

## Commercial DNA Probes for Mycobacteria Incorrectly Identify a Number of Less Frequently Encountered Species<sup>∇†</sup>

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**Although commercially available DNA probes for identification of mycobacteria have been investigated with large numbers of strains, nothing is known about the ability of these probes to identify less frequently encountered species. We analyzed, with INNO LiPA MYCOBACTERIA (Innogenetics) and with GenoType Mycobacterium (Hein), 317 strains, belonging to 136 species, 61 of which had never been assayed before. INNO LiPA misidentified 20 taxa, the majority of which cross-reacted with the probes specific for *Mycobacterium fortuitum* and the *Mycobacterium avium*-*Mycobacterium intracellulare*-*Mycobacterium scrofulaceum* group. GenoType misidentified 28 taxa, most of which cross-reacted with *M. intracellulare* and *M. fortuitum* probes; furthermore, eight species were not recognized as members of the genus *Mycobacterium*. Among 54 strains investigated with AccuProbe (Gen-Probe), cross-reactions were detected for nine species, with the probes aiming at the *M. avium* complex being most involved in cross-reactions.**

In the last 20 years, commercially available DNA probes have increased the quality standard for identification of nontuberculous mycobacteria (NTM) in diagnostic laboratories worldwide.

DNA probes targeted at identification of mycobacteria were first developed by Gen-Probe (San Diego, CA) more than 20 years ago (4). The introduction of the solid-phase reverse hybridization technique led, several years later, to the development of the line probe assay. Innogenetics (Gent, Belgium) (29) offered the first suitable commercial kits for simultaneous identification of large clusters of mycobacterial species, followed, a few years later, by Hain (Nehren, Germany) (13, 29). Various studies have evaluated the sensitivity and specificity of these DNA probe assays with panels of mycobacterial species and have reported satisfactory results (1, 3, 4, 6–8, 10, 12–15, 17, 18, 22–27, 29, 32, 33). With few exceptions, however, the panels investigated included, almost solely, frequently isolated species. Furthermore, in the last few years, taxonomic studies have recognized and described many new mycobacterial species. The major aim of this research was to assess the specificity of the three commercially available DNA probe systems with regard to the mycobacterial groups that had never been evaluated before.

Three hundred seventeen strains, belonging to 136 taxa (species or complexes), 61 of which had not been tested before with the three DNA probe systems, were investigated. Both reference strains ( $n = 80$ ) and clinical isolates ( $n = 237$ ) were included in the study (the list of mycobacteria tested is provided in the supplemental material). The clinical isolates had been identified by sequencing of at least one genetic target

(16S rRNA gene, the spacer interposed between the 16S and 23S rRNA genes, *hsp65*, and *rpoB*), and only the ones presenting 100% identity with sequences of reference strains present in the GenBank database were included in the panel. Nine species not yet officially recognized, whose sequences are, however, present in GenBank, were also included in the study.

For the assessment of AccuProbe (Gen-Probe), which relies on oligonucleotide probes complementary to 16S rRNA, a restricted panel of mycobacteria was used. Supported by previous studies in which no cross-hybridization with unrelated species was demonstrated (1, 3, 4, 7, 8, 10, 12, 17, 18, 24, 32, 33), we limited the investigation to strains ( $n = 54$  [belonging to 28 taxa]) presenting, in the 16S rRNA, relatedness to one of the species targeted by various AccuProbe kits (see the supplemental material). The evaluation of INNO LiPA Mycobacteria (Innogenetics) (LiPA) and GenoType Mycobacterium (Hain) was extended to all 317 mycobacterial strains.

All the tests were carried out by strictly following the respective manufacturers' instructions. As a consequence, the GenoType CM (GT-CM) kit was tested with all the strains included in the study, while GenoType AS (GT-AS) was assayed only with the ones assigned by GT-CM to the genus *Mycobacterium* without differentiation at the species level.

In Tables 1, 2, and 3, the anomalous reactions that emerged with each of the three systems assessed here are compared with the specificities declared by the manufacturers. The highest number of incorrect outcomes involved the probes aiming at the *Mycobacterium avium* complex (MAC) and at the *Mycobacterium fortuitum* group.

The taxonomic status of MAC-related mycobacteria is still far from being clearly defined, and in the last few years, this complex, traditionally including only *M. avium* and *Mycobacterium intracellulare*, has been enriched by several new species (16, 30, 35). While the cross-hybridization of such novel species with some of the probes aiming at the MAC was not unexpected, the results for other species appeared less comprehensible. *Mycobacterium palustre* was assigned to the MAC by

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TABLE 1. Comparison of the AccuProbe specificities declared by the manufacturer and those observed in this study

| Probe                                    | Mycobacteria for which the probe was found to be specific   |   |
|--|---|---|
|  | Declared by the manufacturer  | Additionally observed in this study   |
| AccuProbe <i>M. avium</i>                | <i>M. avium</i>   |   |
| AccuProbe <i>M. intracellulare</i>       | <i>M. intracellulare</i>  | <i>M. arosiense</i> , <i>M. chimaera</i> , <i>M. nebraskense</i> , <i>M. saskatchewanense</i>   |
| AccuProbe <i>M. avium</i> complex        | <i>M. avium</i> , <i>M. intracellulare</i> , MAC spp. other than <i>M. avium</i> and <i>M. intracellulare</i> | <i>M. arosiense</i> , <i>M. chimaera</i> , <i>M. colombiense</i> , <i>M. nebraskense</i> , <i>M. palustre</i> , “ <i>M. paraffinicum</i> ,” <i>M. saskatchewanense</i> , <i>M. vulneris</i> |
| AccuProbe <i>M. tuberculosis</i> complex | <i>M. tuberculosis</i> complex  | <i>M. holsaticum</i>  |
| AccuProbe <i>M. kansasii</i>             | <i>M. kansasii</i>  |   |
| AccuProbe <i>M. gordonae</i>             | <i>M. gordonae</i>  |   |

AccuProbe (16, 28, 30, 35), *Mycobacterium saskatchewanense* was identified as *M. intracellulare* by AccuProbe and by GT-CM, and LiPA misidentified *Mycobacterium nebraskense* as *M. intracellulare* and *Mycobacterium heidelbergense* as a member of the MAC. