The identification of mycobacteria from solid media and directly from VersaTREK Myco bottles using the Sherlock Mycobacteria Identification HPLC system

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ABSTRACT

The Sherlock Mycobacteria Identification HPLC system correctly identified to the species level 61 (67.8%) of 90 isolates growing on solid media, and 73 (45.3%) of 161 isolates directly from positive VersaTREK Myco bottles. When these data were re-analysed with a revised database, correct identifications increased to 91.1% and 83.2%, respectively. All *Mycobacterium tuberculosis* isolates were identified correctly, regardless of the inoculum source or database used. The use of the revised database with isolates obtained directly from positive VersaTREK Myco bottles allows the identification of most isolates within clinically relevant time-frames.

Keywords Diagnosis, HPLC, identification, mycobacteria, *Mycobacterium tuberculosis*, VersaTREK

Original Submission: 20 May 2005; **Revised Submission:** 24 August 2005; **Accepted:** 23 September 2005

Clin Microbiol Infect 2006; 12: 478–481
10.1111/j.1469-0691.2006.01373.x

The use of continuously monitored liquid-based culture systems for mycobacteria has resulted in decreased times to detection for positive cultures. In addition, various species of clinically relevant, non-tuberculous mycobacteria are now isolated routinely with increasing frequency [1–3]. Several investigators have used nucleic acid probes to identify mycobacteria directly from positive culture bottles or tubes, but these probe-based identifications are restricted to a few species [4–6]. High-performance liquid chromatography (HPLC) is an alternative identification method, but is generally performed from solid media and requires manual inspection of mycolic acid chromatographic profiles, as well as extensive expertise. The purpose of the present study was to investigate the use of a commercially available HPLC system with pattern recognition software for the identification of mycobacteria obtained from solid media and directly from positive broth culture bottles.

Clinical specimens received for mycobacterial culture were processed by standard laboratory protocols and inoculated on to Middlebrook 7H11 agar plates (BBL, Sparks, MD, USA) and into VersaTREK Myco bottles containing growth supplement and PVNA (polymyxin B, vancomycin, nalidixic acid, amphotoricin B) antibiotic mixture (TREK Diagnostic Systems, Cleveland, OH, USA). These bottles were incubated in the ESP Culture System II (TREK Diagnostic Systems) and the VersaTREK system when available. Bottles were monitored continuously for growth and an aliquot was removed for identification when a positive culture was detected. All clinical isolates were identified initially using nucleic acid probes as described previously [5]. If the isolates could not be speciated using probes, they were identified by biochemical testing [7]. New clinical isolates were tested by HPLC in real-time as the cultures became positive. In total, 90 isolates were tested from solid media (45 from concurrent positive cultures and 45 from subcultured frozen stock cultures), and 161 isolates were tested directly from positive VersaTREK Myco bottles (120 from concurrent positive cultures and 41 seeded from subcultured stock cultures).

For the HPLC assay, a sterile applicator stick was used to pick up a barely visible amount of growth from solid media for processing, or the pellet from 3 mL of broth, centrifuged at 3000 *g* for 30 min, was used from a positive VersaTREK Myco bottle. Saponification, extraction and derivatisation were performed according to the manufacturer's instructions (MIDI Inc., Newark, DE, USA).

An Agilent 1100 Series HPLC system (Agilent Technologies, Wilmington, DE, USA) with a C18 reverse phase column was used to analyse the mycolic acid content of the samples. A calibration standard (in duplicate) and the specimen samples

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were injected into the column and eluted with a gradient of methanol/isopropanol. Each specimen required *c.* 13 min for elution and data analysis. The entire procedure was completed within 8 h. At the beginning of each run, the calibration standard provided mycolic acid reference retention times, which were used to calculate equivalent carbon length values. Sample chromatographic profiles were then compared with existing profiles contained in the database. Reports include an identification for each isolate, along with a similarity index (SI). An acceptable identification required an SI of ≥0.5, with a minimum of 0.2 SI separation from the next closest species identification.

All samples were analysed with the current US Food and Drug Administration-approved library (Library 1.02) and the updated database (Library 1.03). Both results were compared with identifications made with nucleic acid probes and/or conventional biochemical tests. Five isolates, three *Mycobacterium scrofulaceum*, one *Mycobacterium interjectum* and one *Mycobacterium xenopi*, were re-tested by the New York City Department of Health with a different HPLC method because of apparent discrepancies between the MIDI HPLC and conventional identification results.

