Evaluation of direct detection of *Mycobacterium tuberculosis* in clinical samples using the BD ProbeTec ET system

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ABSTRACT

الأهداف: تقييم مدى فعالية استخدام نظام بيكتون ديكينسون بروب تيك إي تي أثناء التقصي عن عصيات السل الرئوي في العينات التنفسية والغير تنفسية ومقارنة نتائج هذا النظام بنتائج الفحص المجهري، وزراعة الخلايا.

الطريقة: أُجريت هذه الدراسة الاستطلاعية في مستشفى النساء والولادة، المدينة المنورة، المملكة العربية السعودية وذلك خلال الفترة من أكتوبر 2008م إلى أكتوبر 2009م. شملت هذه الدراسة 70 عينة تنفسية وغير تنفسية ويُشتبه إصابتها بالسل الرئوي. لقد قمنا بتحليل نتائج العينات بواسطة الطرق التالية: الفحص المجهري، وزراعة الخلايا (في الوسط السائل والجامد)، واستخدام نظام بيكتون ديكينسون بروب تيك إي تي (DB ProbeTec ET™ system).

النتائج: لقد تمت دراسة 70 عينة (47 عينة تنفسية، و23 عينة غير تنفسية). أشارت النتائج إلى أنه قد تم الحصول على 12 عينة (92.3%) إيجابية من خلال نظام بروب تيك وذلك من أصل 13 عينة مصابة بعصيات السل الرئوي والتي تم عزلها من زراعة الخلايا، فيما كانت نتيجة عينة واحدة إيجابية في هذا النظام ولكنها لم تنمو في الوسط المزروع. وكانت نتائج عينتان شاذة (نتائج سلبية وإيجابية خاطئة). لقد أظهرت نتائج تقييم هذا النظام درجة حساسية تصل إلى 92.3%، ودرجة دقة بنسبة 98%، وكانت درجة توقع النتائج الإيجابية 92.3%، ودرجة توقع النتائج السلبية 98% وذلك لجميع العينات التنفسية وغير التنفسية، حيث كانت نتائج تقييم النظام للعينات التنفسية كالتالي: 88% لدرجة الدقة، و100% لدرجة الحساسية وتوقع النتائج الإيجابية، فيما كانت 97.3% لدرجة توقع النتائج السلبية. وكانت نتائج تقييم النظام للعينات غير التنفسية كالتالي: 100% لدرجة الحساسية، و93.3% لدرجة الدقة، و80% لدرجة توقع النتائج الإيجابية، و100% لدرجة توقع النتائج السلبية.

خاتمة: أثبتت الدراسة أن استخدام طريقة بيكتون ديكينسون بروب تيك إي تي تعد سريعة ودقيقة أثناء التقصي عن عصيات السل الرئوي وذلك بالمقارنة مع طريقة زراعة الخلايا المعتادة، وتتضح مدى فعالية هذا النظام مع العينات التنفسية التي كانت نتائجها سلبية.

Objectives: To evaluate the performance of the semi-automated BD ProbeTec ET system for the direct detection of *Mycobacterium tuberculosis* complex (MTBC) in comparison with microscopy, and culture for respiratory and non-respiratory specimens.

Methods: The study was conducted in the Maternity and Children's Hospital, Madina, Saudi Arabia from October 2008 to October 2009. A single center prospective study of 70 suspected tuberculosis samples were subjected to microscopy, culture (solid and liquid), and the DB ProbeTec ET system.

Results: A total of 70 specimens were studied; 47 respiratory, and 23 non-respiratory. Twelve (92.3%) ProbeTec positive results were obtained from 13 MTBC isolates from culture, while one specimen was BD ProbeTec ET positive, but yielded no growth on culture. Two samples gave anomalous results (false negative and positive results). The evaluated system showed sensitivity of 92.3%, specificity of 98%, positive predictive value of 92.3%, and negative predictive value of 98% for all specimens, while 88% sensitivity, 100% specificity, 100% positive predictive value, and 97.3% negative predictive value in cases of respiratory specimens, and 100% sensitivity, 93.3% specificity, 80% positive predictive value, and 100% negative predictive value in cases of non-respiratory specimens.

