

Mycobacteriology

Rapid detection of *Mycobacterium tuberculosis* in sputum by Patho-TB kit in comparison with direct microscopy and culture

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Abstract

The usefulness of a new rapid diagnostic test (Patho-TB) using antibodies specific to mycobacterial antigens was evaluated for the rapid discrimination between pulmonary tuberculosis (TB) and non-TB pulmonary diseases on sputa. One hundred sputa collected from 79 active TB patients and from 21 patients with non-TB pulmonary diseases (asthma and chronic obstructive pulmonary disease) were enrolled into the study and tested for the presence of *Mycobacterium tuberculosis* by Ziehl–Neelsen smear, Patho-TB kit, and Löwenstein–Jensen culture. The sensitivity, specificity, positive predictive value, and negative predictive value of the Patho-TB test were 95%, 100%, 100%, and 84%, respectively. Patho-TB test is simple, quick, and easy to perform. Its sensitivity, specificity, and positive predictive value are satisfactory. Therefore, it could be used as a screening test in poorly equipped laboratories of TB endemic areas.

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1. Introduction

Tuberculosis (TB) is still a serious health problem throughout the world with approximately 9.2 million new cases per year and 1.7 million deaths reported in 2006 by the World Health Organization (2008a, b).

Conventional microscopy is the most commonly used technique in the routine diagnosis of TB. However, sensitivity of sputum Ziehl–Neelsen (ZN) stained does not exceed 60% to 70% as compared with sputum culture (Kim et al., 1984; Levy et al., 1989). In addition, direct microscopic examination is time consuming and requires sophisticated equipment and trained technicians. A delay in the diagnosis together with misdiagnosed TB cases contributes to *Mycobacterium tuberculosis* (MT) transmission and mortality (Lienhardt et al., 2001; MacIntyre et al., 1995). Hence, a sensitive and specific diagnostic test for the rapid identification of patients with active TB would facilitate early treatment and prevention of transmission.

Antibody-based tests for TB diagnosis have been developed for more than 2 decades (Cho et al., 1990; Krambovitis et al., 1984; Sada et al., 1983; Yáñez et al., 1986). They are attractive because of their technical simplicity, rapidity, and low cost. In addition, they permit to avoid the microscopic examination step, which may be difficult to achieve in poorly equipped hospital laboratories.

In this study, the usefulness of the Patho-TB kit for the diagnosis of active pulmonary TB was assessed and compared with conventional bacteriologic techniques.

2. Materials and methods

The present study was carried out between April and August 2008 in the laboratory of microbiology and immunology of Farhat Hached Teaching Hospital of Sousse, Tunisia.

2.1. Study groups

2.1.1. Active pulmonary TB patients

The 79 TB patients included in this study were admitted at the respiratory diseases unit of Farhat Hached Teaching Hospital of Sousse, Tunisia, for highly suspected pulmonary

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Table 1
Characteristics of TB group (TB group) and control group (non-TB)

Study groups	No. of subjects	Sex, male/ female	Mean age (range) (years)
TB group	79	58:21	42 (23–74)
Non-TB	21	17:4	52.5 (25–78)
Asthma	6	5:1	56 (25–78)
COPD	15	13:2	49 (27–72)

COPD = chronic obstructive pulmonary disease.

TB on the basis of clinical and radiologic data. Of these, 58 were male and 21 were female, and their age ranged from 23 to 74 years. In all of them, active pulmonary TB was confirmed by the demonstration of MT in sputum ZN-stained smears and culture on Löwenstein-Jensen (LJ) medium (American Thoracic Society, 2000). The bacilli load in stained smears was assessed according to the recommendations of the American Thoracic Society (2000) by using the following scale: ZN⁻, no acid-fast bacilli (AFB) per 300 fields (at magnitude $\times 100$); ZN \pm , 1 to 2 AFB per 300 fields; ZN⁺, 1 to 9 AFB per 100 fields; ZN2⁺, 1 to 9 AFB per 10 fields; ZN3⁺, 1 to 9 AFB per fields; ZN4⁺, 9 AFB per field. According to Tazir et al. (1979), the 5 positive bacilli load categories mentioned above correspond to 100, 2.10^3 , 30.10^3 , 100.10^3 , and 300.10^3 mycobacteria/mL, respectively.

2.1.2. Control patient group

This group consisted of 21 non-TB patients, including 15 with chronic obstructive pulmonary disease (13 males and 2 females, with age ranging from 25 to 78 years) and 6 with asthma (5 males and 1 female, with age ranging from 27 to 72 years) (Table 1). In all of these patients, sputum direct examination and culture were negative for MT.

