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TB culture,PCR ,Lipoarabinomannan in diagnosis of tuberculosis

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Keywords:

Tuberculosis, Methods of TB culture,PCR ,Lipoarabinomannan

Abstract:

Background:

Tuberculosis (TB) has been a major health problem in developing countries.

Rapid diagnosis of *Mycobacterium tuberculosis* infection plays a critical role in controlling the spread of tuberculosis. Conventional methods may take up to several weeks or longer to produce results. This study aimed to compare of different methods like culture on bio F-M media,MP BacT/ALERT bottles, and urine lipoarabinomannan by ELISA

Subjects and methods:

This study was done on 100 patients suspected to have tuberculosis, cases divided into two groups :(**Group A):** 68 clinically suspected pulmonary TB cases**. (Group B)**: 32 clinically suspected extrapulmonary TB cases. All samples from group A and B were subjected to direct staining by Zeihl-Neelsen stain, Culture of the samples on Lowenstein Jensen media, Bio FM media and automated MP BacT/ALERT bottles and detection of urine lipoarabinomannan by ELISA.

Results:

in suspected pulmonary tuberculosis cases the Sensitivity and Specificity of Bio FM were 87.2%, 100% respectively, Sensitivity and Specificity of MP BacT/ ALERT were 74.4%, 100% respectively, Sensitivity and Specificity of urine lipoarabinomannan were 20.5%, 96.9% respectively and in suspected extrapulmonary tuberculosis cases were the Sensitivity and Specificity of Bio FM were 87.5%, 100% respectively, Sensitivity and Specificity of MP BacT/ ALERT were were 50 %, 100% respectively, Sensitivity and Specificity of urine lipoarabinomannan were 12.5%, 100% respectively.

Conclusion:

-The sensitivity of both solid (Lowenstein Jensen) and liquid (Bio FM) media were the same and better than MP BacT/ALERT .Bio FM media and MP BacT/ALERT show shorter time than Lowenstein Jensen for detection of mycobacterial growth .Detection of urine lipoarabinomannan by ELISA is insensitive for the diagnosis of TB,

Introduction

Tuberculosis, or TB, is an infectious bacterial disease caused by Mycobacterium tuberculosis,, which most commonly affects the lungs(Pulmonary TB) but can affect other sites as well (Extrapulmonary TB). It is estimated that 2 billion of the world's population are latently infected with Mycobacterium tuberculosis with a resultant 9.6 million cases of active tuberculosis (TB) and 1. 5 million deaths annually.

Mycobacterial culture, which is regarded as the diagnostic gold standard, needs 10–100 viable bacilli per ml sputum and is therefore much more sensitive but requires a maximum incubation time of 6–8 weeks

Lowenstein Jensen culture (LJ) is the most widely used in low-income countries, it is an egg based medium developed from Jensen's modification of Lowenstein's formula. The inoculation time of the bacilli is up to 8 weeks

Bio-FM is an enriched Middlebrook 7H9 medium, optimized for rapid mycobacterial growth whose selectivity is enhanced by a selective VCA (Vancomycin, Colistin and Amphotericin B) supplement, containing a colored indicator that allows the detection of positive cultures which turn into a dark blue to violet color. The results are confirmed by microscopy after ZN staining ***(Essa et al., 2013)***

During lasts decades there have developed automated systems for detection of growth in different microorganisms in liquid medium. Most automated systems are based on different technologies, such as colorimetric methods that detect bacterial CO2 production like BacT/ALERT 3D system***(Adamik et al., 2021)***

As a strategy for rapid TB diagnosis, the detection of Mycobacterium tuberculosis antigens has been explored over several decades. Lipoarabinomannan (LAM), a 17.5 kD glycolipid component of the outer cell wall of mycobacteria is an attractive diagnostic target**.** LAM is released when Mycobacterium tuberculosis is lysed by the host immune system filtered by the kidneys and can be detected in the urine as a potential same day diagnostic test for tuberculosis***(Agha et al., 2013).***

To overcome problems of conventional methods, diagnostic methods for tuberculosis using nucleic acid based amplification have improved TB diagnosis, thus making early diagnosis and treatment for tuberculosis possible ***(Lim et al., 2014)*** .

