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ORIGINAL ARTICLE

# Comparative Study between Line Probe Assay by MTBDR*plus* (Hain PCR) and XpertMTB/RIF assay (GeneXpert PCR) for the Detection of *Mycobacterium Tuberculosis* Complex Directly from Clinical Samples and from Cultured isolates

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***Key words:***

***GeneXpert, Hain, MTBC, VersaTREK***

## ABSTRACT

***Background:*** *Tuberculosis remains a major public health problem worldwide. Nucleic acid amplification tests (NAATs) have been introduced to overcome conventional methods' low sensitivity and diagnostic delays. These rapid tests such as the line probe assay (LPA) and XpertMTB/RIF assay can provide results within hours directly on specimens and thus enable prompt and appropriate treatment, decrease morbidity and mortality, and interrupt transmission.* ***Objectives:*** *The aim of this study was to evaluate the efficiency and reliability of MTBDRplus and XpertMTB/RIF assay for the detection of Mycobacterium tuberculosis complex (MTBC) in smear-positive and smear-negative pulmonary and extrapulmonary specimens in comparison to the gold standard VersaTREK liquid culture system.* ***Methodology and Results:*** *In this study, a total of 130 samples were obtained from tuberculous patients. The percentages of the MTBC detection in AFB-negative samples by Hain PCR was 0.0%,while, the percentages of the MTBC detection in AFB-positive (+1), (+2), (+3) and(+4) samples by Hain PCR were 0.0%,55.6%, 75% and 93.8%. The percentage of the MTBC detection in AFB-negative samples by GeneXpert PCR was higher than those which were detected by Hain PCR (30.4% vs 0.0%) with high significant difference. Mean while, the percentage of the MTBC detection in AFB-positive (+1) samples by GeneXpert PCR was higher than those which were detected by Hain PCR (100% vs 0.0%) with high significant difference. The percentage of the MTBC detection in total AFB-positive samples by GeneXpert PCR was higher than those which were detected by Hain PCR (90.5% vs 54.8%) with high significant difference. The sensitivity, specificity and accuracy of Gene Xpert PCR were 64.9%,0.0% and 56.9% while, they were40.4%,100% and 47.6% by Hain PCR. The degree of detection of MTBC by GeneXpertPCR was correlated with the results of AFB staining with high significant difference.* ***Conclusion****: our findings showed the superior sensitivity of the Xpert MTB/RIF compared to the MTBDRplus in the detection of MTBC. However, the molecular drug susceptibility testing results should always be confirmed by phenotypic methods.*

## INTRODUCTION

Tuberculosis (TB) remains a major public health problem, accounting for more than 9.4 million incident cases and 1.3 million deaths every year, worldwide[1].

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The World Health Organization (WHO) had issued 10 policy statements for improving diagnosis of TB, including the use of commercial and non-commercial drug-susceptibility testing (DST) methods and implementation of molecular methods[2].

Nucleic acid amplification tests (NAATs) have been introduced to overcome conventional methods low sensitivity and diagnostic delays. The NAATs's sensitivities are high for specimens that are acid-fast bacilli (AFB)-positive microscopically, but lower for AFB-negative specimens, taking in consideration that the identification of mutations associated with drug-resistance requires additional NAATs[3].

These rapid tests such as the line probe assay (LPA) and XpertMTB/RIF (GeneXpert) assay can provide results within hours directly from specimens without culture and thus provide rapid and appropriate treatment, decrease morbidity and mortality, and interrupt transmission[4] .

Currently, two commercial line probe assays (based on reverse-hybridisation DNA strip technology) exist, the INNO-LiPA RIF/TB (Innogenetics, Ghent, Belgium) and GenoType MTBDR (HainLifeScience GmbH, Nehren, Germany). The LiPA test can simultaneously detect *Mycobacterium tuberculosis* complex(MTBC) and the presence of a mutation in the *rpo*B gene, which confers resistance to rifampicin[5] .

The GenoTypeMTBDR assay (Hain PCR) has an additional advantage over the LiPA because it can detect both rifampicin (RMP)- and isoniazid (INH)-resistance. The MTBDR*plus*assay identifies mutations in the *rpo*B gene as well as mutations in the *kat*G gene for high-level isoniazid-resistance. The MTBDR*plus*, the second-generation assay, also detects mutations in the *inh*A gene that confers resistance to low-levels of isoniazid[6] .

The XpertMTB/RIF assay (GeneXpert, Cepheid, Sunnyvale, CA) detects the presence of MTBC-DNA and its susceptibility to rifampicin in a single reaction[7,8]. Mono-resistance to RMP is not common, however, >90% of RMP resistant isolates also exhibit resistance to isoniazid (INH). Therefore, the detection of RMP-resistance may serve as a surrogate marker for multi-drug resistant TB(MDR-TB)[9].

