

## Original Research Article

# Evaluation of the Diagnostic Performance of Anyplex II™ MTB/MDR/XDR Detection Assay for Detection and Antimicrobial Susceptibility testing of *Mycobacterium tuberculosis* from Sputum Specimens

Dalia Saad ElFeky\* and Amal ElShimy

Department of Medical Microbiology and Immunology, Faculty of Medicine,  
Cairo University, Egypt

\*Corresponding author

## ABSTRACT

The present study aim to evaluate the diagnostic performance of Anyplex™II MTB/MDR/XDR Detection assay for simultaneous detection of *Mycobacterium tuberculosis* and its resistance to anti-tuberculosis drugs. Sputum specimens were collected from 46 patients with symptoms and radiological findings suggestive of active pulmonary tuberculosis and tested for the presence *Mycobacterium tuberculosis* by ZN-smear microscopy, culture and the Anyplex assay. Drug susceptibility testing was done by direct and indirect proportion method (PM) as well as by the Anyplex assay. *Mycobacterium tuberculosis* was detected in 39 cases (84.8%) by culture, and in 36(78.3%) and 45 (97.8%) cases by smear microscopy and the Anyplex assay, respectively. All discrepant results between culture and the Anyplex assay were among the smear-negative cases. None of the isolates had rifampicin resistance. Isoniazid resistance was detected in 6 isolates by the indirect PM and in 5 isolates by the direct PM and the Anyplex assay. Sensitivity and specificity of the Anyplex assay for the detection of *Mycobacterium tuberculosis* were 100% and 14.6%, respectively, and were 83.3% and 100%, respectively, for detection of INH resistance. Anyplex™II MTB/MDR/XDR Detection assay could be useful for diagnosis of pulmonary tuberculosis in smear-positive cases and can reliably detect INH resistance.

## Keywords

Anyplex™II  
MTB/MDR/XD  
R Detection  
assay, Sputum  
specimens,  
*Mycobacterium  
tuberculosis*,  
Drug  
susceptibility,  
Proportion  
method

## Introduction

Globally, in 2013, 3.5% of new and 20.5% of previously treated tuberculosis (TB) cases were estimated to have had multidrug-resistant (MDR)-*Mycobacterium tuberculosis* (*M. tuberculosis*); defined as resistance to at least isoniazid (INH) and rifampicin (RIF). On average, an estimated 9% of patients with MDR-TB had extensively drug resistant TB (XDR-TB),

defined as MDR-TB with additional resistance to any fluoroquinolone (FLQ) and to at least one of the three injectable second-line drugs: amikacin (AMK), kanamycin (KAN) and/or capreomycin (CAP) (WHO, 2014). Successful treatment outcome for MDR-TB depends on reliable rapid and accurate diagnosis as well as drug susceptibility testing of first-line and

second-line anti-tuberculosis drugs (Said *et al.*, 2012).

Traditionally, acid fast bacilli (AFB) smear microscopy has been the initial method for diagnosis of TB, due to its speed, simplicity, and low cost. However, its low sensitivity limits the usefulness of this technique. Culture remains the gold standard for diagnosis; however, it takes 3 to 8 weeks for confirmation (Lim *et al.*, 2014).

Molecular diagnostics for *M. tuberculosis*, offer the potential for sensitive, specific, and timely diagnoses of TB as well as identifying mutations conferring resistance to the most important first-line TB antibiotics, INH and RIF (Barnard *et al.*, 2012). These methods have recently been endorsed by the World Health Organization (WHO). The Xpert MTB/RIF assay was endorsed for use on patient material, regardless of smear gradation, while the GenoTypeMTBDRplus (version 1) has been limited for use on smear-positive patient material (Barnard *et al.*, 2012; Perry *et al.*, 2014)