The results obtained with isolates grown on 7H 11 medium are shown in Table 1. Of the 90 isolates, encompassing 18 species, 61 (67.7%) were identified correctly by HPLC, which is similar to the rate of correct results (75%) reported previously with use of this system [8]. Another three isolates were identified correctly, but there was < 0.2 SI difference between the first and second identifications. Four additional isolates were identified correctly to the species complex. It is of note that *M. scrofulaceum* (contained within the *Mycobacterium avium–Mycobacterium intracellulare–M. scrofulaceum* complex) was not contained in Library 1.02. However, only two of the 16 *M. scrofulaceum* isolates were identified accurately as part of this complex. The Library 1.02 database identified correctly only 73 (45.3%) of the isolates obtained directly from positive Myco bottles (Table 2). Another seven isolates were identified correctly, but with < 0.2 SI separation between the first two choices. Another 58 isolates were identified correctly to the species complex.

With the Library 1.03 database, the overall agreement increased to 91.1% for isolates from solid media (Table 1), and to 83.2% for isolates from the VersaTREK Myco bottles (Table 2). Among isolates from solid media, all of the *M. avium* complex isolates were identified correctly, as were 15 of the 16 isolates of *M. scrofulaceum*. Among isolates obtained from Myco bottles, 62 of 81 *M. avium* complex isolates were identified correctly, with an additional nine isolates with < 0.2 SI between the top two choices. Fifteen of the 18 *M. scrofulaceum* isolates were also identified. In all cases, isolates of *Mycobacte-*

Table 1. Identification of mycobacterial isolates grown on solid media with the MIDI HPLC system

				Library 1.02			
Organism	***n***	**Stock culture**	**Real-time**	**No. correct**	**No. correct < 0.2 SI**	**Correct to complex**	**Library 1.03 No. correct**
M. abscessus[a]	9	1	8	9			9
M. asiaticum	1	1	0	1			1
M. avium complex	25	4	21	20	2	2	25
M. bovis BCG	1	0	1	1			1
M. celatum	3	3	0	3			3
M. chelonae[b]	3	0	3	3			3
M. fortuitum	1	1	0	1			1
M. gordonae	2	0	2	2			2
M. haemophilum	8	7	1	7	1		7
M. interjectum	2	1	1	0			1
M. kansasii	2	2	0	2			2
M. malmoense	1	1	0	1			1
M. mucogenicum	2	2	0	0			0
M. scrofulaceum	16	16	0	0[c]		2	15
M. simiae	2	2	0	0			0
M. terrae complex	2	0	2	2			2
M. tuberculosis	4	0	4	4			4
M. xenopi	6	4	2	5			5
Totals	90	45	45	61 (67.8%)	3	4	82 (91.1%)

[a]Identified as *M. abscessus/chelonae*.
[b]Identified as *M. chelonae/abscessus*.
[c]*M. avium–M. intracellulare–M. scofulaceum* complex only in Library 1.02.
SI, similarity index.

Table 2. Identification of mycobacterial isolates grown in VersaTREK Myco bottles with the MIDI HPLC system

Organism	*n*	Stock	Real-time	Library 1.02 No. correct	Library 1.02 No. correct < 0.2 SI	Library 1.02 Correct to complex	Library 1.03 No. correct	Library 1.03 No. correct < 0.2 SI
M. abscessus[a]	6	1	5	6			6	
M. asiaticum	1	1	0	1			1	
M. avium complex	81	3	78	16	7	55	62	9
M. bovis BCG	1	1	0	1			1	
M. celatum	2	2	0	2			2	
M. chelonae[b]	2	0	2	2			2	
M. fortuitum	2	1	1	2			2	
M. gordonae	9	1	8	9			9	
M. haemophilum	1	1	0	1			1	
M. interjectum	2	1	1	1			1	
M. kansasii	2	2	0	2			2	
M. malmoense	1	1	0	1			1	
M. marinum	2	2	0	2			2	
M. mucogenicum	2	2	0	0			0	
M. scrofulaceum	18	18	0	0[c]		3	15	
M. simiae	2	2	0	0			0	
M. terrae complex	1	0	1	1			1	
M. tuberculosis	20	0	20	20			20	
M. xenopi	6	2	4	6			6	
Totals	161	41	120	73 (45.3%)	7	58	134 (83.2%)	

[a]Identified as *M. abscessus/chelonae*.
[b]Identified as *M. chelonae/abscessus*.
[c]*M. scrofulaceum* not included in Library 1.02.
SI, similarity index.

rium tuberculosis were identified accurately; however, the system cannot differentiate among the species in the *M. tuberculosis* group, with the exception of *Mycobacterium bovis* BCG.

