

Mycobacteriology

Evaluation of the Speed-oligo[®] Mycobacteria assay for identification of *Mycobacterium* spp. from fresh liquid and solid cultures of human clinical samples[☆]

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Abstract

We evaluated the ability of a novel DNA strip assay (Speed-oligo[®] Mycobacteria) to differentiate mycobacterial species. It is based on polymerase chain reaction targeting 16S rRNA and 16S-23S rRNA regions and double-reverse hybridization on a dipstick using probes bound to colloidal gold and to the membrane. We blindly tested its capacity to identify 182 acid-fast bacilli grown on fresh liquid (BacT/Alert, MGIT) and solid (Lowenstein–Jensen) cultures (from Spanish mycobacteriology laboratories), previously identified by means of Genotype[®] Mycob.CM/AS or Gen-Probe[®] AccuprobeMTC, and 11 collection strains of mycobacteria-related organisms. Discrepancies were resolved by 16S rRNA sequencing. Results were interpreted by identification of 7 specific bands for the following: *Mycobacterium* sp., *M. fortuitum*, *Mycobacterium avium*–*intracellulare* complex, *Mycobacterium tuberculosis* complex, *Mycobacterium kansasii*, *M. gordonae*, and *M. abscessus*–*chelonae* complex. No cross-reactivity was observed with any mycobacteria-related organism. Concordant results were obtained for 177/182 bacilli (97.2%). There was only 1 major discrepancy, misidentification of *Mycobacterium marinum* as *M. kansasii*, verified by sequencing.

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1. Introduction and purpose

The number of validly described mycobacterial species has markedly increased over recent years (Brown-Elliott et al., 2002; Rhodes et al., 2003, 2005; Roux et al., 2009; Tortoli, 2003; Tortoli et al., 2009; Van Ingen et al., 2009a). The identification of mycobacteria responsible for a disease

and the differentiation between environmental and pathogenic species are important diagnostic issues with ramifications for the treatment of patients (Esteban et al., 2008; Glassroth, 2008; Griffith et al., 2007; Piersimoni and Scarparo, 2008; Roux et al., 2009).

The introduction of molecular biologic methods has greatly improved the speed and accuracy of the identification process. New DNA techniques for the identification of mycobacteria have been developed over the past 20 years, including DNA sequencing (Hall et al., 2003; Padilla et al., 2004), pyrosequencing (Heller et al., 2008), polymerase chain reaction (PCR)-restriction fragment length polymorphism assays (Telenti et al., 1993), real-time PCR assays (Richardson et al., 2009), and oligonucleotide arrays (Fukushima et al., 2003).

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Commercially available and extensively evaluated systems include DNA probes targeted to the identification of mycobacteria, such as AccuProbe (Gen-Probe, San Diego, CA), which relies on oligonucleotide probes complementary to 16S rRNA (Cloud et al., 2005; Scarparo et al., 2001; Wu et al., 2007), and DNA strip assays based on reverse hybridization of a PCR product to a nitrocellulose strip with immobilized probes, such as INNO LiPA Mycobacteria (Innogenetics, Ghent, Belgium) (Lebrun et al., 2005; Makinen et al., 2002; Miller et al., 2000) and GenoType Mycobacterium CM/AS (Hain Lifescience, Nehren, Germany) (Makinen et al., 2002; Richter et al., 2006; Russo et al., 2006; Sarkola et al., 2004; Tortoli et al., 2010) assays. These 2 assays permit identification of 16 and 35 different taxa (species or complexes) in 1 and 2 strips, respectively.

Mycobacteria typically isolated in clinical specimens represent a limited number of species (Couto et al., 2009; Ergin et al., 2000; Etxebarrieta et al., 2002; Martin-Casabona et al., 2004; Perez-Martinez et al., 2008; Scarparo et al., 2001; Sorlozano et al., 2009; Van Ingen et al., 2009b; Wu et al., 2008). Hence, the application of simple methods to rapidly identify this group of mycobacteria would meet most of the diagnostic needs of a clinical mycobacteria laboratory.