The Anyplex MTB/NTM real-time detection assay (Seegene) is a test suitable for the direct detection and discrimination of both MTB and NTM. It is validated for a wide range of sample types. Result interpretation is automated and can be reported within 3.5 hours of sample receipt ***(Sawatpanich et al., 2022)***

Subjects and Methods

This study was done on 100 patients suspected to have tuberculosis (68 cases pulmonary and 32 cases extrapulmonary), Age of the patients ranged from 15-76 years they included 62 male and 38 female from patients admitted to chest department in Assiut University Hospital, outpatient from TB clinic and orthopedic operation room

Cases divided into two groups :(**Group A):** 68 clinically suspected pulmonary TB cases**. (Group B)**: 32 clinically suspected extrapulmonary TB cases.All patients were subjected to the following: full history taking, clinical examination and chest x ray

Methods

Samples :(Pulmonary TB) sputum (53) and bronchoalveolar lavage(15).(Extrapulmonary) different kinds of clinical samples including pleural fluid (11), pus (6), urine (6) and stool (5), ascetic fluid (3), bone tissue (1), were collected.

All samples from group A and B were subjected to the following:

Microbiological tests: Direct staining by Zeihl-Neelsen stain, Culture of the samples on Lowenstein Jensen media, Bio FM media and automated MP MP BacT/ALERT bottles

Specific test: lipoarabinomannan by ELISA for urine samples from group A, B and 22 healthy control group cross matched age and sex with cases groups

Specimens collected from contaminated sites were liquefied, decontaminated and concentrated by using the modified petroff´s method but Specimens collected from sterile sites were concentrated by centrifugation (3000 g for 15 min) without prior decontamination.

Smear Preparation

Smears were prepared from all samples and examined for the presence of acid-fast bacilli (AFB) using Zeihl-Neelsen´s.

Inoculation on LJ Medium "Gold Standard"

Then 0.5 mL were inoculated in the culture medium (LJ) and incubated at 37˚C for 8 weeks. The readings of cultures were done weekly for 8 weeks.

Inoculation in MP Bottle for BacT/ALERT 3D (bioMérieux)

The MP bottle contained 10 mL of liquid medium (7H9 Middebrook) with casein, serum bovine albumin and catalase, 0.5 ml of the digested and decontaminated sample was inculated in a bottle then 0.5 mL of antibiotic supplement MB/BacT (amphotericin B, azlocillin, nalidixic acid,polymyxin B, trimethoprim, vancomycin) was added to reduce the incidence of other bacteria contamination,in case of sterile Specimens 0.5 mL of reconstitution fluid and 0.5 mL of samples was inoculated in a bottle and incubated in the BacT/ALERT 3D system for 4 weeks all samples were identified as positive by the instrument BacT/ALERT 3D the ZN staining was performed to confirm positive results.

Inoculation on Bio –FM medium

Specimens culturing was done on Bio-FM medium.it is an enriched Middlebrook 7H9 medium, optimized for rapid mycobacterial growth whose selectivity is enhanced by a selective VCA (Vancomycin, Colistin and Amphotericin B) supplement, containing a colored indicator that allows the detection of positive cultures which turn into a dark blue to violet color. The results are confirmed by microscopy after ZN staining,

Samples were incubated for 5–6 weeks at 37 _C. Reading cycle was performed by the following way: 2–4 weekly readings for 3–4 weeks, then twice a week for another 2 weeks.

Examination of the sediment and liquid medium

–The bottom of the tubes and the liquid medium were carefully examine

–If signs of growth were present:

–Dark blue/violet grains or small flakes that have settled at the bottom of the tube: presumption of MTB

Bio-FM

Lipoarabinomannan by ELISA

Urine sampels were collected in a sterile plastic container and centrifuged for 20-mins at the speed of 2000-3000 rpm, and then the supernatant was taken and stored at -20 ℃, until processing. Lipoarabinomannan was measured in urine by ELISA (Human LAM ELISA kit. (WKEA). The kit uses Purified Human LAM antibody to coat microtiter plate wells, make solid- phase antibody, then added LAM to the wells. Combined LAM antibody with which the enzyme was labeled, becomes the antibody–antigen–enzyme-antibody complex. After washing completely, substrate was added and the substrate becomes blue in color In the HRP enzyme-catalyzed reaction, the reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of LAM in the samples is then determined by comparing the O.D. of the samples to the O.D. of the control samples, the cutoff point of lipoarabinomannan was at 0.35

