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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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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)

liquid media. All isolates were identified by 16S rDNA sequencing as described previously [4,6]. *Mycobacterium chelonae* and *Mycobacterium abscessus* were differentiated further by PCR–restriction enzyme analysis of the *hsp65* gene [7].

Bacteria were lysed by heating at 95°C for 15 min, and the GenoType CM and AS assays were performed according to the manufacturer's instructions. The protocols included methods for PCR amplification, hybridisation and detection, and interpretation of the results. In addition to species-specific probes, each test strip has a conjugate control line to ensure that reactive conjugate and substrate have been added, a universal control probe that detects mycobacteria and other Gram-positive bacteria with a high G + C content, and a genus control line indicating the presence of mycobacteria. Only bands with intensities at least as strong as that of the universal control were considered when interpreting the results. All isolates were first analysed with the GenoType CM test strip. If the CM strip result indicated *Mycobacterium* spp. or *Mycobacterium marinum/M. ulcerans*, the identification process was continued with the AS test strip.

The combination of the GenoType CM and AS tests gave concordant results with 211 (96%) of the 219 isolates when compared with the sequencing results (Table 1). Of the 219 isolates, three were not mycobacteria, i.e., sequencing identified *Gordona* sp., *Actinomadura* sp. and *Kocuria* sp. These isolates were identified correctly as acid-fast bacilli or were negative by the Genotype CM kit. Of the eight isolates that gave discrepant results, one *Mycobacterium avium*, one *Mycobacterium intracellulare* and one *Mycobacterium tuberculosis* isolate were negative with the GenoType CM test. The strips for these three isolates showed a positive hybridisation pattern with the conjugate control, but both the universal control and the genus control were negative. Since the 16S rDNA PCR and sequencing were successful when performed from the same lysates, the GenoType PCR and hybridisation tests were repeated, yielding very faint universal control and genus control, as well as species-specific, bands on the strips, perhaps indicating that the amplification reaction with the GenoType primers was not efficient. Weak hybridisation caused by heterogeneity in the probe-binding region is also a possibility.

Two isolates identified as *M. intracellulare* by sequencing were identified as *Mycobacterium scrofulaceum* by GenoType. These two species are related closely, and the discrepancy may result from the differences in the target gene regions used by the two methods. In addition, two *M. kansasii* isolates and one *M. simiae* isolate reacted only with the genus probe, but not with the species-specific probes. However, as stated in the kit instructions, it is possible that certain subtypes may not be detected because of the high variability of the bacterial genomes.

In total, 192 (88%) of the 219 isolates were identified to the species level with the CM kit. This is in agreement with reports published previously [4,5]. *M. marinum* and *M. ulcerans* cannot be differentiated by the CM kit, and therefore the two *M. marinum/M. ulcerans* isolates, as well as the 19 isolates that reacted only with the genus probe, were analysed further with the AS kit. Eight (38%) of these 21 isolates (four *M. lentiflavum*, two *M. marinum* and two *M. simiae*) were identified to the species level with the AS test. No further identification was obtained for the remaining 11 isolates. None of the isolates were suspected originally to be *M. haemophilum*, and thus they were not cultured on specific blood media that would support the

**Table 1.** Comparison of identification of *Mycobacterium* spp. (acid-fast bacillus (AFB)-positive isolates) by DNA sequencing, GenoType Mycobacterium (GT) common mycobacteria (CM) and additional species (AS) assays

| Sequencing result | *n* | GT-CM and AS correct | GT incorrect |
|---|---|---|---|
| *M. avium* | 43 | 42 | 1 negative |
| *M. abscessus* | 2 | 2 | |
| *M. bohemicum* | 3 | 3[a] | |
| *M. chelonae* | 3 | 3 | |
| *M. fortuitum* | 9 | 9[b] | |
| *M. gordonae* | 23 | 23 | |
| *M. intracellulare* | 28 | 25 | 1 negative; 2 *M. scrofulaceum* |
| *M. interjectum* | 2 | 2 | |
| *M. kansasii* | 2 | 0 | 2 *Mycobacterium* spp. |
| *M. lentiflavum* | 4 | 4 | |
| *M. malmoense* | 4 | 4 | |
| *M. marinum* | 2 | 2 | |
| *M. neoaurum* | 1 | 1[a] | |
| *M. nonchromogenicum* | 1 | 1[a] | |
| *M. peregrinum* | 1 | 1[c] | |
| *M. septicum* | 1 | 1[c] | |
| *M. simiae* | 3 | 2 | 1 *Mycobacterium* sp. |
| *M. terrae* | 4 | 4[a] | |
| *M. tuberculosis* | 75 | 74 | 1 negative |
| *M. xenopi* | 3 | 3 | |
| *M. vaccae* | 1 | 1[a] | |
| *M. wolinskyi* | 1 | 1[a] | |
| *Gordona* sp. | 1 | 1 | 1 AFB |
| *Actinomadura* sp. | 1 | 1 negative | |
| *Kocuria* sp. | 1 | 1 negative | |
| Total | 219 | 211 | 8 |

[a]Correct GenoType result is *Mycobacterium* sp., since no species-specific probe is available in the test strip.
[b]Seven isolates were positive with the *M. fortuitum* I probe, and one with the *M. fortuitum* II probe.
[c]*M. peregrinum*, *M. septicum* and *M. alvei* cannot be differentiated by GT.

growth of this species. Therefore, it was considered that an identification as *M. malmoense/M. haemophilum* with the CM assay indicated the presence of *M. malmoense*, although *M. malmoense* and *M. haemophilum* could have been differentiated further by the AS assay.

In Finland, one GenoType CM strip costs 25 Euros (September 2005), rising to a total cost of 50 Euros if an additional AS strip is needed. This is somewhat more expensive than sequencing (*c.* 20 Euros per reaction), but the GenoType assay is faster and can be performed without any expensive instrumentation.

In conclusion, the GenoType CM assay is a rapid and reliable method for identification of mycobacteria in a clinical laboratory. Although the additional information provided by the GenoType AS assay is insufficient to warrant its uniform use, it is believed that laboratories without sequencing facilities would benefit from the availability of this test.

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# RESEARCH NOTE

## Urease genes in non-O157 Shiga toxin-producing *Escherichia coli*: mostly silent but valuable markers for pathogenicity

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## ABSTRACT

The distribution of *ureC* was investigated among 294 *Escherichia coli* isolates, comprising 72 strains from the *E. coli* standard reference collection (ECOR), 62 strains from the diarrhoeagenic *E. coli* (DEC) collection, and 160 clinical isolates of Shiga toxin-producing *E. coli* (STEC). The *ureC* gene was more frequent among STEC isolates harbouring *eae* than among those lacking *eae* ($p < 0.0001$). All clinical STEC isolates of serogroups O111 and O145 contained *ureC*, but only two of 294 isolates expressed urease activity. The silencing of urease expression could not be linked to a stop codon in *ureD*. The frequent occurrence of *ure* genes in *eae*-positive STEC isolates makes them valuable markers for virulence.

**Keywords** *eae*, *Escherichia coli*, intimin, Shiga toxin, urease, *ureC*, virulence

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Shiga toxin-producing *Escherichia coli* (STEC) strains cause diarrhoea, haemorrhagic colitis and the haemolytic uraemic syndrome. STEC strains associated with severe disease are also termed enterohaemorrhagic *E. coli* (EHEC) in order to differentiate them from STEC strains,

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