

EVALUATION OF A NEW MOLECULAR OLIGOCHROMATOGRAPHIC ASSAY FOR DIRECT MYCOBACTERIAL DETECTION IN HUMAN RESPIRATORY CLINICAL SPECIMENS

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Objective

To evaluate a novel assay, namely “**Speed-oligo® DIRECT MYCOBACTERIUM TUBERCULOSIS**” (SPO DMTB), based on a PCR method attached to a dipstick, for direct detection of *Mycobacterium* spp. and specific identification of *Mycobacterium tuberculosis* complex (MTC) in human respiratory clinical specimens.

Table 1

	Set A: Prospective sample					
	Total (AFB+)	MTC culture (AFB+)	NTM culture (AFB+)	AFB + & culture -	AFB - & culture - & other specimen NTM +	AFB - & culture - Contaminated culture (AFB+)
Patients	270 (8)	15 (7)	3 (1)	1*		236
Specimens	327 (12)	23 (10)	7 (1) ^a	1	3	274

* TB patient under treatment.
^a One AFB (-) and culture (-) specimen collected during the same episode from a patient with others 4 NTM cultures positives was considered false negative and included here.

Table 2

	Set B: Selected positive sample				
	Total	AFB +	MTC culture	NTM culture	Culture - & other specimen MTC +
Patients	29	29	16	12	1
Specimens	44	44	21	20	3

AFB: Acid fast bacilli. MTC: *Mycobacterium tuberculosis* complex. NTM: Nontuberculous mycobacteria.

Methods

A total of 382 N-acetylcysteine-4%NaOH decontaminated specimens in 3 different sets were blindly tested with SPO DMTB:

Set A: 327 fresh prospective respiratory specimens; expectorated sputum (n: 289), bronchoscopic specimens: BAS (n: 29), BAW (n:4) and gastric aspirates (n: 5), from 270 patients suspected of having a type of mycobacterial disease (Table 1).

Set B: Test performance was evaluated using 44 selected respiratory smear positive specimens with different load of acid-fast bacilli (AFB/ field 200X): 13 low (≤ 1) and 31 heavy (>1) positive samples. Specimens from 29 patients included 17 MTB (16 culture-positive and 1 culture-negative specimens collected under healing process) and 12 with NTM lung disease (20 culture-positive specimens): *M. fortuitum* (3), *M. abscessus* (1), *M. intracellulare* (2), *M. shimoidei* (2), *M. chelonae* (1), *M. malmoense* (1), *M. avium* (1), *M. goodii* (1) (Table 2).

Set C: To investigate the cross-reactivity, sputa artificially inoculated with 11 mycobacterium related organisms (MrO); *Corynebacterium amycolatum*, *C. xerosis*, *Streptomyces ambofaciens*, *S. gardneri*, *S. sampsonii*, *N. brasiliensis*, *N. asteroides*, *N. farcinica*, *N. nova*, *Rhodococcus equi* and *Propionibacterium acnes*.

Clinical specimens previously decontaminated were microscopically examined for AFB and cultured in MGIT® or Bact/Alert® and L-J. The isolates were identified by Mycob.CM® assay or AccuProbe® hybridization probes.

The SPO DMTB assay was performed in 4 steps:

Mechanical DNA extraction (cell disruption 5 min)

Amplification (multiplex PCR): The used targets were: human gene *RNAseP* (amplification control for PCR inhibitors detection), 16S rRNA (*Mycobacterium* genus-specific sequence) and *IS6110* (MTC-specific fragment)

PCR detection: PCR product was added to a dipstick which includes probes bound to colloidal gold and to the membrane (5 min incubation)

Reading of the result (visual or automatic) : presence of visible lines (Figures 1 & 2)

Results

Positive mycobacterial cultures:

Set A: 23 MTC; 6 NTM; 273 negative; 5 negative with another specimen from the same patient MTC positive; 1 negative with another specimen from the same patient NTM positive; and 19 contaminated (Table 3).

SPO DMTB:

This assay showed no cross-reactivity with all the MrO strains tested.

No result (INV) could be obtained for 13 culture-negative specimens due to the absence of the amplification control line (poor specimen collection or presence of PCR inhibitors).

The **sensitivities** were:

- 0.92 for **smear-positive** specimens (0.76 for low-positive and 0.97 for heavy-positive)
- 0.94 for MTC positive
- 0.83 for NTM positive
- 0.46 for **smear-negative** specimens
- The **overall** sensitivity in set A was:
- 0.86 for MTC positive
- 0.74 for NTM positive

The **specificity** was 0.99.

The **positive predicted value** was 0.92.

The **negative predicted value** was 0.96 (prevalence 0.12).

Conclusion

SPO DMTB produces reliable results, and may be a valuable tool for rapid diagnosis of mycobacterium lung infection.

Table 3

Set A N: 327	MTC + n: 23			NTM + n: 6			NEG n: 273			NEG & other MTC + n: 5			NEG & other NTM + n: 19			CONT n: 19
	NEG	Low Pos	Heavy Pos	NEG	Low Pos	Heavy Pos	NEG	Low Pos	Heavy Pos	NEG	Low Pos	Heavy Pos	NEG	Low Pos	Heavy Pos	
AFB	10	5	8	3	2	1	273	2	0	3	0	1	0	1	0	19
SPO DMTB	MTC 6	MTC 5	MTC 8	NEG 0	NEG 0	NTM 1	INV 12	NTM 1	MTC 1	INV 1	MTC 1	MTC 3	NTM 1			0

Figure 1

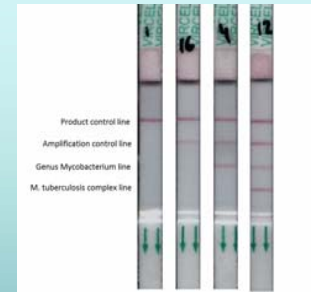


Figure 2



The use of the automatic reading system produced individual reports for each sample. Figures show the intensity value and the qualitative result for each test line in defined samples.