Seegene (South Korea) has developed the Anyplex II™ MTB/MDR/XDR Detection assay, a novel method of multiplex real-time PCR for the simultaneous detection of *M. tuberculosis* and MDR and XDR resistance alleles. In the Anyplex™ II MTB/MDR/XDR Detection assay, the *M. tuberculosis* target sequences and each drug-resistant target mutation are amplified and detected specifically by the corresponding oligos by applying the Seegene's dual priming oligonucleotide™ (DPO™) and Tagging Oligonucleotide Cleavage Extension (TOCE™) technologies without additional downstream processing. DPO™ is a tool for blocking extension of non-specifically primed templates, while TOCE™ is a novel real-time readout

technology based on melting temperature analysis that provides enhanced multiplexing capabilities. Combining the DPO™ and TOCE™ technologies enables the simultaneous real-time detection of multiple point mutations with high specificity. The Anyplex II™ MTB/MDR/XDR real-time PCR assay was released in the market in 2012 and the WHO recommended its use in reference labs (Boyle and Pai, 2013).

The main aim of this study was conducted to evaluate the diagnostic performance of Anyplex™II MTB/MDR/XDR Detection assay for detection of *M. tuberculosis* from sputum specimens of patients with suspected active pulmonary TB and its resistance to anti-tuberculosis drugs against the standard culture and proportion method.

## **Patients and methods**

The study has been approved by the Research and Ethical Clearance Committee at the Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University. A total of 46 patients were enrolled in this study selected from those presenting to outpatient clinics of two chest hospitals in Cairo and Giza, Egypt, from October 2013 till May 2014. According to the WHO definition, all selected patients were new cases (WHO, 2009) presenting with respiratory symptoms and had radiological findings suggestive of active pulmonary TB without prior antituberculosis treatment.

## **Specimen collection**

After taking an individual informed consent from patients or their representatives, three early morning sputum specimens on 3 consecutive days were collected from each patient (Lagrange *et al.*, 2012). The

specimens were screened by Zeil-Neelsen (ZN)-smear microscopy (direct ZN smear) for AFB before being packed (WHO, 2003) and transported in a cold chain to the lab of the Tuberculosis Research Unit in the Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University; where further processing of the specimens was performed.

### **Specimen processing**

All the procedures were conducted in a Biosafety Level 2 lab. All precautions were taken to prevent contamination.

**Pretreatment of sputum specimens:** The specimens were homogenized, digested and decontaminated according to the standard *N*-acetyl-L-cysteine–NaOH–Na citrate decontamination concentration method using a 2% final concentration of NaOH (Richter *et al.*, 2012). Briefly, equal volume of NALC-NaOH (0.5% NALC, 4 % NaOH, and 1.47% trisodium citrate) was added to each specimen in the sputum container, vortexed for 1 minute, and incubated for 15 minutes at room temperature. Then each specimen was divided into 2 aliquots; one of them (tube 1) contained 1.5 mL of the specimen, while the second tube (tube 2) contained the rest of the specimen. Both tubes were undergone the rest of the decontamination concentration process. Finally, after the last centrifugation step, the pellet of tube 2 was re-suspended in 1mL of sterile distilled water (Mattei *et al.*, 2009) to be used for ZN -smear preparation (concentration method) and as inoculum for culture on Löwenstein-Jensen(LJ) slants. The pellet of tube (1) was used for DNA extraction.

**Culture on LJ medium:** Aliquots of 200µL of all processed specimens (tube 2) were inoculated onto 2 drug-free LJ slants (Dang *et al.*, 2012). Isolates on LJ medium were

identified as mycobacteria by ZN-smear microscopy and as *M. tuberculosis* by colony morphology, rate of growth (Hill *et al.*, 2004), in addition to the standard biochemical tests; niacin production and nitrate reduction (WHO, 2009).

**Drug susceptibility testing (DST) using the proportion method (PM):** Direct and indirect PM were performed against the first-line anti-tuberculosis drugs; INH and RIF on drug-containing LJ medium (WHO, 2003). These drugs were chosen to detect possible MDR-TB and pre existing resistance to these drugs in general population (Dang *et al.*, 2012). INH and RIF were obtained as chemically pure powder (Sigma-Aldrich, Belgium). INH was dissolved in sterile distilled water, while RIF was dissolved in ethylene glycol. Stock solutions were prepared and stored at –20°C for no more than 3 months (Martin *et al.*, 2008) to be used for preparation of the fresh drug-containing LJ slants. Drug-containing LJ medium (LJ-INH and LJ-RIF) were prepared according to the published guidelines (WHO, 2003) with INH and RIF at concentrations of 0.2µg/mL and 40.0 µg/mL, respectively (WHO, 2003; Mulenga *et al.*, 2010). An isolate was considered resistant to a drug if growth on the drug containing medium was more than 1% of that on the drug free medium (WHO, 2003). A fully susceptible strain H37Rv and a known MDR *M. tuberculosis* strain supplied by WHO reference TB laboratory of the Middle East (Cairo, Egypt) were used as Quality control for both biochemical reactions and DST using the PM (Brady *et al.*, 2008).

**Direct PM:** Only smear positive specimens were tested by this method (CLSI, 2011). Aliquots of 200 µL of each processed specimens (tube 2) were inoculated onto one LJ-INH slant and one LJ-RIF slant (Mello *et al.*, 2007). The 2 drug-free LJ slants already

used for primary isolation were used as the control. Both drug-free and drug-containing inoculated slants were incubated at 37°C for 8 weeks or until growth of colonies was observed. They were first inspected after 48 hours and then weekly. If there is no growth by 8 weeks or in case of contamination, the cultures were discarded (WHO, 2003; Kidenya *et al.*, 2013).

**Indirect PM:** Isolates of *M. tuberculosis* on drug-free LJ medium were tested by the indirect PM. As early as adequate growth was attained, suspension from *M. tuberculosis* isolates were prepared and adjusted to McFarland 1 turbidity standard. Standard dilutions of  $10^{-2}$  and  $10^{-4}$  were then prepared (Shamputa *et al.*, 2004). Aliquots of 200  $\mu$ L of the  $10^2$  suspensions were used to inoculate drug-containing LJ tubes, while 200  $\mu$ L aliquots of the  $10^{-2}$  and  $10^{-4}$  suspensions were used to inoculate two drug-free control LJ tubes. The initial reading of the tubes was performed on day 28 of incubation. If the result was “sensitive”, a second final reading was made on the 42nd day (WHO, 2003).

#### **Anyplex™ II MTB/MDR/XDR Detection assay**

Sputum specimens were examined by the Anyplex™ II MTB/MDR/XDR Detection assay for the simultaneous detection of *M. tuberculosis* and its resistance to first-line anti-tuberculosis drugs (INH and RIF) and second-line anti-tuberculosis drugs (FLQ and injectable drugs) using the CFX96™ Real-time PCR System (Bio-Rad). It covers 7 mutations causing INH resistance in the *katG* gene and *inh A* promoter region, 18 mutations causing RIF resistance in the *rpoB* gene, 7 mutations causing FLQ resistance in the *gyrA* gene, and 6 mutations causing injectable drug resistance in the *rrs* gene and *eis* promoter region.

**DNA Extraction:** The pellet of tube 1 were undergone DNA extraction according to the manufacturer’s instructions using the DNA extraction solution included in the Anyplex™ II MTB/MDR/XDR Detection kit.

**Real-time PCR:** Each sample was tested in two separate reactions (MTB/MDR and MTB/XDR) according to the manufacturer’s instructions. One reaction is used for amplification and detection of MTB and MDR-TB; and the second for amplification and detection of MTB and XDR-TB. Negative control (RNase-free water), positive control (mixture of clones of all positive targets and internal control) and wild-type control (mixture of clones of wild-type MTB targets and internal control) were included in each test run.

The wild-type control is designed to be exhibited the same result pattern with drug-susceptible *M. tuberculosis* sample and the drug-resistant result of unknown samples is analyzed on the basis of the result of wild-type control.

