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Evaluation of INNO-LiPA MYCOBACTERIA v2: Improved Reverse Hybridization Multiple DNA Probe Assay for Mycobacterial Identification

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INNO-LiPA MYCOBACTERIA (Innogenetics, Ghent, Belgium) is a reverse hybridization DNA probe assay that has been recently improved by increasing the number of identifiable mycobacterial species to 16. Our assessment, performed with 197 mycobacteria belonging to 81 taxa, revealed 100% specificity and sensitivity for 20 out of 23 probes. The probes specific for *Mycobacterium fortuitum* complex, for the *Mycobacterium avium-intracellulare-scrofulaceum* group, and for *Mycobacterium intracellulare* type 2 cross-reacted with several mycobacteria rarely isolated from clinical specimens. The overall sensitivity was 100%, and the overall specificity was 94.4%.

Among nontuberculous mycobacteria only a few species have never been reported as being responsible for human disease (14). The identification, at species level, of mycobacteria isolated from clinical specimens is therefore of clinical relevance.

It is nowadays universally accepted that the future of bacterial identification lies in genetic methods; among them the DNA probes have been very successful in recent years, thanks to their availability in commercial kits. The AccuProbe system (Gen-Probe, San Diego, Calif.), the first to be brought out, is highly specific and sensitive (10); it covers, however, only a limited number of species. The development of the line probe assay has been an important innovation, and the INNO-LiPA MYCOBACTERIA (LiPA) (Innogenetics, Ghent, Belgium) has been the first commercial DNA probe test able to identify many of the more frequently isolated species (16).

The aim of this study was to evaluate a new version of LiPA which adds eight species to the ones identifiable with the previous version (8).

To test the new LiPA, we used 197 strains retrieved from our laboratory collection. Fifty-three of them were reference strains; the others had been identified by high-performance liquid chromatography (HPLC) (2) supported by a wide panel of biochemical tests (7) and, in many cases, by genetic sequencing of 16S ribosomal DNA (rDNA) as well (5).

To test the specificity of LiPA, we included 11 non-*Mycobacterium* strains in our panel, including eight of *Nocardia* spp., one of *Gordona* spp., one of *Tsukamurella* spp., and one of *Rhodococcus equi*, and at least one isolate of every mycobacterial species not identifiable by LiPA available in our collection.

For the species targeted by the probes present in both the previous and present versions of the kit and investigated in depth in several previous studies (6, 8, 9, 11, 12, 16), only a limited number of strains were tested. For the others the number of strains tested was approximately proportional to the frequency with which they are isolated in our laboratory. However, whenever an unexpected result was obtained, other strains of the same species were added, when available, to check the finding.

The statistical evaluation of sensitivity and specificity was made separately for the genus- and the species-specific probes and, for the latter, both including and excluding the strains which had been added later to the panel.

Almost all the strains were tested from subculture on solid medium (Middlebrook 7H11); only two strains of *Mycobacterium genavense* were processed from liquid culture.

The test was performed according to the manufacturer's recommendations, using *Auto*-LiPA (Innogenetics) automated instrumentation. The procedure does not differ, apart from minor details in the automated steps performed by *Auto*-LiPA, from the one used by the previous version of LiPA, which has been described previously (16).

None of the 11 strains not belonging to the genus *Mycobacterium* reacted with any of the probes present on the strip.

As in the previous kit, all mycobacterial strains were positive with the probe specific for the genus *Mycobacterium* (MYC) (Table 1), which has been reformulated in the new kit.

Both the nonmodified probes, specific for *Mycobacterium chelonae* complex (MCH-1,2,3), *Mycobacterium kansasii* (MKA-1,2,3), *Mycobacterium scrofulaceum* (MSC), *Mycobacterium tuberculosis* complex (MTB), and *Mycobacterium xenopi* (MXE), and the newly added ones, specific for *Mycobacterium celatum* (MCE), *M. genavense* (MGV), *Mycobacterium simiae* (MSI), *Mycobacterium marinum-Mycobacterium ulcerans* (MMU), *Mycobacterium malmoense* (MML), *Mycobacterium haemophilum* (MHP), and *Mycobacterium smegmatis* (MSM), hybridized with all the strains belonging to the respective species.

As the manufacturer had stated that there were minor changes in the probe specific for *Mycobacterium gordonae* (MGO), "with the aim of improving its specificity," we in-

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TABLE 1. Results obtained from testing 208 bacterial strains with INNO LiPA MYCOBACTERIA v2