GeneXpert uses hemi-nested real-time PCR to amplify an *M. tuberculosis*-specific sequence of the rpoB gene. The RMP resistance is detected by determining the region of the rpoB gene which is probed with molecular beacons[10] .The assay can be carried out in a nearly fully automated manner, including bacterial lysis, nucleic acid extraction and amplification, and amplicon detection[11] .The test is carried out within 2 h in a disposable cartridge. The only manual step is the mixing of a bactericidal buffer with the sample prior to addition to the cartridge. This pre-amplification step reduces the viability of MTBC organisms, making the assay suitable for use near patients in settings with limited bio-containment facilities[12] .

The aim of this study was to evaluate the efficiency and reliability of the MTBDR assay (Hain PCR) and GeneXpert PCR for the detection of *M. tuberculosis* bacilli in smear-positive and smear-negative pulmonary and extra-pulmonary specimens in comparison to the gold standard conventional liquid culture and phenotypic susceptibility testing methods.

## METHODOLOGY

This is a prospective study which was conducted in the Clinical and Molecular Microbiology Laboratory at King Abdulaziz University Hospital (KAUH), Jeddah , KSA, from June 2014 to June 2015.

**1- Patients**:

This study was carried out on 130 diagnosed tuberculous patients. All patients were subjected to full history taking, full clinical examinations with radiological and routine laboratory investigations.

**2- Methods:**

**A- Preparation of Specimens**: Each specimen (except CSF which was used directly) was digested and decontaminated by ready to use N- acetyl- l- cysteine (NALC) 3% NaOH method ***(Nac-PACTMEA3, Alpha Tec, Inc, Vancouver, Washington, USA)*** as described by the manufacturer and concentrated by centrifugation at 3000 rpm for 20 minutes[13].The supernatant of each sample was removed and the sediment was resuspended in maximum of 1 to 1.5 ml of phosphate buffer. Part of the resultant resuspended pellet of any specimen was used for detection of MBTC isolates by the smear microscopy using fluorochrome stain (Fluo-RAL-Auramine staining kit for Mycobacteria detection by RAL-automated staining system-R.A.L. instruments, Montesquieu-33650 MARTILLAC-France) with examination under the fluorescent microscope and the rest of the re-suspended pellet was used for culture in the VersaTREK liquid culture system as well as for direct MTBC detection by GeneXpert PCR and by Hain PCR.

**B-Detection of MTBC directly from decontaminated clinical sample by;**

**1) The GeneXpertDx system:**

***(Cepheid 904 Caribbean Drive Sunnyvale, CA 94089-1189-USA)***

***a- Sample sediment procedure:***

Each MTB/RIF cartridge was labeled by the sample ID, then 1 ml of the total resuspended pellet was transferred to a conical screw-capped tube for the Xpert MTB/RIF by a pipette, followed by a transfer of 2 ml of the Xpert Sample Reagent (SR) to the re-suspended sediment, then vortex for 10 seconds was done. The conical screw-capped tube was incubated for 10 minutes at room temperature, then vortex was done and re-incubated for additional 5 minutes. The liquefied sample was transferred to the sample chamber of the Xpert MTB/RIF cartridge and the cartridge was loaded into the GeneXpertDx Instrument. The test was started and the running software was was GX 4.0[11] .

***b- Sample preparation and extraction of nucleic acids:***

The GeneXpert System completely integrates and automates the sample preparation and performs all the complex steps of nucleic acid extraction in its advanced microfluidic cartridge[11,14].

***c- Amplification of the extracted nucleic acids:***

The GeneXpert System modules performe rapid heating and cooling cycles required for highly reliable real-time PCR in the reaction tube of the cartridge in order to quickly create enough copies of the sample nucleic acids for reliable measurement[11,14].

***d- Detection of a target gene sequence:***

The GeneXpert System' s multiple optics can detect the presence of multiple target nucleic acids in the same cartridge as soon as the target was detected[11,14].

***e- Results Interpretation:***

The GeneXpert Insturment system generated the results from measured fluorescent signals and embedded calculation algorithms. The detection of MTBC (very low, low, medium, or high) was dependent on the number of the TB bacilli in the sample and also confered RIF resistance detected or not detected[11,14].

**2) GenoType (MTBDR*plus*) by HainLifescience GmbH;**

***(Hardwiesenstrabe 1, 72147 Nerhren, Germany)***

The GenoType MTBDR plus VER 2.0 is a qualitative in vitro test for the extraction of bacteria DNA with identification of MTBCand resistance to RMP by detecting *rpo*B gene (coding for the β-subunit of the RNA polymerase) mutations associated with rifampicin-resistance and for detection of the *kat*G gene (coding for the catalase peroxidase) and the promoter region of the *inh*A gene (coding for the NADH enoyl ACP reductase) associated with isoniazid (INH)-resistance from smear-positive or negative clinical specimens and cultivated samples[15,16,17].

***a- Specimen preparation:***

Clinical specimens must be processed as before. After decontamination, the cell pellet should be resuspended in maximum of 1 to 1.5 ml of phosphate buffer. Higher volumes might hamper the sensitivity of the test.

