testing. Dried tube specimens serve as a simple and practical sample type for Xpert MTB/RIF proficiency-testing. DTS can also be used for quality control of Xpert MTB/RIF reagents and verification of GeneXpert instruments used for Xpert MTB/RIF testing.

The DTS preparation process requires a (1) a functional tuberculosis (TB) laboratory facility with infrastructure and equipment that meet international TB containment laboratory safety standards and (2) competent TB laboratory staff trained on successful completion of specimen processing and Xpert MTB/RIF testing procedures. Laboratories planning to produce DTS must first conduct a DTS production readiness assessment to ensure that quality specimens can be produced in a safe work environment.

1. **SCOPE**

This procedure details the production and validation of DTS proficiency testing panels for use with the Xpert MTB/RIF assay.

1. **RESPONSIBILITIES**
2. **TB laboratory Staff**
3. Complies with instructions outlined in this SOP.
4. Manages freezer isolates and inactivated DTS stock for DTS panels for Xpert MTB/RIF testing.
5. Documents and reviews DTS preparation, pre-testing, and validation results.
6. Notifies supervisor or designee of inactivation verification and validation failures or other issues.
7. Complies with remedial or corrective action as necessary to ensure quality of DTS preparation and validation.
8. **TB** **Laboratory Supervisor**
9. Ensures TB laboratory staff involved are trained and competent in performing this and the Xpert MTB/RIF testing procedures.
10. Confirms all TB laboratory staff are current on safety and security requirements for working under BSL3 conditions.
11. Oversees DTS preparation and validation.
12. Reviews DTS inactivation verification and validation results.
13. Reviews DTS preparation and validation documentation.
14. Provides feedback to laboratory staff on DTS preparation and validation testing.
15. Assigns a designee for supervisory responsibilities during absence.
16. **Laboratory Director**
17. Reviews and approves SOP for DTS preparation for Xpert MTB/RIF testing and related documents.
18. **Laboratory QA Manager**
19. Ensures that the SOP for DTS preparation for Xpert MTB/RIF testing and related documents are updated, reviewed and available to the end user.
20. **REAGENTS AND MEDIA**
21. Xpert MTB/RIF assay kit (Cepheid)
22. Sterile phosphate buffer pH 6.8 (refer to SOP for preparation procedure)
23. Sterile saline (refer to SOP for preparation procedure)
24. Food coloring
25. Bactec MGIT 7 ml tubes (BD #245116)
26. Bactec MGIT 960 supplement (BD #245116)
27. Middlebrook 7H11 agar plates (7H11)
28. Middlebrook 7H9 broth (7H9)
29. Glycerol
30. **EQUIPMENT**
31. GeneXpert Dx System
32. GeneXpert instrument
33. Computer
34. Barcode wand reader
35. Printer
36. Class II Biological Safety Cabinet (BSC)
37. Hot oven set at 80-85oC, with calibrated external thermometer
38. Incubator set at 35-37oC with calibrated external thermometer
39. BD MGIT 960 instrument with UPS
40. Barcode label printer
41. Vortex mixer
42. Trolley cart
43. Timers
44. Autoclavable metal discard pan with cover or any solid waste discard container
45. Plastic biotransport container
46. Microcentrifuge tube racks
47. Graduated beakers
48. Rack for 16 mm tubes
49. Plastic rack for 50 ml conical tubes
50. Plastic rack for 2-ml cryovials
51. Plastic cryoboxes for 2-ml cryovials
52. Metal can for petri dishes (with cover)
53. Repeat pipettor
54. Automatic pipette, P1000
55. Automatic pipette, P200
56. Automatic pipette, P20
57. Automatic pipette, P10
58. Pipette aid
59. **SUPPLIES**
60. N95 respirators
61. 4-ml cryovials with external thread caps
62. 2-ml Nalgene cryovials with external thread caps
63. 2-ml microcentrifuge tubes, sterile, skirted, screw-cap with O-ring
64. Barcode labels
65. Cryo-Babies labels 1.5
66. 3-ml graduated sterile transfer pipettes
67. Extended length filtered 100-200 µl pipette tips
68. Bench liners
69. Clorox: Prepare working solution daily (final concentration of the active ingredient, sodium hypochlorite or chlorine, is no less than 0.5%).
70. 70% ethanol
71. Biohazard discard bags
72. Large plastic specimens transport bags, sealable
73. 3 mm glass beads, sterile
74. 16 x 100 mm glass tubes with screw-cap, sterile
75. 50-ml plastic conical tubes, sterile
76. Disposable 10 µl loop, sterile
77. Heat-shrink plate seals or air-permeable tape
78. Pipette tips, 20 µl, sterile and filtered
79. Pipette tips, 200 µl, sterile and filtered
80. Pipette tips, 1000 µl, sterile and filtered
81. Repeating pipettor tips, 5ml or 10 ml, sterile, individually wrapped
82. 10-ml serological pipettes
83. 25-ml serological pipettes
84. Disposable nitrile gloves
85. Disposable lab coats
86. Disposable hair covers
87. Disposable shoe covers
88. **SAMPLES**
89. **Use well-characterized mycobacterial strains to use for DTS preparation**. Isolates may be obtained from proficiency testing (or EQA) samples from WHO or supranational laboratories or from reputable commercial sources (e.g. ATCC). Prepare permanent stocks of these strains to ensure continuous supply for DTS preparation. Mycobacterial strains frozen at -80oC in 7H9 with 15% glycerol can be stored indefinitely. Verify purity of the isolate before preparing permanent stocks.
90. **Obtain strains for DTS preparation**:
91. At least 3 strains of pansusceptible *M. tuberculosis* (MTB) (e.g. ATCC 27294 or H37rv)
92. At least 4 strains of RIF resistant *M. tuberculosis* with different mutations in the *rpoB* gene associated with RIF resistance. **Do not select MDR MTB** (i.e., resistant to INH and Rifampin).
93. At least one strain of M. *bovis* and/or *M. africanum*.
94. At least 2 species of non-tuberculous mycobacteria (NTM) (e.g., *M. fortuitum*, *M. gordonae*, *M. intracellulare*, and M. kansasii).
95. **Prepare permanent stocks of mycobacterial reference strains**
96. Label two Lowenstein-Jensen (L-J) agar slants for each isolate.
97. Invert thawed isolates several times to mix.
98. Using sterile transfer pipet, inoculate L-J slants with 2-3 drops of the isolate.
99. Incubate inoculated L-J slants at 35-37oC for 1-3 weeks. Incubate in slanting position and make sure tube caps are ¼ turn loose.
100. Examine slants for growth twice on the first week and once on each succeeding week. Tighten caps after the first week of incubation to avoid drying of the media and incubate tubes in upright position.
101. Rapidly growing NTM will exhibit good growth in one week.
102. MTB will grow in 2-3 weeks.
103. When growth is observed, note colonial morphology. Make sure there is pure growth of mycobacteria, with no contaminants and morphology is consistent with the isolate.
104. In a 50-ml sterile plastic conical tube, prepare suspension of the colonies of each isolate in 10 ml of 7H9 broth. Make a suspension equivalent to turbidity of at least 2.0 McFarland standard. Tighten cap and vortex vigorously to break up the clumps. Allow tubes to stand for 10 minutes for aerosol to settle.
105. Add equal volume (10 ml) of sterile 30% glycerol into each of the tube. Invert 3-4 times to mix well.
106. Using sterile transfer pipette, aliquot 0.5 ml of the broth culture with glycerol into labeled cryovials. Place vials in labeled cryoboxes.
107. Label each vial with name of the isolate, ATCC or EQA number, passage number (#1), and date prepared.
108. Each isolate should have at least 20 stock freezer vials. This number is sufficient for 5 years of DTS production assuming there are 2 PT events or rounds per year.
109. Store freezer vial stock at -70 to -80oC freezer.
110. Use the last vial of the permanent stock to prepare another batch (passage #2) of stock freezer vials for another 5 years by repeating step 8.3.
111. Maintain an inventory of freezer vial stocks in the freezer.
112. **SAFETY PRECAUTIONS**
113. **General Safety Precautions**
114. All TB laboratory personnel must receive appropriate safety training prior to working in the laboratory. Staff training should always include information on the safest methods to use for culture procedures to prevent generation and inhalation of aerosols.
115. Follow standard safety practices at all times when inside the TB laboratory (e.g., proper handwashing, no eating or drinking, do not use cellphones).
116. All personnel working in the BSCs should be trained and assessed to ensure they follow correct working practices before they routinely perform testing in the BSC.
117. Wear appropriate personal protective equipment (PPE) at all times when inside the TB laboratory, according to the laboratory’s risk assessment and SOP.
118. BSC must be certified at least annually and whenever moved to ensure HEPA filters are functioning properly and airflow rates meet specifications.
119. Perform regular maintenance (daily, weekly, and monthly) of the BSC. Document on the maintenance log.
120. Refer to Safety Data Sheet (SDS) located in each laboratory room for handling, storage, and first aid information for reagents and chemicals listed in this SOP.
121. Always follow the laboratory’s waste management procedure when preparing all laboratory waste for autoclaving.
122. Strict adherence to safety precautions is required at all times. In the event of workplace safety or medical incident, follow the laboratory’s SOP. Report incident to the supervisor.
123. **Specific Safety Precautions**
124. Aerosolized droplet nuclei containing *Mycobacterium tuberculosis* complex (MTBC) are the primary route of laboratory acquired TB infections. All procedures in this SOP that involves manipulation of mycobacterial cultures, including preparation of permanent stocks of reference strains (section 8.3), must be performed inside a Class II BSC in a BSL3 laboratory, in such a way as to minimize or prevent the formation of infectious aerosols.
125. Wear appropriate PPEs at all times in the BSL3 laboratory: disposable solid-front surgical gown, N95 respirator, disposable gloves, hair cover, dedicated BSL3 shoes (closed toe), and shoe covers.
126. Always verify negative air pressure in the room before working in the BSL3 laboratory using the visual indicator located on entry door. **Do not start work and notify supervisor if negative pressure is not detected**.
127. Always verify BSC is operating within airflow parameters before use. Allow the BSC to run for at least 15 minutes before starting work. After completing work, allow to run for 15 minutes before turning off the BSC. *Note*: Consult BSC User’s Manual and follow manufacturer’s recommendations.
128. Before starting work arrange materials properly inside the BSC and follow correct work practices when working inside the BSC.
129. Perform culture manipulation procedures inside the BSC over disinfectant-soaked paper towels or absorbent surface liners.
130. When pipetting or aliquoting cultures, work with one tube at a time to avoid cross-contamination. Use one pipette for one culture. This precaution is mentioned in the various steps in Section 11.
131. Use disposable sterile inoculating loops when streaking culture plates for isolation.
132. Use disposable sterile transfer pipettes or pipette tips when pipetting liquid cultures.
133. Dispose of used pipettes and loops in a container with appropriate concentration of disinfectant (container must be placed inside the BSC). Allow contact time of at least one hour before disposing the soaked items in the biohazard waste bag.
134. When work is completed, surface decontaminate all items before removing from the BSC.
135. Wipe the interior surface of the BSC with disinfectant before and after each use with sufficient contact time.
136. Minimize or prevent the formation of aerosols.
137. Do not forcibly expel infectious liquids cultures from a pipette.
138. Do not expel air from a pipette into infectious liquids.
139. When using a pipette to add a reagent to infectious liquid, place the pipette against the inner wall of the tube and gently expel the fluid.
140. Always avoid disrupting a bubble or film in an open culture tube. This may be avoided by replacing the cap, gently tapping the top of the tube, setting the tube aside and allowing any generated aerosols to settle before reopening.
141. Following vortexing or shaking broth cultures, leave the tube undisturbed for at least 10 minutes to allow the aerosols to settle before opening.
142. Never vortex an open culture tube; always ensure that screw caps are securely fastened to tubes before vortexing or shaking. Do not vortex tubes with cotton plugs or rubber stoppers.
143. Do not mix or suspend broth cultures by repeatedly filling and fully emptying a pipette.
144. Allow vortexed culture tubes to stand for at least 10 minutes to minimize the spread of aerosols.
145. Only insert the disposable tip of a micropipette into a tube. Never insert the barrel of a micropipette into a culture tube.
146. **Cepheid GeneXpert**
147. Wear disposable gloves and laboratory coat when handling inactivated samples, cartridges, and reagents. Wash hands thoroughly after handling samples and test reagents.
148. Add sample reagent to inactivated samples and inoculate Xpert MTB/RIF cartridges inside a Class II BSC.
149. Treat used cartridges as if capable of transmitting infectious agents and dispose of them as biological waste according to the laboratory’s waste management SOP.
150. Wear disposable gloves and lab coat for monthly maintenance cleaning procedures.
151. Use care when applying cleaning solution on the GeneXpert instrument. Do not allow cleaning solutions to come in contact with the AC power components.
152. DTS Rehydration Specific Precautions
153. Add Xpert MTB/RIF Sample Reagent (SR) to inactivated samples and inoculate Xpert MTB/RIF cartridges inside a Class II BSC.
154. **Spill Management**
155. All TB laboratory personnel must read, understand, and acknowledge the SOP for handling biohazard spills prior to working in the TB laboratory.
156. If a spill occurs in the TB laboratory outside the BSC, staff in the immediate vicinity must be notified and evacuated if in danger.
157. A biological spill response to MTBC is based in a risk assessment. If unsure how to handle a spill (not sure if minor or major), evacuate the room and consult the supervisor and safety officer.
158. When BSL3 spill require evacuation from the room/area, do not return until 99% (minor spill – 2 hours; major spill – minimum 4 hours to overnight) of the airborne particles have been removed. *Note:* TB containment laboratories must have at least 6-12 air exchanges per hour.
159. Biohazard spill kits are located inside and outside the laboratory to facilitate rapid response.
160. Prevent breakage by using the sealed biotransport carrier and a trolley cart when transporting isolates outside the BSL3 laboratory.
161. Do not use hands to handle broken glass. Use forceps to place broken glass in the designated sharps container.
162. **QUALITY CONTROL**

