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Use of the GenoType Mycobacterium CM and AS Assays To Analyze 76 Nontuberculous Mycobacterial Isolates from Greece

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Seventy-six nontuberculous mycobacterial isolates obtained from patients living in Greece were analyzed with the GenoType Mycobacterium CM (for common mycobacteria) and AS (for additional species) assays. GenoType correctly identified all but one of the mycobacterial species. For this species, additional probes should be designed and added to the strip.

Nontuberculous mycobacteria (NTM) can cause opportunistic infections to immunocompromised patients. The incidence of NTM infections has dramatically increased over the past few decades due to the parallel increase in the immunocompromised population. Since treatment and quarantine measures depend on the species responsible for the infection, rapid identification of mycobacteria at the species level is of great relevance.

Since they offer improved speed and accuracy in mycobacterial identification, molecular biological methods have replaced conventional ones (1, 4, 7, 8). Sequencing of the 16S rRNA gene is regarded as the gold standard method, whereas the most widely used in clinical laboratories is the AccuProbe test (GenProbe, San Diego, CA), which is a DNA probe assay targeting 16S rRNA. Sequencing is a laborious, time-consuming method that requires expensive equipment, whereas AccuProbe has a limited identification spectrum.

DNA strip technology based on the reverse hybridization of PCR products to their complementary probes has been used for the simultaneous detection and identification of mycobacteria (4). GenoType Mycobacterium (Hain Lifescience GmbH, Nehren, Germany) is a commercial DNA strip assay used for the detection and identification to the species level of mycobacteria obtained from positive liquid or solid cultures. It comprises two kits: the GenoType CM (for common mycobacteria) and GenoType AS (for additional species) assays, providing probes for 14 and 16 species, respectively.

Mycobacteria isolated from different geographical regions have been reported to exhibit intraspecies variations (2, 3, 5). In order to evaluate the ability of this assay to identify mycobacteria isolated from people living in Crete, Greece, we performed analysis of 76 NTM isolated over a 5-year period. To our knowledge, this is the first such study in Greece.

Mycobacterial strains. Seventy-six consecutive NTM isolates from different patients isolated over a 5-year period (2000 to 2004) were analyzed. Our Mycobacteriology Laboratory is a level III facility and is established within the Heraklion University Hospital, the only tertiary hospital on the island of Crete. Samples were collected from inpatients and outpatients living on the island, regardless of their nationality. In parallel, 11 NTM strains obtained from the Institute Pasteur (Paris, France) were used as additional controls. These control strains belonged to the following species: *M. simiae M. szulgai*, *M. gastri*, *M. avium*, *M. intracellulare*, *M. kansasii*, *M. peregrinum*, *M. malmoense*, *M. phlei*, *M. fortuitum*, and *M. xenopi*.

The majority of the isolates had been identified to the species level using either conventional methods (phenotypic characteristics and biochemical tests) or AccuProbe (Table 1). Bacteria were incubated at Lowenstein-Jensen medium at 37°C and were suspended in 300 μl of distilled water. DNA was prepared, as proposed by the manufacturer, by incubation of bacteria at 95°C for 20 min, followed by incubation in an ultrasonic bath for 15 min and centrifugation at full speed for 5 min. A total of 5 μl of the supernatant was used for the PCR.

GenoType assay. The GenoType assay was performed according to the manufacturer's instructions, using the reagents provided with the kits. The GenoType protocol consists of PCR amplification, hybridization of the PCR products to the strips, detection, and interpretation of the results.

GenoType CM permits the identification of the following mycobacterial species: *M. avium* subspecies, *M. chelonae*, *M. abscessus*, *M. fortuitum*, *M. gordonae*, *M. intracellulare*, *M. scrofulaceum*, *M. interjectum*, *M. kansasii*, *M. malmoense*, *M. marinum-M. ulcerans*, *M. peregrinum*, the *M. tuberculosis* complex, and *M. xenopi*. GenoType AS provides probes for a series of additional NTM, namely, *M. simiae*, *M. mucogenicum*, *M. goodie*, *M. cellatum*, *M. smegmatis*, *M. genavense*, *M. lentiflavum*, *M. heckeshornense*, *M. szulgai*, *M. phlei*, *M. hemophilum*, *M. kansasii*, *M. ulcerans*, *M. gastri*, *M. asiaticum*, and *M. shimoidei.*

Identification of mycobacterial isolates as determined by conventional methods, Accuprobe, and GenoType are shown in Table 1. We could not identify 18 (23.9%) and 41 (53.9%) of the isolates by conventional methods and AccuProbe, respectively. GenoType failed to identify 12 isolates (15.8%). Along with the positive conjugate and universal control, these 12 rapid growers gave positive bands 10 and 12 when the kits GenoType CM and AS were used, respectively (Fig. 1). Apart from these 12 isolates, all other GenoType identifications were in accordance with the identification obtained by conventional

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TABLE 1. Identification of mycobacterial isolates by conventional methods, AccuProbe, and GenoType

NTM	No. of strains identified by:		
	Conventional methods[a]	AccuProbe	GenoType
M. gordonae	23	23	23
M. scrofulaceum			1
M. kansasii	1	1	1
M. avium		8	9
M. intracellulare		3	2
M. fortuitum	19		19 (type I)
M. peregrinum			6
M. chelonae	3		3
M. fortuitum-M. chelonae complex	12		12[b]
Others	18 (23.68%)	41 (53.94%)	

[a] Presumptive identification.
[b] CM band 10, AS band 12.

methods. In addition, seven isolates were only identified by GenoType (one *M. scrofulaceum* and six *M. peregrinum* isolates).