Conclusion: The ProbeTec ET is a rapid and specific method for direct detection of MTBC in clinical specimens compared with the 'gold standard' of culture, especially in patients with smear-negative non-respiratory specimens.

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It was estimated that the global pandemic of tuberculosis (TB) has caused disease in 8 million and killed 1.6 million people in 2006.[1] Tuberculosis is an increasing health problem worldwide, especially in developing countries. The spread of HIV/AIDS and the emergence of multidrug-resistant TB are contributing to the worsening impact of this disease.[2] The primary TB diagnosis mainly depends on microscopic examination and detection of acid-fast bacilli (AFB) in the clinical specimen. The AFB smear results are available in hours or less, but the technique has poor sensitivity and cannot distinguish between different species of mycobacteria; however, it remains the international standard for TB diagnosis.[3,4] A definitive diagnosis of TB is still dependent on the isolation of *Mycobacterium tuberculosis (M. tuberculosis)* by cultivation. However, cultivation on solid media, such as that of Löwenstein-Jensen (LJ), is both time-consuming, taking up to 6 to 8 weeks, and insensitive.[5] Nonradioactive broth-based culture methods were recently introduced. The BACTEC MGIT 960 system (MGIT; BBL Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) is a fluorescence-based, continuously monitoring detection system that measures bacterial growth by determining oxygen consumption.[6-8] The BD ProbeTec ETTM system (Becton Dickinson, Oxford, UK) is a semi-automated real-time system, which allows simultaneous amplification and detection of *M. tuberculosis* target DNA IS6110 using amplification primers and a fluorescently labelled probe, and has been consistently reported to have an excellent performance.[9] The aim of this study is to assess the performance of the newly developed semi-automated BD ProbeTec ET system for the direct detection of the *M. tuberculosis* complex (MTBC) in comparison with microscopy, and culture for respiratory and non-respiratory specimens.

Methods. The study was conducted in the Central Tuberculosis Laboratory, the Maternity and Children's Hospital, Madina, Kingdom of Saudi Arabia (KSA) from October 2008 to October 2009. All specimens in this study were nonselective and were routinely sent to the Central Tuberculosis Laboratory from different hospitals in the Madina region. These specimens included 47 respiratory, and 23 non-respiratory specimens. The study was conducted after approval from the ethical committee of the Maternity and Children's Hospital, Madina, KSA. All specimens were processed following conventional methods for mycobacterial isolation, digestion, and decontamination by the N-acetyl-L-cysteine-NaOH procedure using the MycoPrep specimen digestion/decontamination kit (BBL MycoPrep, Becton Dickinson, Franklin Lakes, NJ, USA). Briefly, equal volumes of the freshly prepared MycoPrep NALC-NaOH solution were added to the sputum specimen, mixed on vortex, and left to stand at room temperature for 15 minutes. The mixture was completed to double its volume with sterile phosphate buffer pH 6.8 and centrifuged at 3000 x g for 15 minutes. The supernatant was decanted, and the sediment was used for AFB microscopy (Ziehl-Neelsen [ZN] stain, which is the routine method in our laboratory), and for cultures (one solid medium [Löwenstein-Jensen, Saudi Prepared Media Laboratories, Riyadh, KSA]) and one liquid medium (Mycobacteria Growth Indicator Tube [MGIT], BACTEC MGIT 960, Becton Dickinson, Franklin Lakes, NJ, USA) with 0.1 and 0.5 ml, and incubated at 37°C for 8 and 6 weeks. The LJ cultures were examined twice per week, whereas the BACTEC-MGIT 960 is an automated system that provides continuous monitoring. The initial acid fast smear was prepared and graded according to the recommended procedures of the Centers for Disease Control.[5] Smears from suspected colonies were stained with ZN stain for acid-alcohol fast bacilli. All mycobacterial isolates were identified using conventional methods of identifications (based on their rate of growth and pigmentation on LJ) and on biochemical tests. The Becton Dickinson ProbeTec ET System was used for identification of MTBC. The BD ProbeTec ET testing was carried out according to the manufacturer's recommendations, and is described elsewhere.[9] Briefly, a 500 µL aliquot of treated sediment was added to 1 mL of sample wash buffer and centrifuged for 3 minutes at 12,200 x g. The supernatant was discarded, and the pellet was heated at 105°C for 30 minutes and then resuspended in 100 µL of sample lysis buffer. This mixture was sonicated for 45 minutes at 65°C. Finally, 600 µL of sample neutralization buffer was added. For each run, one positive and one negative control was prepared. Samples and controls were distributed randomly in the sample rack. Inhibition of amplification is monitored by the internal amplification control (IAC), which runs as a duplex test along with the target in the same microwell.

