EVALUATION OF THE GEN-PROBE AMPLIFIED *MYCOBACTERIUM TUBERCULOSIS* DIRECT TEST FOR THE ROUTINE DIAGNOSIS OF PULMONARY TUBERCULOSIS

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SUMMARY

A total of 624 respiratory specimens from 543 patients (418 Belgian, 110 Rwandan, and 15 Colombian patients) were tested for the presence of *Mycobacterium tuberculosis* by the Mycobacterium Tuberculosis Direct Test (MTDT, Gen-Probe). Compared to culture,the MTDT on 497 samples of sputum or broncho-alveolar lavage from Belgium had a sensitivity, specificity and positive and negative predictive value of 86.4%, 96.0%, 50.0% and 99.3% respectively. The pooled results for Rwanda (112 specimens) and Colombia (15 specimens) were 97.8%, 65.7%, 88.2%, 92% respectively. After resolution of discrepant results by taking into account the clinical data, the results for the Belgian patients were 86.9%, 96.2%, 52.6%, 99.3% respectively, and for the Rwandan-Colombian patients 98.1%, 100%, 100% and 92% respectively.

Results could be improved by testing more than one specimen from each patient and the inclusion of an internal control to detect inhibitors of the reaction. Culture remains necessary for drug susceptibility tests and the isolation and identification of non-tuberculous mycobacteria.

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INTRODUCTION

The traditional approach for the laboratory diagnosis of tuberculosis consists of microscopy and culture. Microscopy lacks sensitivity and specificity while culture requires several weeks for detection and identification of tuberculosis bacilli.

Efforts have been made to improve the laboratory diagnosis of tuberculosis by the introduction of nucleic acid amplification techniques such as the polymerase chain reaction amplifying specific regions of mycobacterial DNA, while Gen-Probe (Gen-Probe Inc. San Diego, CAL, USA) developed the Mycobacterium Tuberculosis Direct Test (MTDT) based on an isothermal amplification of ribosomal RNA from bacilli belonging to the *M. tuberculosis* complex, followed by the detection of amplicon with an acridinum ester-labelled DNA probe. The test can be performed in less than 5 hours.

A number of investigators from industrialised countries (1-7) have already reported the detection of *M. tuberculosis* in respiratory specimens using the MTDT. We present here an evaluation of the MTDT on respiratory specimens from Belgium, with a low prevalence of tuberculosis, and from two high prevalence countries, Rwanda and Colombia.

MATERIALS AND METHODS

A total of 624 consecutive sputum or broncho-alveolar lavage specimens from 543 pa-

TABLE 1: NUMBER OF SPECIMENS AND PATIENTS FROM 3 AREAS, WITH RESULTS OF SMEAR EXAMINATIONS AND CULTURES.

	Number of specimens	Number of patients	Smear positive specimens n	Smear positive specimens %	Culture positive specimens n	Culture positive specimens %
Belgium	497	418	10	2.0	22	4.4
Rwanda-Colombia	127	125	89	70.0	92	73.6
TOTAL	624	543				

TABLE 2: RANGE OF RLU* VALUES.

RLU	n	Percentage
0 - 14,999	444	71.0
15,000 - 24,999	35	5.6
25,000 - 29,999	5	0.8
cut-off		
30,000 - 34,999	2	0.3
35,000 - 99,999	7	1.1
100,000 - 249,999	5	0.8
250,000 - 999,999	11	1.8
1,000,000-1,999,999	7	1.2
> 2,000,000	108	17.3

*RLU : Relative Light Units

TABLE 3: RESULTS OF CULTURE AND DIRECT SMEAR EXAMINATION COMPARED WITH THE MTDT.

	Belgium culture				Rwanda and Colombia culture			
MTDT	pos		neg		pos		neg	
	Sm+	Sm-	Sm+	Sm-	Sm+	Sm-	Sm+	Sm-
pos	8	11	1	18	82	8	5	7
neg	1	2	0	456	2	0	0	23
Total	22		475		92		35	
% Sensitivity	86.4				97.8			
% Specificity	96.0				65.7			
% PPV	50.0				88.2			
% NPV	99.3				92.0			

Sm+: direct smear examination positive. Sm-: direct smear examination negative. PPV: positive predictive value. NPV: negative predictive value

tients collected between January and June 1993, were examined: 497 from 418 Belgian, 112 from 110 Rwandan and 15 from Colombian patients (Table 1).