The objective of the present study was to evaluate the capacity of a rapid and simple new commercial assay, Speedoligo[®] Mycobacteria (Vircell, Spain) (SpO-M) to identify the most frequently isolated human mycobacteria. It is based on a PCR plus a rapid test device that yields results within 1 h. For this purpose, we examined whether the identification of fresh clinical mycobacterial isolates using this new oligochromatic test matches the results of Genotype[®] Mycobacterium CM/AS (Hain-LifeScience) (GnT-CM/AS) and/or AccuprobeMTC Gen-Probe[®] (BioMérieux, Madrid, Spain) (AcP-M) assays. Discrepancies were resolved by 16S rRNA sequencing. The applicability of the assay for routine mycobacterial laboratory testing is discussed.

2. Materials and methods

2.1. Strains assayed

We evaluated the ability of the new assay to differentiate mycobacterial species in 190 primary mycobacteria cultures: i) 185 solid and liquid positive cultures for acid-fast bacteria: BBL-MGIT (BD, Madrid, Spain) ($n = 24$, 12.6%), BacT/Alert MP (BioMérieux) ($n = 75$; 39.5%), and Lowenstein–Jensen ($n = 91$; 47.9%) inoculated with 160 specimens (134 respiratory, 26 extrapulmonary) from 130 patients living in southeastern Spain (Almería, Cadiz, Granada, Jaen, and Malaga); these positive cultures correspond to almost all of the cultures sent from 7 level II mycobacteriology laboratories (ATS, 1990; Kubica et al., 1975; Shinnick and Good, 1995) in the public health system to a level III Regional Reference Laboratory (Hospital Costa del Sol, Marbella, Spain) for identification

and/or susceptibility testing between February and October 2008; and ii) 5 negative-contaminated cultures.

The test sample comprised 61 primary cultures with *Mycobacterium tuberculosis* complex (MTC), including 35 identified with AcP-M and 26 with GnT-CM/AS; 117 with ≥ 1 nontuberculous mycobacteria (NTM), identified using GnT-CM/AS (*Mycobacterium avium* [$n = 41$], *Mycobacterium intracellulare* [$n = 15$], *M. gordonae* [$n = 15$], *M. chelonae* [$n = 11$], *M. abscessus* [$n = 9$], *M. fortuitum* [$n = 7$], *Mycobacterium kansasii* [$n = 6$], *M. simiae* [$n = 3$], *Mycobacterium scrofulaceum* [$n = 2$], *Mycobacterium marinum* [$n = 2$], *M. avium* plus *M. gordonae* [$n = 2$], *M. heckeshornense*, *M. lentiflavum*, *M. mucogenicum*, *M. chelonae* plus *M. gordonae* [$n = 1$]); 7 primary cultures containing acid-fast bacilli only identified to the genus level of mycobacteria by using GnT-CM/AS; and 5 negative-contaminated cultures: BacT/Alert MP ($n = 2$), MGIT ($n = 1$), and Lowenstein–Jensen ($n = 2$) (Table 1).

To assess the specificity of the test, we included 11 IVAMI (Instituto Valenciano de Microbiología) collection strains of mycobacteria-related organisms: *Corynebacterium* sp. ($n = 2$): *C. amycolatum* IVAMI 4023656, *C. xerosis* NCTC 7243; *Streptomyces* sp. ($n = 3$): *S. ambofaciens* IVAMI 4021542, *S. gardneri* IVAMI 4022939, *S. sampsonii* IVAMI 4023161; *Nocardia* sp. ($n = 4$): *N. brasiliensis* NCTC 10300, *N. cyriacigeorgica/asteroides* IVAMI 4022938, *N. farcinica* IVAMI 4016593; *Rhodococcus equi* CECT 555 ($n = 1$); and *Propionibacterium acnes* IVAMI 4023683 ($n = 1$) (Kwon et al., 1995; Portevin et al., 2004; Saito and Tomioka, 1984).