Seegene Anyplex MTB/NTM TB PCR

For DNA extraction, 100 µl of DNA extraction solution was added and mixed with vortexing. Then the sample was allowed to boil at a heat block of 100°C for 20 min and the mixtures were subjected to 5 min of centrifugation at 15,000 ×g. A 5 µl aliquot of supernatant was added to 15 µl of master mix solution which contain 10 µl 2 × Anyplex PCR master mixes, 3 µl methoxypsoralen (8-MOP), and 2 µl 10× M. tuberculosis/NTM oligonucleotide mix. Amplification and detection were performed on CFX 96TM Realtime PCR System (Bio-Rad Laboratories) for all sample extracts. MTBC detection targeted the IS6110 and MPB64 genes, while NTM detection was based on amplification and detection of a section of the 16S rRNA gene. In addition to the in-house extraction controls, each run included positive and negative amplification controls provided in the kit and an internal control in the master mix to detect inhibition. Result interpretation was performed automatically using the instrument's software according to threshold and cutoff values outlined by the manufacturer

Data analysis:

Data collected and analyzed by computer program SPSS" ver. 21" Chicago. USA. Data expressed as mean± SE, number and percentage. Mann-Whitney was used to determine significant for the numeric variables while Chi square test was used to determine the significance for categorical variables. Pearson correlation was used to determine the correlation between variables in the same group. The ROC curve was used for determination of the sensitivity and the specificity for the different cut-off values of the tumor markers.

Results:

This study was done on 100 patient, 68 cases suspected to have pulmonary tuberculosis they include 44(64.7%) male and 24(35.3%) female with age ranged from 17 to 77 years with mean age±S.D 47.35± 14.58 years and 32 cases suspected to have extrapulmonary tuberculosis they include18 (56.2%) male and 14 (43.8%) female with age ranged from 24 to 78 years with mean age±S.D 44.81± 15.24 years.

Table I Positive results among studied cases by different methods.

	Z- N stain	LJ culture	BacT /ALERT	Bio FM	LAM	PCR
	Positive no (%)	Positive no (%)	Positive no (%)	Positive no (%)	Positive no (%)	Positive no (%)
Pulmonary N=68	40 (58.8%)	39 (57.4%)	29 (42.6%)	34 (50%)	9 (13.2%)	49 (72.1%)
Extrapulmonary N=32	6 (18.8%)	8 (25%)	4 (12.5%)	7 (21.9%)	1 (3.1%)	13 (40.6%)

Table II Comparison of the Bio FM media with Lowenstein Jensen culture

			Lowenstein Jensen culture		Sensitivity	Specificity	PPV	NPV
			Positive	Negative				
Bio FM media	**pulmonary**	**Positive**	TP = 34	FP = 0	87.20 %	100.0 %	100 %	85.3 %
		Negative	FN = 5	TN = 29				
	Extrapulmonary	**Positive**	TP = 7	FP = 0	87.50 %	100 %	100 %	96.0 %
		Negative	FN = 1	TN = 24				

Table III Comparison of MP BacT/ALERT with Lowenstein Jensen culture

			Lowenstein Jensen culture		Sensitivity	Specificity	PPV	NPV
			Positive	**Negative**				
MP BacT/ALERT	**pulmonary**	**Positive**	TP =29	FP = 0	74.4 %	100 %	100 %	74.4 %
		Negative	FN = 10	TN = 29				
	Extrapulmonary	**Positive**	TP = 4	FP = 0	50 %	100 %	100 %	85.7 %
		Negative	FN = 4	TN = 24				

There was statistically significant differences between the LJ groups and Bact/ Alert group (P^1=0.001) and between the LJ groups and Bio FM media group (P^2=0.007)but there was no statistically significant differences between Bio FM media group and BacT/ALERTgroup (P^3=0.205).TableIV

Table IV Comparison between Lowenstein Jensen culture, Bio FM media and MP BacT/ALERT as regard to mean detection time and duration ranges in days

Mean duration of illness	LJ Positive	MP BacT/ALERT Positive	Bio FM media Positive	P Value
Min.	7	7	7	P^1 0.001^{**}
Max.	56	42	42	
Mean	25.06	16.33	19.07	P^2 0.007^{**}
S.D.	11.29	9.58	8.82	P^3 0.205^{ns}

ns : No statistically significant difference (p>0.05),* Statistically significant difference (p<0.05)