***b- DNA Extraction by GenoLyse® kit:***

A total of 1 ml of the re-suspended pellet was directly applied by spinning for 15min at approximately 10,000×g. The supernatant was discarded and bacteria resuspend in 100-300µl of water molecular biology grade by vortexing and incubated for 10 min at 95°C in water bath and for 15 min in an ultrasonic bath, then spined down for 5 min at full speed. Finally, 5µl of the supernatant was directly used for PCR[15,16,17].

***c- Amplification:***

All reagents needed for amplification such as polymerase were included in the amplification mixes A and B (AM-A and AM-B) and were optimized for this test. For each sample, 10µl AM-A, 35µl AM-B and 5µl DNA solution with final volume of 50µl. A total of 45µl of the master mix was aliquoted in each of prepared PCR tubes and 5µl of water (molecular biology grade) was added to one aliquot (negative control) and 5µl of DNA was added to each aliquot except for negative control. A thermal cycler from HainLifescience with the respective pre-installation was used and protocol "MDR DIR. SCR" was selected for clinical samples[15,16,17].

***d- Hybridization:*** was done as recommended by the manufacturer instructions[17].

***e- Evaluation and interpretation of the results of GenoTypeMTBDRplus:***

The developed strips were pasted in the designated fields by aligning the bands of conjugate control (CC) and amplification control (AC) with the respective lines on the evaluation sheet which was provided with the kit.The MTBC and the resistance statuswere determined with the help of the interpretation chart[17].

**C- Inoculation of VersaTREK 528:**

***(Trek Diagnostic Systems , Inc. Westlake. Ohio, USA)***

**a- Bottle Preparation and Inoculation:**

***(Remel™ Oxoid™Versa TREK and Sensititre™ products)***

Antibiotic Inhibitor (supplement) bottle was reconstituted according to the manufacturer's instructions. A total of 1ml of Myco growth supplement was aseptically added to each Myco broth bottle to be used for culture, then, 0.5 ml of reconstituted antibiotic inhibitor supplement was also added to each bottle. Finally, up to 1.0 ml of concentrated clinical specimen was added and the bottle was vortexed. After that, the connector was placed on the top of the bottle which was loaded into the VersaTREK instrument and incubated till signaled positive (the maximum incubation time for the bottles is 42 days ) [18].

**D- Classification of positive isolates by;**

**1) The GeneXpertDx system*:***

***(Cepheid 904 Caribbean Drive Sunnyvale, CA 94089-1189-USA)***

As we used the 1 ml of the resuspended pellet of the processed sample, we can use 1 ml from the positive culture bottle to follow the same steps as before for the detection of MTBC and also to detect *rpo*B gene mutations associated with rifampicin (RMP)-resistance[11,14].

**2) GenoType (MTBDR*plus*) by HainLifescience GmbH;**

***(Hardwiesenstrabe 1, 72147 Nerhren, Germany)***

***a- Specimen preparation:***

A total of 1 ml from positive liquid culture medium from VersaTREK used as starting material for DNA extraction to follow the same steps as before.A thermal cycler from HainLifescience with the respective pre-installation was used and protocol "MDR CUL. SCR" was selected for cultivated samples[17].

**3) GenoType (Mycobacterium CM) by HainLifescience GmbH;**

***(Hardwiesenstrabe 1, 72147 Nerhren, Germany)***

The GenoType Mycobacterium Common Mycobacterium (CM) permits the identification of many non-tuberculous Mycobacteria (NTM) as *M. avium*ssp., *M. intracellulare, M. chelonae ….etc.*

***a- Specimen preparation:***

A total of 1 ml from positive liquid culture medium from VersaTREK was used as starting material for DNA extraction to follow the same steps as before for detection of MTBC except for the composition of amplification mix of GenoType MTBDR plus which contain the following per tube; 35 µl PNM, 5 µl 10x polymerase amplification buffer for HotStarTaq (contains 15 mM $Mgcl_2$), 2 µl 25 mM $Mgcl_2$, 0.2 µl (1 U) HotStarTaq, 3µl of water (molecular biology grade) and 5µl DNA solution. The final $Mgcl_2$ concentration in this amplification mix was 2.5 Mm.

A thermal cycler from HainLifescience with the respective pre-installation was used and protocol "HOT 30" was selected for cultivated samples[19].

***b- Evaluation and interpretation of results GenoType (Mycobacterium CM):***

The developed strips were pasted in the designated fields by aligning the bands CC and UC with the respective lines on the evaluation sheet which was provided with the kit. The NTM species were determined with the help of the interpretation chart[19].

**E- Susceptibility testing for Anti-TB drugs by VersaTREK:**

***(Remel™ Oxoid™ Versa TREK and Sensititre™ products)***

***a-Preparation of anti-tuberculous drugs*:**

Rifampicin, isoniazid, ethambutol, streptomycin and pyrazinamide were reconstituted according to manufacturer's instructions. A total of 1.0 ml of the positive culture was taken and 9.0 ml of distilled water was added and vortex was done, then 0.5 ml of this diluted positive culture was used for inoculation of each mycobroth bottles[20].