2.1.1. Genotype[®] Mycob. CM/AS assay

This assay was performed as recommended by the manufacturer. Briefly, 35 μL of a primer-nucleotide mixture (provided with the kit), amplification buffer containing 2.5 mmol/L MgCl_2 , 1.25 U of hot start *Taq* polymerase (QIAGEN, Hilden, Germany), and 5 μL of the heat-inactivated suspension in a final volume of 50 μL , was used for amplification under the following conditions: 15 min of denaturing at 95 °C, 10 cycles of 30 s at 95 °C and 120 s at 58 °C; 20 cycles of 25 s at 95 °C, 40 s at 53 °C, and 40 s at 70 °C; and a final extension for 8 min at 70 °C. Hybridization and detection were performed in an automated washing and shaking device (Profiblot; Tekan, Maennedorf, Switzerland). The program was started after mixing 20 μL of the amplification products with 20 μL of denaturing reagent (provided with the kit) for 5 min in separate troughs of a plastic well; 1 mL of prewarmed hybridization buffer was automatically added, followed by placement of the membrane strips in each trough. The hybridization procedure was performed at 45 °C for 0.5 h, followed by 2 washing steps. For colorimetric detection of hybridized amplicons, streptavidin conjugated with alkaline phosphatase and substrate buffer were added. After a final washing step, strips were air-dried and fixed on paper. Each CM and AS strip contains 17 probes, including amplification

Table 1

Concordance Speed-oligo[®] Mycobacteria and Gen-Probe[®] AccuprobeMTC or Genotype[®] Mycob.CM/AS

Cultures (n)	Gen-Probe [®] AccuprobeMTC	Speed-oligo [®] Mycobacteria	Congruent Speed-oligo M taxa identification in different types of cultures, n (%)			Total concordance (%)
			MGIT	B/A MP ^b	LJ ^b	
35	Positive (MTC)	<i>M. tuberculosis</i> complex		25	10	35 (100)
46 ^a	Negative (NTM)	NTM (GnT-CM/AS confirmed)		35	11	46 (100)
19	Genotype [®] Mycob.CM/AS 8 <i>M. abscessus</i> 11 <i>M. chelonae</i>	Speed-oligo [®] Mycobacteria <i>M. chelonae/abscessus</i> complex		5	3	8 (100)
15	<i>M. gordonae</i>	<i>M. gordonae</i>	7	3	13	11 (100)
6	<i>M. kansasii</i>	<i>M. kansasii</i>	1	2	3	15 (100)
26	<i>M. tuberculosis</i> complex	<i>M. tuberculosis</i> complex	1	2	3	6 (100)
7	<i>M. fortuitum</i>	<i>M. fortuitum</i>	4	4	18	26 (100)
56	39 <i>M. avium</i>	<i>M. avium/intracellulare/</i> <i>scrofulaceum</i> complex	1	2	4	7 (100)
	15 <i>M. intracellulare</i>		1	18	20	39 (100)
	2 <i>M. scrofulaceum</i>		3	5/6	5/6	13/15 (86.6)
6	1 <i>M. heckeshornense</i>	<i>Mycobacterium</i> sp. (NTM) (taxa not included)		0/1	1	1/2 (50)
	1 <i>M. lentiflavum</i>				1	6 (100)
	1 <i>M. mucogenicum</i>		1			
	3 <i>M. simiae</i>		3			
7	<i>Mycobacterium</i> spp.(NTM)	<i>Mycobacterium</i> sp. (NTM)		2	5	7 (100)
2	<i>M. marinum</i>	<i>M. kansasii</i>			0/2	0/2 (0)
1	<i>M. chelonae</i> + <i>M. gordonae</i>	<i>M. abscessus/chelonae</i> + <i>M. gordonae</i>		1		1 (100)
2	<i>M. avium</i> + <i>M. gordonae</i>	<i>MAI-scrofulaceum</i> + <i>M. gordonae</i>		1	1	2 (100)
182	Total <i>Mycobacterium</i> sp.	Total <i>Mycobacterium</i> sp.	22/22 (100)	69/71 (97.2)	86/89 (96.6)	177/182 (97.2)
5	Negative (contaminated)	Negative	1	2	2	5 (100)

