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Evaluation of the Speed-Oligo Mycobacteria assay for the identification of nontuberculous mycobacteria

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Nontuberculous mycobacteria (NTM) causing human infectious disease have become increasingly common. Rapid and accurate identification to the species level is, therefore, critical. The Speed-Oligo Mycobacteria assay is an oligochromatographic method that was made available recently for the identification and differentiation of mycobacteria. The present study aimed to evaluate the performance of the Speed-Oligo Mycobacteria assay for the identification of NTM. We examined a total of 62 strains (9 type strains, 19 reference strains and 34 clinical isolates) belonging to 13 different species (*Mycobacterium intracellulare*, *M. fortuitum, M. gordonae, M. kansasii, M. marinum, M. peregrinum, M. scrofulaceum, M. abscessus*, *M. bovis* BCG, *M. chelonae*, *M. avium*, *M. malmoense* and *M. xenopi*). The Speed-Oligo Mycobacteria assay was performed according to the manufacturer's instructions. Discrepant results between Speed-Oligo Mycobacteria and the original identification were reassessed by the Speed-Oligo Mycobacteria assay and resolved by the GenoType Mycobacterium CM assay and by sequencing of 16S rRNA and protein-encoding genes. We found 93.5 % (58/62) concordance for the identification of NTM as compared with the original identification. Three strains were erroneously identified by Speed-Oligo Mycobacteria: one *M. kansasii* strain was identified as *Mycobacterium tuberculosis* complex, and one *M. chelonae* strain and one *M. peregrinum* strain were both identified as *Mycobacterium abscessus*. Moreover, one *M. chelonae* strain was not identified by Speed-Oligo Mycobacteria since it did not react with any species-specific probe. For these strains, sequencing of the genes *hsp65,* 16S rRNA and *rpoB* and the GenoType Mycobacterium CM assay were performed. The Speed-Oligo Mycobacteria assay can be a useful tool for the rapid and easy identification of the most common NTM. If applied in clinical practice it could reduce diagnostic delays and contribute to correct clinical and better management of infections caused by NTM.

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INTRODUCTION

Nontuberculous mycobacteria (NTM) are ubiquitous environmental micro-organisms. They are considered opportunistic pathogens in humans, and several species are associated with

Abbreviation: NTM, nontuberculous mycobacteria.

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respiratory infections, lymphadenitis, skin and soft-tissue infections, and disseminated diseases (Foo *et al.*, 2009; Babalik *et al.*, 2012). The increasing prevalence of disease due to NTM is related both to more intense interactions of humans with certain types of environment and to changes in population demographics, including a growing number of people who are immunosuppressed as a result of HIV/AIDS, malignancy or medical intervention (Heifets, 2004).

Since clinical management, antimicrobial therapy and infection control measures differ depending on the infecting mycobacterial species, a rapid and accurate diagnostic tool for the reliable identification of NTM is essential (Hofmann-Thiel *et al.*, 2011). Conventional biochemical tests used to identify mycobacteria are complex, time consuming and can generate misidentification. The introduction of molecular methods has greatly improved the speed and accuracy of the identification process (Singh *et al.*, 2013).

Recently, a new DNA line probe assay for the differentiation of mycobacteria was made available as a commercial test. The Speed-Oligo Mycobacteria assay (Vircell) is an oligochromatographic method based on PCR targeting 16S rRNA gene and 16S–23S rRNA regions and double reverse hybridization on a dipstick using probes bound to colloidal gold and a membrane. This assay identifies the *Mycobacterium* genus, *Mycobacterium tuberculosis* complex and 13 different species of NTM (Quezel-Guerraz *et al.*, 2010; Hofmann-Thiel *et al.*, 2011). The present study aimed to evaluate the performance of the Speed-Oligo Mycobacteria assay for the identification of NTM.

METHODS

Strains. A total of 62 mycobacterial strains (9 type strains, 19 reference strains and 34 clinical isolates) belonging to 13 different species were used in this study (Tables 1, 2 and 3) and were obtained from the CCUG collection (http://www.ccug.se), from the clinical laboratory of St Luc Hospital, Brussels, and from the WHO supernational reference laboratory for tuberculosis, Milano, Italy. The clinical isolates were previously identified by 16S rRNA gene sequence analysis.