Statistically high significant difference (p<0.01),* Statistically very high significant difference (p<0.001)

Table V Comparison of urine lipoarabinomannan with Lowenstein Jensen culture

			Lowenstein Jensen culture		Sensitivity	Specificity	PPV	NPV
			Positive	**Negative**				
LAM by ELISA	**pulmonary**	**Positive**	TP =8	FP = 1	20.5 %	96.9 %	88.9 %	47.5 %
		Negative	FN = 31	TN = 28				
	Extrapulmonary	**Positive**	TP = 1	FP = 0	12.5 %	100 %	100 %	77.4 %
		Negative	FN = 7	TN = 24				

Table VI Comparison of PCR results with Lowenstein Jensen culture

			Lowenstein Jensen culture		Sensitivity	Specificity	PPV	NPV
			Positive	**Negative**				
PCR	**Pulmonary**	**Positive**	TP =39	FP = 10	100 %	65.5 %	79.6 %	100 %
		Negative	FN = 0	TN = 19				
		Positive	TP = 8	FP = 5	100	79.2	61.5	100

	Extrapulmonary				%	%	%	%
		Negative	FN = 0	TN = 19				

Discussion:

Bio FM broth is also a manual system based on Middlebrook 7H9 broth optimised for mycobacterial growth and containing a coloured indicator that allows the detection of positive cultures which become a dark blue to violet colour ***(Ramarokoto et al., 2007).***

In the present study, in suspected pulmonary tuberculosis cases the Sensitivity and Specificity of Bio FM were 87.2%, 100% respectively and in suspected extrapulmonary tuberculosis cases were 87.5%, 100% respectively.As regard to the mean detection time and duration ranges in days, here there was statistically significant differences between the LJ Positive groups and Bio FM media Positive group (P=0.007).

This results are in agreement with ***Essa et al., 2013*** who reported the detection rate (94% of the total cases gave positive results on Lowenstein Jensen compared with 92% on Bio-FM) but the mean detection time of cases on Bio- FM was highly significantly shorter than that of cases on Lowenstein Jensen (12.58 ± 8.622 days for Bio-FM versus 20.62 ± 9.640 days for Lowenstein Jensen (P <0.001), and with ***Ramarokoto et al., 2007*** who found no significant difference in detection rate between Lowenstein Jensen compared with that on Bio-FM. It was 96.58% and 92.3% for Lowenstein Jensen and Bio-FM respectively but also the mean detection time of cases on Bio-FM was highly significantly shorter than that of cases on Lowenstein Jensen (12.42 days versus 20.7 days (P <0.001).

It is possible for all laboratories performing mycobacterial culture to use the Bio FM medium,unlike the other culture systems in liquid medium, it does not require costly specific equipment; the Bio FM system is entirely manual, with visual reading of cultures, which is therefore simple, although only qualitative ,due to its ease of use and the rapid growth time of mycobacteria on Bio FM medium so it can be used for early and rapid detection of M. tuberculosis ***(Ramarokoto et al., 2007).*** Furthermore, the cost of the Bio FM is three to four times higher than for LJ, and it can therefore not be used for all samples routinely analyzed in low income countries. Bio FM could be used in combination with LJ for bacteriological diagnosis of extrapulmonary TB, which is usually the most difficult to diagnose using smear microscopy, and the association of two methods, a liquid and a solid medium, would be the optimal solution for mycobacterial detection.It could also be useful in those countries that are unable to make LJ medium, as well as in laboratories that do not have large volumes of samples to process and which cannot justify using a costly system such as BACTEC or BacT/ALERT3D system.***(Heifets et al., 2000).***

During lasts decades there have developed automated systems for detection of growth in different microorganisms in liquid medium,most automated systems are based on different technologies, such as colorimetric methods that detect bacterial CO2 production like BacT/ALERT 3D system, ***(Martinez et al., 2014)*** BacT/ ALERT 3D system, is a colorimetric fully automated non-radiometric liquid culture system This instrument is approved by Food and Drug administration in 1996 ***(Li et al., 2022)***

In the present study, in suspected pulmonary tuberculosis cases the Sensitivity and Specificity of BacT/ ALERT were 74.4%, 100% respectively and in suspected extrapulmonary tuberculosis cases were 50 %, 100% respectively. As regard to the mean detection time and duration, there was statistically significant differences between the LJ Positive groups and BacT/ALERTPositive group (P=0.001).