***b- Sensitivity by Myco-Susceptibility kit:***

Eight new Mycobroth bottles were prepared for each positive MBTC culture according to manufacturer's instructions[20].

***c- Sensitivity for Myco-Pyrazinamide (PZA) Kit :***

Two Myco broth bottles were prepared according to manufacturer's instructions[20].

***d- Results Interpretation:***

The isolate in a drug-containing bottle is considered to be resistant when its time for detection is equal to, or within three days from the detection time of the drug-free control bottle [20].

**Statistical analysis**

Data were analyzed using Statistical Package for Social Sciences (SPSS) software, version 18. Chi square test was utilized to test for the association and/or difference between categorical variables. Fisher exact test was used when appropriate. Continuous variables were presented as mean, standard deviation and range. Student t test was used to compare between two groups. P value less than 0.05 was considered statistically significant.

## RESULTS

In this study, a total of 130 samples (sputum;66, tracheal aspirate;22, bronchial wash;8, cerebrospinal fluid;2, cyst;12, tissue;12, peritoneal fluid;2 and pleural fluid;6)were obtained from tuberculous patients who were admitted to King Abdulaziz University Hospital (KAUH), Jeddah , KSA. MTBC were detected in 104 samples and NTM were detected in 26 samples.

Our results revealed that, the percentages of the MTBC detection in AFB- negative samples and AFB-positive (+1) samples by Hain PCR were 0.0% and 0.0% respectively, while, the percentages of the MTBC detection in AFB-positive (+2), (+3) and(+4) samples by Hain PCR were 55.6%, 75% and 93.8%Table 1.

The percentage of the MTBC detection in AFB-negative samples by GeneXpert PCR was higher than those which were detected by Hain PCR (30.4% vs 0.0%) with high significant difference Table 2.

The percentage of the MTBC detection in AFB-positive (+1) samples by GeneXpert PCR was higher than those which were detected by Hain PCR (100% vs 0.0%) with high significant difference Table 2.

The percentage of the MTBC detection in total AFB-positive samples by GeneXpert PCR was higher than those which were detected by Hain PCR (90.5% vs 54.8%) with high significant difference Table 2.

The percentage of the MTBC detection in total samples by GeneXpert PCR was higher than those which were detected by Hain PCR (69.2%% vs 35.4%) with high significant difference Table 2.

The percentage of the MTBC detection in AFB-negative samples by VersaTREK was higher than those which were detected by Hain PCR (95.7% vs 0.0%) with high significant difference, while, the percentage of the MTBC detection in AFB-positive (+1) samples by VersaTREK were higher than those which were detected by Hain PCR (53.8% vs 0.0%) with high significant difference and the percentage of the MTBC detection in AFB-positive (+2) samples by Versa TREKwere higher than those which were detected by Hain PCR (88.9%vs 55.6%) with high significant difference Table 3.

The percentages of the MTBC detection in AFB-positive (+3) and (+4) samples by VersaTREK were higher than those which were detected by Hain PCR (100% vs 75%) and (100% vs 93.8%) with no significant difference. The percentage of the MTBC detection in the total AFB-positive samples by VersaTREK were higher than those which were detected by HainPCR (83.3% vs 54.8%) with high significant difference and the percentage of the MTBC detection in the total samples by VersaTREK were higher than those which were detected by Hain PCR (87.7% vs 35.4%) with high significant difference Table 3.

The percentage of the MTBC detection in AFB-negative samples by VersaTREK was higher than those which were detected by GeneXpertPCR (95.7% vs 30.4%) with high significant difference. However, the percentage of the MTBC detection in AFB-positive (+1) samples by VersaTREK was lower than those which were detected by GeneXpertPCR (53.8% vs 100%) with high significant difference Table 4.

The percentages of the MTBC detection in AFB-positive (+2), (+3) and (+4) samples by VersaTREK were higher than those which were detected by GeneXpertPCR with no significant difference and the percentage of the MTBC detection in the total AFB-positive samples by VersaTREK were higher than those which were detected by GeneXpertPCR (90.5% vs 83.3%) with no significant difference. However, the percentage of the MTBC detection in the total samples by VersaTREK were higher than those which were detected by GeneXpertPCR (87.7% vs 69.2%) with high significant difference Table 4.