Speed-Oligo Mycobacteria assay. This method identifies the *Mycobacterium* genus, *M. tuberculosis* complex (*M. tuberculosis*, *Mycobacterium bovis*, *Mycobacterium microti*, *Mycobacterium africanum*, *Mycobacterium caprae*) and 13 different species of NTM (*Mycobacterium abscessus*, *Mycobacterium marinum/Mycobacterium ulcerans* complex, *Mycobacterium kansasii*, *Mycobacterium xenopi*, *Mycobacterium intracellulare*, *Mycobacterium avium*, *Mycobacterium scrofulaceum*, *Mycobacterium malmoense*, *Mycobacterium gordonae*, *Mycobacterium interjectum*, *Mycobacterium chelonae*, *Mycobacterium fortuitum* and *Mycobacterium peregrinum*). For DNA extraction, a loopful of bacteria from Löwenstein–Jensen medium was resuspended in 150 µl of Vircell sample solution and incubated at 95 °C for 60 min. The bacterial suspension was then centrifuged at 12 000 ***g*** for 5 min and the supernatant was used directly for PCR or stored at −20 °C. For PCR, 15 µl of ready-to-use reconstituted PCR mix (provided with the kit) and 10 µl of DNA sample were added to PCR tubes. PCR conditions were 1 min of denaturation at 92 °C, 40 cycles consisting of 20 s at 92 °C, 30 s at 55 °C, 30 s at 72 °C, and a final extension of 1 min at 72 °C. The PCR product was denatured by heating at 95 °C for 1 min and immediately cooled on ice. Then, 10 µl of denatured PCR product was added to 35 µl of preheated (55 °C) hybridization solution (provided with the kit) in a 1.5 ml tube. The test strip was immediately inserted and results were interpreted after 10 min of incubation at 55 °C. Positive (*M. tuberculosis* DNA) and negative (deionized water) controls were always included in each test run. These controls were provided by the manufacturer. The visual interpretation of the results was based on the presence or absence of different bands and compared with reference bands according to the manufacturer's instructions. The entire process took approximately 150 min.

Resolution of discrepancies. Discrepant results between Speed-Oligo Mycobacteria and the original identification methods were re-examined by the Speed-Oligo Mycobacteria assay and resolved by the GenoType Mycobacterium CM assay (Hain Lifescience) and by sequencing of *hsp65*, 16S rRNA and *rpoB* genes.

GenoType Mycobacterium CM assay. The GenoType Mycobacterium CM assay was divided into three steps: DNA extraction from cultured material, multiplex amplification with biotinylated primers and a reverse hybridization. The whole procedure was performed as recommended by the manufacturer.

PCR amplification and sequencing. For the PCR amplification of the *hsp65*, 16S rRNA and *rpoB* genes we used the primers described by Telenti *et al.* (1993), Harmsen *et al.* (2003) and Adékambi *et al.* (2003), respectively. PCR products were purified by using a NucleoFast 96 PCR clean-up kit (Macherey-Nagel). Sequencing reactions were performed by using a BigDye terminator cycle-sequencing kit (Applied Biosystems) and purified using the BigDye X Terminator Purification kit (Applied Biosystems). For sequencing, the primers used were: Tb11 5′-ACCAACGATGGTGTGTCCAT and Tb12 5′-CTTGTCGAACCG-CATACCCT (for the *hsp65* gene), 16S27f 5′-AGAGTTTGATCMTGG-CTCAG and BKL1 5′-GTATTACCGCGGCTGCTGGCA (for the 16S

Table 1. Results of Speed-Oligo Mycobacteria analysis of nine taxonomic type strains

Species	Strain number	Speed-Oligo identification results
M. intracellulare	CCUG 28005^T	*M. intracellulare*
M. abscessus subsp. *bolletii*	CCUG 50184^T	*M. abscessus*
M. abscessus subsp. *abscessus*	ATCC 19977^T	*M. abscessus*
M. fortuitum	CCUG 27973^T	*M. fortuitum*
M. gordonae	CCUG 21801^T	*M. gordonae*
M. kansasii	CCUG 20997^T	*M. kansasii*
M. marinum	CCUG 20998^T	*M. marinum*
M. peregrinum	DSM 43271^T	*M. peregrinum*
M. scrofulaceum	CCUG 29045^T	*M. scrofulaceum*

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Table 2. Results of Speed-Oligo Mycobacteria analysis of 19 reference strains

