# Comparison and evaluation of diagnostic techniques of *mycobacterium tuberculosis* in Iraq

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(*Submitted: 10 December 2016 – Revised version received: 05 January 2017 – Accepted: 20 January 2017 – Published online: 26 March 2017*)

**Objective** Diagnostic tests devoted to the rapid, sensitive, and specific identification of the causative agent are key components of successful wellness plans directed at tuberculosis control. This study focusses on rapid and accurate detection of tuberculosis cases among Babylon population.
**Methods** The sputum samples were collected from 60 patients suspected have suffering from tuberculosis infection, in the Specialized Chest and Respiratory Center, Hilla City and Department of Medical Microbiology, College of Medicine, Babylon University, Hilla-Iraq during the period from February to June 2015. Molecular detection of Mycobacterium tuberculosis in patients' sputum samples using real-time PCR, and gene X-pert for suspected TB-infected patients. The clinical signs were recorded for each patient, including night sweating, fever, loss of weight, and history of cough.
**Results** Gene X-pert MTB/RIF technique, real-time PCR recording the high sensitivity for AFB positive smear (100%) for both, AFB negative smear (66%, and 58%), respectively. AFB sensitivity was (16.6%), and the specificity was (100%) for all in the present study.
**Conclusion** The comparison between advance technique (Gene X-pert and real-time PCR) and classical technique (AFB) for the diagnosis of MTB, shows that genetic technique is the best with high sensitivity and specificity.
**Keywords** tuberculosis, real-time PCR, gene-X-pert

## Introduction

Tuberculosis (TB) has been one of the oldest infectious diseases affecting human. It was identified 4000 years ago, from the Middle East and Europe, as the cause of death, suggesting that this disease has been already a widespread health problem back then. In a detailed history, Hippocrates wrote about patients with wasting away associated with coughing and chest pain, often with blood in sputum. These symptoms had been allowed Hippocrates to diagnose TB, which at that fourth dimension has been called "consumption". The occurrence of descriptions of patients with these signs indicated that the disease was already well entrenched in early times.[1,2]

TB is a communicable and deadly infectious disease caused by mycobacteria, essentially *Mycobacterium tuberculosis.*[3] TB affects 8.8 million people each year, most of whom dwell in low economic society.[4] Additionally, an estimated 2 billion people are believed to be latently infected, providing a great reservoir.[5] Tuberculosis typically attacking the lungs (as pulmonary TB) but can affect the central nervous system, the lymphatic system, the circulatory system, the genitourinary system, the gastrointestinal system, bones, joints, and even the skin (extra pulmonary TB).[6] Although in that respect other mycobacteria such as *Mycobacterium bovis*, *Mycobacterium africanum, Mycobacterium canetti, and Mycobacterium microti* also can cause tuberculosis, these species are less common. The usual symptoms of pulmonary tuberculosis are a chronic cough with blood-tinged sputum, fever, night sweats and weight loss. Tuberculosis considers an immunological disease and the pathology of disease mediates by the host immune response.[7]

Diagnostic tests devoted to the rapid, sensitive, and specific identification of the causative agent are key components of successful wellness plans directed at disease control. Moreover, the precise determination of *Mycobacterial burden* might be beneficial for fast assessment of patient response to standard therapy, particularly in those patients suspected of harboring antibiotic resistant *M. tuberculosis* strains.[8]

Chest radiographs may be applied to exclude pulmonary TB disease in a person with a normal immune system who has a positive TST (tuberculin skin test) reaction or IGRA (IFN-γ release assay) and has no symptoms or signs of TB disease.[9] Gain techniques for the diagnosis of tuberculosis have attracted considerable interest in diagnosis, particularly with the hope of reducing the time taken to find and identify *Mycobacterium tuberculosis* in respiratory and non-respiratory specimens.[10] Real-time PCR has further advantages, including precision, reproducibility, accuracy, quality control procedures and reduced pollution. In addition, real-time PCR eliminates the need for electrophoresis after the cycling reaction. Furthermore, this technique cuts the analysis time for 3 or 4 days, which is important in lengthy microbiological diagnoses, such as those needed for TB.[11] The Gene X-pert system, a real-time PCR that simultaneously detects both *Mycobacterium tuberculosis* complex (MTBC) and rifampin resistance, was developed.[12] In contrast to some real-time PCR instruments, the X-pert MTB/RIF is an on-demand assay described as a simple method that can be performed by personnel with minimal grooming and can provide answers within 2 h.[13]

The detection of rifampin resistance, as a surrogate for multidrug-resistant TB (MDR-TB), directly from smear-positive respiratory specimens from patients bearing a high risk of MDR-TB has recently been advocated by the World Health Organization.[14] Chemotherapy of drug susceptible TB consists of three or four drug regimen, administered for 6 months. The long duration of therapy solutions in poor compliance leading to the emergence of multi-drug resistant forms of *M. tuberculosis*.[15]

## Materials and Methods

### Study Population

A total of 60 patients suspected with tuberculosis infection, in the Specialized Chest and Respiratory Center, Hilla City during the period from February to June 2015. The clinical signs were recorded for each patient, including the night sweating, fever, loss of weight, and history of dry cough. Checklist sheets were drawn out for each patient including age, gender, radiological finding, and incidence area. Sputum samples were collected from TB according to the stated procedure.