Each Xpert MTB/RIF cartridge includes a sample processing control (SPC) and probe check control (PCC)

1. The SPC ensures the sample was correctly processed. The SPC contains non-infectious *Bacillus globigii* spores in the form of a dry spore cake that is included in each cartridge to verify adequate processing of MTB. The SPC verifies that lysis of MTBC has occurred if the organisms are present, sample processing is adequate, and detects sample-associated inhibition of the real-time PCR assay.
2. The SPC should be positive in a negative sample and can be negative or positive in a positive sample.
3. The SPC passes if it meets the validated acceptance criteria.
4. The result will be “Invalid” if the SPC is not detected in a negative test.
5. PCC: The GeneXpert Dx System measures the fluorescence signal form the probes to monitor bead rehydration, reaction tube filling, probe integrity and dye stability prior to start of PCR reaction.
6. PPC passes if it meets the assigned acceptance criteria.
7. **PROCEDURE FOR PREPARATION OF DRIED TUBE SPECIMENS**

***Note:*** *Determine procedure workflow and schedule to complete all procedure steps. Prepare and label materials (i.e., media, reagents, supplies) needed to move from one procedure step to another. Preparation and labeling of materials may be done in a BSL2 space. Move labeled materials to the TB containment room (using a laboratory cart) where manipulation of cultures and isolates are done.*

*Record media and reagents lot number and expiration dates on the* ***Reagents and Media Log*** *(Appendix A.1).*

1. **Grow reference strains in broth culture (MGIT tube)**
2. Select at least eight strains of mycobacterial species from the permanent stocks in freezer vials (from section 8.3).
3. 2 strains of pansusceptible MTB
4. 4 strains of RIF resistant MTB
5. 2 species of NTM
6. Record isolate name and complete all needed information on the **DTS Stock Preparation Log (Appendix A.2**).
7. By the end of the DTS preparation procedure, five strains will be selected for preparation of PT panels. Each panel will contain five DTS sample tubes.
8. Label 4 MGIT tubes for each MTB isolate. Label 2 MGIT tubes for each NTM isolate (total of 28 MGIT tubes).
9. Using a repeat pipettor, add 0.8 ml of MGIT supplement to each MGIT tube.
10. Using 1-ml sterile graduated transfer pipette, transfer 0.25 ml of the isolate to its labeled MGIT tube. Tighten cap and gently invert tube 2-3 times to mix.

*Note:* Work with one tube at a time to avoid cross-contamination.

1. Load inoculated tubes into the MGIT 960 instrument.
2. Unload tubes that were flagged positive by the MGIT 960 instrument.
3. Place tubes in a rack.
4. Write date of positivity on each MGIT tube.
5. Print unloaded positives, initial, and file in the laboratory binder.
6. Incubate unloaded positive MGIT tubes in a 35-37oC incubator for 4 to 6 days.

After the 4-6 days incubation, store MGIT cultures at 2-8oC while waiting for the other cultures to flag positive. The wait should not be longer than 30 days.

1. Proceed to next step (11.2) to check purity of mycobacterial culture.
2. **Check purity of mycobacterial culture**
3. Place rack of positive MGIT cultures inside the BSC.
4. Set timer: Vortex each tube for one minute. Allow tubes to stand for 10 minutes.
5. Label one 7H11 plate for each MGIT culture.
6. Using a sterile 10 µl disposable loop, transfer one loopful of the MGIT culture onto one 7H11 plate. Streak plate for isolation. Discard loop into discard container with disinfectant. Complete this step one MGIT culture tube at a time to avoid cross-contamination.
7. Seal the plates and place in the metal can with cover.
8. Place metal can with the inoculated 7H11 plates in an incubator set at 35-37oC.
9. Proceed to next step (11.3) to inactivate mycobacterial cultures.

*Interpretation of purity check plates*: Incubate plates for up to 3 weeks. Check plates for growth at least once per week. Examine growth to confirm that the morphology of the colonies is consistent with the selected species of mycobacteria. Discard MGIT (or stock) cultures that grow mixed cultures on the 7H11 plate and/or colonial morphology is not consistent with the expected morphology for the selected species of mycobacteria. Record results on the **Purity Check Log (Appendix A.3).**

***Note:*** *Work for Steps* ***11.3 – 11.6*** *may be performed in one day. If these steps cannot be completed in one day, work may be spread over 2 days; however, Steps 11.4-11.6* ***must*** *be completed on the same day. Example of work schedule:*

*Day 1 – perform step 11.3 (heat-inactivate culture) then store inactivated MGIT cultures at 2-8oC.*

*Day 2 – perform Steps 11.4-11.6*

1. **Heat -inactivate culture to kill the mycobacteria**
2. Heat-inactivate all positive MGIT cultures at the same time.
3. Check oven temperature reading is at 80-85oC.
4. Place rack of MGIT cultures inside the oven.
5. Close oven door tightly and wait for temperature to stabilize at 80-85oC.

Note: Oven temperature typically drops when door is opened; wait for temperature to reach 80oC before starting the timer.

1. Start timer for 30 minutes. Record oven temperature and time on the inactivation verification worksheet.
2. After 30 minutes has elapsed, verify oven temperature is 80-85oC. Record temperature and time on the **Inactivation Verification Log (Appendix A.4)**. Do not open the oven.
3. Set timer for additional 30 minutes.
4. Once a total of one hour has elapsed, verify temperature is 80-85oC. Record temperature and time on the **Inactivation Verification Log (Appendix A.4)**.
5. Remove rack of MGIT culture tubes from the oven.
6. Allow tubes to cool to room temperature. Label rack “heat-inactivated cultures” and date of inactivation.
7. Proceed to the next step (11.4) to prepare stock solutions of the heat-inactivated cultures.
8. **Prepare stock solution of the heat- inactivated culture**
9. Place rack of inactivated MGIT culture tubes inside the BSC. Make sure the cultures have been allowed to cool to room temperature (step 11.3.10)
10. For each MGIT culture, prepare one tube containing 5-10 sterile 3 mm glass beads.
11. Add the glass beads to the MGIT culture tube. Work with one culture tube at a time to avoid cross-contamination.
12. Gently remove the cap from the inactivated MGIT culture tube.
13. Hold MGIT tube at 45-degree angle and slowly pour in 5-10 pieces of sterile 3 mm glass beads while holding the mouths of the two tubes together. Discard the bead tube in designated waste container (do not re-use).
14. Tighten cap of the MGIT tube and vortex tube for 5 minutes. Make sure a full vortex is obtained.
15. Allow tubes to sit for 10 minutes undisturbed, for clumps to settle.
16. Label one 50-ml sterile conical tube for each isolate.
17. Using sterile transfer pipette, carefully pipet off all the liquid above the beads and transfer fluid to the labeled 50-ml conical tube. Work with one culture tube at a time to avoid cross-contamination.
18. Combine the liquids from the same isolate in the same conical tube.
19. The combined suspensions of each isolate will be the **stock solution** to use for performing inactivation verification and for preparing dilutions.