Species	Strain number	Speed-Oligo identification results	GenoType CM	Sequencing identification (% similarity)*		
				16S rRNA gene	*rpoB* gene	*hsp65* gene
M. avium	CCUG 27851	*M. avium*				
M. intracellulare	CCUG 28000	*M. intracellulare*				
M. bovis BCG	PANEL 13 ISP	*M. tuberculosis* complex				
M. chelonae	PANEL 5 ISP	No band	*M. chelonae*	*M. chelonae* (100 %)[1]	*M. chelonae* (99 %)[2]	*M. chelonae* (100 %)[3]
M. abscessus	CCUG 41449	*M. abscessus*				
M. chelonae	ATCC 14472	*M. abscessus*	*M. abscessus*	*M. abscessus* (100 %)[4] *M. chelonae* (100 %)[7]	*M. abscessus* (96 %)[5] *M. chelonae* (96 %)[8]	*M. abscessus* (99 %)[6] *M. chelonae* (93 %)[9]
M. fortuitum	CCUG 31556	*M. fortuitum*				
	PANEL 10 ISP	*M. fortuitum*				
	PANEL 9 ISP	*M. fortuitum*				
	PANEL 4 ISP	*M. fortuitum*				
	PANEL 2 ISP	*M. fortuitum*				
M. gordonae	CCUG 21799	*M. gordonae*				
	PANEL 8 ISP	*M. gordonae*				
M. kansasii	CCUG 32245	*M. tuberculosis* complex	*M. kansasii*	*M. kansasii* (99 %)[10]	*M. kansasii* (97 %)[11]	*M. kansasii* (92 %)[12]
M. malmoense	PANEL 1 ISP	*M. malmoense*				
M. peregrinum	CCUG 28064	*M. peregrinum*				
	PANEL 11 ISP	*M. peregrinum*				
	ATCC 700686	*M. abscessus*	*M. peregrinum*	*M. peregrinum* (100 %)[13] *M. septicum* (100 %)[16]	*M. peregrinum* (99 %)[14] *M. septicum* (98 %)[17]	*M. peregrinum* (99 %)[15] *M. septicum* (98 %)[18]
M. scrofulaceum	CCUG 28164	*M. scrofulaceum*				

*Percentage similarity towards the following type strain sequences are given: 1, ATCC 35752^T (AY457072); 2, ATCC 35752^T (AY457072); 3, ATCC 35752^T (AY457072); 4, ATCC 19977^T (AY457071); 5, ATCC 19977^T (JF346875.1); 6, ATCC 19977^T (JF491290.1); 7, ATCC 35752^T (AY457072); 8, ATCC 35752^T (AY1471163.1); 9, ATCC 35752^T (DQ869273.1); 10, ATCC 12478^T (NR_121712.1); 11, ATCC 12478^T (EU591500.1); 12, ATCC 12478^T (AF547849.1); 13, ATCC 14467^T (AY457069); 14, ATCC 14467^T (JF712876.1); 15, ATCC 14467^T (AY457069); 16, ATCC 700731^T (AY457070); 17, ATCC 700731^T (AY147167.1); 18, ATCC 700731^T (AY496142.1).

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Table 3. Results of Speed-Oligo Mycobacteria analysis of 34 clinical isolates

Species	Isolate number	Speed-Oligo identification results
M. avium	H-12, 2012/46	*M. avium*
M. intracellulare	H-17, 2012/6	*M. intracellulare*
M. avium/intracellulare	2013-28, 2013-14	*M. intracellulare*
M. bovis	H-8, 2008/13, 2007/40	*M. tuberculosis* complex
M. chelonae	2010/51, 2013-50	*M. chelonae*
M. abscessus	P-1, 52495	*M. abscessus*
M. chelonae/abscessus	2012/13, 98203, 2012/56	*M. abscessus*
M. fortuitum	4/44, 2011/12, 2011/15	*M. fortuitum*
M. gordonae	2012/52, 2013-32, 2013-29, 2013-10	*M. gordonae*
M. kansasii	T1-5, 2000/75, 2008/10	*M. kansasii*
M. malmoense	2-81	*M. malmoense*
M. marinum	P-8 11266, 2009/25, 2010/46	*M. marinum*
M. peregrinum	2-77	*M. peregrinum*
M. scrofulaceum	H-18	*M. scrofulaceum*
M. xenopi	H-24, 2012/47	*M. xenopi*

rRNA gene), and MycoF 5′-GGCAAGGTCACCCCGAAGGG and MycoR 5′-AGCGGCTGCTGGGTGATCATC (for the *rpoB* gene). Sequencing was performed using an ABI Prism 3130 XL Genetic Analyzer (Applied Biosystems). Sequence assembly was performed using the BioNumerics 5.1 software package (Applied Maths). Sequences were aligned and compared with those available in the GenBank database by BLAST analyses.