2.2. Microscopy and culture

Before testing, the sputa were decontaminated according to Kubica's protocol (David et al., 1989). Microscopy and culture of decontaminated sputum pellet were achieved using the conventional ZN technique (auramine and confirmation by fuchsin) (David et al., 1989). Specimens were cultured on LJ media and incubated at 37 °C for 16 weeks (American Thoracic Society, 2000; David et al., 1989).

2.3. Patho-TB test

The Patho-TB test (Anda-RT mycobacteria Patho-TB; ANDA biologicals, Strasbourg, France) was achieved on the same day as microscopy and culture. It was performed according to the manufacturer's instructions. Sputa were decontaminated using Kubica's method. (All the reagents needed for the achievement of the Kubica's method were supplied in the Patho-TB kit.) Subsequently, 200 μ L of the decontaminated sputum was solubilized by adding 800 μ L of the dissolving solution and boiled for 20 min to pass through the filter. The dissolved sputum was then poured onto the filtering cartridge. Once the sample absorbed, the prefilter was

washed. Then, the prefilter is discarded and the filter washed. TB bacilli, when present in the sample, were immobilized on the filter, and the added rabbit immunoglobulin G (IgG) antibodies react with all MT antigens (extracted by immunochromatographic protocol), leading to the formation of antigen–antibody complexes, which were revealed in the last step by the addition of a gold conjugate. Positivity of the test is evidenced by a red–pink color in the middle of the filter.

2.4. Statistical analysis

The sensitivity, specificity, positive predictive value, and negative predictive value of Patho-TB test were calculated using Epi Info 6 software (Centers for Disease Control and Prevention, Atlanta, GA). Culture of MT on LJ media was used as the “gold standard”.

3. Results

Results of bacteriologic examination and Patho-TB test for all 79 investigated TB patients are given in Table 2. All sputum samples were positive for MT by microscopic examination and culture.

The distribution of the bacilli load in all specimens is shown in Table 2. Of 79 sputum samples, 75 (95%) were positive in Patho-TB test. The bacilli loads of the 4 false-negative samples are given in Table 2. Mycobacterial concentration of these cases were 30.10^3 mycobacteria/mL (1 case ZN⁺), 100.10^3 mycobacteria/mL (2 cases ZN3⁺), and 300.10^3 mycobacteria/mL (1 case ZN4⁺).

None of the 21 controls specimens was positive in Patho-TB test.

The sensitivity, specificity, positive predictive value, and negative predictive value of the Patho-TB test were 95%, 100%, 100% and 84%, respectively.

4. Discussion

Bacilli-positive TB patients are the most potent sources of MT transmission in the community (American Thoracic

Table 2
Results of smear direct examination and culture of various sputum samples tested

	Tuberculous specimens		Nontuberculous specimens	
	Positive	Negative	Positive	Negative
Culture on LJ media	79	0	0	21
ZN score				
ZN ⁻	0		0	21
ZN \pm	0		0	
ZN ⁺	20		0	
ZN2 ⁺	16		0	
ZN3 ⁺	14		0	
ZN4 ⁺	29		0	
Patho-TB test	75	4	0	21

Society, 2000; Beggs et al., 2003; Horna-Campos et al., 2007). Therefore, early detection of MT in clinical samples is important in the control of TB (Carpentier et al., 1995). Direct microscopic examination of clinical samples stained by ZN technique is the most commonly used method for the diagnosis of TB (American Thoracic Society, 2000). Its sensitivity is, however, suboptimal because ZN staining is unlikely to detect less than 5000 bacilli/mL of sample (Jenkins, 1994). Therefore, the development of a simple and inexpensive test, which compares favorably with conventional procedures in terms of sensitivity, would be much helpful in the diagnosis of TB. Over the last decades, new tests based on the detection of mycobacterial antigens in clinical samples were developed (Cho et al., 1990; Krambovitis et al., 1984, 1986; Sada et al., 1983, 1992; Yáñez et al., 1986). The reported studies aimed at the demonstration of MT antigens in cerebrospinal fluid (Krambovitis et al., 1984; Sada et al., 1983), serum (Krambovitis et al., 1986; Sada et al., 1992), and sputum (Cho et al., 1990; Yáñez et al., 1986) by using either homemade techniques (Cho et al., 1990) or commercial kits (Fabre et al., 2007; Nilanjan et al., 2009). The overall performance of these tests was considered satisfactory (Tables 3 and 4).