Taxon[f]	No. of strains	Genus-specific probe	Hybridizing probe(s)	
			Expected	Unexpected
M. intracellulare	5	+	MAIS, MIN-1	
	11	+	MAIS, MIN-2	
M. fortuitum	16	+	MFO	
M. avium[a]	13	+	MAIS, MAV	
MAC	11	+	MAIS	
M. celatum[b]	9	+	MCE	
M. gordonae[c]	8	+	MGO	
M. simiae	8	+	MSI	
M. xenopi	6	+	MXE	
M. smegmatis	5	+	MFO, MSM	
M. bohemicum	4	+		
M. genavense	4	+	MGE	
M. lentiflavum	4	+		
M. terrae	4	+		
M. tuberculosis complex[d]	4	+	MTB	
M. chelonae complex	1	+	MCH-1	
	1	+	MCH-1, MCH-2	
	4	+	MCH-1, MCH-3	
M. interjectum[e]	3	+		
M. kansasii	1	+	MKA-1	
	2	+	MKA-2	
M. malmoense	3	+	MML, MAIS	
M. marinum	3	+	MMU	
M. thermoresistibile	3	+		MFO
M. agri	2	+		MFO
M. asiaticum	2	+		
M. mucogenicum	2	+		
M. szulgai	2	+		
M. alvei	1	+		MFO
M. gastri	1	+	MKA-3	
M. haemophilum	1	+	MHP, MAIS	
M. mageritense	1	+		MFO
"*M. paraffinicum*"	1	+		MAIS, MIN-2
M. scrofulaceum	1	+	MAIS, MSC	
M. senegalense	1	+		MFO
M. simiae-like	1	+		MAIS
M. ulcerans	1	+	MMU	
M. aikiense, *M. austroafricanum*, *M. aurum*, *M. botniense*, *M. branderi*, *M. brumae*, *M. chitae*, *M. chlorophenolicum*, *M. chubuense*, *M. confluentis*, *M. conspicuum*, *M. cookii*, *M. diernhoferi*, *M. doricum*, *M. duvalii*, *M. elephantis*, *M. fallax*, *M. farcinogenes*, *M. flavescens*, *M. gadium*, *M. gilvum*, *M. hassiacum*, *M. heckeshornense*, *M. hiberniae*, *M. holsaticum*, *M. intermedium*, *M. komossense*, *M. murale*, *M. neoaurum*, *M. nonchromogenicum*, *M. novocastrense*, *M. obuense*, *M. palustre*, *M. parafortuitum*, *M. peregrinum*, *M. phlei*, *M. porcinum*, *M. poriferae*, *M. pulveris*, *M. rhodesiae*, *M. senegalense*, *M. shimoidei*, *M. silvaticum*, *M. sphagni*, *M. tokaiense*, *M. triviale*, *M. tusciae*	1	+		
Nocardia sp.	7	−		
Corynebacterium sp., *Gordona* sp., *Rhodococcus equi*, *Tsukamurella* sp.	1	−		

[a] Including one strain of *M. avium* subsp. *paratuberculosis* and one strain of *M. avium* subsp. *silvaticum*.
[b] Including eight strains of *M. celatum* type 1 and one strain of *M. celatum* type 3.
[c] Including strains of three different sequevars (5), strains of two HPLC profiles (3), and one unpigmented strain.
[d] Including one strain of "*M. canettii*" and one of *M. bovis* subsp. *caprae*.
[e] Including both HPLC variants of *M. interjectum* (15).
[f] All strains are *Mycobacterium* spp. unless otherwise identified.

cluded all the available variants of this species in the present evaluation: at least one strain for each of the three described sequevars of 16S rDNA (4), representatives of both HPLC profiles characterizing *M. gordonae* (1), and a rare unpigmented isolate as well. All of them were identified correctly.

A large number of the strains that we tested belonged to the *Mycobacterium avium* complex (MAC). Within this group, all the strains reacted with the MAIS probe; in addition, *M. avium* (13 strains), including the subspecies *Mycobacterium avium* subsp. *paratuberculosis* and *Mycobacterium avium* subsp. *silvaticum*, reacted with the MAV line, and *Mycobacterium intracellulare* (five strains) reacted with MIN-1. The MAC strains not belonging to *M. avium* or *M. intracellulare* (11 strains) reacted with the genus-specific line and with the MAIS probe only. Eleven MAC strains belonging to the internal transcribed spacer sequevar MAC-A (3), whose taxonomic place is still

uncertain and which had been identified as *M. intracellulare* by AccuProbe and as MAC by the previous LiPA, were also positive with the new MIN-2 line.

One strain of the newly described species *Mycobacterium palustre*, which cross-reacts with the MAC-specific AccuProbe (13), did not produce such an anomalous result with LiPA.

Several cross-reactions were detected, however. The probe MFO, specific for the *Mycobacterium fortuitum* complex and *M. smegmatis*, also stimulated reactions from *Mycobacterium thermoresistibile* (three strains); *Mycobacterium agri* (two strains); and *Mycobacterium alvei*, *Mycobacterium mageritense*, and *Mycobacterium senegalense* (one strain each). One strain not belonging to any officially recognized species, related in terms of 16S rDNA to *M. simiae*, and unquestionably not belonging to the MAC hybridized with the MAIS line probe. One strain of "*Mycobacterium paraffinicum*" reacted with the MAIS probe and, although weakly, with MIN-2 but was, in contrast, negative with AccuProbe MAC.

In our assessment, the overall specificity and sensitivity of the new LiPA were both 100% for the genus-specific probe. For the other probes the total specificity was 92.2% (as, among 208 strains, 10 unexpected hybridizations were obtained, in adjunct to the expected 109) while the sensitivity was 100%. The specificity value was negatively affected by our decision to test further strains of the very rare species that we found cross-reacting; once two strains of *M. thermoresistibile* and one of *M. agri*, added a posteriori, were excluded from the evaluation, the specificity rose to 94.4%.

In conclusion, the substantial enlargement of the spectrum of species identifiable and the introduction of the MIN-2 line, which resolves the ambiguity of the strains identified as *M. intracellulare* by AccuProbe and as MAC by the previous LiPA, appear to be the major bonuses of the improved kit. Some caution is, however, warranted, because of the cross-reactions detected, in particular when a strain is attributed to the species *M. fortuitum* based only on the hybridization with the specific probe.

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