^a Included in the GnT-CM/AS and SpO-M comparative analysis.^b B/A MP = BacT/Alert MP; LJ = Lowenstein–Jensen.

and hybridization controls to verify test procedures. With the CM assay, 15 patterns can be obtained from 23 species (10 individually and 13 in combination); with the AS assay, 16 patterns can be obtained from 18 species (12 individually and 2 in combination). The CM assay shows the same pattern for both *M. marinum* and *M. ulcerans*, but these species can be distinguished by serial use of the AS assay; therefore, the *M. marinum*–*M. ulcerans* pattern in this assay was considered to represent individual species. A total of 23 patterns attributable to 22 individual species and 8 patterns representing 15 species in combinations of 2 to 4 species can be obtained. Each assay also includes 2 patterns with an amplification control specific for the genus *Mycobacterium* and 1 pattern for the detection of all known mycobacteria and members of the group of Gram-positive bacteria with a high G + C content.

2.1.2. AccuprobeMTC assay

This assay was performed in L-J or MP/BacT Alert bottles. To reduce false-negative results associated with an inadequate mycobacterial cell mass in liquid cultures, we centrifuged a 1.5-mL portion of liquid medium at 12 000 × g for 10 min in a sterile screw-cap microcentrifuge tube, and the pellet was used for a hybridization test. The DNA probe was used according to the kit instructions. Samples

producing signals >30 000 relative light units (RLU) (Selan et al., 1992) were considered positive. When the initial test gave a negative result (<30 000 RLU) or a mixed culture was suspected, the sample was sent to the reference center for species identification by means of the GNT-M assay.

2.1.3. Oligochromatographic assay (Speed-Oligo[®] Mycobacteria)

Bacteria were concentrated by centrifugation at 12 000 × g for 15 min in 1 mL of liquid media. The pellet was resuspended with 300 μL of Vircell sample solution. Bacterial growth on solid media was collected with an inoculation loop and resuspended with 300 μL of Vircell Sample Solution. In both cases, the bacterial suspensions were incubated for 20 min at 95 °C using a thermoblock in a biosafety cabinet. Next, the bacterial suspensions were centrifuged for 5 min at 12 000 × g, and 10 μL of the supernatant were used for the PCR.

The SpO-M assay is a PCR-based method coupled to a dipstick device that enables rapid mycobacteria identification in culture samples. The PCR mix (supplied in lyophilized format) contains PCR reagents and specific oligo pairs for mycobacteria amplification. A multiple PCR was performed. A DNA fragment encoding 16S rRNA