RESULTS

Sixty-two mycobacterial strains were included in the study. A concordance of 93.5 % (58/62) was found in the identification of the NTM between the Speed-Oligo Mycobacteria assay and the routine identification methods (Tables 1, 2 and 3). The reference strain *M. chelonae* Panel 5 ISP was not identified by this assay because its DNA did not react with any species-specific probe available in the test. However, the GenoType Mycobacterium CM assay identified this strain as *M. chelonae*. This strain showed sequence similarities of 100 % for the 16S rRNA and *hsp65* genes, and 99 % for the *rpoB* gene with the type strain *M. chelonae* ATCC 35752^T. Another *M. chelonae* reference strain, ATCC 14472, was identified as *M. abscessus* by the Speed-Oligo Mycobacteria assay. In this case, the GenoType Mycobacterium CM assay identified this strain correctly as *M. abscessus*. This strain shared 100 % 16S rRNA gene sequence similarity with *M. abscessus* ATCC 19977^T and *M. chelonae* ATCC 35752^T, 96 % *rpoB* gene sequence similarity with *M. abscessus* ATCC 19977^T and *M. chelonae* ATCC 35752^T, and 99 % and 93 % *hsp65* gene sequence similarity with *M. abscessus* ATCC 19977^T and *M. chelonae* ATCC 35752^T, respectively. The third discordance was *M. kansasii* CCUG 32245, which was erroneously identified by Speed-Oligo Mycobacteria as *M. tuberculosis* complex. The GenoType Mycobacterium CM assay identified this strain correctly as *M. kansasii*. We found a sequence similarity of 99 % for the 16S rRNA gene, 97 % for the *rpoB* gene and 92 % for *hsp65* gene sequences with the type strain *M. kansasii* ATCC 12478^T. The last discordant strain, *M. peregrinum* ATCC 700686, was identified as *M. abscessus* by Speed-Oligo Mycobacteria. The GenoType Mycobacterium CM assay identified this strain as *M. peregrinum*. This strain showed sequence similarities of 100 % for the 16S rRNA gene with *M. peregrinum* ATCC 14467^T and *M. septicum* ATCC 700731^T, 99 % and 98 % similarity for the *rpoB* gene sequence with *M. peregrinum* ATCC 14467^T and *M. septicum* ATCC 700731^T, and 99 % and 98 % similarity for the *hsp65* gene sequence with *M. peregrinum* ATCC 14467^T and *M. septicum* ATCC 700731^T type strains (Table 2). The Speed-Oligo Mycobacteria assay therefore correctly identified 100 % (9/9) of the type strains, 78.9 % (15/19) of the reference strains and 100 % (34/34) of the clinical isolates evaluated.

DISCUSSION

The recently introduced commercial test Speed-Oligo Mycobacteria allows detection of mycobacteria including *M. tuberculosis* complex and 13 of the most frequently isolated species of NTM (Fig. S1, available in the online Supplementary Material) (Quezel-Guerraz *et al.*, 2010). Its major advantage compared with other line-probe tests comprises its direct application to denatured amplification products. No washing steps are needed compared with similar methods. This dipstick test requires only two pipetting steps, thereby decreasing the hands-on time and the risk of cross-contamination. Results can be obtained within 2 h 30 min with a total hands-on time of 30 min (Hofmann-Thiel *et al.*, 2011; Lara-Oya *et al.*, 2013).

In the present study, 93.5 % of the strains tested with the Speed-Oligo Mycobacteria assay showed concordant results. Previously, O'Donnell *et al.* (2012) obtained

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98 % concordance between Speed-Oligo Mycobacteria and the AccuProbe assay. In our study, three strains were misidentified by the Speed-Oligo Mycobacteria assay: *M. chelonae* ATCC 14472 was identified as *M. abscessus*, *M. kansasii* CCUG 32245 as *M. tuberculosis* complex and *M. peregrinum* ATCC 700686 as *M. abscessus*. These misidentifications should be taken into account since they could influence treatment decisions and disease outcome. Each species of NTM has a different profile of antimicrobial susceptibility (Pang *et al.*, 2011). Moreover, the Speed-Oligo Mycobacteria failed to identify one strain of *M. chelonae*, Panel 5 ISP, which did not react with any probe. This may be due to variability of the bacterial genome of this strain (Lara-Oya *et al.*, 2013).

The species discrepancies found in our study were also found in previous studies. Quezel-Guerraz *et al.* (2010) evaluated the ability of Speed-Oligo Mycobacteria to differentiate mycobacterial species and detected misidentification of *M. marinum* as *M. kansasii*. Hofmann-Thiel *et al.* (2011) also found misidentification of *M. peregrinum* as *M. fortuitum* and of *M. marinum* as *M. kansasii*.

Speed-Oligo Mycobacteria is a rapid, easy-to-perform and inexpensive method. However, its major current limitation is that it allows the identification of a relatively small spectrum of mycobacterial species only and, therefore, some NTM commonly found in the routine clinical laboratory, such as *Mycobacterium goodii*, *Mycobacterium szulgai* and *Mycobacterium lentiflavum*, may not be identified. This could be solved if additional probes were incorporated into the membrane strip in order to cover more species (Wu *et al.*, 2007).

We conclude that the Speed-Oligo Mycobacteria test can be considered as a valid tool for the rapid and easy identification of the most frequently occurring species of NTM. If applied in clinical practice, this assay could reduce diagnostic delays and contribute to a targeted therapy and better management of infections caused by NTM. The limitation of this study was the limited number of clinical isolates evaluated. However, evaluation under routine conditions in a multicentre study could prove, more reliably and robustly, the accuracy of this method.

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