

Accuracy of a new rapid antigen detection test for pulmonary tuberculosis

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ABSTRACT

Background and Objectives: Tuberculosis (TB) is a major problem in the world. Treatment and control of TB needs detection of the *Mycobacterium tuberculosis* (MT) in the proper samples. While smear doesn't have enough sensitivity, culture and PCR are expensive, time consuming and unavailable in many centers. Recent development of a rapid TB antigen detection test (PrTBK) at Pasteur Institute of Iran could give a simple way for diagnosis of TB in about two hours. In this test the antigen-antibody complex will change color when gold conjugated mouse anti-rabbit antibody detects specific MT cell wall antigen in suspected samples.

Materials and Methods: We evaluated the diagnostic accuracy of PrTBK for diagnosis of pulmonary TB in comparison with smear, culture and PCR techniques in 56 consecutive samples (47 BAL and 13 sputum samples) obtained from patients with clinical suspicion of active TB.

Results: Twentynine patients (52%) were female and seven patients were HIV positive. PrTBK was positive in 17 culture positive and 4 culture negative samples (100% sensitivity, 89% specificity and 92% accuracy in comparison with culture method). In two out of four patients with negative culture who were positive for PrTBK, PCR and anti-tuberculosis drugs trial therapy responses were in favor of tuberculosis. If we take this finding into account, the accuracy of PrTBK will rise.

Conclusion: High sensitivity and accuracy of PrTBK test enable us to initiate treatment on the basis of this convenient and rapid test.

Keywords: Pulmonary tuberculosis, Diagnosis, Rapid test

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INTRODUCTION

Tuberculosis is considered as a major cause of morbidity and mortality worldwide. According to the 2014 WHO report, approximately 9 million people developed TB during 2013, more than half (56%) were in the South-East Asia and Western Pacific Region (1). Diagnosis of tuberculosis (TB) is based on clinico-radiological data and confirmatory *Mycobacterium tuberculosis* studies. Direct smear sputum microscopy is the primary method for diagnosing pulmonary tuberculosis but it lacks enough sensitivity and only about 44% of all new cases are detected by this method (2). Although the sensitivity and specificity of culture method are high but its procedure is slow and time-consuming and needs special laboratory equipment (3-5). Nucleic acid amplification tests (NAATs) can be performed in one day and has specificity and sensitivity comparable to culture methods. Regrettably amplification targets of NAAT are not fully standardized and the diagnostic accuracy is highly variable which needs experienced personnel and expensive equipment (6-9). Considering noticeable improvement in TB standardized care in recent years, lack of a rapid, accurate and cost-effective diagnostic test is a major obstacle for TB control. Recent development of Pasteur rapid TB antigen detection kit (PrTBK) at Pasteur institute of Iran could give a rapid and inexpensive way for diagnosis of TB in two hours. The aim of this study was to evaluate accuracy of this new test.

MATERIALS AND METHODS

Setting and design. Samples (sputum or broncho-alveolar lavage; BAL) were collected from 56 patients suspected to pulmonary tuberculosis by their pulmonologist between September 2008 and May 2010 in Imam Khomeini Medical center, a referral TB center in Tehran, Iran. For obtaining BAL samples, bronchoscopy was done under premedication with fentanyl and midazolam. BAL was done by instillation of 100ml normal saline. Samples were given specific codes and sent simultaneously to Pasteur institute of Iran for direct smear, Lowenstein-Jensen (LJ) culture, PCR and PrTBK. The same samples were also sent to another referral TB laboratory in Tehran (Zarifi) for smear and culture tests. PrTBK was performed on BAL and sputum samples according to

new TB rapid test instructions.

The study was approved by Ethical Committee of Tehran University of Medical Sciences. The results of PrTBK were compared with culture and PCR techniques using chi-square test. The LJ culture was used as the gold standard test.

Culture. Clinical samples (sputum and BAL) were cultured on Lowenstein-Jensen slant media after decontamination according to the CDC methodology for isolation and identification of mycobacteria (10). The grown colonies were characterized by colony characteristics and biochemical reactions (10).