This results are in agreement with and with ***Rahaman et al.,2015*** who reported that Sensitivity and specificity of BacT/ALERT MP culture were 76% and 85%, and with ***Naveen and peerapur, 2012*** who reported that the sensitivity, specificity, positive predictive value and the negative predictive value of the MB/BACT in comparison to LJ was 69.5%, 94.3%, 95% and 66.7% respectively MB/BACT is a safer and quicker method, as it is an automated method which involves liquid media and as it does not involve any radioactive material and the mean duration of the isolation on LJ and MB/BACT was 30.81 days and 18.70 days respectively, and with ***Dionne et al.,2007*** who reported that sensitivity for mycobacterial recovery was 65% for the MP BacT/ALERT and recovery time of mycobacterium tuberculosis by MP BacT/ALERT is 16.9 days, and with ***Parrish et al., 2009*** reported that sensitivity of Mycobacterium sp. recovery was 66.6% .

our results are controversary with ***Amer et al.,2016*** who reported that BacT/ALERT 3D system show Sensitivity 100% Specificity 85.7% The mean times to detection of mycobacteria by BacT/ALERT 3D system and LJ medium were 14.2 and 24.3 days, respectivelyand with ***Sorlozano et al.,2009*** who reported that sensitivity values for the BacT/ALERT 3D system range from 78% to 99% and with ***Piersimoni et al., 2001*** who reported that cultured 67 cases on Lowenstein Jensen in comparison MP/BacT ALERT 3D System ,62 cases gave positive results on Lowenstein Jensen with a detection rate of about 92.53%and with ***Parrish et al., 2009*** reported that the time to detection by the BacT/ALERT MP system was 25.2 days.

One of the disadvantages of culture in liquid medium is that it does not provide visible colonies increasing the time required for confirmation of the result. However, the direct testing of positive BacT/ALERT MP broth medium by PCR allows for the accurate and rapid identification of M. tuberculosis. Especially that ZN staining from the bottle failed to confirm the positive signal in 15.6% of positive samples. the application of PCR assay directly on positive liquid media of automated systems allows confirmation of the results and fast identification of M. tuberculosis.***(Amer et al., 2016).***

BacT/ALERT 3D system requires one person who is good at computer basics and is trained, in order to feed the data of the sample and to take the bar code reading. Even though the cost of each MB/BACT bottle is costlier as compared to the conventional LJ medium and the MB7H10 agar, it is cost effective in identifying the growth 1-2 weeks earlier and with even minimum amount of growth.***(Li et al., 2022)***

In the present study, we found that in suspected pulmonary tuberculosis cases, Sensitivity and Specificity of lipoarabinomannan (LAM by ELISA) were 20.5%, 96.9% respectively and in suspected extrapulmonary tuberculosis cases, Sensitivity and Specificity were 12.5%, 100% respectively.

These results are in agreement with ***Daley et al., 2009*** found that when positivity on either LJ or BACTEC was considered, LAM sensitivity was 17.8%, with a specificity of 87.7% .Compared to positivity on both LJ and BACTEC, LAM sensitivity was 5.8%, with a specificity of 88.8%. Compared to the clinical diagnosis, LAM sensitivity was 20.0%, with a specificity of 83.3%.

seven studies, assessing test accuracy in microbiologically confirmed cases only, estimates of sensitivity ranged from 13% to 93% and specificity from 87% to 99%. When results were stratified by HIV status in five studies, mean sensitivity in HIV-negative patients was 14% (range 7–24%) and in HIV-positive patients 51% (32–69%). The sensitivity of the test was 3–53% higher in HIV-positive than HIV-negative subgroups and was highest with advanced immunosuppression***(Minion et al., 2011)***

Nicol et al., 2014 found that ELISA was positive in 21 of 535 (4%) children, including two of 89 (2%) with definite tuberculosis, ten of 250 (4%) with possible tuberculosis, and nine of 196 (5%) with not tuberculosis. With culture-confirmed tuberculosis as the reference standard, sensitivity was 2.3% and specificity 95.7%.

our results are controversary with ***Agha et al., 2013*** who reported that Urine LAM test had sensitivity, specificity, PPV, NPV, and an accuracy of 81.2%, 95.7%, 97.2%, 73.8%, and 86.4% respectively. In another study ***martinson et al.,2011*** reported that sensitivity and specificity of a positive LAM for culture-confirmed tuberculosis were 65% and 86% respectively.