Note: At this point, there will be eight conical tubes of stock solutions, one for each of the eight isolates selected at the beginning of the procedure (step 11.1.1).

1. Label each tube of the stock solutions with the assigned stock number (e.g., stock # 1801 corresponding to stock #1 for year 2018). **See DTS Stock Preparation Log (Appendix A.2).**
2. Proceed **immediately** to the next step (11.5) to check viability of the stock solutions of each isolate.
3. **Check viability of mycobacteria in the stock solution for inactivation verification**
4. Prepare one MGIT tube for each heat-inactivated stock solution. Label tubes with stock solution number, name of the isolate, and date of inoculation.
5. Using a repeat pipettor, add 0.8 ml of MGIT supplement to each MGIT tube.
6. Invert heat-inactivated stock solution 2-3x to mix.
7. Using 1-ml sterile transfer pipette, transfer 0.5 ml of the stock solution of each isolate to its labeled MGIT tube. Cap tightly and invert MGIT tube 2-3 times to mix. Work with one stock solution at a time to avoid cross-contamination.
8. Load inoculated MGIT tubes in the MGIT instrument.
9. Leave tubes in the instrument for two 42-day cycles (total of 84 days) or until flagged positive by the MGIT instrument.
10. Scan out negative tubes when the first 42-day cycle is completed. Print Unloaded Negative Report from the MGIT instrument. File report in the DTS Preparation binder.
11. Scan tubes back into MGIT 960 instrument to start the second 42-day cycle.
12. Scan out negative tubes when the second 42-day cycle is completed. Print Unloaded Negative Report from the MGIT instrument. File report in the DTS Preparation binder.
13. Scan out positive MGIT tubes, if any. Print Unloaded Positive report. File report in the DTS Preparation binder.
14. Discard the stock solution for any viability test that is flagged positive by the MGIT instrument. Growth in MGIT tube indicates that the heat-inactivation procedure failed and the organism is still viable and must not be used to prepare DTS panels.
15. Record MGIT results for each 42-day cycle of the viability check on the **Inactivation Verification Log (Appendix A.4)**.
16. Proceed **immediately** to the next step (11.6) to prepare dilutions of the stock solutions for DTS pre-testing.
17. **Prepare dilution of the stock solution of the isolates for DTS pre-test**
18. Prepare materials and place inside the BSC.
19. Five cryovials (4-ml capacity) for each stock solution. Label each vial with name of isolate, stock number, and aliquot number or letter (A-E).
20. Eight 50-ml sterile conical plastic centrifuge tubes, label with name of isolate, stock solution number, dilution (1:10), and date.
21. Sterile saline solution
22. Food coloring
23. Prepare a 1:10 dilution of each heat-inactivated stock solution (from Step 11.4).
24. Pipet 4.5 ml of sterile saline to each labeled 50-ml conical tube.
25. Add 5 µl of food coloring to each 50-ml conical tube.
26. Using 1-ml sterile transfer pipette, transfer 0.5 ml of stock solution to the labeled 50-ml conical tube. Vortex tube for 30 seconds. Work with one stock solution at a time to avoid cross-contamination. Allow tubes to stand for 10 minutes after vortexing (set timer) to allow for settling of large clumps to achieve a more homogenous aliquots.
27. Prepare 5 DTS tubes for each of the 1:10 diluted stock solution.
28. Uncap labeled 4-ml cryovials inside the BSC and place caps in a clean plastic zipper bag.
29. Using a repeat pipettor, pipet 100 µl of the diluted stock into the 5 labeled cryovials. Keep pipette tip in upper 1/3 of the fluid when aspirating the diluted stock to avoid disrupting larger clumps that settled to the bottom of the tube. Work with one diluted stock at a time to avoid cross-contamination.
30. Discard the remaining 1:10 dilution.
31. Store remaining stock solutions in the refrigerator set at 2-8oC.
32. Allow aliquot tubes to sit open inside the BSC in the TB containment laboratory for 7-10 days.
33. After 7-10 days, check if specimen at the bottom of the tubes is dry, then tightly cap all tubes. Ensure that tubes are visually dry before capping.
34. Store DTS in the dark at room temperature while waiting for pre-testing.
35. Proceed to next step (11.7) for DTS pre-testing.
36. **Perform DTS pre-test to determine which stock solutions to include in the DTS panel**
37. Test the five aliquots from the 1:10 dilution of each stock with Xpert MTB/RIF assay following this procedure:
38. Add 2.5 ml of SR to each DTS sample to be tested. Tightly recap samples.

Note: Work with one DTS at a time to avoid cross-contamination.

1. Shake vigorously 20 times. One back and forth movement is a single shake.
2. Incubate the samples for 10 minutes (set timer) at room temperature.
3. Shake the samples vigorously again 20 times.
4. Incubate for an additional 5 minutes (set timer) at room temperature.
5. Add 2.0 ml of the sample to labeled Xpert MTB/RIF cartridge. Scan and load cartridges in the GeneXpert instrument.

Note: Follow the laboratory’s SOP for adding sample to the cartridge and for loading the cartridges in the instrument.

1. Once pre-testing is complete, print test reports.
2. From the printed test results, enter pre-test data into the **DTS Pre-test Results Worksheet (Appendix A.5)**.
3. Proceed to next step (11.8) to prepare the PT panel.
4. **Prepare the DTS PT panel**
5. Using data from the DTS **Pre-test Results Worksheet (Appendix A.5)**, determine which heat-inactivated stock solutions to include in the DTS panel. Select stocks based on these criteria:
6. With a mean cycle threshold (Ct) for Probe A in the medium to low range (16-23 Ct) and a standard deviation ≤ 3.
7. With 100% accuracy of results (i.e., all 5 aliquots for each isolate gave the expected result).

|  |  |
| --- | --- |
| Isolate | Expected result |
| Pansusceptible MTB | MTB detected; RIF resistance not detected |
| RIF resistant MTB | MTB detected; RIF resistance detected |
| NTM | MTB not detected |

1. Select five inactivated stock solutions.
2. Select a combination of:
3. Pan-susceptible *M. tuberculosis*
4. Rifampin resistant *M. tuberculosis* with varying probe patterns.
5. *M. bovis* or *M. africanum*
6. NTM
7. Do not use stocks that failed the purity check and heat inactivation verification or those that did not meet the criteria in Step 11.8.1.
8. Record stock number of the solutions selected for DTS panel on **DTS Stock Preparation Log (Appendix A.2)**
9. Determine the number of 4-ml cryovials (aliquots) needed per stock. Add at least 15% extra for panel validation and for other needs like for QC and instrument verification.

For example: If there are 78 sites enrolled in the PT program, then 78 panels are needed.

78 x 15% = 11.7 (round up to 12) 78 +12 = 90 (at least 90 aliquots must be prepared to ensure enough aliquots are available for validation and for all the 78 testing sites enrolled in the Xpert MTB/RIF PT program).

There are 5 stock solutions to aliquot: 90 tubes per stock x 5 = 450 total number of cryovials needed.

Place tubes in racks and label racks with isolate name and stock number.

1. Prepare all materials and place them inside the BSC in the TB containment laboratory.
2. Selected stock solutions (from Step 11.6.4.4)
3. 4-ml cryovials on labeled racks
4. Sterile saline solution
5. Food coloring
6. Select type of dilution to use for DTS sample aliquots. There are two options to dilute stocks for DTS aliquots.
7. **Use 1:10 dilution of inactivated stock solution when mean CT for Probe A is 16-17 on pretest**. Refer to Table 1 for calculations.

Table 1. Preparation of 1:10 Dilutions (example of calculations for 250 and 400 aliquots)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **A.** No. of aliquots per stock | **B.** Total volume of 1:10 diluted stock required  **[(A x 0.1\* ml) + 5 ml]** | **C.** Volume of stock solution vortexed with beads  **[(B/10) + 0.5 ml]** | **D.** Volume of vortexed stock added to saline for 1:10 dilution **(B/10)** | **E.** Volume of saline for 1:10 dilution (**B-D)** | **F.** Volume of food grade dye for 1:1000 dilution  **(B/1000)** |
| 250 | 25 ml + 5 ml = 30 ml | 3.0 + 0.5 = 3.5 ml | 3 ml | 27 ml | 30 μl |
| 400 | 40 ml + 5 ml = 45 ml | 4.5 + 0.5 = 5.0 ml | 4.5 ml | 40.5 ml | 45 μl |

**\***0.1 ml (100 μl) is the volume of diluted stock solution aliquot for each DTS tube

1. **Use entire volume of inactivated stock solution when** **mean Ct for Probe A is 18-23 on pre-test**. Dilute stock with saline only enough to produce the desired number of aliquots plus 5 ml for pipetting error. Refer to Table 2 calculations.