The development of automated pattern recognition software that analyses HPLC peak retention times and characteristics allows this methodology to be used in routine clinical microbiology laboratories. Library 1.02 was developed from isolates grown on 7H10 agar. However, this proved to be inadequate when used with isolates obtained from liquid media. The major improvements seen in the revised database were the inclusion of *M. scrofulaceum* in the *M. avium–M. intracellulare–M. scrofulaceum* complex and the refinement in the identification of *M. avium* isolates. The other non-tuberculous mycobacterial species were identified correctly in 42 of 49 cases from solid media, and in 57 of 62 cases from the Myco bottles. The *Mycobacterium simiae* isolates were identified as *Mycobacterium lentiflavum/Mycobacterium triplex*, which have been reported to have similar mycolic acid profiles [9]. The two isolates of *Mycobacterium mucogenicum* in the present study were identified as *Mycobacterium fortuitum/Mycobacterium peregrinum*.

The MIDI HPLC system, combined with Library 1.03, is feasible for use in the routine laboratory to identify mycobacterial isolates in real-time. The instrument costs 55 000 Euro; each run requires two standards and two control isolates at a cost of *c.* 11 Euro, each sample costs *c.* 4 Euro, and one calibrator must be re-run after every ten samples. The system obviates the need for using more expensive nucleic acid probes and most routine biochemicals. The MIDI HPLC system with the updated Library is capable of identifying most clinical isolates within clinically relevant time-frames.

ACKNOWLEDGEMENTS

The authors wish to thank G. Osterhout for his work on producing the revised database and for his critical review of the manuscript. This study was not supported by any outside source or company. This paper was presented, in part, at the 15th European Congress for Clinical Microbiology and Infectious Disease (Copenhagen, 2005).

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RESEARCH NOTE

Evaluation of a novel strip test, GenoType Mycobacterium CM/AS, for species identification of mycobacterial cultures

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ABSTRACT

A novel DNA strip assay, GenoType Mycobacterium AS, was evaluated for its ability to identify 219 mycobacterial isolates in combination with the GenoType Mycobacterium CM assay. The results were compared with those obtained by conventional 16S rDNA sequencing. The Genotype test correlated well (96%) with sequencing. However, with the CM kit alone, it was possible to identify most (88%) of the isolates found in clinical specimens, and the AS kit provided very little additional information.

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Keywords DNA strip assay, Genotype Mycobacterium AS, identification, *Mycobacterium* spp., 16S rDNA sequencing

Original Submission: 4 July 2005; **Revised Submission:** 8 September 2005; **Accepted:** 5 October 2005

Clin Microbiol Infect 2006; 12: 481–483
10.1111/j.1469-0691.2006.01380.x

Non-tuberculous mycobacteria are isolated increasingly from patient specimens in countries where the incidence of tuberculosis is low [1], and there is a need for rapid methods for their identification. Two DNA line probe assays, INNO-LiPA Mycobacteria (Innogenetics, Ghent, Belgium), targeting the 16S–23S rDNA spacer region, and Genotype Mycobacterium common mycobacteria (CM) (Hain Lifescience, Nehren, Germany), targeting the 23S rDNA region, have been developed for this purpose. These tests are based on reverse hybridisation of PCR amplicons to membrane-bound probes covering the species-specific variable regions of the target genes. The assays are reported to be sensitive (100%) and specific (94.4–100%) for identification of most common mycobacteria [2–5].

The GenoType Mycobacterium additional species (AS) kit supplements the CM kit and allows identification of 16 additional mycobacterial species (*Mycobacterium asiaticum*, *Mycobacterium celatum*, *Mycobacterium gastri*, *Mycobacterium genavense*, *Mycobacterium goodii*, *Mycobacterium haemophilum*, *Mycobacterium heckeshornense*, *Mycobacterium kansasii*, *Mycobacterium lentiflavum*, *Mycobacterium mucogenicum*, *Mycobacterium phlei*, *Mycobacterium shimoidei*, *Mycobacterium simiae*, *Mycobacterium smegmatis*, *Mycobacterium szulgai/Mycobacterium intermedium* and *Mycobacterium ulcerans*). This test has been available in Europe since 2004, but an evaluation from a clinical laboratory has not been published previously. The aim of the present study was to assess the usefulness of the GenoType Mycobacterium CM and AS tests for the routine identification of mycobacteria isolated from patient specimens in Finland.

All new patient isolates (n = 219) sent to the Finnish Mycobacterial Reference Laboratory during October–December 2004 were included in the study. The isolates were received either on solid Löwenstein–Jensen media, or in BACTEC-MGIT (BD Biosciences, Franklin Lakes, NJ, USA) or MB/BacT (bioMérieux, Marcy l'Etoile, France)