#### **Statistical analysis**

Data were statistically described in terms of frequencies (number of cases) and percentages. Comparison between the study methods was done using McNemar test and agreement was tested using kappa statistic. Accuracy was represented using the terms sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy. *P*-values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows (2006).

## Results and Discussion

Sputum samples from 46 new patients suspected to have active pulmonary TB were enrolled in the study. The patients were from both sexes with age ranging from 16-83 years old.

### Detection of *M. tuberculosis*:

Standard culture on LJ medium was considered the gold standard method for detection of *M. tuberculosis* in the current study (Barnard *et al.*, 2012; Lim *et al.*, 2014). Comparisons between the results of detection of *M. tuberculosis* from sputum specimens by ZN-smear microscopy and the Anyplex assay with culture are shown in table 1, while accuracy indices of the these methods used are presented in table 2.

### Culture on LJ medium (Table 1)

Thirty-nine sputum specimens (84.8%) were positive for *M. tuberculosis* by culture. The turnaround time (TAT) for detection of growth on LJ medium ranged from 14-35 days with a mean of  $19.4 \pm 6.1$ .

### ZN-smear microscopy (Table 1)

Direct ZN-smear microscopy was positive in 32 specimens (69.6%) which increased to 36 (78.3%) after decontamination and concentration of the specimens (concentration method). Only results of the concentration method were considered for further analysis and for calculation of the accuracy indices. All of the smear-positive cases were culture positive. Three out of the 10 smear-negative cases were positive by both culture and the Anyplex assay, while the remaining 7 were culture negative. There was a statistically significant strong agreement between ZN-smear microscopy & culture results ( $kappa=0.79$ ,  $p-value < 0.001$ ).

### Anyplex™ II MTB/MDR/XDR Detection assay (Table 1)

The assay was positive for *M. tuberculosis* in 45 specimens (97.8%). Thirty-nine of these positive cases were culture positive, while six cases were culture negative; all of these 6 cases were smear negative. There was weak statistically significant agreement between the Anyplex assay and culture ( $kappa=0.22$ ,  $p-value = 0.017$ ). Concordant results between the Anyplex assay and culture were recorded in all smear-positive cases with sensitivity, PPV and accuracy of the assay for detection of *M. tuberculosis* in these cases of 100% for each, while specificity couldn't be calculated due the absence of negative cases. On the other hand, the sensitivity, specificity, PPV, NPV and accuracy of the assay for detection of *M. tuberculosis* in smear-negative cases were 100%, 14.29%, 33.3%, 100% and 40%, respectively. There was no statistically significant agreement between the Anyplex assay and culture ( $kappa=0.091$ ,  $p-value=0.490$ ) in smear-negative cases.

### Drug susceptibility results of *M. tuberculosis*

Results of DST of *M. tuberculosis* isolates in the current study by the different methods used in the study are shown in table 3. Indirect PM (Figure 1) was considered the gold standard for detection of resistance of *M. tuberculosis* to first-line antituberculosis drug; INH and RIF (Bergmann *et al.*, 2000; Gupta and Anupurba, 2010). Accuracy indices of the direct PM and the Anyplex assay are presented in table 4.

### Indirect PM:

RIF resistance was not detected in the isolates by any of the three methods used in the study. A total of 6 cases had INH

resistance detected by the indirect PM. Four out of these six cases had INH resistance detected additionally by both the direct PM and the Anyplex assay. While in the remaining 2 cases, INH resistance was detected additionally by the Anyplex assay in one case, and by the direct PM in the other.

#### **Direct PM:**

INH resistance was detected in 5 cases by both the direct and indirect PM. There was a statistically significant strong agreement (97.2%) between direct and indirect PM ( $kappa=0.893$ ,  $p-value<0.001$ ). The TAT for detection of resistance by the direct PM ranged from 21-35 days with a mean of  $25.2 \pm 6.3$ .

#### **Anyplex™ II MTB/MDR/XDR Detection assay:**

INH resistance was detected in 5 isolates by both the Anyplex assay and indirect PM; 3 of them had mutation in the *inh A* gene (Figure 2); while 2 isolates have mutation in the *kat G* gene. There was a statistically significant strong agreement (97.4%) between the Anyplex assay and indirect PM ( $kappa=0.894$ ,  $p-value<0.001$ ). The TAT for detection of resistance by the Anyplex assay was about 6 hours.

None of the *M. tuberculosis* strains isolated in the current study were MDR, and accordingly none of them were tested for susceptibility to second-line drugs by the PM, however, we use the Anyplex assay for detection of pre-existing resistance to second-line antituberculosis drugs in general population, which took on average an additional 1 hour. While no resistance to injectable drugs was detected in the isolates, 3 isolates (6.5%) had FLQ resistance (Figure 3); one of these isolates was also resistant to

INH as detected by both anyplex assay and indirect PM.

The current study was conducted to evaluate the performance of the Anyplex™ II MTB/MDR/XDR Detection assay against conventional methods for detection of *M. tuberculosis* and its resistance to antituberculosis drugs. Although culture is the gold standard for diagnosis of TB, however it gives results within 4-6 weeks (American Thoracic Society, 2000). The current study was conducted on 46 patients presenting with respiratory symptoms and had radiological findings suggestive of active pulmonary TB without prior antituberculosis treatment. Sputum specimens were positive for *M. tuberculosis* in 39 cases (84.8%) by culture on LJ medium. The TAT for detection of growth on the standard LJ culture medium was from 14-35 days with a mean of  $19.4 \pm 6.1$ . The high rate of recovery of *M. tuberculosis* from sputum specimens in this study could be attributed to that the specimens were from patients suspected clinically and radiologically to have active pulmonary TB.

The diagnosis of TB in developing countries is based mainly on AFB smear microscopy (Runa *et al.*, 2011). Although rapid and inexpensive, this method is limited by its poor sensitivity; 45%--80% with culture-confirmed pulmonary TB cases (CDC, 2009). In the current study, ZN-smear microscopy were positive for AFB in 32 cases (69.6%) by direct ZN smear which increased after concentration to 36 cases (78.3%). The differences between the direct and concentration method were reported in other studies (Runa *et al.*, 2011) and could be explained by the fact that the concentration method facilitates the detection of AFB. The overall sensitivity, specificity, PPV, NPV and accuracy of the ZN-smear microscopy (concentration

method) for detection of *M. tuberculosis* in the current study were 92.31%, 100%, 100%, 70% and 93.48%, respectively. Lower accuracy indices were reported in other studies (Runa *et al.*, 2011; Perry *et al.*, 2014). The higher sensitivity of the ZN-smear microscopy in the current study could be explained by that most of the positive specimens detected in the study had a high bacillary load, while the high specificity may reflect the low incidence of non-tuberculous mycobacteria (NTM) in our population.

Molecular methods for diagnosis of *M. tuberculosis* have improved TB diagnosis, thus making early diagnosis and treatment possible (Lim *et al.*, 2014). In the current study, 45 cases (97.8%) were positive for *M. tuberculosis* by Anyplex II™ MTB/MDR/XDR real-time PCR assay. The Anyplex assay had excellent sensitivity (100%) for detection of *M. tuberculosis*, which is better than the ZN-smear microscopy. The TAT for the Anyplex assay was around 6 hours, which is much shorter than the culture with the adding advantage of detection of drug resistance. However, the assay had poor specificity (14.29%). The performance of the Anyplex assay was excellent among the smear-positive specimens (concordant results with culture), however, the low specificity of the assay was among the smear-negative specimens (6 false positive results). This finding is compatible with the fact reported from the previous studies that the PCR method is reliable in smear-positive specimens (Lim *et al.*, 2014). The positivity of PCR in culture-negative samples obtained in the current study were reported in other studies for the Anyplex MTB/NTM assay (Perry *et al.*, 2014) and for a manual PCR protocol (Runa *et al.*, 2011). In accordance with the current study, one of these studies (Perry *et al.*, 2014) reported that the false positive results

were from patients with a recent history of TB infection. This finding of false positivity could be explained either by laboratory cross-contamination or possibly by higher sensitivity of the Anyplex assay than the LJ culture method. As the Centre for Disease Control and Prevention (CDC) recommends that clinicians should interpret all laboratory results for TB diagnosis on the basis of the clinical situation (CDC, 2009), the latter explanation could be proposed as the specimens were collected from patient with clinical and radiological findings suggestive of active pulmonary tuberculosis.

