GXP: Practical Session I

10 July 2019

TASK™
APPLIED SCIENCE

General & Safety Procedures

- GeneXpert system only to be handled by trained persons
- Before starting, read the operator manual
- Read and follow instructions in product insert of Xpert MTB/Rif cartridge

- Process all clinical specimens as per the biosafety standards:
  - Treat all sputum specimens, incl. used cartridges, as potentially infectious
  - Wear protective gloves and laboratory coats when handling specimens and reagents.
  - Wash hands thoroughly after handling specimens and test reagents.
  - All benches to be decontaminated after work, or immediately after a spill, with appropriate mycobactericidal disinfectant followed by 70% alcohol
  - Dispose of used Xpert MTB/RIF cartridges according to infectious waste material disposal guidelines.

- Do not:
  - Open Xpert MTB/Rif cartridge lid except when adding sample
  - Reuse Xpert MTB/Rif cartridges

Materials & System Requirements

System:

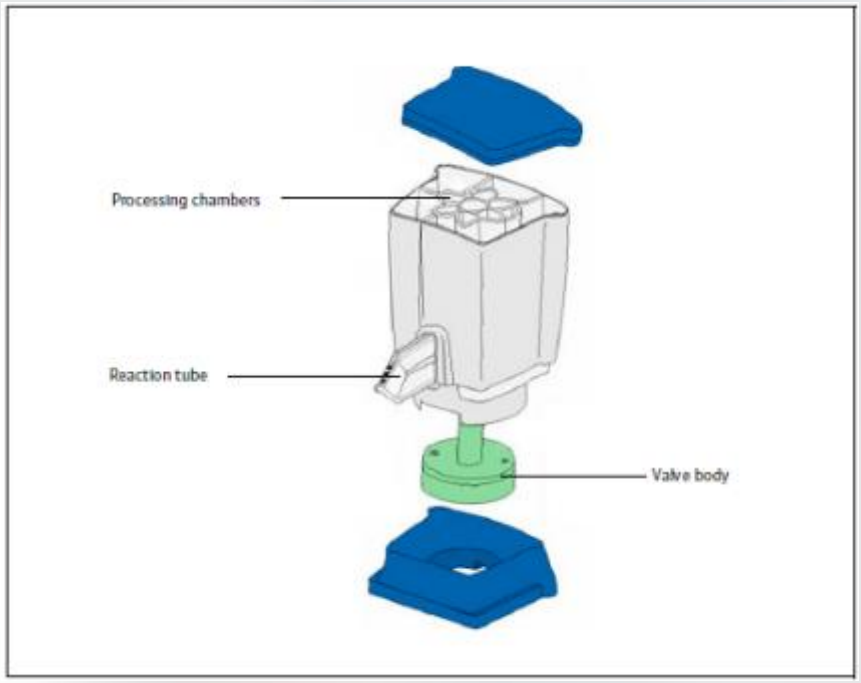
- GeneXpert System equipped with GX2.1 software / computer / printer / barcode wand-reader and operator manual (Cepheid Inc, Sunnyvale, USA)
  - Available in a one, two, four, or 16-module configuration

- Class II biological safety cabinet (BSC)

Materials & System Requirements

GeneXpert Cartridge & Reagents:

- Single-use disposable XpertMTB / RIF cartridges:
  - Sample extraction, amplification & detection all carried out within this self-contained cartridge

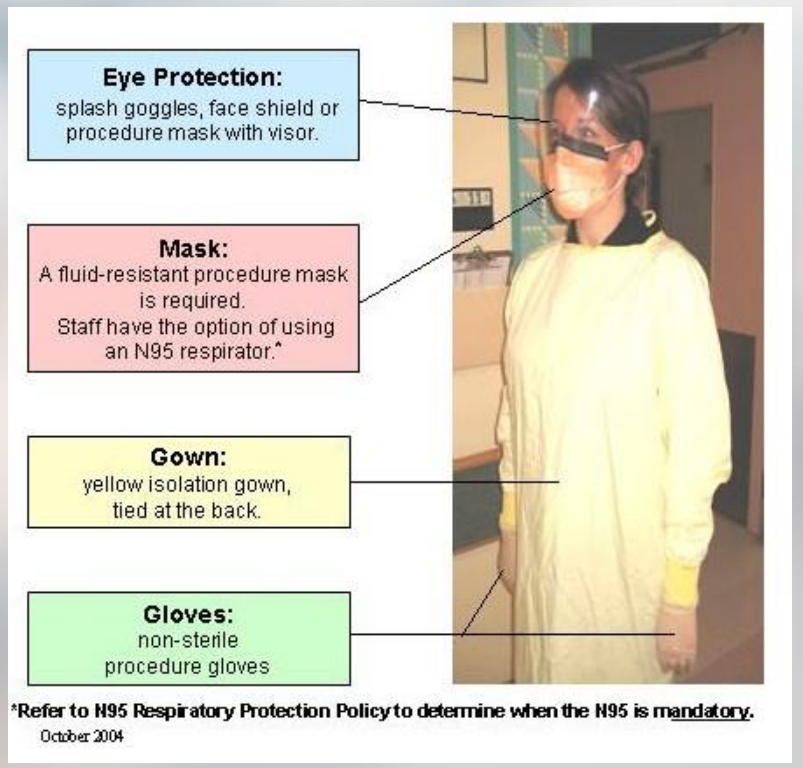


- Sample reagent (provided in Xpert MTB/RIF kit), 8ml volume pack per each cartridge. The sample reagent solution is clear, but may range from colorless to golden yellow.