Table 2. Dilution of entire stock calculations (example of calculations for 250 and 500 aliquots)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **A.** No. of aliquots per stock | **B.** Total volume of diluted stock required  **[(A x 0.1\* ml) + 5 ml]** | **C.** Volume of stock solution | **D.** Volume of stock added to saline | **E.** Volume of saline for dilution  (**B-D)** | **F.** Volume of food grade dye for 1:1000 dilution  **(B/1000)** |
| 250 | 25 ml + 5 ml = 30 ml | Entire volume of stock \_\_ ml | Entire volume of stock above beads (9 ml) | 30 ml – 9 ml = 21 ml | 30 ml/1000 = 30 μl |
| 500 | 50 ml + 5 ml = 45 ml | Entire volume of stock \_\_ ml | Entire volume of stock above beads (9 ml) | 55 ml – 9 ml = 46 ml | 55 ml/1000 = 55 μl |

**\***0.1 ml (100 μl) is the volume of diluted stock solution aliquot for each DTS tube

1. Prepare dilution of stock solutions. Note: The type of dilution must first be selected (Step 11.8.5)
2. Transfer the required volume of stock solution (C) to a labeled sterile 16 mm x 100 mm tubes with 5-10 sterile 3-mm beads.
3. Vortex each tube for 5 minutes ensuring a full vortex is obtained.
4. Allow tubes to stand for 10 minutes undisturbed.
5. Transfer the required volume of vortexed stock (D) from above the beads to a 50-ml plastic conical tube. Do not push pipette tips into the beads or disturb the beads
6. Add required volume of sterile saline (E) to the tube. Depending on total volume, dilutions may need to be split between two 50-ml conical tubes.
7. Add blue food coloring at a concentration of 1:1000 to each tube. Refer to Table 1 and Table 2 for the appropriate volume of the food coloring.
8. Vortex each dilution tubes for 30 seconds.
9. Aliquot diluted stock solutions to 4-ml cryovials
10. Remove caps from cryovials inside the BSC and place caps in plastic zipper bag. Write the stock number and isolate name on the bag.
11. Using a repeat pipettor, aliquot 100 μl of each diluted stock solution to the cryovials. When aspirating solution, always keep pipette tip in upper 1/3 of the solution to avoid disrupting larger clumps that have settled to the bottom of the conical tube. Always prime pipette 2-3 times by expressing back into dilution tube before beginning to aliquot.
12. Return any remaining stock to storage at 2-8oC.
13. Allow aliquot tubes to sit open inside the BSC in the BSL3 laboratory for 7-10 days.
14. After 7-10 days, check if specimen at the bottom of the tubes is dry, then tightly cap all tubes. Ensure that tubes are visually dry before capping.
15. Place all DTS aliquot tubes in labeled large plastic zipper bags. Store at room temperature in the dark until validation testing. DTS tubes must remain inside the TB containment laboratory until the inactivation verification is completed and passed.
16. Determine and randomly select the number of aliquots to be used for DTS panel validation. Perform panel validation testing (Step 11.9).
17. Check viability test results for the stock solutions. If stock solutions have passed the viability test (i.e., no growth is detected after 84 days), and the laboratory supervisor has reviewed and signed the **Inactivation Verification Log (Appendix A.4)**, the DTS aliquots may be brought into the BSL2 area for labeling, packing, and storage.
18. Check panel validation results. If panel validation passed, proceed to labeling and packing of DTS aliquots.
19. **DTS Panel Validation**
20. When preparing DTS for the first time, validate 10% of the prepared aliquots.
21. Once data has been collected from 3 previous panels, use data to calculate the mean standard deviation. Use Table 3 to determine the number of aliquots (sample size) needed to perform a robust validation according to the historical variability observed in the previous 3 panels.

Table 3. Sample Size Calculation

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **One Variance Power Analysis** | | | | | |
| Numeric Results when H0: S0 = S1 versus Ha: S0 ≠ S1 | | | | | |
|  | | | | | |
| **Power** | **N** | **SO (Standard deviation)** | **SI** | **Alpha** | **Beta** |
| 0.95223 | 7 | 1.0000 | 3.0000 | 0.05000 | 0.04777 |
| 0.95094 | 8 | 1.1000 | 3.0000 | 0.05000 | 0.04906 |
| 0.96256 | 10 | 1.2000 | 3.0000 | 0.05000 | 0.03744 |
| 0.95403 | 11 | 1.3000 | 3.0000 | 0.05000 | 0.04597 |
| 0.95519 | 13 | 1.4000 | 3.0000 | 0.05000 | 0.04481 |
| 0.95133 | 15 | 1.5000 | 3.0000 | 0.05000 | 0.04867 |
| 0.95232 | 18 | 1.6000 | 3.0000 | 0.05000 | 0.04768 |
| 0.95459 | 22 | 1.7000 | 3.0000 | 0.05000 | 0.04541 |
| 0.95562 | 27 | 1.8000 | 3.0000 | 0.05000 | 0.04438 |
| 0.95386 | 33 | 1.9000 | 3.0000 | 0.05000 | 0.04614 |
| 0.95203 | 41 | 2.0000 | 3.0000 | 0.05000 | 0.04797 |
|  |  | 2.1000 | 3.0000 | 0.05000 |  |
|  |  | 2.2000 | 3.0000 | 0.05000 |  |
|  |  | 2.3000 | 3.0000 | 0.05000 |  |
|  |  | 2.4000 | 3.0000 | 0.05000 |  |
|  |  | 2.5000 | 3.0000 | 0.05000 |  |
|  |  | 2.6000 | 3.0000 | 0.05000 |  |
|  |  | 2.7000 | 3.0000 | 0.05000 |  |
|  |  | 2.8000 | 3.0000 | 0.05000 |  |
|  |  | 2.9000 | 3.0000 | 0.05000 |  |
|  |  | 3.0000 | 3.000 | 0.05000 |  |

1. Each year thereafter, re-calculate the sample size needed for validation using the mean standard deviation of probe A (for all samples positive for MTBC) from the previous three panels. See table below for example.

**Example:** If the mean standard deviations for Probe A for all MTBC positive samples from the previous three panels are equal to 1.09, 1.73, and 1.83 the mean standard deviation for probe A for all 3 panels would equal 1.55. Round 1.55 to 1.6. Find 1.6 in the S0 column of the Table 3.The number of aliquots (N) to test is 18. For this year, 18 samples would be tested for validation, regardless of the number of aliquots or panels prepared.

Table 4. Example of Sample Size Calculation

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| A. Mean SD of Probe A for 2016-C Panel | B. Mean SD of Probe A for 2017-A Panel | C. Mean SD of Probe A for 2017-B Panel | D. Mean SD for last 3 panels | E. Correspond.  sample size according  to table |
| (2016-C-1 SD  +2016-C-2 SD  +2016-C-3 SD  +2016-C-5 SD\*) / 4  = Mean SD for 2016-C | (2017-A-1  + 2017-A-2  +2017-A-3  +2017-A-4\*) / 4  = Mean SD for 2017-A | (2017-B-1  + 2017-B-2  + 2017-B-4  + 2017-B-5\*) / 4  = Mean SD for 2017-B | (A + B + C) / 3  = Mean of the  mean SD from  previous 3 panels | 1.55 (D) rounded to  one decimal point  is 1.6 |
| (1.15  + 1.01  +1.09  +1.12) / 4  = 1.09 | (1.35  + 2.54  + 1.87  + 1.17) / 4  = 1.73 | (1.79  + 1.64  + 1.90  + 1.99) / 4  = 1.83 | (1.09  + 1.73  + 1.83) / 3  = 1.55 | Find 1.6 in chart above  and see corresponding  value in N column =18 |
| \*2016-C-4 sample is  a NTM | \*2017-A-5 sample is  a NTM | \*2017-B-3 sample is  a NTM |  |  |

1. Perform DTS panel validation
2. Randomly select the 10% or number of DTS aliquots calculated from Table 3.
3. Label selected DTS aliquots with the name of the isolate, stock number and serial number. Example: H37Rv-1801-1, H37Rv-1801-2, H37Rv-1801-3… (Up to 18, the calculated sample size (N) from the example above)
4. Test samples. Follow procedure for Xpert MTB/RIF testing of DTS samples.
5. If the GeneXpert reports an uninterpretable result (error, invalid, or no result), repeat testing with a new aliquot of DTS sample.
6. Once validation testing of the DTS panel is completed, print test reports.
7. Enter all results on the Xpert MTB/RIF **DTS Panel Validation Worksheet (Appendix B)**. The mean cycle threshold (Ct), standard deviation (SD), and % CV for each probe will be calculated for each validated sample.
8. **METHOD PERFORMANCE SPECIFICATIONS**
9. This procedure is only to be used for preparing DTS samples for proficiency testing, quality control, and instrument verification using the Xpert MTB/RIF assay.
10. DTS samples are stable for 6 months when stored at a consistent 20oC or 2-8oC and protected from light.
11. **CALCULATIONS**
12. Calculation of Ct values is performed by the GeneXpert DX System from measured fluorescent signals and an embedded calculation algorithm. Results are displayed in the “View Results” window and on the printed reports.
13. Lower Cycle Threshold (Ct) values represent a higher starting concentration of DNA template.
14. Calculations are done on Excel spreadsheets for Ct mean, standard deviation, and percent CV.
15. **INTERPRETATION OF RESULTS**
16. **Test Results**

See the laboratory’s SOP or Xpert MTB/RIF manufacturer’s package insert for test results interpretation.

1. **Panel Validation Results**
2. The level of detection results target is in the low to medium range (16-23 Ct).

Refer to Table 5.

1. The sample validation result is considered valid if:
2. SD is ≤ 3
3. Mean Ct of probe A or probe C (if probe A is absent) is ≤ 23.

Note: In certain rifampicin-resistant isolates, a probe may exhibit incomplete fallout, i.e., the probe fails to bind (Ct = 0) in most but not all circumstance. The SD for these probes is not a true reflection of the variability in the sample preparation.

Table 5. Xpert MTB/RIF Semi-Quantitative Results

|  |  |
| --- | --- |
| **MTB Result** | **Ct Range** |
| High | < 16 |
| Medium | 16-22 |
| Low | 22-28 |
| Very Low | >28 |

1. Troubleshooting
2. Increased cycle threshold results
3. If the SPC internal control Ct results for a single aliquot ≥ 34.1, then it may be removed from the validation results and the test repeated using a fresh aliquot.
4. Blakemore et al. found that when the SPC internal control Ct result is above 34.0 considerable variation (increase) in Ct and detection results were noted.
5. Unexpected results
6. When an unexpected result is encountered (unless the expected results is “TB Not Detected”), add an additional 2 ml of SR to the remaining original sample and re-run within 4 hours of original sample preparation.
7. If the retested result matches the expected result, replace the unexpected result with the retested result.
8. The sample fails validation if the retested result matched the original unexpected result.
9. The sample fails validation if repeat testing is not possible, or more than 4 hours has elapsed since original sample preparation, or there is no way to determine if the unexpected results were due to sample or cartridge failure.
10. The sample fails validation if TB is detected when the expected result is “TB not Detected” for one or more aliquots.
11. Select another stock sample to replace the failed sample, dilute, aliquot, and validate as described above.
12. **RESULTS REVIEW AND APPROVAL**
13. Submit all validation results to the supervisor for review and approval. Do not start labeling and packing pending approval of the supervisor.
14. After review and approval, begin labeling and packing of the DTS samples.
15. Label each tube with the year, panel letter, and sample number.