Belgian specimens were processed locally either by the N-acetyl-L-cysteine-sodium hydroxide method (NALC)(8), or the Petroff method (9), and 0.1 ml was inoculated in BACTEC 12 B medium (Becton Dickinson)(10) or on two Löwenstein-Jensen tubes. Smears for Ziehl-Neelsen or auramine-rhodamine staining were made after decontamination. The remainder of the samples was frozen at -20°C and later transported frozen to the Institute of Tropical Medicine, Antwerp (ITM). Sputum specimens from Rwanda were transported at ambient temperature to ITM within 3-7 days of collection and stored at -20°C until further processing as above. Sputum specimens from Colombia were mixed with an equal volume of cetylpyridinium chloride 1% (CPC) (11), kept at ambient temperature, and delivered to ITM within 10 days.

The MTDT was performed following the manufacturers' instructions. Results above 30,000 Relative Light Units (RLU) were considered positive.

RESULTS

Table 1 shows that the percentage of culture positive specimens is about 17 times higher among those from high prevalence countries when compared with those from Belgium. Compared with the microscopic examinations, the proportion of culture positive specimens is significantly higher in Belgium (4.4% vs 2.0%), but is comparable (73.6% vs 70.0%) for the specimens from Rwanda and Colombia.

Table 2 shows the range of RLU measured for all specimens in the MTDT. Only 7 specimens (1.1%) produced results within the range of 5,000 RLU below or above the manufacturers' recommended cut-off value of 30,000.

Table 3 shows the comparison between culture and direct smear examination results and the MTDT. MTDT was negative for 3 smear positive samples, one from Belgium and 2 from Rwanda. The sensitivity of the MTDT compared with culture, varied from 86.4 for Belgian spe-

TABLE 5: ANALYSIS OF 31 CULTURE NEGATIVE, MTDT POSITIVE SPECIMENS.

Specimen n°	Origin	Smear result(1)	MTDT result (RLU)	Repeat test same specimen	Decont. method	Clinical information	MTDT result on second specimen	Conclusion
7771	B	5AFB	828,933	ND	a	HIV infection	NA	TP (?)
7781	B	-	45,819	-	a	HIV infection	-	FP
7825	B	-	225,493	-	a	bilateral infiltration	NA	FP
7886	B	-	+	-	a	skin test++	NA	TP (?)
7948	B	-	42,632	-	b	sarcoidosis	-	FP
7960	B	-	+	ND	a	skin test++	-	TP (?)
8017	B	-	+	+	a	haemoptysis	NA	FP
8042	B	-	168,333	-	a	emphysema	NA	FP
8183	B	-	50,235	ND	a	RX:pneumopathy	NA	FP
8184	B	-	+	ND	a	normal	NA	FP
8224	B	-	39,694	ND	b	TB in the past	-	FP
8226	B	-	+	ND	b	active TB	(1)	TP
8232	B	-	33,308	-	b	HIV infection	NA	FP
8238	B	-	172,974	ND	b	opacity lung top	-	FP
8246	B	-	208,965	-	b	opacity lung top	-	FP
8295	B	-	43,571	-	b	RX:pneumopathy	NA	FP
8355	B	-	+	ND	a	normal	NA	FP
8356	B	-	37,538	ND	a	normal	NA	FP
8426	B	-	31,970	-	b	skin test++	-	TP (?)
7986	C	-	+	ND	a	active TB	NA	TP
7994	C	1 AFB	+	ND	a	active TB	NA	TP
7776	R	-	+	ND	a	active TB	NA	TP
7778	R	1+	298,657	ND	a	active TB	NA	TP
7785	R	-	819,915	ND	a	active TB	NA	TP
7786	R	-	+	ND	a	active TB	NA	TP
7787	R	1 AFB	+	ND	a	active TB	NA	TP
7775	R	-	584,719	ND	a	active TB	NA	TP
7976	R	-	+	ND	a	active TB	NA	TP
8303	R	1+	+	ND	a	active TB(2)	NA	TP
8473	R	5 AFB	529,941+	ND	a	active TB	NA	TP
8315	R	-	+	ND	a	active TB	NA	TP

AFB = acid fast bacilli; + RLU = > 1.10^6 RLU; a = Petroff method; b = NALC; 1+ = 1-9 AFB/100 fields (12); B = Belgium; C = Colombia; R = Rwanda; FP = false positive; TP = true positive; (1) previous specimen not MTDT tested was culture positive; (2) after 4 months treatment; ? = questionable; NA = not available; ND = not done.

second specimen for 2 of these patients was not available.

