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 diagnosis of tuberculosis and detection of rifampin (RIF) resistance are essential for early disease management. The GeneXpert MTB/RIF assay is a novel integrated diagnostic device for the diagnosis of tuberculosis and rapid detection of RIF resistance in clinical specimens. We determined the performance of the MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in smear-positive and smear-negative pulmonary and extra pulmonary specimens obtained from suspected tuberculosis cases. Xpert is a cartridge-based, fully automated, real-time nucleic acid amplification test for rapid detection of M. tuberculosis and RMP resistance, which is a good marker for multi- drug-resistant TB (MDR-TB). Results are obtained within 3 h, which helps reduce the length of hospital- isolation and the number of deaths due to TB.

Objectives

To have a best diagnostic test in extra pulmonary precious samples like organ aspirates, CSF, Tissues etc where other investigations are inconclusive.Detect the MTB in initial stage of infection along with drug sensitivity to prevent spread of MDR TB especially in developing countries.To detect MTB in HIV positive patients as there is more involvement of extra pulmonary sites which is rapidly progressive.

Principle

Xpert MTB/RIF Molecular Beacon Assay

Common rpo B Mutation

- Rifampin binds to the beta subunit of the RNA polymerase, preventing transcription.
- Mutations in the rpoB gene prevent the binding of rifampin.
- As documented by WHO, RIF resistance is most commonly seen in multi-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
- FLUIDS-Cerebrospinal fluid, ascitic, peritoneal, pleural, BAL, liver aspirates,
- Tissues,
- PUS
- EBUS (Endobronchial ultrasound) specimens
- Urine

Materials and Methods

- Prepare the Mycoprep phosphate buffer as needed, by pouring contents of one packet into a 500ml volumetric flask and fill to line with purified water .Transfer the buffer solution to a screw capped container and, with cap loosened ,autoclave with 121degrees for 15 min. Cool to room temperature and tighten cap.
- Using caution not to spill, loosen screw-cap on the Myco prep Reagent bottle. Locate ampule in bottle, squeeze excess air from the bottle and tighten cap. With bottle in the upright position, squeeze the bottle until the ampule breaks. (Note: the 150mL bottle contains two ampoules that must be broken) Shake gently to dissolve the NALC. Avoid excessive agitation.
- In a biological safety cabinet, using a sterile aerosol free 50ml centrifuge tube with screw cap, add equal amounts of specimen and activated NALC NaOH solution (app 10 ml each).
- Cap the Centrifuge tube and mix on a vortex type mixer until specimen is liquefied. If specimen is especially viscous. Add more NALC-NaOH solution and repeat mixing.
- Allow mixture to stand at room temperature for 15 min with occasional gentle shaking.
- Avoid Over treating the Specimen.
- Add the prepared phosphate buffer to the 50mL mark on the centrifuge tube and mix. Centrifuge for 15-20 min at 3000xg.
- Carefully decant all of the supernatant fluid.
- Add a small quantity of phosphate buffer of PH 6.8(e.g., 0.5 to 2.0 mL) and resuspend the Sediment.
- Use the suspension for the preparation of Smears 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 7days possible)
- Control sample is sufficiently homogenized.
- Ensure there are no visible particles transferred to the cartridge.
- Don't dilute samples additionally, as TB bacilli Concentration will already be low.
- Non-sterile samples (e.g. like gastric fluid, Lymph node aspirate) might need an additional decontamination procedure.
- When possible perform culture concurrently as sensitivity is higher for all sample types.
- Ensure adequate biosafety procedures, Do NOT use Xpert MTB/RIF on blood samples.

Results

- Out of 125 samples 90/125[72%] were smear negative & gene expert negative 32/125[25.6%] were smear positive & gene expert positive, 15/125 [12%] were smear negative gene expert positive.
- These 20 samples were 3 are BAL samples and 2 are EBUS samples, 5 CSF samples, Tissues -10 samples..
- In smear the sensitivity and specificity was 67%and 75% as compared to the combined sensitivity and specificity of the Xpert assay were calculated to be 87.3% and 97%, respectively.
- Addendum to the abstract sent-In extra pulmonary samples were other 5 CSF samples were received of which all were smear negative Genxpert positive were 3(60%) and 2 were negative, tissue samples of 10 samples 7 were expert positive(70%) 3 were negative and all were negative for AFB stain.

Interpretation of Results

- The results are interpreted by the Gene Xpert DX system from measured fluorescent signals and embedded calculation algorithms and will be displayed in the "View Results" Window.
- Lower Ct values represent a higher starting concentration of DNA template
- Higher Ct values represent a lower concentration of DNA template. MTB detected MTB target DNA is detected.
- MTB Detected-The MTB result will be displayed as High, Medium, Low or Very low depending on the Ct value of the MTB target present in the sample. The table lists the Ct value ranges for the displayed MTB results.
- MTB Result Ct (Threshold cycle)
  High <16
  Medium 16-22
  Low 22-28
  Very low >28

Studies from Journals

- AMERICAN SOCIETY FOR MICROBIOLOGY-The combined sensitivity and specificity of the Xpert assay were calculated to be 77.3% and 98.2%, respectively.
- EUROPEAN JOURNAL- In lymph node tissues or aspirates, Xpert pooled sensitivity was 83.1% (95% CI 71.4–90.7%) versus culture and 81.2% (95% CI 72.4–87.7%) versus CRS. In cerebrospinal fluid, Xpert pooled sensitivity was 80.5% (95% CI 59.0–92.2%) against culture and 62.8% (95% CI 47.7–75.8%) against CRS. In pleural fluid, pooled sensitivity was 46.4% (95% CI 26.3–67.8%) against culture and 21.4% (95% CI 8.8–33.9%) against CRS. Xpert pooled specificity was consistently >98.7% against CRS across different sample types.
- WHO study- Gen xpert -98.2%sensitivity in smear positive pulmonary samples
- 72.5% sensitivity in smear negative pulmonary samples
- Specificity is 99.2%

Advantages of Gene Xpert

- Sample extraction can be done on board
- Precise pipetting is not required
- Hemi nested PCR targeting 5 probes of the rpoB gene.
- Detects MTB complex
- RIF sensitivity -97.6%
- RIF specificity-98.1%

Conclusion

- The MTB/RIF test is less dependent on the user's skills, and routine staff with minimal training can use the test. It has a short turnaround time and simultaneously detects M.tuberculosis and RIF resistance in less than 3 h.
- No chance of cross contamination. As per the study Gene xpert is more sensitive in detecting from extra pulmonary samples useful in detecting TB in case of low bacilli load where it cannot be detected by sputum AFB smear.
- Claimed to be more sensitive in CSF samples.

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