Example: 2018-A-1, 2018-A-2, 2018-A-3 2018-A-4, 2018-A-5 (5 DTS tubes per PT panel)

1. Pack 5 DTS tubes, 5 transfer pipettes, PT Kit instructions, and PT Result Form in a resealable plastic zipper bag.
2. Ship packed panels to the testing sites.
3. **DOCUMENTATION**
4. Record observations, and results on the DTS Preparation Worksheets (Appendix A). File completed worksheets in the DTS Preparation binder.
5. Reagents and Media Log
6. DTS Stock Preparation Log
7. Purity Check Log
8. Inactivation Verification Log
9. Pre-Test Results Worksheet
10. Record all panel validation results and summary in the **DTS Panel Validation Worksheet** (Appendix B) and file in the DTS Preparation binder.
11. File MGIT 960 unloaded positive and negative reports in the DTS Preparation binder.
12. **SAMPLE RETENTION AND STORAGE**
13. Retain and store spare PT panels at 20oC or at 2-8oC in the dark until PT results of participating sites have been received and evaluation reports have been sent to participating sites.
14. The remaining panels may be used by the NTRL for quality assurance testing such as module verification testing and Xpert MTB/RIF new lot QC.
15. **REFERENCES**
16. Helb, D., et al. (2010). "Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology." J Clin Microbiol. 48(1): 229-237.
17. Parekh, B. S., et al. (2010). "Dried tube specimens: a simple and cost-effective method for preparation of HIV proficiency testing panels and quality control materials for use in resource-limited settings." J Virol Methods. 163(2): 295-300.
18. Scott, L. E., et al. (2011). "Dried culture spots for Xpert MTB/RIF external quality assessment: results of a phase 1 pilot study in South Africa." J Clin Microbiol. 49(12): 4356-4360.
19. Blakemore, R., et al. (2011). "A multisite assessment of the quantitative capabilities of the Xpert MTB/RIF assay." Am J Respir Crit Care Med 184(9): 1076-1084.
20. WHO TB Laboratory Biosafety Manual. 2012. Geneva, Switzerland.
21. **RELATED DOCUMENTS**
22. SOP: Operation and Maintenance of the GeneXpert Instrument
23. SOP: Operation and Maintenance of MGIT 960 Instrument
24. GeneXpert Dx System Operations Manual 2010
25. Xpert MTB/RIF Assay Package Insert 2015-5
26. Laboratory Safety Manual
27. DTS Preparation Readiness Assessment Checklist
28. Xpert MTB/RIF PT Kit Instructions
29. Xpert MTB/RIF DTS PT Testing Job Aid
30. Xpert MTB/RIF PT Result Form
31. Xpert MTB/RIF Participant’ Evaluation and Results Summary
32. **APPENDICES**
33. Appendix A. DTS Preparation Worksheets (available as an Excel workbook containing 5 worksheets)
34. Reagents and Media Log
35. DTS Stock Preparation Log
36. Purity Check Log
37. Inactivation Verification Log
38. Pre-test Results Worksheet
39. Appendix B. DTS Panel Validation Worksheets

B.1 Panel Validation Summary

B.2 Xpert MTB/RIF Validation Results

**20.1. Appendix A.1: Reagents and Media Log**

Name of Laboratory: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Preparation Worksheet 1. Reagents and Media Used for DTS Preparation** | | | | | |
| **DTS preparation date:** | |  |  |  | **Panel 2018-A** |
| **#** | **DTS Preparation step** | **Media/Reagent** | **Lot number** | **Expiry date** | **Tech initials Comments** |
| 1 | Growth of mycobacterial strains | MGIT tubes |  |  |  |
| PANTA supplement |  |  |  |
|  |  |  |  |  |  |
| 2 | Purity check | Middlebrook 7H11 agar plate |  |  |  |
|  |  |  |  |  |  |
| 3 | Inactivation verification | MGIT tubes |  |  |  |
| PANTA supplement |  |  |  |
|  |  |  |  |  |  |
| 4 | DTS Pre-testing | Sterile saline solution |  |  |  |
| Blue food coloring |  |  |  |
| Xpert MTB/RIF cartridge |  |  |  |
|  |  |  |  |  |  |
| 5 | Panel preparation | Sterile saline solution |  |  |  |
|  |  | Blue food coloring |  |  |  |
|  |  |  |  |  |  |
| 6 | Panel validation | Xpert MTB RIF cartridge |  |  |  |

**20.1. Appendix A.2: DTS Stock Preparation Log**

Name of Laboratory: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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| **Preparation Worksheet 2. DTS Stock Preparation Log** | | | | | | | | | | | | | | |
| **Year:** | 2018 | |  | |  | |  | |  | |  | | |  |
| **Sequence #** | | **Stock number** | | **Isolate name** | | **Date prepared** | | **Prepared by** | | **Date stock used** | | **Used for what purpose?** | **Used by (Tech initials)** | |
| 1 | | 1801 | |  | |  | |  | |  | |  |  | |
| 2 | | 1802 | |  | |  | |  | |  | |  |  | |
| 3 | | 1803 | |  | |  | |  | |  | |  |  | |
| 4 | | 1804 | |  | |  | |  | |  | |  |  | |
| 5 | | 1805 | |  | |  | |  | |  | |  |  | |
| 6 | | 1806 | |  | |  | |  | |  | |  |  | |
| 7 | | 1807 | |  | |  | |  | |  | |  |  | |
| 8 | | 1808 | |  | |  | |  | |  | |  |  | |
| 9 | | 1809 | |  | |  | |  | |  | |  |  | |
| 10 | | 1810 | |  | |  | |  | |  | |  |  | |
| 11 | | 1811 | |  | |  | |  | |  | |  |  | |
| 12 | | 1812 | |  | |  | |  | |  | |  |  | |
| 13 | | 1812 | |  | |  | |  | |  | |  |  | |
| 14 | | 1814 | |  | |  | |  | |  | |  |  | |
| 15 | | 1815 | |  | |  | |  | |  | |  |  | |
|  | |  | |  | |  | |  | |  | |  |  | |
|  | |  | |  | |  | |  | |  | |  |  | |
| \*Assign a stock number for each selected mycobacterial strain. Assign stock numbers in sequential order as DTS stocks are prepared.  \*Stock number is a 4-digit number beginning with the last 2 digits of the year and 2 sequential numbers (e.g., first stock number of 2018 is 1801). | | | | | | | | | | | | | | |
|  | | | | | | | | | | | | | | |
| Supervisor review: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | | | | | | | | | | | |
| Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | | | | | | | | | | | |

**20.1. Appendix A.3: Purity Check log**

Name of Laboratory: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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| --- | --- | --- | --- | --- | --- | --- |
| **Preparation Worksheet 3. Purity Check log** | | | | | | |
| DTS preparation date: | | | | | | |
| **#** | **Isolate name and number** | **Date of inoculation of 7H11 plate** | **Read Date** | **Colony morphology on 7H11 plate** | **Read by (Tech initials)** | **DTS stock number** |
| 1 | Pansusceptible MTB Strain 1 - MGIT # 1 |  |  |  |  | 1801 |
| 2 | Pansusceptible MTB Strain 1 - MGIT # 2 |  |  |  |  |
| 3 | Pansusceptible MTB Strain 1 - MGIT # 3 |  |  |  |  |  |
| 4 | Pansusceptible MTB Strain 1 - MGIT # 4 |  |  |  |  |
| 5 | Pansusceptible MTB Strain 2 - MGIT # 1 |  |  |  |  |  |
| 6 | Pansusceptible MTB Strain 2 - MGIT # 2 |  |  |  |  | 1802 |
| 7 | Pansusceptible MTB Strain 2 - MGIT # 3 |  |  |  |  |  |
| 8 | Pansusceptible MTB Strain 2 - MGIT # 4 |  |  |  |  |  |
| 9 | RIF resistant MTB Strain 1 - MGIT # 1 |  |  |  |  |  |
| 10 | RIF resistant MTB Strain 1 - MGIT # 2 |  |  |  |  | 1803 |
| 11 | RIF resistant MTB Strain 1 - MGIT # 3 |  |  |  |  |  |
| 12 | RIF resistant MTB Strain 1 - MGIT # 4 |  |  |  |  |  |
| 13 | RIF resistant MTB Strain 2 - MGIT # 1 |  |  |  |  |  |
| 14 | RIF resistant MTB Strain 2 - MGIT # 2 |  |  |  |  | 1804 |
| 15 | RIF resistant MTB Strain 2 - MGIT # 3 |  |  |  |  |  |
| 16 | RIF resistant MTB Strain 2 - MGIT # 4 |  |  |  |  |  |
| 17 | RIF resistant MTB Strain 3 - MGIT # 1 |  |  |  |  |  |
| 18 | RIF resistant MTB Strain 3 - MGIT # 2 |  |  |  |  | 1805 |
| 19 | RIF resistant MTB Strain 3 - MGIT # 3 |  |  |  |  |  |
| 20 | RIF resistant MTB Strain 3 - MGIT # 4 |  |  |  |  |  |
| 21 | RIF resistant MTB Strain 4 - MGIT # 1 |  |  |  |  |  |
| 22 | RIF resistant MTB Strain 4 - MGIT # 2 |  |  |  |  | 1806 |
| 23 | RIF resistant MTB Strain 4 - MGIT # 3 |  |  |  |  |  |
| 24 | RIF resistant MTB Strain 4 - MGIT # 4 |  |  |  |  |  |
| 25 | *M. fortuitum* - MGIT # 1 |  |  |  |  |  |
| 26 | *M. fortuitum* - MGIT # 2 |  |  |  |  | 1807 |
| 27 | *M. kansasii* - MGIT # 1 |  |  |  |  |  |
| 28 | *M. kansasii* - MGIT # 2 |  |  |  |  | 1808 |
| \* Morphology Abbreviations: B= Buff, R= Rough, I= Irregular, W= Wet, Y=Yellow, M=Mucoid, S=Spreading | | | | | | |
|  |  |  |  |  |  |  |
| Supervisor review: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | | | |
| Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | | | |

**20.1. Appendix A.4: Inactivation Verification Log**

Name of Laboratory: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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| **Preparation Worksheet 4. DTS Inactivation Verification Log** | | | | | | | | | | | |
| Date of DTS Inactivation : \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | | | Tech Initials: \_\_\_\_\_\_\_\_\_ | | Panel Name: 2018-A | | | |
| **Critical Step Checklist** | | | | | | | | | | | |
| 1. Place MGIT tubes in pre-heated racks inside oven. | | | | | | | |  | |  | |
| 2. Close oven door tightly and wait for temperature to stabilize between 80 and 85°C. Start the timer for 30 minutes, record the time and temperature. | | | | | | | | Start Time: \_\_\_\_\_\_\_\_\_\_ | | Start Temp: \_\_\_\_\_\_\_\_\_\_ | |
| 3. After 30 minutes has elapsed verify oven temperature is 80-85°C, record the temperature and time on the inactivation verification worksheet. Start timer for additional 30 minutes. | | | | | | | | Time at 30min: \_\_\_\_\_\_\_\_\_\_ | | Temp at 30min: \_\_\_\_\_\_\_\_\_\_ | |
| 4. Once a total of 1 hour has elapsed verify temperature is 80-85°C. Remove MGIT tubes from the oven and record the temperature and time removed. | | | | | | | | End Time: \_\_\_\_\_\_\_\_\_\_ | | End Temp: \_\_\_\_\_\_\_\_\_\_ | |
|  | | | | | | | | | | | |
| **Isolate Name** | **DTS Stock Number** | **MGIT inoculation** | | **42-day incubation** | | **84-day incubation** | | **Final Result** | | | Comments\*\* |
| Date | Tech initials | Result (Positive/ Negative) | Date | Result (Positive/ Negative) | Date | Positive/ Negative | Date | Tech initials |
|  |  |  |  |  |  |  |  |  |  |  |  |
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| \*File copies of all unloaded positive and negative MGIT 960 Reports directly behind this report in the DTS Preparation Binder for the current year. | | | | | | | | | | | |
| \*\*If culture is positive, indicate date all related inactivated stock and DTS samples were discarded | | | | | | | | | | | |
|  | | | | | | | | | | | |
| Supervisor review: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | | | | | | | | |
| Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | | | | | | | | |

**20.1. Appendix A.5: Pre-test Results Worksheet**

Name of Laboratory: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Preparation Worksheet 5. Pretest Results Worksheet Year: 2018** | | | | | | | | | | |
|  | **Isolate name \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Stock #\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_** | | | | | | | | | |
|  |  | **Results** | | | **Cycle Threshold** | | | | | |
| **Aliquot** | **Date Tested** | **MTB Detected** | **Rif Resistance** | | **Probe D** | **Probe  C** | **Probe E** | **Probe B** | **SPC** | **Probe  A** |
| A |  |  |  | |  |  |  |  |  |  |
| B |  |  |  | |  |  |  |  |  |  |
| C |  |  |  | |  |  |  |  |  |  |
| D |  |  |  | |  |  |  |  |  |  |
| E |  |  |  | |  |  |  |  |  |  |
| SD |  |  |  | | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| Mean |  |  |  | | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| %CV |  |  |  | | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
|  |  |  |  | |  |  |  |  |  |  |
|  | **Isolate name \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Stock #\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_** | | | | | | | | | |
|  |  | **Results** | | | **Cycle Threshold** | | | | | |
| **Aliquot** | **Date Tested** | **MTB Detected** | **Rif Resistance** | | **Probe D** | **Probe C** | **Probe E** | **Probe B** | **SPC** | **Probe A** |
| A |  |  |  | |  |  |  |  |  |  |
| B |  |  |  | |  |  |  |  |  |  |
| C |  |  |  | |  |  |  |  |  |  |
| D |  |  |  | |  |  |  |  |  |  |
| E |  |  |  | |  |  |  |  |  |  |
| SD |  |  |  | | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| Mean |  |  |  | | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| %CV |  |  |  | | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
|  |  |  |  | |  |  |  |  |  |  |
|  | **Isolate name \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Stock #\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_** | | | | | | | | | |
|  |  | **Results** | | | **Cycle Threshold** | | | | | |
| Aliquot | **Date Tested** | **MTB Detected** | | **Rif Resistance** | **Probe D** | **Probe C** | **Probe E** | **Probe B** | **SPC** | **Probe  A** |
| A |  |  | |  |  |  |  |  |  |  |
| B |  |  | |  |  |  |  |  |  |  |
| C |  |  | |  |  |  |  |  |  |  |
| D |  |  | |  |  |  |  |  |  |  |
| E |  |  | |  |  |  |  |  |  |  |
| SD |  |  | |  | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| Mean |  |  | |  | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| %CV |  |  | |  | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |

*Note: Results will be entered for each isolate used in the PT panel.*

**20.2. Appendix B.1: Panel Validation Summary**

Name of Laboratory: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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| --- | --- | --- | --- | --- | --- |
| **Validation Worksheet 1. Panel Validation Summary** | | | | | |
| **Panel/sample ID** | **Isolate Name** | **Stock Number** | **Date Diluted and Aliquoted** | **Tech initials** | **Comments** |
| 2018-A-1 | MTB H37rv | 1801 |  |  |  |
| 2018-A-2 | MTBC s522L | 1804 |  |  |  |
| 2018-A-3 | MTBC 35810 | 1805 |  |  |  |
| 2018-A-4 | *M. fortuitum* | 1807 |  |  |  |
| 2018-A-5 | *M. kansasii* | 1808 |  |  |  |
|  |  |  |  |  |  |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Panel/ Sample ID** | **MTB Detected** | **Rif Resistance** | **Probe D** | **Probe C** | **Probe E** | **Probe B** | **SPC** | **Probe A** |
| **SD** | 2018-A-1-1801 | Medium | Not detected |  |  |  |  |  |  |
| **Mean** |  |  |  |  |  |  |
| **%CV** |  |  |  |  |  |  |
| **SD** |  |  |  |  |  |  |  |  |  |
| **Mean** |  |  |  |  |  |  |
| **%CV** |  |  |  |  |  |  |
| **SD** |  |  |  |  |  |  |  |  |  |
| **Mean** |  |  |  |  |  |  |
| **%CV** |  |  |  |  |  |  |
| **SD** |  |  |  |  |  |  |  |  |  |
| **Mean** |  |  |  |  |  |  |
| **%CV** |  |  |  |  |  |  |
| **SD** |  |  |  |  |  |  |  |  |  |
| **Mean** |  |  |  |  |  |  |
| **%CV** |  |  |  |  |  |  |

**20.2. Appendix B.2: Xpert MTB/RIF Validation Results**

Name of Laboratory: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Validation Worksheet 2. Xpert MTB/RIF Validation Results** | | | | | | | | | |
| **Panel/Sample ID: MTB** | | | | | | | | | |
|  |  | **Results** | | **Cycle Threshold** | | | | | |
| **Aliquot #** | **Date Tested** | **MTB Detected** | **Rif Resistance** | **Probe D** | **Probe C** | **Probe E** | **Probe B** | **SPC** | **Probe A** |
| 1 |  |  |  |  |  |  |  |  |  |
| 2 |  |  |  |  |  |  |  |  |  |
| 3 |  |  |  |  |  |  |  |  |  |
| 4 |  |  |  |  |  |  |  |  |  |
| 5 |  |  |  |  |  |  |  |  |  |
| 6 |  |  |  |  |  |  |  |  |  |
| 7 |  |  |  |  |  |  |  |  |  |
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| 13 |  |  |  |  |  |  |  |  |  |
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| SD |  |  |  | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| Mean |  |  |  | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |
| %CV |  |  |  | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! | #DIV/0! |

## 8.7. Xpert MTB/RIF Proficiency Testing Standard Operating Procedure and Forms

**STANDARD OPERATING PROCEDURE**

1. **TITLE**

Xpert MTB/RIF Proficiency Testing

1. **PURPOSE**
2. Proficiency testing (PT) is an essential element of the testing site quality assurance.
3. PT schemes are inter-laboratory comparisons that are organized regularly to assess the performance of analytical laboratories and the competence of the analytical personnel.
4. Performance on PT has been found to be an indicator of the quality of patient testing.
5. **SCOPE**
6. This procedure provides instructions to testing sites for proper handling and analysis of Xpert MTB/RIF PT samples, and reporting and review of proficiency testing results.
7. In this SOP, “testing site” includes both traditional laboratories and point of care or other clinical testing sites where the Xpert MTB/RIF is being performed.
8. **RESPONSIBILITIES**
9. Testing Site Staff
10. Participate in proficiency testing activities of the testing site.
11. Comply with instructions outlined in this SOP.
12. Review Participant’s Evaluation Report from PT provider.
13. Comply with remedial or corrective action as necessary to ensure quality results.
14. Testing Site Supervisor
15. For the purposes of this SOP, the laboratory supervisor refers to person(s) who direct and manage the activities, and with authority over a testing site.
16. Ensure that the testing site participates in a proficiency testing program.
17. Ensure all staff involved are trained and competent in performing this procedure.
18. Ensure that all testing personnel are given the opportunity to analyse PT samples.
19. Ensure that PT materials are processed and reported in a timely manner.
20. Oversee testing of PT samples.
21. Review PT sample results prior to submission to the PT provider.
22. Review and sign PT Participant’s Evaluation Report and provide feedback to the staff.
23. Investigate PT failures and institute remedial or corrective action as necessary.
24. Prepare Annual Summary of PT Results.
25. Ensure that all PT reports and other related documents are properly filed and managed.
26. Assign a designee as necessary to support PT testing and results review.
27. Ensure that this SOP and other related documents are updated, reviewed, and available to the staff and test operators.
28. **MATERIALS**
29. Xpert MTB/RIF PT Samples
30. Refer to Xpert MTB/RIF Testing SOP for list of reagents, equipment, and supplies
31. **SAFETY PRECAUTIONS**
32. All testing personnel must receive appropriate safety training prior to working with patient specimens and PT samples.
33. Xpert MTB/RIF PT samples are presumed infectious and must be handled using universal precautions.
34. Wear appropriate personal protective equipment (laboratory coat and disposable gloves) when handling PT samples
35. Wash hands thoroughly after handling samples and test reagents.
36. Use proper technique to minimize aerosols when working with any sample.
37. Do not shake specimens vigorously (may introduce bubbles which affect the test)
38. Do not process grossly leaking specimens
39. Manipulate specimens with care
40. Work space must have adequate ventilation.
41. Waste management
42. Immerse used transfer pipettes and sticks in discard container containing dilution of appropriate disinfectant, placed on the work station.
43. Discard empty specimen containers in infectious waste container.
44. Discard used Xpert MTB/RIF cartridges in infectious waste container.
45. Contaminated wastes (e.g., pipettes, specimens, cartridges) should be removed from the testing site in sealed disposal bags.
46. Discard wastes according to the testing site’s waste management SOP.
47. Clean work bench with paper towels soaked with appropriate disinfectant before starting and after completing work.
48. Clean spills with appropriate disinfectant according to the testing site’s SOP for handling spills.
49. Strict adherence to safety precautions is required at all times. In the event of workplace safety incident, follow the testing site’s SOP.
50. **QUALITY CONTROL**
51. Each Xpert MTB/RIF test includes a Sample Processing Control (SPC) and Probe Check Control (PCC). Refer to Xpert MTB/RIF testing SOP for the purpose of each control and interpretation of results.
52. External controls should be used in accordance with local or national requirements as applicable.
53. **PROCEDURE**

This section provides instructions for receiving and handling PT samples, testing, and recording and reporting PT results.