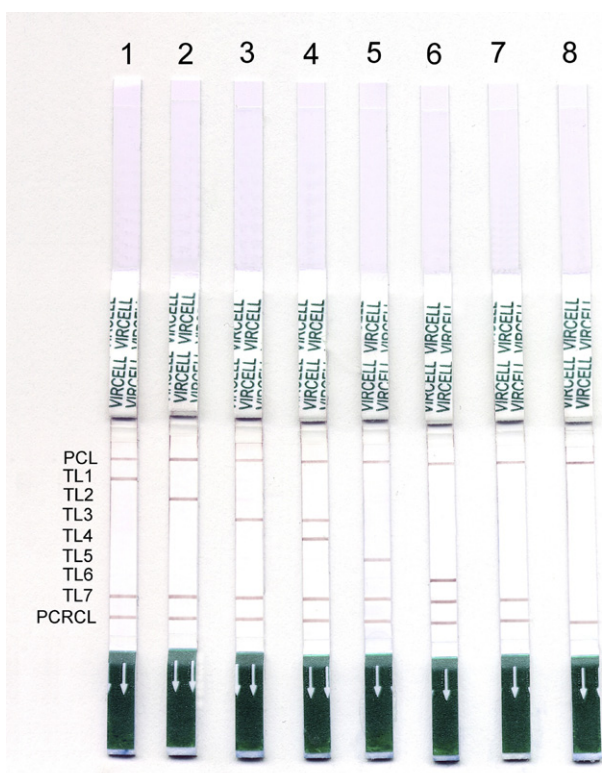


Fig. 1. Hybridization results of different mycobacteria: a PCR amplification control line (PCRCL) and a product control line (PCL) are included in the strip. A generic mycobacteria genus band (TL7) and 6 specific mycobacteria identification test lines (TL1 to TL6) are included in the test. Both control lines appear in all mycobacteria hybridizations (PCL and PCRCL). TL1 + TL7 appears for *M. chelonae*/*M. abscessus* complex (1), TL2 + TL7 appears for *M. gordonae* (2), TL3 + TL7 appears for *M. kansasii* (3), TL3 + TL4 + TL7 appears for *M. tuberculosis* complex (4), TL5 + TL7 appears for *M. avium*/*M. intracellulare*/*M. scrofulaceum* complex (5), TL6 + TL7 appears for *M. fortuitum* (6), and only TL7 appears when a different species of mycobacteria is present in the sample (7). Finally, only PCR and PCRCL appear in negative samples and samples with a microorganism other than mycobacteria (8).

(rDNA) was amplified for *Mycobacterium* genus detection, and 16S-23S rDNA spacer region was amplified for specific *Mycobacterium* spp. detection. A noncompetitive internal amplification control was coamplified simultaneously with the target sequence, including a plasmid with specific oligo pair in the PCR mix. This control allowed ruling out the inhibition of PCR reaction due to the presence of inhibitory substances in the sample, thermal cycler malfunction, incorrect PCR mixture, or poor DNA polymerase activity (Hoorfar et al., 2004; Rådström et al., 2003). For the PCR test, the lyophilized PCR mix was resuspended following the manufacturer's instructions, and 10 μ L of the sample was added to 15 μ L of PCR mix. The procedure was carried out in a Labcycler instrument (Sensoquest, Göttingen, Germany) with the following amplification conditions: 92 $^{\circ}$ C/1 min, 40 cycles of 92 $^{\circ}$ C/20 s + 55 $^{\circ}$ C/30 s + 72 $^{\circ}$ C/30 s, 72 $^{\circ}$ C/1 min, and 95 $^{\circ}$ C/1 min. PCR products were denatured by heating in the thermocycler at 95 $^{\circ}$ C for 1 min.

PCR products were detected by using the dipstick according to kit instructions. Briefly, 5 μ L of denatured PCR product was diluted in 40 μ L of running solution and placed in a thermal block set at 55 $^{\circ}$ C. Amplification products were hybridized on a dipstick using 3 specific probes bound to colloidal gold and 9 probes immobilized in the membrane. When placed in contact with the dipstick, the PCR product flows into the strip and reacts, in a first instance, with the gold-probes. The PCR product-gold probes complex then reaches the specific lines with the nitrocellulose-probe and a second hybridization takes place. At the top of the strip, a third control line monitors the flow of the liquid along the strip. This line reacts with the excess of colloidal gold. The final reading was visually accomplished after 5 min of incubation. Reactivity was confirmed by visualization of a red line (Fig. 1). The entire process takes 120 min. Results were interpreted by identification of 7 specific bands for each of the following: 1) *Mycobacterium* sp., 2) *M. fortuitum*, 3) *M. avium*-*intracellulare* complex, 4) *M. tuberculosis* complex, 5) *M. kansasii*, 6) *M. gordonae*, and 7) *M. abscessus*-*chelonae* complex.

2.2. Genotypic characterization

Comparative analysis was done i) when mycobacterial species were only classified to genus level by SpO-M and GnT-CM/AS systems; ii) to resolve major discrepancies, when both tests classified the same isolate in different taxa; and iii) to resolve minor discrepancies, when a group species included in the SpO-M test was only identified to genus level.

Definitive genotypic identification was performed by 16S rRNA gene PCR and sequencing of the first -bp gene using universal bacterial primers E8F (5'-AGAGTTTGAT-CCTGGCTCAG-3') and E533R (5'-TTACCGCGGCTG-CTGGCA-3') (Baker, 2003).