The sensitivity, specificity and accuracy of GeneXpert PCR were 64.9% ,0.0% and 56.9% Table 5. While, the sensitivity, specificity and accuracy of Hain PCR were40.4%,100% and 47.6% Table 6.However, the sensitivity, specificity and accuracy of post-culture Hain for detection of rifampicin- and isoniazid-resistance were100%,100% and 100% and the sensitivity, specificity and accuracy of post-culture Hain for detection of MTBC or NTM were 100%,100% and 100% Table 7**.**Finally, the degree of detection of MTBC by GeneXpertPCR was correlated with the results of AFB staining with high significant difference Table 8**.**

**Table 1. Detection of MTBC by Hain PCR directly from samples.**

| **AFB staining results** | **Hain PCR** | | | | **X2** | **P value** | **sign** |
|---|---|---|---|---|---|---|---|
| | **Detected** | | **Not detected** | | | | |
| **AFB-ve (46)** | 0(0.0%) | | 46(100%) | | 38.99 | <0.001 | HS |
| | | | 28 MTBC | 18 NTM | | | |
| **+1 (26)** | 0(0.0%) | | 26(100%) | | 17.88 | <0.001 | HS |
| **+2 (18)** | 10(55.6%) | | 8(44.4%) | | 2.24 | 0.13 | NS |
| | 4 NTM | 6 MTBC | | | | | |
| **+3 (8)** | 6(75%) | | 2(25%) NTM | | 58.5 | 0.015 | S |
| **+4 (32)** | 30(93.8%) | | 2(6.2%) | | 63.25 | <0.001 | HS |
| | 2 NTM | 28 MTBC | | | | | |
| **Total AFB +ve (84)** | 46(54.8%) | | 38(45.2%) | | 1.46 | 0.22 | NS |
| | 6 NTM | 40 MTBC | 2 NTM | 36 MTBC | | | |
| **Total samples (130)** | 46(35.4%) | | 84(64.6%) | | --- | --- | ---- |
| | 6 NTM | 40 MTBC | 68 False -ve | 16 True –ve | | | |

**-ve= negative +ve= positive sign= significance**

**Table 2.**Comparison between Hain PCR and GeneXpert PCR for detection of MTBC according to AFB staining results.

| **AFB staining results** | **Hain PCR** | | | | **GeneXpert PCR** | | | **X2** | **P value** | **sig** |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Detected** | | **Not detected** | | **Detected** | **Not detected** | | | | |
| **AFB -ve (46)** | 0(0.0%) | | 46(100%) | | 14(30.4%) | 32(69.6%) | | 16.51 | <0.001 | HS |
| | | | 28 MTBC | 18 NTM | | 14 MTBC | 18 NTM must be –ve | | | |
| **+1 (26)** | 0(0.0%) | | 26(100%) | | 26(100%) | 0(0.0%) | | 26.21 | <0.001 | HS |
| **+2 (18)** | 10(55.6%) | | 8(44.4%) | | 14(77.8%) | 4(22.2%) NTM must be –ve | | 2.00 | 0.15 | NS |
| | 4 NTM | 6 MTBC | | | | | | | | |
| **+3 (8)** | 6(75%) | | 2(25%) NTM | | 6(75%) | 2(25%) NTM must be –ve | | 0.00 | 0.71 | NS |
| **+4 (32)** | 30(93.8%) | | 2(6.2%) | | 30(93.8%) | 2(6.2%) NTM must be –ve | | 0.00 | 0.69 | NS |
| | 2 NTM | 28 MTBC | | | | | | | | |
| **Total AFB +ve (84)** | 46(54.8%) | | 38(45.2%) | | 76(90.5%) MTBC | 8(9.5%) NTM must be –ve | | 62.1 | <0.001 | HS |
| | 6 NTM | 40 MTBC | 2 NTM | 36 MTBC | | | | | | |
| **Total samples (130)** | 46(35.4%) | | 84(64.6%) | | 90(69.2%) | 40(30.8%) | | 29.85 | <0.001 | HS |
| | 6 NTM | 40 MTBC | 68 False -ve | 16 True –ve | | 14 MTBC | 26 NTM | | | |

**Table 3. Comparison between Hain PCR and VersaTREK for the detection of MTBC according to AFB staining results.**

| AFB staining results | Hain PCR | | | | VersaTREK | | X2 | P value | sig |
|---|---|---|---|---|---|---|---|---|---|
| | Detected | | Not detected | | Detected | Not detected | | | |
| **AFB -ve (46)** | 0(0.0%) | | 46(100%) | | 44(95.7%) | 2(4.3%) these samples were +ve by GeneXpert | 84.21 | <0.001 | HS |
| | | | 28 MTBC | 18 NTM | | | | | |
| **+1 (26)** | 0(0.0%) | | 26(100%) | | 14(53.8%) | 12(46.2%) they were +ve by GeneXpert | 19.16 | <0.001 | HS |
| **+2 (18)** | 10(55.6%) | | 8(44.4%) | | 16(88.9%) | 2(11.1%) They were +ve by GeneXpert | 12.61 | <0.001 | HS |
| | 4 NTM | 6 MTBC | | | | | | | |
| **+3 (8)** | 6(75%) | | 2(25%) NTM | | 8(100%) | 0(0.0%) | 2.29 | 0.13 | NS |
| **+4 (32)** | 30(93.8%) | | 2(6.2%) | | 32(100%) | 0(0.0%) | 2.06 | 0.15 | NS |
| | 2 NTM | 28 MTBC | | | | | | | |
| **Total AFB +ve (84)** | 46(54.8%) | | 38(45.2%) | | 70(83.3%) | 14(16.7%) | 16.04 | <0.001 | HS |
| | 6 NTM | 40 MTBC | 2 NTM | 36 MTBC | | | | | |
| **Total samples (130)** | 46(35.4%) | | 84(64.6%) | | 114(87.7%) | 16(12.3%) | 75.4 | <0.001 | HS |
| | 6 NTM | 40 MTBC | 68 False -ve | 16 True –ve | | | | | |