1. **Receiving and Handling Proficiency Testing Samples (Pre-analytical)**
2. The testing staff are responsible for receiving PT samples.
3. Note the date of receipt of your shipment.
4. Immediately inspect and reconcile the contents of the shipment with the accompanying paperwork.
5. Are the PT samples and accompanying documents (e.g., Kit instructions, Report Form) complete?
6. Was there delay in transport?
7. Are the quality and appearance of the specimens acceptable?
8. Notify supervisor of any shipment or specimen problems. The supervisor will contact the PT provider for resolution of the problem.
9. Note the due date of the results.
10. Log-in PT sample in the assigned register (or computer). Each PT sample must be assigned a separate specimen number.
11. Label PT samples with the specimen number.
12. Store PT samples until testing. Refer to PT Kit Instructions for storage and stability information.
13. **Testing of PT Samples (Analytical)**
14. The supervisor will assign the staff to perform testing of the PT samples.
15. PT samples are assigned to the testing staff on a rotation basis.
16. PT samples are tested by the same personnel who routinely perform the procedure.
17. PT samples are tested and reported by trained and competent staff.
18. PT samples are tested in the same space and using the same PPE and other biosafety precautions for testing patient samples.
19. Complete PT sample testing and submit results within the timeframe given by the PT program.
20. Integrate PT samples into the routine workflow and test in the same manner as patient specimens using the same method (Xpert MTB/RIF Assay).
21. Always refer to the PT provider’s PT Kit Instructions for sample rehydration and special testing instructions.
22. Analyse specimens at correct temperature. If shipment was stored in the refrigerator, samples may need to come to room temperature before testing.
23. If there is more than one instrument used for testing patient samples, testing of PT samples can be rotated among primary instruments for different PT rounds or events.
24. There is no need to order multiple PT kits for the purpose of Xpert MTB/RIF testing on multiple instruments.
25. Do not refer PT samples to a reference laboratory or other testing site for testing.
26. Only accept PT panels from the PT provider. If you receive panels for other testing sites do not test but contact the PT provider immediately.
27. Do not communicate with other testing sites regarding PT samples and results prior to the date of submission of results.
28. **Recording and Reporting PT Results (Post-analytical)**
29. The testing staff is responsible for recording PT result.
30. As soon as testing is completed, record results on the specimen register and on the PT Result Form, following PT provider’s instructions.
31. The supervisor and another individual will review results on the register, instrument print-out, and the PT Result Form for accuracy, completion, and clerical errors.
32. The supervisor and staff who processed or tested the PT samples should sign and date the Attestation Statement on the PT Result Form.
33. The supervisor or designee will send the report to the PT provider according to the PT provider’s instructions.
34. Retain a copy of the completed PT Results Form.
35. Retain specimens until the Participant’s Evaluation Report from the PT provider is received in the laboratory, for investigation of unacceptable PT results, if any.
36. PT records must not be shared with and should not be accessible to personnel of other testing sites until after the deadline for submission of results.
37. **REVIEW OF PT EVALUATION REPORTS**
38. The testing site supervisor is responsible for reviewing PT reports (PT Participant’s Evaluation) and discussing the results with the testing site staff in a timely manner.
39. Review PT results within 10 days of receipt of results from the provider.
40. Review PT results with the staff, which may be done during scheduled staff meetings.
41. Review the accompanying commentaries that will provide continuing education to the staff.
42. Document discussions of PT evaluation reports in the staff meeting minutes or on the PT Participant’s Evaluation Report.
43. The testing site supervisor will ensure that unacceptable PT results are investigated.
44. Each PT result is evaluated to be “acceptable” or “unacceptable” as scored against peer groups by the PT provider.
45. A failed PT event or round is a less than 80% performance for Xpert MTB/RIF assay.
46. Investigate any unacceptable result even if considered a successful event, as this may detect system problems (See Section 10)**.**
47. Ungraded PT results
48. PT challenges may not be graded for many reasons including lack of consensus, late or lack of submission of results, and incorrect completion of results form.
49. Identify the PT result with an ungraded response and review all participant statistics for any explanatory information.
50. Investigate ungraded results determined to be unacceptable upon review in the same manner as graded unacceptable results.
51. **PROFICIENCY TESTING FAILURES OR UNACCEPTABLE RESULTS**
52. The testing site supervisor will discuss unacceptable PT results with the staff.
53. The testing site should make every effort to find the cause(s) of an unacceptable results and design process improvements to prevent re-occurrences.
54. Identify the cause of the unacceptable result through systematic evaluation of the pre-analytic, analytic, and post-analytic phases of testing.
55. Question the staff who processed the specimen and performed the analysis, and review the PT kit instructions to assure the PT samples were handled correctly.
56. Review all recorded data surrounding the PT event, e.g., PT Result Form, instrument print-outs, specimen register, and PT specimen labels. Look for obvious transcription errors including transposed results.
57. Review instrument calibration records, reagent lot records, and storage temperature logs.
58. Use the PT Failure Investigation Form to ensure that possible critical steps in the investigative process were not overlooked.
59. Remedial or corrective action may need to be considered to remove the cause of unacceptable result.
60. Review and monitor the effectiveness of the preventive action taken.
61. Document results of investigation and action taken on the PT Failure Investigation Form (Appendix A).
62. **DOCUMENTATION**
63. The testing site supervisor and staff will ensure that all PT-related documents and records are filed in a timely manner and organized systematically for easy retrieval and review.
64. Compile all PT documents and records in the PT binder per event or round.
65. Completed (signed) PT Result Form
66. PT Participant Evaluation Report from the PT provider, signed and dated by supervisor.
67. PT Failure Investigation Form, completed and signed, if there are any unacceptable results.
68. Instrument reports print-out, Xpert MTB/RIF worksheets.
69. PT Kit Instructions and other documents from the PT provider.
70. The testing site supervisor is responsible for preparing an annual summary of PT scores using the standard form (Appendix B).
71. The annual summary allows easy “at a glance” review of the site’s PT performance, by the supervisor and external assessors.
72. File the completed annual summary form in the PT binder.
73. The testing site supervisor and staff will ensure that the PT Tracking Form (Appendix C) are updated in a timely manner.
74. File the PT Tracking Form in the PT binder.
75. All PT records must be maintained at the testing site for at least two years.
76. **PROCEDURAL NOTES**
77. In the proficiency testing process, the testing sites receive sample from a PT provider.
78. Testing sites are provided challenge samples at regular interval, typically 2-3 times yearly.
79. The testing sites participating in the program analyse the samples and return their results to the PT provider.
80. Results are evaluated and analysed by the PT provider, and the testing sites are provided with information (PT Participant’s Evaluation Report) about their performance and how they compared with other participants.
81. The testing sites use the information regarding their performance to make appropriate changes and improvements.
82. The educational purposes of proficiency testing are best served by a rotation that allows testing personnel to be involved in the PT activities.
83. PT records must be retained and can be an important part of the competency and continuing education documentation in the personnel files of the individuals.
84. To be successful, PT instructions must be followed carefully, all paperwork completed accurately, and results submission deadlines are met.
85. PT is valuable only if the information received is directed to improvement at the testing site.
86. PT have some limitations and it is not appropriate to use PT as the only means for evaluating the quality of the testing site.
87. On-site assessment of the testing site (internal/external assessments), using standardized checklist can give a true picture of a testing site’s overall performance, and offer real-time guidance for improvements that are needed.
88. **RELATED DOCUMENTS**
89. SOP: Xpert MTB/RIF Assay
90. Testing Site Safety Manual
91. **REFERENCES**
92. Laboratory Quality Management Systems Handbook, 2011. WHO/CDC/CLSI.
93. Tuberculosis Laboratory Biosafety Manual, 2012. WHO
94. Strengthening Laboratory Management towards Accreditation (SLMTA) training/mentoring toolkit. CDC and ASCP.
95. Medical Laboratories Requirements for Quality and Competence. ISO 15189:2012
96. Cumitech 3B Quality Systems in the Clinical Microbiology Laboratory, 2005. American Society for Microbiology (ASM), Washington D.C., USA.
97. Astles JR, Stang H, Alpasch T. et al. CLI requirements for proficiency testing: the basics for laboratory professionals. *Med Lab Observ*, 2013:45(9):8.
98. Educational Commentary, 2014 2nd Test Event. American Proficiency Institute. Michigan, U.S.A.
99. College of American Pathologists (CAP) All Common Checklist, 2016. Illinois, U.S.A.
100. **APPENDICES**
101. Appendix A. PT Failure Investigation Form
102. Appendix B. Annual Summary of Xpert MTB/RIF PT Result
103. Appendix C. Xpert MTB/RIF PT Tracking Form