### AFB Smear

The slides were flooded with carbol fuchsin dye. The slides are heated slowly until it is steaming with direct flame for at least 5 minutes. The smear was decolorized with a decolorizing agent and left until the color of the solution is gone. Then it was washed with tap water. Methylene blue was applied to slides by which the dye covered the entire slides surface and left for maximum 60 seconds than washing with water. Microscopically examination: The positive acid fast bacilli smear appears as pink or red rods in a blue background.[16] The number of AFB was calculated by country number of cell in smear according to standard method.[17]

## Real Time PCR Assay

### Extraction of DNA

The required number of 1.5 ml disposable polypropylene micro centrifuge tubes was developed, including single tube for Negative Control of Extraction (Negative Control, c-). Ten microliter of the MTB IC (Internal Control) and 300 µl of Lysis Solution was added to each pipe. One hundred microliter of samples was added to the appropriate tubes using pipette tips with aerosol barriers. The control was prepared as follows: Add 100 µl of Negative Control C- to the labeled Cneg. Vortex the tubes and incubated for 5 min at 65˚C. Centrifuged for 10 s. Four hundred microliter of Prec solution was added and mixed by vortex. Centrifuged all tubes at 13,000 rpm for 5 min and using a micropipette with a plugged aerosol barrier tip, carefully removed and discarded supernatant from each tube without disturbing the pellet. Changed tips between the tubes. Five hundred microliter of Wash Solution was added into each tube. Vortex vigorously rinsed to ensure pellet washing. Centrifuged all tubes at 13,000 rpm for 60 s using a micropipette with a plugged aerosol barrier tip. Carefully removed and discarded supernatant from each tube without disturbing the pellet. Changed tips between the tube. Two hundred microliter of Wash Solution 4 was added to each tube. Vortex vigorously rinsed to ensure the pellet washing. Centrifuge all tubes at 13,000 rpm/min for 60 s and using a micropipette with a plugged aerosol barrier tip. Carefully removed and discarded supernatant from each tube without disturbing the pellet. The tubes were incubated with open caps at 65˚ C for 5 min. Resuspended the pellet in 50 µl of RE- buffer (the elution volume can be increased upwards to 90 µl). Incubate for 5 min at 65˚C and vortex periodically. The tubes were centrifuged at 13000 g for 60 s. The supernatant contains RNA/DNA ready for amplification. If amplification is not performed on the same day of extraction, the processed samples can be stored at 4˚C for a maximum period of 5 days or frozen at –20˚C for a long time.

### Amplification Mix Preparation

In the new sterile tube each sample was prepared 10* (n+1) µl of PCR-mix-1, 5* (n+1) µl of PCR Buffer Flu, 0,5* (N+1) µl Taq DNA Polymerase and 0,5* (N+1) µl of UDG Enzyme. Vortex rinsed gently and centrifuged briefly. Fifteen microliter of Reaction Mix was added to each tube. Ten microliter of extracting DNA was added to appropriate tube. Two controls were prepared for each panel: 10 µl of DNA-buffer was added to the tube labeled Amplification Negative Control. 10 µl of C-MTB&IC was added to the tube labeled Amplification Positive Control. The tubes were ready to insert in to the thermalcycler. Data analysis: The fluorescent single intensity was detected in two channels: *Mycobacterium tuberculosis* was detected on the FAM (Green) channel, IC DNA on the JOE (Yellow) / HEX/Cy3 channel.

## Gene X-Pert MTB/RIF Assay

### Preparing the Sputum Samples

Each X-pert MTB/RIF cartridge was labelled with the sample ID. In a leak-proof sputum collection container, a. The lid of the sputum collection container was carefully opened. b. Approximately 2 times the volume of the SR was poured to the sputum (2:1 dilution, SR:sputum). c. The lid was replaced and secured. d. Shaked vigorously 10 to 20 times by vortex for at least 10 s.

The sample was incubated for 10 minutes at room temperature, and then shaken the specimen vigorously 10 to 20 times or vortex for at least 10 seconds. The sample was incubated at room temperature for an additional 5 minutes.