**-ve= negative +ve= positive sign= significance**

**Table 4.Comparison between GeneXpert and VersaTREK for detection of MTBC according to AFB staining results.**

| AFB staining results | GeneXpert PCR | | | VersaTREK | | X2 | P-value | sig |
|---|---|---|---|---|---|---|---|---|
| | Detected | Not detected | | Detected | Not detected | | | |
| **AFB -ve (46)** | 14(30.4%) | 32(69.6%) | | 44(95.7%) | 2(4.3%) these samples were +ve by Gene expert | 41.99 | <0.001 | HS |
| | | 14 MTBC | 18 NTM must be –ve | | | | | |
| **+1 (26)** | 26(100%) | 0(0.0%) | | 14(53.8%) | 12(46.2%) they were +ve by Gene xpert | 15.6 | <0.001 | HS |
| **+2 (18)** | 14(77.8%) | 4(22.2%) NTM | | 16(88.9%) | 2(11.1%) They were +ve by Gene xpert | 0.8 | 0.37 | NS |
| **+3 (8)** | 6(75%) | 2(25%) NTM | | 8(100%) | 0(0.0%) | 1.2 | 0.27 | NS |
| **+4 (32)** | 30(93.8%) | 2(6.2%) NTM | | 32(100%) | 0(0.0%) | 2.06 | 0.49 | NS |
| **Total AFB +ve samples (84)** | 76(90.5.5%) MTBC | 8(9.5%) NTM | | 70(83.3%) | 14(16.7%) | 1.85 | 0.25 | NS |
| **Total samples (130)** | 90(69.2%) | 40(30.8%) | | 114(87.7%) | 16(12.3%) | 13.02 | <0.001 | HS |
| | | 14 MTBC | 26 NTM | | | | | |

**-ve= negative +ve= positive sign= significance**

**Table 5. Sensitivity, specificity and accuracy of Gene Xpert PCR.**

| GeneXpert PCR | VersaTREK Detected | VersaTREK Not detected |
|---|---|---|
| **Detected; 90(69.2%)** | 74(64.9%) | 16(100%) |
| **Not detected; 40(30.8%)** | 40(35.1%) | 0(0.0%) |
| **Total; 130(100%)** | 114(100%) | 16(100%) |

**Sensitivity = 64.9% Specificity =0.0 % Accuracy = 56.9%**

**Table 6.Sensitivity, specificity and accuracy of Hain PCR.**

| Hain PCR | VersaTREK Detected | VersaTREK Not detected |
|---|---|---|
| **Detected; 46(35.4%)** | 46(40.4%) | 0(0.0%) |
| **Not detected; 84(64.6%)** | 68(59.6%) | 16(100%) |
| **Total; 130(100%)** | 114(100%) | 16(100%) |

**Sensitivity = 40.4% Specificity =100% Accuracy = 47.6%**

**Table 7.The sensitivity, specificity and accuracy of post-culture Hain for the detection ofMTBC & NTM and the detection of rifampicin- & isoniazid-resistance of MTBC isolates compared to VersaTREK.**

| Technology | Rifampicin 88 of MTBC | | Isoniazid 88 of MTBC | | Total samples (130) | |
|---|---|---|---|---|---|---|
| | Sensitive | Resistant | Sensitive | Resistant | Detected | Not detected |
| **VersaTREK** | 84(95.5%) | 4(4.5%) | 80(91%) | 8(9%) | 114(87.7%) MTBC or NTM | 16(12.3%) Neither MTBC nor NTM |
| **Post-culture Hain** | 84(95.5%) | 4(4.5%) | 80(91%) | 8(9%) | 114(87.7%) MTBC or NTM | 16(12.3%) Neither MTBC nor NTM |

**Sensitivity = 100% Specificity =100% Accuracy = 100%**

**Table 8.Correlation between AFB staining results and degree of detection of MTBC by GeneXpert PCR.**

| AFB staining results | Not detected(40) | Low detection (48) | Medium detection (12) | High detection (30) |
|---|---|---|---|---|
| **-ve(46)** | 32(69.6%) | 14(30.4%) | 0.0% | 0.0% |
| **+1(26)** | 0.0% | 26(100%) | 0.0% | 0.0% |
| **+2(18)** | 4(22.2%) | 8(44.4%) | 6(33.3%) | 0.0% |
| **+3(8)** | 2(25%) | 0.0% | 2(25%) | 5(50%) |
| **+4(32)** | 2(6.2%) | 0.0% | 4(12.5%) | 26(81.2%) |

**r=0.82 p<0.001 (HS)**

## DISCUSSION

Culture-based methods have been greatly improved over the past decades and remain the "gold standard" for TB diagnosis. However, the time to positivity is dependent on the replication rate of *Mycobacterium tuberculosis* complex as well as the bacillary load in the specimen with delaying of results for weeks[21]. Furthermore, an additional test is required to confirm the presence of MTBC. To address the associated diagnostic delay, molecular tests have been developed with the aim of providing both a diagnosis of the presence of MTBC and identifying mutations conferring resistance to the most important first-line anti-TB drugs as RIF and/or INH[22].

