Evaluation of the GeneXpert MTB/RIF Assay for Rapid Diagnosis of Tuberculosis and Detection of Rifampin Resistance in Pulmonary and Extra-pulmonary Specimens and comparison with smear ZN staining technique-Hospital based study.

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Introduction
Mycobacterium tuberculosis remains one of the most significant causes of death from an infectious agent. The rapid detection of tuberculosis and detection of rifampin resistance (RIF) is essential for early drug management. The GeneXpert MTB/RIF assay is a novel integrated diagnostic device for the diagnosis of tuberculosis and rapid detection of rifampin resistance in clinical specimens. We determined the performance of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in sputum-positive and smear-negative pulmonary and extra-pulmonary specimens obtained from suspected tuberculosis cases. Xpert is a card-based, fully automated, real-time nucleic acid amplification test for rapid detection of M. tuberculosis and RMP resistance, which is a good marker for multidrug-resistant TB (MDR-TB). Results are obtained within 3 h, which helps reduce the length of hospitalization and the number of deaths due to TB.

Objectives
To have a best diagnostic test in extra pulmonary specimen samples like sputum aspirate, CSF, pleural aspirates and other investigations are invasive. Hence the Xpert MTB/RIF may be a better choice to avoid spread of MDR-TB especially in developing countries. To detect MTB in HIV-positive patients as there is immune suppression of extra-pulmonary sites which is rapidly progressive.

Principle

Xpert MTB/RIF Molecular Beacon Assay

Common rpoB mutation

Rifampin binds to the beta subunit of the RNA polymerase, preventing transcription. Mutations in the rpoB gene prevent the binding of rifampin.

As determined by WHO, RIF resistance is almost uniformly seen in drug-resistant (MDR-TB) strains and has a reported frequency of greater than 95% in such isolates.

Extra Pulmonary samples

- Gastric aspirate
- Lymph node aspirate
- Pleural effusion
- CSF, pleural, blood, BAL, liver aspirates, Tissues, Pus
- EBUS (Endobronchial ultrasound) specimens
- Urethral Gram stain and culture
- Add the pre-probe phosphatase buffer to the blind, mark on the centrifuge tube and mix. Centrifuge for 15-20 mins at 3000 rpm.
- Carefully decant all of the supernatant fluid.
- Add a small volume of phosphate buffer (10 X 1000 µl) and reconstitute the pellet.
- Use the suspensions for the preparation of smear and the performance of Mycobacteriological procedures.

Good Laboratory Practices

- Ensure there is a sufficient sample volume.
- Process samples as soon as possible (storage at 4°C for 72h possible).
- Control samples are successfully processed.
- Ensure there are no visible particles transferred to the smear.
- Don’t dilute sample additionally, as submission concentration will already be low.
- Non-smear samples (e.g., like gastric fluid, lymph node aspirate) might need an additional decontamination procedure.
- When possible, perform culture (currently as sensitivity is higher for all sample types).
- Use aerobic incubation procedures.
- Xpert MTB/RIF assay is not used in extra-pulmonary samples. Do NOT use Xpert MTB/RIF on blood samples.

Results

- Out of 125 samples, 93 (74.4%) were smear negative & gene probe positive sensitive 32 (25.6%) were smear positive & gene positive, resistant 15 (12%) were smear negative gene positive.
- Of 20 samples from blood, 15 were EBCS samples, 3 were EBUS samples, 2 were BAL samples, 3 were CSF samples. Enrols 4 days.
- To ensure the specificity and sensitivity was 89% and 75% as compared with culture sensitivity and specificity of the Xpert assay were calculated to be 87.5% and 97.5% respectively.

Advantages of Gene Xpert

- Sample collection can be done on board
- Precise pipetting is not required
- Manual PCR tagging, 3 probes of the spoligotype
- Detects MDR
- RIF sensitivity: 97.6%
- RIF sensitivity: 99.9%

References