The data were analyzed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 12.

Results. In this study, a total of 70 specimens (47 respiratory and 23 non-respiratory) from 55 males and 15 females were collected. Of these, 53 samples were culture negative, and 17 samples were culture positive for AFB: 13 isolates were identified to be MTBC, whereas the remaining 4 strains were classified as NTM (nontuberculous mycobacteria). In total, out of these

70 specimens, 7 were positive by all methods (Z-N smear, culture on both LJ and MGIT broth, and BD ProbeTec ET (**Table 1**). Of these, 8 specimens were smear positive; 6 respiratory specimens and 2 non-respiratory specimens) while 57 were smear negative; 40 respiratory specimens and 17 non-respiratory specimens. All smear positive specimens were also BD ProbeTec ET and culture positive. Out of 57 smear negative specimens, 4 were BD ProbeTec ET and culture positive, and one specimen was BD ProbeTec ET positive, but yielded no growth on culture (**Table 2**). Analysis of the 2 samples that gave anomalous results showed that one sample was culture positive for MTBC organisms but negative in the ProbeTec system (false negative). This sample remained negative on repeat testing. The samples found to give growths of NTM organisms, were also found to be negative in the ProbeTec system. One sample was considered to be false-positive (yielded no growth on culture). One sample was smear-positive but culture and ProbeTec negative, despite prolonged incubation in both liquid and solid culture systems. This finding probably represents the presence of non-viable environmental mycobacteria. **Table 3** shows the overall evaluation parameters of the ProbeTec system compared to MGIT culture.

Table 1 - Combination of *Mycobacterium tuberculosis* complex positivity in the various mycobacterial diagnostic tools included in this study.

Smear	MGIT	DB ProbeTec ET	LJ media	Number
N	P	N	P	1
N	N	P	N	1
P	P	P	P	7
N	P	P	P	4
P	N	P	P	1

N - negative, P - positive, MGIT - Mycobacteria growth indicator tube, LJ - Löwenstein-Jensen

Table 2 - Detailed results of BD probeTec assay compared to Mycobacteria growth indicator tube culture for *Mycobacterium tuberculosis* complex specimens.

Specimen types	Smear results	No.	BD ProbeTec results	Culture results	
				Positive	Negative
Respiratory	Smear positive	6	Positive	5	0
			Negative	0	1
	Smear negative	40	Positive	2	0
			Negative	1	37
Non-respiratory	Smear positive	2	Positive	2	0
			Negative	0	0
	Smear negative	17	Positive	2	1
			Negative	0	14
Total	Smear positive	8	Positive	7	0
			Negative	0	1
	Smear negative	57	Positive	4	1
			Negative	1	51

Table 3 - The BD probeTec results validated against culture outcome.