There are few published data on the performance characteristics of Anyplex II™ MTB/MDR/XDR real-time PCR assay. Studies have reported sensitivity and specificity for MTB detection of 82.9% and 99.4% for the Anyplex plus MTB/NTM/MDR-TB Detection kit (Kim and Lee, 2015) and sensitivities, specificities, PPV and NPV of 100%, 90%, 94% and 100%, respectively for the Anyplex MTB/NTM assay (Perry *et al.*, 2014). Both kits are similar versions of the Anyplex assays. In addition, some studies showed that the performance characteristics of the Anyplex MTB/NTM assay were comparable with those for other commercial molecular methods (Lim *et al.*, 2014; Perry *et al.*, 2014)

In Egypt, the prevalence of the disease is 27 per 100.000 population are infected with *M. tuberculosis* with 3.4% and 15% of the new and retreatment cases respectively have MDR TB (WHO, 2014). In the current study, none of the isolates were MDR (no detectable RIF resistance), however, 15.4% of the isolates (6 isolates) were resistant to INH as detected by the gold standard indirect PM. Contrary to our results, some studies reported rates of resistance to INH and RIF in Egypt of 20.7% and 16.3%,

respectively, while rate of MDR-TB was 14.8% (Abdullal, 2015). The lower drug resistance rate in this study may be explained by that the specimens were from new cases of TB. Although, none of the isolates in the current study were MDR, however, the anyplex assay diagnoses FLQ resistance in 6.5% of the cases, while none of the isolates had injectable drug resistance. These results which were not confirmed by

the standard PM. Similar rate of FLQ resistance was reported in one study, while 1.5% of the isolates had injectable drug resistance, although none of the isolates in that study were XDR (Abdullal, 2015). The inappropriate use of fluoroquinolones as empiric monotherapy of pneumonias is suspected to lead to development of FLQ-resistant *M. tuberculosis* (CDC, 2009).

**Table.1** Comparison of results of ZN-smear microscopy and Anyplex assay with culture

	Culture			
	Positive (n=39)		Negative (n=7)	
<b>ZN-smear microscopy</b>	N	%	N	%
Positive(n=36)	36	92.3%	0	0%
Negative(n=10)	3	7.7%	7	100%
<b>Anyplex™ II assay</b>				
Positive(n=45)	39	100%	6	85.7%
Negative(n=1)	0	0%	1	14.3%

**Table.2** Accuracy indices of ZN-smear microscopy and Anyplex assay for detection of *M. tuberculosis*

Test	Sensitivity	Specificity	PPV	NPV	Accuracy
<b>ZN-smear microscopy</b>	92.31	100.00	100.00	70.00	93.48
<b>Anyplex™ II assay</b>	100%	14.29%	86.67%	100%	87%

**Table.3** Results of drug susceptibility testing of *M. tuberculosis*

	<b>Direct PM (n=36)</b> N (%)	<b>Indirect PM (n=39)</b> N (%)	<b>Anyplex™ II assay (n=45)</b> N (%)
<b>INH<sup>1</sup>:</b>			
Resistant	5 (13.9 %)	6 (15.4%)	5 (11.1 %)
Sensitive	31(86.1%)	33 (84.6%)	40 (88.9%)
<b>RIF<sup>2</sup>:</b>			
Resistant	0 (0%)	0 (0%)	0 (0%)
Sensitive	36 (100%)	39 (100%)	45 (100%)
<b>FLQ<sup>3</sup>:</b>			
Resistant	NA*	NA*	3 (6.5%)
Sensitive			43 (93.5%)
<b>Injectable Drugs:</b>			
Resistant	NA*	NA*	0 (0%)
Sensitive			45 (100%)