PCR. PCR detection was based on *IS6110* gene (11). DNA extraction was done with "High Pure PCR Template Preparation Kit" (Roche) and PCR was done with *Mycobacterium tuberculosis* FLASH PCR Kit (DNA Technology, Russia).

TB-rapid-test preparation. Antibodies against pathogenic TB strains were obtained by inoculation of rabbits with cell wall proteins purified from sensitive and resistant TB strains obtained from collection of Pasteur Institute of Iran. To remove the non-TB specific immunoglobulins, antibody solution was passed through several absorption processes with sputa of patients who were TB negative through culture and PCR tests (12).

Digestion and decontamination of samples. Samples were decontaminated by "KUBICA" standard method. Solutions used for the KUBICA method were supplied in PrTBK package. 200 µl decontaminated sputum was solubilized by adding 800 µl dissolving solution and boiled for 20 min prior to passage through the filter. The dissolved sputum was then poured onto filtering cartridge (12).

PrTBK test. PrTBK test is based on a filtering device made from a cartridge fitted with adsorbing pads topped with a 0.45 µm nitrocellulose membrane. A small disposable funnel is used to concentrate the aliquot and channel the dissolved sputum in the middle of absorbing area, focusing the fluids in a central area, 0.3 cm in diameter. This method improves the sensitivity of the test and saves the reagents. Samples (200 ml) were allowed to pass through the filter buffer. Then the pre-filter was washed with two drops of washing solution. Thereafter, the funnel and the

pre-filter were removed and a second wash was done with two drops of washing solution. Three drops of antibody solution was added. After absorption and washing, three drops of the gold conjugate solution was added. Antibodies at the surface of the membrane were then reacted with gold-conjugated protein A diluted in PBS. Excess gold was removed by twice washing. After the last liquid washing and absorption, the results were read immediately. Positive controls consisted of 3×10^8 live bacilli suspended in 0.1 ml either negative sputum or PBS equal to McFarland standard 1.0. Negative controls were 0.1 ml either PBS or sputa proven negative by microscopy, PCR analysis as well as culture test. A red-pink color at central region of the filter will indicate the presence of tuberculosis bacilli in the processed sample (12).

Clinical and radiological assessments. Patients were managed by their physicians based on mycobacterial culture and PCR results. TB clinical probability was evaluated by two pulmonologists based on patients' clinico-radiological data before sample evaluation. Patients' CXR and chest CT-scan were evaluated by two radiologists unaware of clinical data for

determining the radiological probability of TB. We reviewed patients' records of anti-TB prescription, further work up for other diagnosis and response of the patients to anti-TB agents after six months for evaluation of cases with discordant culture, PCR and PrTBK results. The Ethics committee of Tehran University of Medical Sciences has approved the study and informed consent was obtained from patients for evaluation of their sputum and BAL samples.

RESULTS

A total of 56 samples (43 BAL and 13 sputum samples) were studied. The mean age of patients was 53 years (in the range of 18 and 83 years). Twenty nine patients (52%) were female and seven patients were HIV positive. Culture results were the same by the two TB laboratories (Pasteur Institute and Zarifi Lab). Table 1 shows the performance of PrTBK in comparison with culture test. Table 2 shows follow-ups of 4 culture negative and positive PrTBK patients.

Table 1. Performance of TB-rapid test compared to LJ culture method

PrTBK test	Culture		Sensitivity (%)	Specificity (%)	Accuracy (%)
	Negative	Positive			
Positive	17	4	100	89.6	92.7
Negative	0	35			

Table 2. Clinico-radiological data of culture negative but PrTBK positive patients

Patients codes	N.R	GH.SH	M.R	M.E
PCR	Positive	Positive	Negative	Negative
CS.1	High	Moderate	Moderate	Very low
CS.2	High	High	moderate	Very low
RS	High/high	Moderate/high	High/high	Very low
Anti-TBR	Yes	Yes	No	No
Follow-up	Death	Partial response	Incomplete follow up	Death
Co-morbidity	Lung cancer	Bronchiectasis	Vasculitis	Esophageal cancer

CS.1 and 2: Clinical probability by clinicians#1 and #2; RS: Radiological probability of TB in chest X-ray and chest CT-scan; Anti-TBR: Anti tuberculosis treatment response;

DISCUSSION

The accuracy of this new TB antigen rapid detection test (PrTBK) was 92.7% with a sensitivity of 100% and a specificity of 89.6% in comparison to conventional LJ culture in our study. The high accuracy of PrTBK enables us to initiate treatment on the basis of this convenient and rapid test in suspicious TB patients as a point-of-care test. Of four patients with negative culture and positive PrTBK test, PCR and anti-tuberculosis trial response of two patients were in favor of tuberculosis diagnosis (Table 2). If we take into account of these findings, the accuracy of PrTBK will rise.