Parameter	All specimens (%)	Respiratory specimens (%)	Non respiratory specimens (%)
Positive predictive value	(92.3)	(100)	(80)
Negative predictive value	(98.0)	(97.3)	(100)
Sensitivity	(92.3)	(88.8)	(100)
Specificity	(98.0)	(100)	(93.3)

Discussion. The laboratory detection of mycobacterial infection is commonly based on acid-fast staining and culture on solid and liquid media. Staining is a rapid test, but is not very sensitive, particularly in non-respiratory specimens, and is unable to distinguish between different species of mycobacteria.[10] Although culture is the gold standard, and it is specific and more sensitive than smear, it is quite slow and several weeks are required for reporting the results.[5] It is necessary to have methods with high sensitivity and specificity that provide rapid and accurate results. The most promising diagnostic tools for rapid and accurate diagnosis are molecular techniques. Strand displacement amplification (SDA) is a molecular technique based on isothermal amplification of DNA, using a 2-enzyme system (restriction enzyme and DNA polymerase). Later, a semi-automated BD ProbeTec SDA system was evaluated.[11,12] Further technical modifications led to the introduction of a new automated instrument, BD ProbeTec ET, which detects DNA in real-time using exponential SDA. The BD ProbeTec ET system is based on the simultaneous amplification of the target DNA and detection by fluorescent energy transfer for the direct detection of MTBC in clinical samples.[6,9, 12-14]

For evaluation of BD ProbeTec ET in this study, we found that the BD ProbeTec ET has an overall sensitivity of 92.3%, specificity of 98%, and positive predictive value of 92.3%, and negative predictive value

Table 4 - Comparative results of several parameters of BD probeTec.

Reference	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Jesús de la Calle et al, 2003[19]	(93.7)	(98.7)	(83.3)	(99.5)
Rusch-Gerdes & Richter, 2004[15]	(90.3)	(96.9)	(78.3)	(98.9)
Barber, 2008[17]	(85.0)	(100)	(100)	(99.0)
Current work	(92.3)	(98.0)	(92.3)	(98.0)

of 98%. **Table 4** shows a comparison between our results and previous works.[15-17]

The rate of culture positivity of all specimens included in our study was approximately 18.5%, and 17.4% in cases of non-respiratory specimens. High TB-culture positivity detection rates of around 52% have been reported in cases of non-respiratory specimens in some studies, while others calculated only 10% culture positivity rates of all specimens included in their study, and 5% in case of non-respiratory specimens.[15,16] The performance values for smear-negative non-respiratory specimens demonstrate that the BD ProbeTec ET assay also can be used for these specimens to achieve highly sensitive and specific results. Although Johansen et al,[16] have included a high percentage of culture positive specimens (more than 50%) they could obtain only 40.3% sensitivity with the BD ProbeTec ET. They explained such low sensitivity due to the increase of NaOH% during the decontamination procedure may easily result in an increase of the pH of the decontaminated specimens, which may have a different influence on cultures and amplification techniques.

In an attempt to analyze possible reasons for false BD ProbeTec results, the 2 specimens with discrepant results were reanalyzed in a second run using the residual specimens that had been stored in the deep freezer (–20°C), the false-negative result was negative also after repetition. In this case presumably unequal distribution of bacilli may be responsible for negative results. Concerning the false-positive result, it was still positive. Possible explanation for the false-positive result in the first run may be an unknown procedural error, since this was at the beginning of the establishing of the procedure. This was presumed by Bergmann et al,[14] who pointed out that false-positive rates were related to lack of attention to detail on the part of technical personnel. Another explanation is that BDProbeTec ET system detects mycobacterial DNA and may detect viable and non-viable mycobacteria.

McHugh et al[18] previously reported that there is cross-reactivity between the target (IS6110) and mycobacteria other than tuberculosis as they found that the specificity of the SDA was notably low (89%). They estimated that such cross-reactivity may contribute to the decreased specificity of the test. This view is supported by the observation that specificity against culture is good in those samples that are likely to have fewer competing bacteria (cerebrospinal fluid, fine needle aspiration, and pleural fluid) as compared to pus and respiratory specimens, also, the inhibitory samples are frequently a problem in the application of molecular techniques so, re-testing of such samples after freezing and thawing is often successful in removing inhibition. We want to emphasize that the small number of samples limited our study and it will be more informative if we could collect more samples.

In conclusion, this study demonstrates that ProbeTec ET is a rapid and specific method for direct detection of MTBC in clinical specimens compared with the 'gold standard' of culture, especially in patients with smear-negative non-respiratory specimens. Further study with a large number of samples will help in establishing the diagnostic capability of this technique.

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