In our study, a total of 130 samples were obtained from tuberculous patients who were admitted to King Abdulaziz University Hospital (KAUH), Jeddah , KSA.

Our results revealed that the percentages of the MTBC detection in AFB-negative samples and total AFB-positive samples byHain PCR were 0.0% and 54.8% respectively Table 1. Barnard et al.,[22]found that 14% to 16% of smear-negative specimens were detected byHain PCR.

In our study, the percentage of the MTBC detection in AFB-negative samples by GeneXpert PCR was higher than those which were detected by Hain PCR (30.4% vs 0.0%) with high significant difference and the percentage of the MTBC detection in total AFB-positive samples by GeneXpert PCR was higher than those which were detected by Hain PCR (90.5% vs

54.8%) with high significant difference Table 2. However, Scott et al.,[23]found that, the percentage of the MTBC detection in AFB-negative samples by GeneXpert PCR was higher than those which were detected by Hain PCR (61% vs 28%) and the percentage of the MTBC detection in total AFB-positive samples by GeneXpert PCR was higher than those which were detected by Hain PCR (96% vs 94%). The increased percentages of MTBC detection in their study than our study may be explained by the type of the patients' specimens which were sputa only with high assay sensitivity in their study than the patient specimens in our study which were pulmonary and exra-pulmonary specimens.

Our study revealed that, the percentage of the MTBC detection in AFB-negative samples by VersaTREK was higher than those which were detected by HainPCR (95.7% vs 0.0%) with high significant difference, while the percentage of the MTBC detection in the total AFB-positive samples by VersaTREK were higher than those which were detected by HainPCR (83.3% vs 54.8%) with high significant difference Table 3. Scott et al.,[23]found that,the percentage of the MTBC detection in AFB-negative samples by liquid culture (MGIT) was higher than those which were detected by MTBDR*plus* (100% vs 28%) and the percentage of the MTBC detection in total AFB-positive samples by liquid culture (MGIT) was higher than those which were detected by MTBDR*plus*(100% vs 94%).

In the current study, the percentage of the MTBC detection in AFB- negative samples by VersaTREK was higher than those which were detected by GeneXpertPCR (95.7% vs 30.4%) with high significant difference. However, the percentage of the MTBC detection in the total AFB-positive samples by VersaTREK were higher than those which were detected by GeneXpertPCR (90.5% vs 83.3%) with no significant difference Table 4. Scott et al.,[23] found that, the percentage of the MTBC detection in AFB-negative samples byliquidculture (MGIT) was higher than those which were detected by GeneXpertPCR (100% vs 61%) and the percentage of the MTBC detection in total AFB-positive samples by liquid culture (MGIT) was higher than those which were detected by GeneXpertPCR (100% vs 96%).

Ioannidis et al.,[24]concluded that, the Xpert MTB/RIF assay demonstrated a high capability to detect MTBC DNA in AFB -negative samples (86.3%) of pulmonary and extra-pulmonary origin. Also, it can detect 100% of detect MTBC DNA in AFB -positive samples of pulmonary and extra-pulmonary origin.

In our study, it was found that the percentage of the MTBC detection in AFB-positive (+1)samples by VersaTREK was lower than those which were detected by GeneXpertPCR (53.8% vs 100%) with high significant difference Table 4. This finding may be explained by the presence of dead TB bacilli due to treatment or previous treatment by anti-TB drugs in some patients included in our study which gave false positive results by GeneXpertPCR with negative results by liquid culture[25]. Meanwhile, four positive samples were not correlated with clinical data for the diagnosis of TB which may be explained by high sensitivity of GeneXpert assay or laboratory cross contamination[26].

In the current study, the sensitivity, specificity and accuracy of GeneXpert PCR were 64.9% ,0.0% and 56.9% Table 5.According to the results of Zeka et al.,[27]for 110 tuberculosis patients diagnosed clinically and microbiologically, the sensitivity of the XpertMTB/RIF test was 70% (77/110) and the specificity was 100% (319/319).

Scott et al.,[23] found that, the sensitivity and specificity GeneXpert PCR compared to liquid culture (MGIT) were 86% and 97% respectively.

In the current study, the sensitivity, specificity and accuracy of Hain PCR were40.4%,100% and 47.6% Table 8. Scott et al.,[23]found that, the sensitivity and specificity GenoTypeMTBDR*plus* compared to liquid culture (MGIT) were 76% and 97% respectively. While other study found that the combined overall sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of smear-positive and smear-negative samples for both extraction methods by MTBDR*plus* compared to clinical findings were 87.6%, 99.2%, 99.4%, and 84.1%, respectively[18].