**15.1. Appendix A: Proficiency Testing Discrepancy Investigation Form**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Name of Site:** | |  | | | **Site PT-ID Number:** | | | | |  |
| The Testing Site Supervisor or Laboratory Director must review and document their review of Proficiency Testing results. All PT discrepancies and failures must be investigated (refer to PT SOP). The results of the investigation and all remedial action must be documented. PT documentation must be maintained at the testing site for a minimum of 2 years. | | | | | | | | | | |
| A PT failure ("Unacceptable" result) occurs when the results reported by your laboratory do not fall within the acceptable range for that particular test or analyte. | | | | | | | | | | |
| **PT Sample/s with "Unacceptable" Results:** | | | | | | | | | | |
| Sample ID | Testing Site Result | | Expected Result | | | Date Tested | | | | Tested By |
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| **Problem/Explanation of Findings:**  \*Attach documents as needed | | | | | | | | | | |
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| **Corrective Action/Preventive Action Taken: \***Attach documents as needed | | | | | | | | | | |
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| Person Performing Investigation Signature: | | | |  | | | | Date: |  | |
| Testing Site Supervisor Signature: | | | |  | | | | Date: |  | |

**Proficiency Testing Discrepancy Investigation Form**

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|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **PT Failure Investigation Checklist** | | | | |
| Yes | No | Clerical | | Suggested Actions |
|  |  | Were the result on your evaluation the same as the results you reported? | | Contact the PT provider to correct their error. |
|  |  | Were the results transcribed onto the forms/online correctly? | | Determine how the error occurred and develop an action plan to prevent future clerical errors. |
|  |  | Were the results reviewed by the supervisor prior to submission? | |
|  |  | Were the results submitted to the PT provider by due date? | |
| Yes | No | PT Samples | | Suggested Actions |
|  |  | Was the PT kit received on time and in good condition? | | **Assign** and train staff on proper kit handling protocol. **Develop** a protocol for notifying testing personnel when a PT kit is received. **Complete** PT testing within 7 days of receipt. |
|  |  | Was the PT kit stored per the PT provider's instructions upon receipt? | |
|  |  | Were testing personnel notified promptly about receipt of PT kit? | |
|  |  | Was PT samples testing initiated in a timely manner? | |
| Yes | No | Testing Procedure | | Suggested Actions |
|  |  | Have personnel been trained on the testing procedure? | | **Train** testing personnel and perform competency assessment. **Develop** a policy for periodic retraining and continuing education. **Review** testing SOP if up-to-date; revise if needed and re-train personnel. **Review** PT Kit Instructions for special handling instructions including rehydration method. **To avoid sample mix-u**p during processing, train staff to ensure PT sample tubes are moved to another spot in the rack after reagent is added or sample is added to the cartridge. |
|  |  | Were testing steps followed correctly, according to PT provider's instructions? | |
|  |  | Was sample rehydrated correctly, according to PT provider's instructions? | |
|  |  | Was sample mixed well and at room temperature when tested? | |
|  |  | Was sample tested within specified time from rehydration? | |
|  |  | Was testing done in the correct tube without sample mix-up? | |
| Yes | No | Instrument | | Suggested Actions |
|  |  | Was instrument calibration performed when it was due? | | **Ensure** that instrument calibration is up-tp-date. **Conduct** regular review (at least monthly) of instrument maintenance records, investigate equipment failure and implement corrective / preventive action. |
|  |  | Was the most recent calibration successful? | |
|  |  | Has there been any recent maintenance on the instrument? | |
|  |  | Was routine maintenance performed as required on the day the samples were tested? | |
|  |  | Were instrument problems noted on the day the samples were tested? | |
| Yes | No | Reagents | Suggested Actions | |
|  |  | Were the reagents used within expiration dates? | **Develop** an inventory system to eliminate expired reagents.  **Monitor** temperature of reagent storage areas and conduct regular review (at least monthly) of temperature logs.  **Record** new expiration date on reagent kits when a new pack is opened and put in-use.  **Never** interchange kit components.  **Use** only lots of reagents that has passed new lot testing. | |
|  |  | Were the reagents stored properly? |
|  |  | Were kit components substituted from other kits? |
|  |  | Was QC performed on lot number used for testing? |

Page 2 of 2

* 1. **Appendix B: Annual Summary of Xpert MTB/RIF Proficiency Testing Results**

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| --- | --- | --- | --- |
| **Name of Site:** |  | **Site PT-ID Number:** |  |

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| **YEAR / PANEL** | **Pass/Fail** | | | | | | | | | | |  | | | | **TOTAL SCORE** | | | |
| Sample #1 | | | Sample #2 | | | Sample #3 | | Sample #4 | | | | Sample #5 | | | **%** | **Pass/Fail\*** | | |
| 2018-A |  | | |  | | |  | |  | | | |  | | |  |  | | |
|  | PT failure investigation results and corrective action documented?\*\* Yes/No: \_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | | | | | | | | | | | | | | | |
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| 2018-B |  | | |  | | |  | |  | | | |  | | |  |  | | |
|  | PT failure investigation results and corrective action documented?\*\* Yes/No: \_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | | | | | | | | | | | | | | | |
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| 2018-C |  | | |  | | |  | |  | | | |  | | |  |  | | |
|  | PT failure investigation results and corrective action documented?\*\*Yes/No: \_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | | | | | | | | | | | | | | | |
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| \*Passing PT score is 80% or higher | | | | | | | | | | | | | | | | | | | |
| \*\*All PT failure investigation results and corrective action must be documented on PT Failure Investigation and Corrective Action Form. | | | | | | | | | | | | | | | | | | | |
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| **Prepared by:** | | | Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | | | | | | | | | | | | | |
| Signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | | | | | | | | | | | | | | | | |
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| **Reviewed by (Site supervisor):** | | | | | | Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | | | | | | | | | | |
| Signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | | | | | | | | | | | | | | | | | | | |

**15.3. Appendix C: Xpert MTB/RIF Proficiency Testing Tracking Form**

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Name of Site:** | |  | | | | | **Site PT-ID Number:** | |  |  |
| The testing site supervisor and staff should ensure that the PT Tracking Form is updated in a timely manner and filed in the PT binder. This form is an easy way to track and document participation in the PT program. | | | | | | | | | | |
| **YEAR** | **EVENT** | **PT KIT** | | | **PT RESULT** | | **PT PARTICIPANT EVALUATION** | | | |
| A, B, C | Date Received | Acceptable (Yes/No) | Received by | Date submitted | Submitted by | Date received | Received by | Review date | Reviewed by |
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# Annex 9. New lot testing SOP

#### Introduction

Conditions during transport & storage of Xpert MTB/RIF test reagents may affect their performance. Reagent test failures could indicate that the new batch of reagents are not fit for use.

The use of positive and negative controls to monitor Xpert MTB/RIF performance is recommended for incoming QC of new batches of reagents. QC testing is done on the module that would have run the next patient sample.

#### Materials and Methods

* Positive & negative control strains:
  + Positive control (e.g., PT samples / sputum sample of know reactivity or simulated sample containing MTBC H37Rv). Dried tube specimens (preferred specimen) can be used for QC testing.
  + Negative control (e.g., PT samples / sputum sample of know reactivity or simulated sample containing *M. avium intracellulare*) or water
* Xpert MTB/RIF reagents (2 cartridges & sample reagent) from new lot
* Xpert MTB/RIF reagents (2 cartridges & sample reagent) from current lot
* MGIT 960 instrument
* Two Mycobacteria Growth Indicator Tube (MGIT) (Becton, Dickinson and Company, Sparks, Maryland)
* MGIT Growth Supplement
* Vortex
* Glass beads
* Transfer pipettes

#### Frequency of testing

Positive and negative controls should be tested with each new batch of Xpert MTB/RIF reagents received in the testing site. Testing of controls must be performed before the current batch of reagents reaches a critical low level. If this is not done, and the new batch fails QC testing, laboratories may be forced to suspend testing due to stock-outs.

#### Procedure – PT samples and sputum samples of known reactivity

1. Follow the procedure as described in the insert received with the PT panel or the Xpert MTB/RIF test processing procedure as described in the test information for use.
2. Inoculate the Xpert MTB/RIF cartridges with 1ml of the positive or negative control material.
3. Perform the Xpert MTB/RIF test and record the results*.*

#### Procedure – simulated samples

1. In the BSC, inoculate each MGIT tube (supplement added) with a control strain.
2. Incubate the MGIT tube in the MGIT instrument until the instrument indicates that the controls are positive.
3. Remove the controls from the instrument and chemical inactivate with 3 ml Xpert Sample Reagent.
4. Dilute the 1:100 control strains and disrupt any clumps by vortexing with the glass beads.
5. Inoculate the Xpert MTB/RIF cartridges with 1ml of the positive and negative controls.
6. Perform the Xpert MTB/RIF test and record the results in the new lot QC testing report form*.*

#### Interpretation of results

The interpretation of the incoming QC of new batches testing is shown below:

1. The positive control is expected to be “MTB detected”
2. The negative control is expected to be “MTB not detected”
3. Evaluate the results for both the new and current lots for both the controls. The results from positive and negative controls from new and current lots must be recorded, and unexpected results must be investigated and monitored for trends over time.
4. If new lot reagents do not produce the desired results, these reagents should not be used. Contact the manufacturer for further troubleshooting. If current lot reagents do not produce the desired results, this suggests a procedural problem. Investigate the problem and repeat the test using the new and current lots before proceeding.
5. Label kits with acceptable QC performance as “Ready for Use” or “QC passed” and receipt date.

# New lot QC testing report form

|  |  |  |
| --- | --- | --- |
| **Document type: Form** | **Xpert MTB/RIF New Lot testing\_form.doc** | Place Logo here |
| **Confidentiality: None** |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Xpert MTB/RIF: Current LOT** | | | | | |
| **Date tested** | **LOT**  **number** | **Positive control result:** | **Negative control result:** | **QC Pass**  **/ Fail\*** | **Initials / Comments** |
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**\*To pass QC, the positive control must be “MTB detected” and the negative control must be “MTB Not detected”.**

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| --- | --- | --- | --- | --- | --- |
| **Xpert MTB/RIF: New LOT** | | | | | |
| **Date tested** | **LOT**  **number** | **Positive control result:** | **Negative control result:** | **QC Pass**  **/ Fail\*** | **Initials / Comments** |
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**\*To pass QC, the positive control must be “MTB detected” and the negative control must be “MTB Not detected”.**

The new LOT (Lot number: \_\_\_\_\_\_\_\_\_\_\_\_\_\_), passed / did not pass new LOT QC testing.

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Signed Date

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