

Evaluation of an oligochromatographic test for identification of mycobacteria most frequently isolated in human from liquid and solid culture media

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Introduction and Purpose

The mycobacteria usually isolated in human specimens are restricted to a limited number of species. The incorporation of techniques for fast and easy handling solves the problems of species identification of mycobacteria in microbiology laboratories.

The purpose of the present study is to evaluate a rapid and simple new commercial assay (Speed-oligo® mycobacteria, Vircell) for the identification of most frequently human mycobacteria based on a PCR plus a rapid test device that provides results in less than two hours.

Material and Methods

Material 1:

A convenience sample of 182 positive cultures for mycobacteria of 129 respiratory, 20 extra respiratory and 5 unknown origins from 124 patients from southeast of Spain. Table 1.

Table 1: Number of patients positive and culture media

MATERIAL 1: Mycobacterium spp.	N	MTC	MOTT	MTC & MOTT
Patients	124	56	67	1
Samples	154	60	94	0
Primary + cultures:	182	61	121	0
- MIGT	22	4	18	0
- BACT/ ALERT	72	29	43	0
- LJ	88	28	60	0

Material 2:

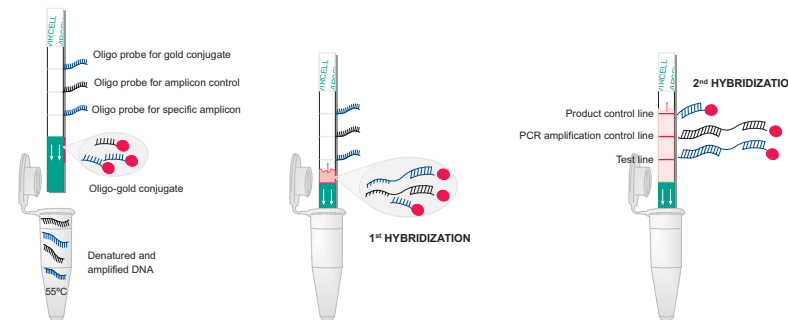
Eleven collection strains of mycobacteria-related organisms: 2 *Corynebacterium* spp., 3 *Streptomyces* spp., 4 *Nocardia* spp., 1 *Rhodococcus equi*, 1 *Propionibacterium acnes* were included to test the specificity of the assay.

Methods:

All the positive culture samples were blindly assayed for identification using Genotype® Mycobacterium CM/AS (Hain-LifeScience) and/or Accuprobe MTC Gen-Probe® (BioMerieux) and later studied with the oligochromatographic test. The discrepancies observed were resolved by rDNA sequencing and PRA methods.

Oligochromatographic test: DNA was extracted by heating and centrifugation without requiring a purification step. The 16S rRNA and 16S-23S rRNA gene spacer regions were used as targets for amplification.

The amplification products were hybridized on a dipstick using specific probes bound to colloidal gold and to the membrane. As a whole, the process took less than 120 minutes. The results were interpreted by identification of 7 bands specific for each of the following categories: 1) *M. chelonae/ abscessus* complex, 2) *M. gordonae*, 3) *M. kansasii*, 4) *M. tuberculosis* complex, 5) *M. avium/ intracellulare/ scrofulaceum* complex, 6) *M. fortuitum* and 7) *Mycobacterium* genus.



Results

All 182 positive cultures and 9 mycobacteria-related organisms were tested. No cross reactivity was observed with any mycobacteria-related organisms. Speed-oligo mycobacteria and reference methods identified all 61 isolates of MTC (*M. tuberculosis* complex), and classified 105 and 117 MOTT (mycobacterium other than tuberculosis) in one of the group-species and 15 and 7 MOTT only to the genus level respectively.

The agreement between both tests was 177/182 (97.2%). Table 2.

Table 2: Concordance between Speed-oligo M and Genotype M CM/AS in identifying different species of mycobacteria

Genotype M CM/AS	Speed-oligo M	MGIT	B/ALERT	LJ	Total CONCORD.
<i>M. tuberculosis</i> complex (28) <i>M. tuberculosis</i> complex (33)*	<i>M. tuberculosis</i> complex (61)	4/4	4/4	20/20	28/28 33/33 (100%)
<i>M. fortuitum</i> (7)	<i>M. fortuitum</i> (7)	1/1	2/2	4/4	7/7 (100%)
<i>M. avium</i> (39) <i>M. intracellulare</i> (15) <i>M. scrofulaceum</i> (2)	<i>M. avium</i> or <i>M. intracellulare</i> or <i>M. scrofulaceum</i> 56	1/1 3/3	18/18 6/5 1/0	20/20 6/5 1/1	53/56 (94.6%)
<i>M. kansasii</i>	<i>M. kansasii</i>	1/1	2/2	3/3	6/6 (100%)
<i>M. gordonae</i> (14)	<i>M. gordonae</i> (14)	1/1	3/3	10/10	14/14 (100%)
<i>M. abscessus</i> (9) <i>M. chelonae</i> (11)	<i>M. abscessus</i> or <i>M. chelonae</i> 20	7/7	6/6 1/1	3/3 3/3	20/20 (100%)
<i>M. chelonae</i> + <i>M. gordonae</i>	<i>M. abscessus</i> - <i>M. chelonae</i> and <i>M. gordonae</i>		1/1		1/1 (100%)
<i>M. avium</i> + <i>M. gordonae</i>	<i>MAI. scrofulaceum</i> and <i>M. gordonae</i>		1/1	1/1	2/2 (100%)
<i>Mycobacterium</i> spp. (MOTT)	<i>Mycobacterium</i> spp. (MOTT)		2/2	5/5	7/7 (100%)

* ACCUPROBE MTC Gen-Probe (BioMerieux)

The discrepancies were distributed in i) minor: some MOTTs not included in the test that were identified as *Mycobacterium* sp: 1 *M. heckeshornense*, 1 *M. lentiflavum* 1 *M. scrofulaceum*, 3 *M. simiae*. Three species of mycobacteria included in the oligochromatographic test identified as *Mycobacterium* sp: 2 *M. intracellulare* and 1 *M. scrofulaceum* and ii) major: 2 *M. marinum* misidentified as *M. kansasii*. Table 3.

Table 3: Minor and major discrepancies between Speed-oligo M and Genotype M CM/AS

Minor discrepancies Speed-oligo M and Genotype M CM/AS					
Genotype M CM/AS	Speed-oligo M	MGIT	B/ALERT	LJ	Total CONCORD.
					True**
<i>M. heckeshornense</i> (1) <i>M. lentiflavum</i> (1) <i>M. mucogenicum</i> (1) <i>M. simiae</i> (3)	<i>Mycobacterium</i> spp. (MOTT) (species not included) (6)	1 3		1 1	
<i>M. intracellulare</i> (2)	<i>Mycobacterium</i> spp. (MOTT) (species included in the test no identified)		1	1	Pending
<i>M. scrofulaceum</i> (1)	<i>Mycobacterium</i> spp. (MOTT) (species included in the test not identified)		1		Pending
Major discrepancies Speed-oligo M and Genotype M CM/AS					
<i>M. marinum</i> (2)	Misidentification <i>M. kansasii</i> (2)			2	<i>M. marinum</i>

** PRA and/or rDNA sequencing



Conclusions

Speed-oligo® Mycobacteria is a fast and sensitive assay for mycobacteria identification. It showed high correlation when results were compared with the standard method. The few observed discrepancies are being resolved by rDNA sequencing and PRA methods.