Amplicons were purified by using the GFX PCR purification kit (Amersham Biosciences), sequenced by a PCR-based reaction using the Big Dye Terminator method according to the manufacturer's instructions, and detected in an AbiPrism 3100 automatic DNA sequencer (Applied Biosystems, Foster City, CA). The sequences obtained were compared with those stored in GenBank using BIBI software (Bio Informatic Bacteria Identification tool: <http://pbil.univ-lyon1.fr/bibi>) (Devulder et al., 2003). Identification to species level was defined as $\geq 99\%$ sequence similarity with the sequence of the type strain of only 1 species.

Sequencing of the *hsp65* gene, using primers described by Telenti et al. (1993), was performed in the case of ambiguous identification results (more than 99% similarity with the sequences of more than 1 species).

3. Results

3.1. Cross-reactivity

The oligochromatographic assay showed no reactivity with the 11 collection strains of mycobacteria-related organisms or with the 5 negative-contaminated cultures.

3.2. Excluded isolates and finally evaluated sample

Three (1.6%) of the 190 initially selected cultures were excluded because 2 of them were not SpO-M tested and another became contaminated prior to the assay. Hence, the final study included the following samples (Table 1): i) 182 solid and/or liquid primary positive cultures of 152 specimens from 124 patients (MGIT, $n = 22$; BacT/Alert MP, $n = 71$; Lowenstein–Jensen, $n = 89$) containing 14 identified complex species of mycobacteria and 7 strains only identified to the genus level, representing most of the mycobacterial taxa isolated in the 7 clinical laboratories (Matin-Casanova et al 2004, López Prieto 2006 [<http://www.gefor.4t.com/micobacterias/gem/poster3.htm>], Rodríguez V et al 2008 [http://www.sademi.com/actividades/publicaciones/libro_resumenes_sademi_08.pdf]); ii) 11 collection strains of mycobacteria-related organisms; and iii) 5 negative-contaminated cultures.

3.3. Agreement between SpO-M and AcP-M/GnT-CM/AS assays

3.3.1. Concordance

The SpO-M assay identified the same complex species in 111 (89.5%) of the patients, in 144 of 152 (94.7%) specimens, and 97.8% of the 182 positive cultures identified by the reference methods. The new test detected all of the MTC in 61 cultures previously identified with AcP-M ($n = 35$) or GnT-CM/AS ($n = 26$). It classified in 2 or 2 NTM complex species the NTM mycobacteria present in 105 (86.7%) of 114 culture media previously identified with GnT-CM/AS assay. All of the 7 NTM isolates identified only to the genus level by mean GnT-CM/AS assay were identified to the same level with the SpO-M assay (Table 1).

The sensitivity was $\geq 99\%$, and the specificity was $\geq 99\%$ for MTC ($n = 61$), *M. chelonae/abscessus* complex ($n = 19$), and *M. goodii* ($n = 15$). The sensitivity was 94.5% and specificity 100% for MAC ($n = 56$). The sensitivity was 100% and specificity 98.8%, with a relatively low positive predictive value of 75%, for *M. kansasii* ($n = 6$).

Analyses in the different cultures showed very similar results ($P = 0.9948$); therefore, the efficacy of mycobacterial

identification by this new test was not affected by the type of culture medium in which it was performed (Table 1).

3.4. Limitations and discrepancies of the SpO-M

3.4.1. Test limitations

Thirteen NTM species not included in the test were only identified to *Mycobacterium* genus level, for example, i) NTM classified to the genus level by GnT-CM/AS ($n = 7$), ii) *M. simiae* ($n = 3$), iii) *M. heckeshornense* ($n = 1$), iv) *M. lentiflavum* ($n = 1$), and v) *M. mucogenicum* ($n = 1$).

3.4.2. Minor discrepancies

Three NTM species were included in the SpO-M test but not perfectly classified: *M. intracellulare* ($n = 2$) and *M. scrofulaceum* ($n = 1$).

3.4.3. Major discrepancies

Isolates were classified in different group species: *M. marinum* ($n = 2$) were identified as *M. kansasii* ($n = 2$) (Table 2).