Youssef et al., 2016 reported that Quantitative urine LAM had a sensitivity of 85.5%, specificity 90%, accuracy 86.1%, positive predictive value 98.1%, and negative predictive value 50%.

The state of circulating LAM may therefore have major implications for urine LAM antigen detection assays ,free non-antibody associated LAM is of a size comparable to myoglobin (17 kd), which rapidly crosses the glomerular basement membrane. Glomerular filtration of systemically circulating LAM has been the premise to date, on which urine LAM has been interpreted as a correlate of pulmonary TB. However, LAM antigen complexed with IgG (150 kd), IgA (370 kd) or IgM (1000 kd) antibodies would be too large to pass through the normal healthy human glomerulus***.(Ricks et al., 2020)***

Therefore in the presence of circulating anti-LAM immunoglobulin, LAM detected in urine might be more likely to reflect local renal involvement with TB rather than distant pulmonary disease***(Lawn et al., 2012).***

In the present study, the results of PCR in suspected pulmonary tuberculosis cases it was found that 49 (72.1%) were positive cases it was found that 48 cases (70.6%) was MTB, 1 case (1.5%) NTM, Sensitivity and Specificity were 100%, 65.5% respectively and in suspected extrapulmonary tuberculosis it was found that 13 (40.6%) were positive it was found that 8 cases (25%) was MTB, 5 cases (15.6%) NTM, Sensitivity and Specificity were 100%, Specificity 79.2% respectively.

These results are in agreement with ***Johar et al., 2014*** who reported that for pulmonary specimens Sensitivity and specificity of PCR was found to be 89% and 71% respectively. For extra-pulmonary specimens Sensitivity and specificity of PCR was 92% and 67%, respectively, and with ***Chakravorty et al .,2005*** who reported that the sensitivity and specificity of PCR were 99.1% and 71.2%, respectively, with culture used as the gold standard, ***Raveendran and wattal ,2016*** reported that the role of PCR in the diagnosis of extrapulmonary has been evaluated previously as an alternative diagnostic tool and has yielded variable results with sensitivities ranging from 42 to100% and specificities ranging from 85 to 100% using various PCR targets.

Lim et al.,2014 who reported that the diagnostic sensitivity and specificity of the Anyplex TB PCR were 87.5% and 98.2% respectively but Specificity in our results was lower, Specificity of PCR results varies between laboratories due to procedural differences, differences in cross-contamination rates and the choice of primers. Also the primary limitation of PCR arises from the absence of a suitable gold standard to assess its efficiency. When culture is used as a gold standard in comparison studies, samples containing non-viable Mycobacteria may lead to a false positive PCR, thereby misleading clinicians**.**

PCR is found to be very rapid and sensitive method to aid in early diagnosis, treatment, and cure of TB, especially extrapulmonary but the drawbacks of PCR are its high cost, specific requirement of infrastructure, equipment, and expertise and lack of treatment monitoring by PCR ***(Nagpal et al., 2016).***

Our results are controversary with ***Wang et al., 2013*** reported that the real-time PCR (RT-PCR) for TB is a rapid and reliable method for the diagnosis of TB. Although TB RT-PCR provides high specificity (up to 100%), the sensitivity has a wide range (42.8–96.7%), depending on the results of smears, cultures and other clinical specimens.

Conclusion:

Culture procedure is considered to be more sensitive and specific than microscopic examination for the detection of mycobacteria.

-The sensitivity of both solid (Lowenstein Jensen) and liquid (Bio FM) media were the same and better than MP BacT/ALERT system.

- Bio FM media and MP BacT/ALERT system show shorter time than Lowenstein Jensen for detection of mycobacterial growth which was very useful to provide faster initiation of treatment and better outcome for the patients.
- Detection of urine lipoarabinomannan by ELISA is insensitive for the diagnosis of TB, although its specificity is adequate.
-The application of the Seegene Anyplex MTB/NTM detection assay in mycobacteriology laboratory plays an important role for rapid diagnosis of mycobacteria.

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