Other study by Barnard et al.,[22] concluded that, the Xpert MTB/RIF and GenoTypeMTBDR*plus* (v2.0) showed sensitivities of 71.2% and 73.1%, respectively, and similar specificities of 100% compared to liquid culture (MGIT).

Our findings on the performance of the Xpert MTB/RIF assay and GenoTypeMTBDR*plus* were not correlating well with those reported by others regarding the effectiveness of the assay in detecting the presence of MTBC bacilli specially in AFB-negative specimens. This could be due to that those studies performed exclusively or mainly on respiratory samples or the higher sensitivity for AFB-negative specimens was attained upon testing of additional samples that increased the initial values in their studies while we included only one specimen for each patient in our study. Also, our study included samples positive for NTM which were not detected by GeneXpert reducing our overall sensitivity. Also, the extraction method may affect the final yield of GenoTypeMTBDR*plus* detection of MTBC.

Makinen et al.,[29] stated that the accuracy of Xpert MTB/RIF assay may vary from region to region due to variation in the circulating *M. tuberculosis* strains.

Our study revealed that, the sensitivity, specificity and accuracy of post-culture Hain for the detection of rifampicin- and isoniazid-resistancewere100%,100% and 100% and the sensitivity, specificity and accuracy of post-culture Hain for detection of MTBC or NTM

were 100%,100% and 100% Table 9.In agreement with our results**,** Ioannidis et al.,[24] found that the MTBDR*plus* and drug-susceptibility testing(DST) results had a 100% agreement regarding RIF-susceptibility results.

Crudu et al.,[18]found that the sensitivity and specificity of detection of rifampin resistance by MTBDR*plus* (v2.0) were 94.3 and 96.0%, respectively. The sensitivity and specificity of detection of rifampin resistance by MTBDR*plus* (v2.0) were 95.8% and 88.9%, respectively. Hillemann et al.,[28] found that the new GenoTypeMTBDR*plus* assay was able to identify RMP-resistance correctly in 98.7% and 96.8% of specimens. The lower sensitivity and specificity of their studies may be due to mis-identification or the use of patients' samples directly to detect susceptibility to RIF and INH by MTBDR*plus*, while in our study we used the positive cultures of the samples to detect susceptibility to RIF and INH by MTBDR*plus***.**

Some cases of resistance also may be caused by mutations not detectable with MTBDR*plus* 2.0, e.g., in other regions of *rpo*B, *kat*G, or unknown genes[30]. Another explanation for these false results could be the existence of mutations resulting in low-level RMP-resistance[31]. A second infection with a nosocomial strain during the stay in the hospital could be a further explanation for discrepant results[18].

The GenotypeMTBDR*plus* is a reliable test when used on clinical isolates, while culture growth requires 2-4 weeks, and susceptibility results are not available at the beginning of therapy. Performing the test on selected clinical specimens may give critical information needed for appropriate treatment in a very short time and can be crucial for avoiding the transmission of drug-resistant *M. tuberculosis* strains. One disadvantage that the line probe tests share with other probe-based assays is that they can identify only the most frequent mutations and otherwise rely on the negative hybridization result with the wild type-specific probe as a marker for additional mutations. So, its results should always be confirmed by phenotypic methods[32].

One of the most important and obvious reason for the use of the Xpert MTB/RIF is the significantly reduced turnaround time (TAT) for detection. Not only is the TAT reduced to 2 to 3 h, but this test can also detect rifampin-resistance simultaneously[33].

Despite false positive and false negative results reported by some studies, Xpert MTB/RIF still showed superior performance among all NAATs. The product detection using automated, more sensitive fluorescence, not visual detection as with the MTBDR*plus* assay[34].TheXpert MTB/RIF test automates DNA extraction, amplification and detection inside a test cartridge that is never re-opened, with little chance of amplicon contamination. Specimen processing is simplified to a single non-precise step that both liquefies and inactivates sputum, which results in a reduction of viable tubercle bacilli of 6 to 8 logs and eliminates the necessity for a biosafety cabinet with no infectious aerosols. These features of simplicity and safety of use could allow for cost-effective and highly sensitive detection of TB and drug-resistance outside reference centers, which would increase access to testing and decrease delays in diagnosis, without the need to build special laboratories equipped with advanced biosafety measures[12].

**In conclusion**; the results of our study demonstrated that the rate of concordance of the Genotype MTBDR*plus* findings with those of conventional methods is satisfactory. Also, our findings further show the superior sensitivity of the Xpert MTB/RIF compared to the Genotype MTBDR*plus* in the detection of MTBC. However, the molecular drug-susceptibility testing results should always be confirmed by phenotypic methods.

**Recommendations**; the Xpert MTB/RIF should be the initial test in the diagnosis of TB and detection of resistance. Further studies are needed to assess the accuracy of the Genotype MTBDR*plus* assays specially in smear-negative samples.

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