<sup>1</sup>Isonizid, <sup>2</sup> Rifampicin, <sup>3</sup> Fluoroquinilones, \* Not Applicable

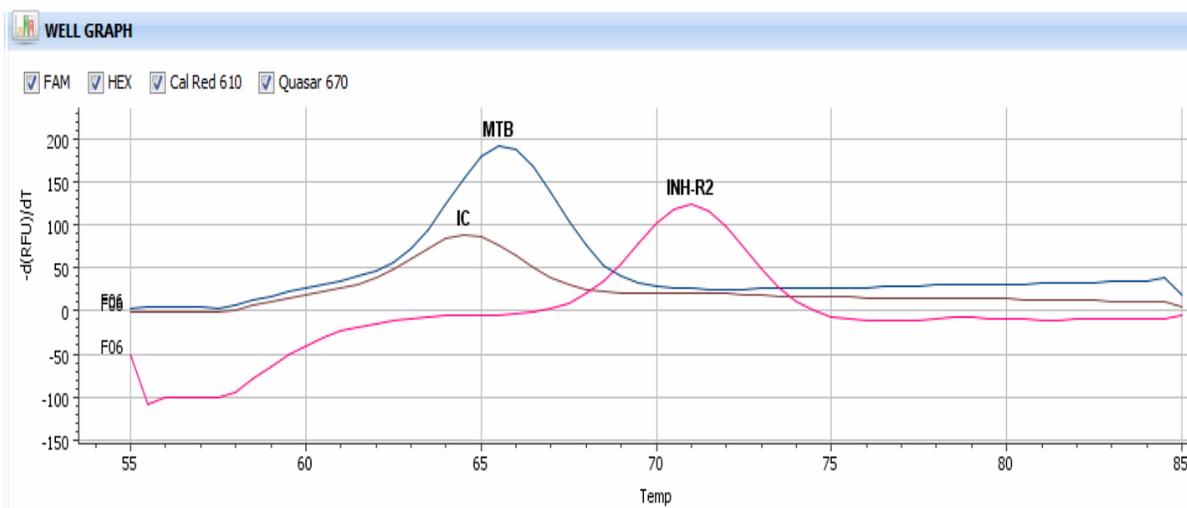
**Table.4** Accuracy indices for direct PM and the Anyplex assay for detection of INH resistance in *M. tuberculosis*

Test	Sensitivity	Specificity	PPV	NPV	Accuracy
<b>Direct PM</b>	83.3%	100%	100%	96.8%	97.2%
<b>Anyplex™ II assay</b>	83.3%	100%	100%	97.06%	97.4%