### The Test Procedure

The computer was turned on, farther Turn on the Gene X-pert instrument. Open the Software shortcut icon of the Gene X-pert in the Windows® desktop by double-click on it. User name and password have been used to log on the Gene-X-pert Dx System software. Click Create, Test, in the Gene X-pert Dx System window. The Scan Sample ID dialog box appeared. In the Sample ID box, scan or type the sample ID. Make sure you type the correct sample ID (sample ID is associated with the test results and is shown in the View Results window and all the reports). The Scan Cartridge Barcode dialog box appears. The barcode on the X-pert MTB/RIF cartridge has been scanned. The Create Test window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date. Click Start Test. Enter your password if requested. The instrument module door with the blinking green light has been opened, and loaded the cartridge. The door was closed. The test starts and the green light stop-blinking.

When the test is finished, the light turns off. Wait until the system releases the door lock at the end of the run, then open the module door and remove the cartridge. Reading the Results: The Gene X-pert Instrument system generates the results from measured fluorescent signals and embedded calculation algorithms. The results can be seen in the View Results window.

## Results

Among 60 samples, only 10 samples which give a positive AFB, 50 samples give a negative AFB result, 39 samples positive,

Table 1. **Results of diagnostic techniques for TB**

| Test | No. of patients | | Total | Sensitivity | | Specificity |
|---|---|---|---|---|---|---|
| | True Positive | False Negative | | | | |
| AFB | 10 (16.7%) | 50 (83.3%) | 60 | 16.6 % | | 100% |
| Real-time PCR | 39 (65%) | 21(35%) | 60 | AFB +ve 100% | AFB –ve 58% | 100% |
| Gene X-Pert | 43 (71.7%) | 17(28.3%) | 60 | AFB +ve 100% | AFB –ve 66% | 100% |

The chi-square statistic is 43.2658. The p-value is < 0.00001. The result is significant at p < 0.005.

21 samples negative results for Real-Time PCR, 43 samples positive, 17 negative results for Gene X-pert respectively as shows in **Table-1**.

## Discussion

Gene X-pert MTB/RIF technique, Real-Time PCR recording the high sensitivity for AFB positive (100%) for both, for AFB negative (66%, and 58%) respectively, AFB sensitivity was (16.6%), and the specificity was (100%) as shown in Table 1. The sensitivity of real-time PCR and X-pert MTB/RIF are given similar results with smears positive, but it is low in real-time technique for smears negative. These are matching with Armand et al.,[18] who suggested that both methods were highly specific and exhibited excellent sensitivity (100%) with smear-positive specimens, but does not match in the sensitivity of the X-pert MTB/RIF test with the smear-negative specimens were more reduced than that of the real-time PCR assay (48 versus 69%, P < 0.005).

American Thoracic Society Workshop,[19] who estimated that the Gene X-pert assay was introduced lately. Its advantage lies in higher sensitivity and specificity for the diagnosis of TB, together with the detection of drug resistance, and in smear-positive specimens. The sensitivity and specificity of PCR are in the range 90–100%, with a positive prognostic value of > 95%, whereas in smear-negative specimens, the sensitivity of PCR was reduced to < 50%.

Our study found that the sensitivity of Gene X-pert for AFB smear- negative was (66%). These results agreed with the work reported by Zeka et al.,[20] who suggested that the X-pert MTB/RIF assay have reported test sensitivities of 57 to 76.9% in cases of smear-negative.

In our study, real-time PCR has a second value of sensitivity (69%) for smear negative after the Gene X-pert. These results were in accordance with those results reported by Raviglion,[21] who proposed that the real-time PCR assay with internal control achieved a sensitivity of 96.2 % and specificity of 99.2%. Templeton et al.,[22] explains that internal control reaction has been included to determine the robustness of the PCR resulting by monitoring the nucleic acid extraction as well as the presence of inhibitors.

Rajpal et al.,[23] stated that direct microscopy may give negative results if the number of AFB less than 5000 bacilli/ml, consistently positive specimens would have to contain 105 bacilli per ml varying with the extent of the wound or the presence citations. The scanty or numerous AFB smear test, which differs according to the number of bacilli in microscopy filed.

In our study, the sensitivity for the diagnosis of *M. tuberculosis* of Gene X-pert and real-time PCR higher than AFB because the Gene X-pert and real-time PCR depends on diagnosis on DNA amplification and have ability to detect less than 10 of acid fast bacilli per ml in a sputum sample while AFB smear depending on the ability of *M. tuberculosis* to take stain and, may give a negative result if there few number of acid fast bacilli less than 5000 bacilli per ml in the field.

## Conclusions

The comparison between advance technique (Gene X-pert and Real time PCR) and classical technique (AFB) for the diagnosis of MTB, show that genetic technique is the best with high sensitivity, and specificity.

## Consent

All authors declare that written informed consent was obtained from the patients for publication of this research article.

## Competing Interests

Authors have declared that no competing interests exist.

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