The incidence of TB is moderate in Tunisia (World Health Organization, 2008b). The aim of the present study was to evaluate the Patho-TB kit for the diagnosis of TB as compared with microscopic examination and culture in our working area. The test was found very appropriate for such purpose because it is simple, rapid, and easy to perform. In addition, it does not require any sophisticated equipment or special skillfulness and offers the opportunity of investigating a large series of samples in a short period (Cho et al., 1990; Fabre et al., 2007; Krambovitis et al., 1986). Moreover, all the reagents needed for decontamination of sputa by the Kubica's method were supplied in the Patho-TB kit. Indeed, Kubica's method can be standardized in different laboratories, and the cost of TB diagnosis will be reduced. Therefore, Patho-TB test could be of great value for rapid diagnosis of TB, in contrast to direct microscopic examination of ZN-stained smears, which is time consuming especially for low-loaded MT slides. Molecular techniques like polymerase chain reaction are laborious and expensive and require highly trained technicians.

In our series of patients, the sensitivity of Patho-TB test was found satisfactory, although slightly lower than that of conventional bacteriologic methods, because 4 confirmed

Table 3
Correlation between results of Phato-TB test and status of disease

Patho-TB test	No. of patients	
	Tuberculous patients (<i>n</i> = 79)	Nontuberculous patients (<i>n</i> = 21)
Positive	75	0
Negative	4	21

Table 4
Results of Phato-TB test according to the grading of the ZN

	Patho-TB test results			
	Positive (%)	Negative (%)	False positive (%)	False negative (%)
ZN score (<i>n</i>)				
ZN- (21)	0 (0)	21 (100)	0 (0)	
ZN± (0)	0 (0)			
ZN+ (20)	19 (95)			1 (5.3)
ZN2+ (16)	16 (100)			0 (0)
ZN3+ (14)	12 (86)			2 (17)
ZN4+ (29)	28 (97)			1 (3.5)
Culture on LJ media (<i>n</i>)				
Positive (75)	71 (95)			4 (5)
Negative (25)	0 (0)	25 (0)		

TB cases were negative in Patho-TB test. In terms of mycobacterial load, these include 1 weakly positive case (classified as ZN+) and 3 strongly positive cases (classified as ZN3+ to ZN4+). These findings suggest that the MT load of the sputa-decontaminated pellet does not affect the result of Patho-TB test. On the other hand, the dilution of the sample pellet does not seem to have an impact on the lowering of the MT density, as far as the result of Patho-TB test is concerned. Indeed, positive results with Patho-TB were obtained at satisfactory percentage (95%) in weakly positive cases (19/20 cases classified as ZN+). False-negative result may be caused by 1 or more of the following factors: i) the incomplete solubilization of the antigen by the solvent supplied in the kit and/or the destruction of the epitopes to be recognized by the antibody as proposed by Pereira Arias-Bouda et al. (2000); ii) Even sputa were decontaminated according to Kubica's protocol (David et al., 1989), the nonwidely homogenization of the pellet by vortex could affect the repartition of mycobacteria; iii) a mutation that affects the gene encoding for the antigen looked for inside the sample tested as suggested by some reports (Banu et al., 2002; Karboul et al., 2008; Talarico et al., 2005, 2008). Indeed, according to previous reports, sequence polymorphism mainly affects the members of the 168 PE/PPE family genes of MT (Fleischmann et al., 2002; Karboul et al., 2008; Liu et al., 2006). In our study, because 3 of the 4 false-negative cases in Patho-TB test had high mycobacterial load (classified as ZN3+ to ZN4+), the most probable explanation of this result can be related to the antigenic variability affecting MT contained in sputa. Hence, IgG supplied in Patho-TB could not recognize these modified antigens. Our findings are quite similar to those reported by Fabre et al. (2007) who used the same Patho-TB kit and by other authors who used assays detecting either lipoarabinomannan antigen (Pereira Arias-Bouda et al., 2000) or different categories of antigens (Nilanjan et al., 2009).

According to some authors (Fabre et al., 2007), the results of the Patho-TB test may be affected by prior treatment of patients. None of our samples, however, were obtained from patients undergoing anti-TB treatment. As far as specificity of Patho-TB test is considered, no false-positive results were

demonstrated in the 21 control patients included in our study, in contrast to some previous findings (Fabre et al., 2007). Further investigation of a large number of non-TB patients is, however, needed to definitely assess the specificity of the test.

Patho-TB test for the detection of MT in sputum is promising and represents a potential candidate to replace the direct microscopy in endemic areas of TB even with limited resources and limited technical expertise.

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