3.5. Sequencing identification of isolates classified only to genus level by both GnT-CM/AS and SpO-M systems

The mycobacteria present on 7 cultures (2 BacT/Alert MP and 5 Lowenstein–Jensen) of 5 different samples were classified only to genus level by SpO-M and GnT-CM/AS. The sequencing analysis results of these nonidentified isolates were *M. terrae* ($n = 3$), *M. arupense* ($n = 2$), and *M. simiae* ($n = 2$). None of them were identifiable to species-group level with SpO-M (Table 3).

3.6. Resolution of minor and major discrepancies

3.6.1. Minor discrepancies

Three NTM complex species were identified with GnT-CM/AS: *M. intracellulare* ($n = 2$, same sample) and *M. scrofulaceum* ($n = 1$) were only identified to genus level by the SpO-M assay. Identification of these isolates by sequencing indicated that they were *M. intracellulare* ($n = 2$) and *M. kumamotoense* ($n = 1$), respectively, hence, confirming only 2 of the 3 GnT-CM/AS results. The following are the major discrepancies: 2 isolates identified as *M. marinum* and confirmed by comparative sequence

Table 2
Limitations, minor and major discrepancies of Speed-oligo[®] Mycobacteria

	GnT-CM/AS ^a	SpO-M ^a	Sequencing identification
Limitations: mycobacteria not included in the test	<i>M. heckeshornense</i> (1) <i>M. lentiflavum</i> (1) <i>M. mucogenicum</i> (1) <i>M. simiae</i> (3)	<i>Mycobacterium</i> sp. (6)	Not tested
Minor discrepancies: micobacteria included in the test but not identified	<i>M. intracellulare</i> (2) <i>M. scrofulaceum</i> (1)	<i>Mycobacterium</i> sp. (2) <i>Mycobacterium</i> sp.(1)	<i>M. intracellulare</i> <i>M. kumamotoense</i>
Major discrepancy: misidentification	<i>M. marinum</i> (2)	<i>M. kansasii</i> (2)	<i>M. marinum</i>

^a SpO-M = Speed-oligo[®] Mycobacteria; GnT-CM/AS = Genotype[®] Mycobacterium CM/AS.

Table 3
Sequencing identification of isolates only classified to genus level by SpO-M and GnT-CM/AS

Sample type	Culture media ^a	SpO-M and GnT-CM/AS identification ^b	Sequencing identification	Species included in GnT-CM/A S	Species included in SpO-M
Sputum	B/A MP	<i>Mycobacterium</i> sp.	<i>M. simiae</i>	Yes	No
	LJ	<i>Mycobacterium</i> sp.	<i>M. simiae</i>	Yes	No
Sputum	B/A MP	<i>Mycobacterium</i> sp.	<i>M. arupense</i>	No	No
	LJ	<i>Mycobacterium</i> sp.	<i>M. arupense</i>	No	No
Sputum	LJ	<i>Mycobacterium</i> sp.	<i>M. terrae</i>	No	No
Sputum	LJ	<i>Mycobacterium</i> sp.	<i>M. terrae</i>	No	No
Sputum	LJ	<i>Mycobacterium</i> sp.	<i>M. terrae</i>	No	No

^a B/A MP = BacT/Alert MP; LJ = Lowenstein–Jensen.

^b SpO-M = Speed-oligo[®] Mycobacteria; GnT-CM/AS = Genotype[®] Mycobacterium CM/AS.

analysis were misidentified as *M. kansasii* with the SpO-M assay (Table 2).

4. Discussion

Rapid identification of mycobacteria is of critical importance for the early administration of appropriate empirical antibiotic therapy and for the effective implementation of public health measures. Moreover, the early detection of environmental mycobacteria can avoid the unnecessary treatment of patients and contacts.