Materials & System Requirements

Other Materials:

- Personal protective equipment (PPE)
- Permanent marker pens
- Sterile (individually packed) disposable transfer pipettes– with single mark for minimum volume of sample transfer to cartridge (provided in Xpert MTB/RIF kit)
- Sterile screw-capped specimen collection containers / cups
- Discard containers for pipettes and sputum containers



Procedures

- Options:
  1. Expectorate sputum sample
  2. Decontaminated sputum pellet

- Different preparation procedures for each

- Only begin testing if sufficient volumes

See below for fresh sputum or re-suspended BAL sediment volumes required for the assay

Specimen type	Minimum volume per test	If repeat testing is required, minimum volume for 2 tests
BAL sediment	0.5 ml	1 ml
Fresh sputum	1 ml	2 ml

Procedure: Expectorated Sputum Samples

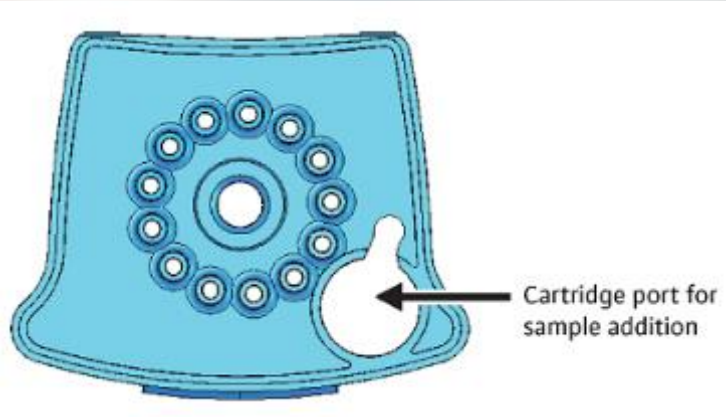
- Unscrew lid of sputum collection container
- Using a plastic disposable pipette, measure and note sputum volume
  (Note: maximum volume on graduated disposable pipette is 3ml)
- Record the volume on the Xpert/Rif worksheet
- Carefully discard the pipette
- Using separate plastic disposable pipette, add sample reagent at 2:1 ratio (2v reagent : 1v sputum)
- Replace the lid of sputum cup tightly
- Shake sputum cup vigorously 10-20 times using back and forth movements in a single shake
- Incubate sample in the sputum cup for 15 minutes at room temperature.
- During the incubation period, shake the sputum cup at least once, as above
- Sputum sample should be liquefied with no visible clumps of sputum after incubation. Particulate matter may exist that is not part of the sample.

Procedure: Decontaminated Sputum Sediment

- Label a 50ml centrifuge tube with the lab number
- Use the sediment obtained from a decontaminated sputum sample
- If sediment volume < 2ml, add 0.01% Tween 80 saline to obtain final volume of 2ml
- Homogenize resuspended sediment by vortexing for 10 seconds
- Aliquot 500ul (0.5ml) of homogenized suspension into the labelled 50ml centrifuge tube, using sterile, disposable 1ml Pasteur pipette; discard the used pipette
- Keep residual 1.5ml of sample in 50ml centrifuge tube and store refrigerated at 4^{o}C for repeat testing if needed
- Add 1.5ml of Xpert MTB/Rif Sample Reagent (SR) to the 0.5ml sediment using sterile, disposable 3ml Pasteur pipette
- Vortex for 10 seconds
- Incubate at room temperature for 10 minutes
- Vortex again for 10 seconds
- Incubate at room temperature for another 5 minutes

Preparing the Cartridge

- Label each cartridge with the laboratory number (as on the sputum cup / centrifuge tube)
  - Write with marker pen on the front side bottom of the cartridge, never on the lid of cartridge / on barcode
- Note: Start test within 30 minutes of adding sample to cartridge
- Using the pipette provided in the Xpert/Rif kit, draw liquefied sample until meniscus of pipette is above the minimum mark (2ml)
  - Avoid touching the pipette & retain the paper cover
- Open cartridge lid & transfer sample into the open port; dispense slowly to minimize risk of aerosol formation
- Put the pipette back into paper cover & discard into bio-hazard waste bin
- Close cartridge lid ensuring the lid snaps firmly into place.
- Remaining liquefied sample may be kept for 12 hours at 2-8°C (repeat testing).
- NB: Load cartridge into instrument & start test within 30 minutes of preparing



Starting the Test

- NB: Before starting the test, ensure the system attached to a working uninterrupted power source (UPS)
- Turn on computer & enter password
- Turn on GeneXpert Dx instrument
- On computer, double-click GeneXpert Dx shortcut icon
- In GeneXpert Dx system window, click “Create Test”
- When “Scan Cartridge Barcode” dialog box appears, either:
  1. Scan barcode of sample ID / lab number, or
  2. Skip or cancel the dialog and select “Manual Entry” & type in sample ID / lab number (which matches cartridge & sputum cup)
- Close door of module firmly (an audible click sound should be heard)
- The test starts and the green light stops blinking; when the test is finished, the light turns off
- Continue with loading next cartridge following the steps described above
- To each specimen in sputum container with screw cap:
- Once run is completed (<2 hours), results are printed automatically
- Wait until the system releases the door lock at end of run, open module door and remove the cartridge & dispose in biohazard waste container

THANK YOU

Wait 1hour 55 minutes