When the GenoType and AccuProbe (available species-kits) results are compared, full agreement was noted except for one case. An isolate identified as *M. intracellulare* with AccuProbe was identified as *M. avium* by GenoType. In addition to the 35 isolates identified by AccuProbe, 29 isolates were only identified by GenoType (1 *M. scrofulaceum*, 19 *M. fortuitum*, 6 *M. peregrinum*, and 3 *M. chelonae* isolates).

One isolate was presumptively identified as *M. fortuitum* by conventional methods. The same sample had initially been tested negative with the AccuProbe-*M. tuberculosis* complex. When the sample was tested with the GenoType CM, positive results were obtained for both *M. fortuitum* type 1 and *M. tuberculosis* complex. This is an interesting phenomenon that has been observed in the past (6). In that study, 12 isolates or 5.5% of the total *M. tuberculosis* isolates tested negative with the AccuProbe-*M. tuberculosis* complex and were identified as *M. tuberculosis* by GenoType. This may be due to the fact that GenoType is a PCR-based assay targeting stable DNA, whereas AccuProbe targets unstable rRNA, thus requiring an ample amount of living bacteria for a positive result (6, 9). It should be emphasized that the ability of GenoType to demonstrate the simultaneous presence of more than one mycobacterial species in the same sample can be of great clinical relevance.

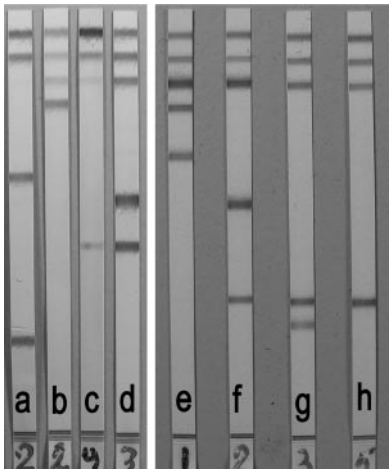

FIG. 1. Examples of the results of GenoType Mycobacterium CM (lanes a to d) and AS (lanes e to h). Lanes (the numbers of positive bands other than the bands 1, 2, and 3 are given in parentheses): a, *M. fortuitum* (bands 7 and 14); b, *M. avium* (band 4); c, acid-fast bacillus with a positive band 10; d, *M. gordonae* (bands 8 and 10); e, *M. simiae* (bands 4 and 6); f, *M. zulgai* (bands 8 and 12); g, *M. gastri* (bands 12 and 13); h, acid-fast bacillus with positive band 12.

Over the last few years, a growing number of NTM isolates, often of newly described species, are being submitted to laboratories for identification. The clinical relevance of these mycobacteria is under constant evaluation. An early identification may lead to the institution of faster treatment and a better prognosis. The majority of laboratories use AccuProbe as the gold standard commercial system for the rapid identification of mycobacteria. However, its limited identification spectrum restricts its impact in current mycobacterial diagnostics. Hence, the need for the development of a molecular assay with a wider spectrum and/or a different technology is intense. GenoType is such a commercially available assay. In the present study, we wanted to assess the usefulness of its application in mycobacterial isolates from patients living in Greece.

Based on our results, GenoType performed well; it identified all of the different species, with the exception of a group of 12 rapid growers that probably belong to the same species, since they gave an identical pattern when both GenoType CM and GenoType AS were used. The exact identity and clinical relevance of these mycobacteria is currently under further investigation. Our results indicate that new probes should be added for the correct identification of this species and, moreover, no intraspecies heterogeneity exists in our region for all other mycobacteria.

One of the three *M. intracellulare* isolates identified by AccuProbe did not give a positive *M. intracellulare* strip pattern with GenoType. This has been noticed in a previous study and with a similar distribution (6). More specifically, in that study, 5 of 16 *M. intracellulare* isolates were not identified as such by GenoType. One of these isolates was positive with both GenoType *M. avium*- and *M. intracellulare*-specific probes, whereas the remaining isolates gave a positive universal control for mycobacteria. In our case, the isolate was identified as *M. avium*. It should be noted that, from a clinical point of view, this difference has no relevance.

In conclusion, GenoType is a reliable, rapid, easy-to-perform, and easy-to-interpret assay. Moreover, it appears to be suitable for use in our region, since it identified all but one mycobacterial species. For this latter species, new probes should be designed and added to the strip.

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