The introduction of molecular sequence-based techniques for mycobacterial identification enabled the recognition and reliable phylogenetic placement of more than 100 species (RIDOM (<http://www.rdna.ridom.de>), National Centre for Biotechnology Information database (<http://www.ncbi.nlm.nih.gov>). However, the most frequently isolated NTM in clinical laboratories are in a small number of well-described species, headed by MAC (Martin-Casabona et al., 2004; Richter et al., 2006). In a multicountry survey (36 099 patients with NTM from 14 countries) covering a 20-year period, the most frequently isolated NTM in laboratories were the *M. avium*–*intracellulare* complex (29.1%), *M. gordonae* (18.8%), *M. xenopi* (19%), *M. kansasii* (10.3%), and *M. fortuitum* (9.8%), accounting for 87.6% of all NTM (Martin-Casabona et al., 2004). These different frequencies of NTM led to the development and the widespread use in microbiologic laboratories of different formats of commercially available molecular assays targeted to the identification of MTC and the most frequent NTM. These techniques include DNA hybridization probes with or without nucleic acid amplification, real-time PCR, and microarray based kits for detecting mycobacterial DNA or RNA, advancing the speed and effectiveness of identification of the most frequent mycobacterial species on different mycobacterial culture media (Lebrun et al., 2005; Musial et al., 1988; Ruiz et al., 2002; Tortoli et al., 2003, 2010) and, recently, directly in clinical samples (Barber, 2008; Bicmen et al., 2007; Couto et al., 2009).

SpO-M is a new assay for the identification of MTC and the most frequently isolated species of NTM. We evaluated this new assay in 182 mycobacterial-positive solid and liquid cultures of 149 specimens from 124 patients: 60 MTC-positive specimens from 57 patients and a further 89 with 1 or 2 NTM from 67 patients. This selection includes the most frequently isolated NTM in the clinical mycobacteriology laboratories of Andalusia, where a similar distribution of frequencies of NTM was observed in comparison to other geographic areas (Couto et al., 2009; Leitritz et al., 2004; Martin-Casabona et al., 2004; Sorlozano et al., 2009).

Overall, 177 (97.2%) of the isolates tested showed concordant results with the oligochromatographic assay. The assay was particularly efficacious for differentiating between MTC and NTM (positive and negative predictive values of 100%). It also demonstrated a high specificity, producing no reactivity with the mycobacteria-related and contaminant organisms tested.

SpO-M identified to species-group level in a single step 91.7% (144/157) of the NTM growing in different culture media, leaving only 8.3% ($n = 13$) of the NTM identified as *Mycobacterium* genus and with only single species sequencing proven misidentification of the 2 isolates from the same patient of *M. marinum*, which were both erroneously classified as *M. kansasii*.

Hence, we detected a specificity problem that produces misinterpretation between *M. marinum* and *M. kansasii*. However, the very different clinical signs of the 2 species mean that confusion about the final diagnosis is unlikely. Thus, *M. kansasii* is a cause of lung disease, and extrapulmonary or disseminated infections are uncommon (Baker and Stonecypher, 2009; Bermudez et al., 1994; Bernard et al., 1999; Etxebarrieta et al., 2002; Loddenkemper et al., 2005; Neuberger et al., 2006; Puerto et al., 2007). In contrast, *M. marinum* causes cutaneous infections as a result of trauma to the skin and subsequent exposure to contaminated freshwater stagnant fish tanks or salt water (Kullavanijaya et al., 1993; Lewis et al., 2003). Infection is acquired by direct inoculation with *M. marinum* through broken skin (De Smet, 2008; Hautmann and Lotti, 1994;

Kullavanijaya et al., 1993). The most typical presentation is a single papulonodular lesion that appears 2 to 3 weeks after inoculation (Falkinham, 1996). Disseminated *M. marinum* infections, including infections in patients with AIDS or persons undergoing systemic steroid therapy, have been unusual (Adhikesavan and Harrington, 2008; Holmes et al., 1999; Parent et al., 1995).

We conclude that SpO-M is a valid tool, with a final manufacturer's recommendation price of 15 € per test, which rapidly identifies the most frequent species of mycobacteria in a single step. It is reliable when applied on either liquid or solid mycobacterial culture media and reduces the turnaround time by shortening the hybridization procedure to 5 min.

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