GXP: Practical Session I

10 July 2019
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, Sunnyvalle, USA)
  - Available in a one, two, four, or 16-module configuration

- Class II biological safety cabinet (BSC)
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.
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

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Minimum volume per test</th>
<th>If repeat testing is required, minimum volume for 2 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL sediment</td>
<td>0.5 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>Fresh sputum</td>
<td>1 ml</td>
<td>2 ml</td>
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</tbody>
</table>
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°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

1. **NB:** Before starting the test, ensure the system attached to a working uninterrupted power source (UPS)
2. Turn on computer & enter password
3. Turn on GeneXpert Dx instrument
4. On computer, double-click GeneXpert Dx shortcut icon
5. In GeneXpert Dx system window, click “Create Test”
6. 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)
7. Close door of module firmly (an audible click sound should be heard)
8. The test starts and the green light stops blinking; when the test is finished, the light turns off
9. Continue with loading next cartridge following the steps described above
10. To each specimen in sputum container with screw cap:
11. Once run is completed (<2 hours), results are printed automatically
12. 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 1 hour 55 minutes