The 14 false positive and 4 questionable results among the Belgian specimens amounted to 3.6% of the total number of specimens (18/497) comparable to the 2.5% false positives as obtained by Pfyffer et al.(4) after discrepancy analysis.

The false positive results must be ascribed to contamination during specimen handling or during amplification. They were equally distributed between the test runs.

In conclusion, the MTDT applied to the low prevalence Belgian population has a sensitivity, specificity and positive and negative predictive values of 86.4%, 96%, 50% and 99.3% respectively; after discrepancy analysis these figures are 86.9%, 96.2%, 52.4% and 99.3%. In a high prevalence population such as Rwanda and Columbia, sensitivity and specificity are 97.8% and 65.7% respectively, but after discrepancy analysis they are 98.1% and 100% respectively.

The MTDT can confirm the presence of *M. tuberculosis* complex bacteria in respiratory samples in less than 5 hours. However our re-

sults illustrate that in order to obtain maximum sensitivity, several specimens from the same patient should be tested, as is the norm for conventional tests (12).

In the eventuality of some culture positive MTDT-negative results, an internal control devised to detect inhibition of the amplification reaction would enhance the quality of the assay (13).

Culture remains necessary for drug susceptibility testing and the isolation and identification of non-tuberculous mycobacteria.

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RESUME

Le MTDT test (Mycobacterium Tuberculosis Direct Test, GenProbe) a été appliqué sur 624 échantillons respiratoires provenant de 543 personnes (418 belges, 100 rwandais, 15 colombiens). Comparé à la culture le test MTDT, sur 497 échantillons belges, a une sensibilité, spécificité et valeur prédictive positive et négative de respectivement 86.4%, 96.0%, 50.0% et 99.3%. Les résultats combinés pour le Rwanda et la Colombie (respectivement 112 et 15 échantillons) étaient de 97.8%, 65,7%, 88.2% et 92.0%. Après discussion des résultats discordants en fonction des données cliniques, les résultats pour les malades belges sont de 86.9%. 96.2%, 52.6% et 99.3% et pour les malades rwandais et colombiens: 98.1%, 100%, 100% et 92.0% respectivement.

Ces résultats pourraient être améliorés en examinant plus d'un échantillon par malade et en incluant dans la réaction un contrôle interne spécifique qui permettrait de détecter des inhibiteurs de la réaction. La culture reste indispensable pour déterminer la sensibilité aux antibiotiques et isoler, ainsi qu'identifier des mycobactéries non tuberculeuses.

SAMENVATTING

De Mycobacterium Tuberculosis Direct Test (MTDT, GenProbe) werd toegepast op 624 respiratoire monsters van 543 patiënten: 418 uit België, 110 uit Rwanda en 15 uit Columbia. Voor 497 bemonsteringen uit België had de MTDT test, vergeleken met kweek, een gevoeligheid, specificiteit, positieve en negatieve predictieve waarde achtereensvolgens van 86.4%,96%,50% en 99.3%. De gezamelijke resultaten voor Rwanda en Columbia (respectievelijk 112 en 15 monsters) waren 97.8%, 65.7%, 88.2% en 92.0%. Na bespreking van en beslissing over de tegenstrijdige resultaten aan de hand van de klinische gegevens, waren de resultaten van de belgische patiënten achtereenvolgens: 86.9%, 96,2%, 52.6% en 99.3% en voor de Rwanda-Columbiaanse patiënten achtereenvolgens 98.1%, 100%, 100%, 92.0%.

Deze resultaten zijn voor verbetering vatbaar indien meer dan één enkel monster van elke patiënt wordt onderzocht en een specifieke interne controle in de reactie wordt verwerkt, waardoor reactie inhibitoren kunnen worden opgespoord. De kweek blijft een noodzaak voor het testen van de antibiotica gevoeligheid en de identificatie van niet tuberculeuze mycobacteriën.

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