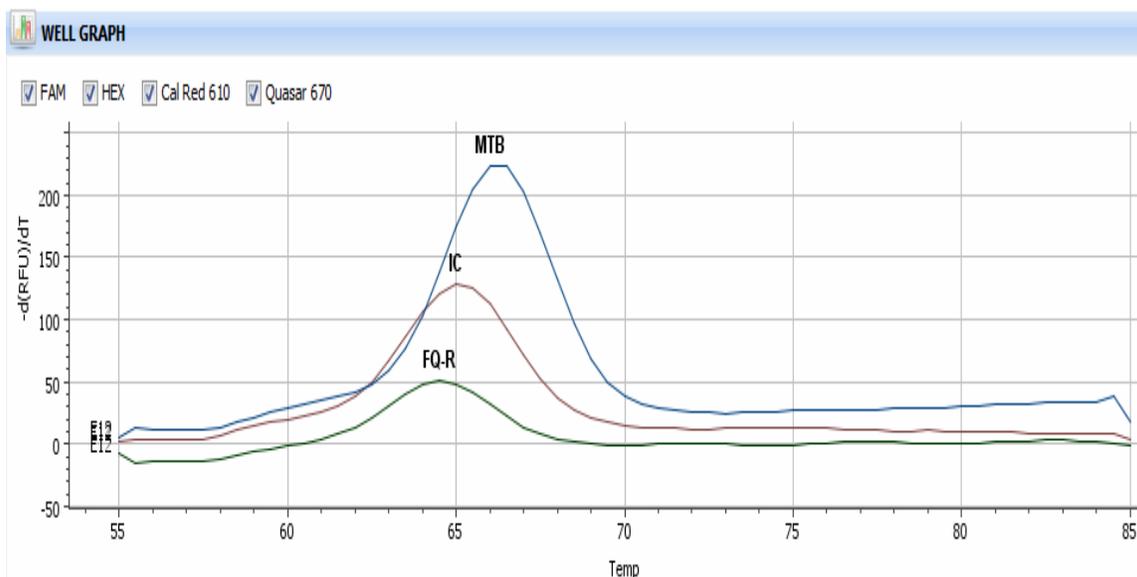
**Figure.1** Drug susceptibility testing of *M. tuberculosis* using the indirect proportion method showing INH-resistant strain. Colony count on INH-LJ slant (on the right) is more than 1% of the colony count on the drug-free LJ slant on the left



**Figure.2** *M. tuberculosis* strain resistant to INH (mutation in *inh A* gene)



**Figure.3** *M. tuberculosis* strain resistant to fluoroquinolones (mutation in *gyrA* gene)



Results obtained with the conventional indirect susceptibility testing methods of *M. tuberculosis* come too late to influence a timely decision on patient management. More rapid tests directly applied on sputum samples are needed (Bwanga *et al.*, 2009). In the current study, 2 methods were used for direct DST of *M. tuberculosis*; the Anyplex II™ MTB/MDR/XDR Detection assay and the direct PM. Both methods had comparable accuracy indices with excellent sensitivity and specificity (83.3% and 100%, respectively). However, the Anyplex assay gave rapid results, while the TAT for detection of resistance by the direct PM ranged from 21-35 days with a mean of 25.2 ± 6.3. Some studies have reported comparable accuracy indices for the direct PM (Gupta and Anupurba, 2010). Other studies have reported almost similar accuracy indices for the Anyplex assays (Anyplex plus MTB/NTM/MDR-TB Detection assay and Anyplex II™ MTB/MDR/XDR Detection assay) for detection of INH resistance (Abdullal, 2015; Kim and Lee, 2015; Molina-Moya *et al.*, 2015). The reported sensitivity and

specificity for the Anyplex assays for detection of RIF resistance in some studies were 100% and 90.3% (Abdullal, 2015), and 100% and 100% (Molina-Moya *et al.*, 2015), while for detection of FLQ resistance were 50.0% and 100%, respectively and 100% and 94.4%, respectively, for both KAN and CAP (Molina-Moya *et al.*, 2015). On the other hand, some studies have shown that the usage of the Anyplex assays on clinical samples produces inconsistent results (Bustamante *et al.*, 2014). These studies (Bustamante *et al.*, 2014; Molina-Moya *et al.*, 2015) showed that the kit was successful in detecting *M. tuberculosis* and specific resistance-causing point mutations from culture samples which may allow for an initial therapeutic approach.

In conclusion, Anyplex II™ MTB/MDR/XDR real-time PCR assay can confirm the diagnosis of pulmonary TB in smear-positive cases much earlier than culture with the added advantage of detection of drug resistance. It had a higher sensitivity and PPV compared with smear microscopy, however, the specificity of the

smear microscopy was much better. As a method of detection of drug resistance, the Anyplex assay had excellent sensitivity and specificity and in addition, had the advantage of detection of drug resistance weeks earlier than the conventional PM.

Limitation of the study include the small number of cases examined; the small number of smear-negative cases included, in addition to the absence of a validation method for testing the sensitivity of the isolates to second-line drugs.

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