An ideal test for active pulmonary tuberculosis should be easily performed with rapid results having high accuracy, low cost and reproducible results in a variety of settings. It should also be able to follow responses to treatment regimens and drug-susceptibility testing and could distinguish *Mycobacterium tuberculosis* from other mycobacteria.

High accuracy, low cost, rapid (in 1 hour) and easy performance of PrTBK for non-specialized laboratories can be considered as its advantages. On the other hand, this test does not give any information about susceptibility of the infection towards antibiotic treatment. In most resource-limited areas direct sputum smear microscopy remains the main approach in diagnosis of pulmonary TB. However, low sensitivity of this method (range from 34 to 80%) remains as an obstacle (3, 13, 14). Fluorescent microscopy has increased sensitivity (about 10%) with similar specificity in comparison to ordinary light microscopy technique (12). The new PrTBK is also a direct observation method with more sensitivity and specificity in comparison to the direct smear microscopy. The simplicity of PrTBK method is comparable to direct smear microscopy and can be used for screening and case detection even in health system fields. Despite of higher cost of PrTBK than direct microscopy its application in TB diagnosis is reasonable and can be extended to remote health posts.

Human errors play important role in performance of direct smear microscopy and cause major differences in calculated sensitivities in comparison to culture. Culture on LJ medium has been set as the gold standard method for a long time with a high specificity (98%) but the sensitivity of this method varies from 80 to 93% (3, 16). It also allows to differentiate species, drug-susceptibility testing and genotyping studies.

However, it has disadvantages such as time-consuming approach (as the main weakness), high cost, false negative results and contamination (2-5% in good quality TB laboratories) (3, 17).

Nucleic acid amplification tests (NAATs) have been used for many years mostly in developed countries. Sensitivity of NAATs, using culture as a gold standard test, is 95% in smear-positive patients (6, 7) and varies between 50-80% in smear-negative patients (4, 8). Although the specificity of NAATs is quite high (6, 18) but it has not been recommended for routine TB diagnosis due to its inconsistent accuracy (8, 9). This technique has several limitations in practice including its high costs (main limitation of its usage in developing countries), false positive results in patients with bronchogenic carcinoma and past medical history of TB, necessity of special expertise and equipment and inability to address drug resistant infections. Whereas PrTBK can address some of these problems in culture and NAAT such as slowness and high cost but cannot provide any information about bacterial drug susceptibility and genotyping.

Due to the possibility of non-specific reactions of killed bacilli cell wall in PrTBK, we used live bacilli on PBS or sputum smear as positive control. The same method with different antibody has been used in Anda-RT mycobacteria Patho-TB (ANDA biologicals, France) with a reported lower accuracy (19-22).

Although the results of the present study on PrTBK is very promising but it needs confirmation in other settings especially in resource-limited areas.

The new recently developed rapid TB detection test is Xpert MTB/RIF that will be performed in less than 2 hours. The pooled reported criteria of Xpert MTB/RIF were 89% sensitivity and 99% specificity but it is very expensive and may be valuable just as an add-on test in the case of AFB smear-negative samples. In comparison to Xpert MTB/RIF, PrTBK is cheaper and easy to perform which can be used for screening in routine diagnostic laboratories and fields (23).

CONCLUSION

The high accuracy of PrTBK with immediate availability of its results proposes applicability of this test in the beginning of TB treatment especially in smear negative patients. Culture would be needed for confirming the results and performing drug susceptibility testing. Easy performance of this test makes it useful

in screening TB infection in routine diagnostic laboratories and health system fields.

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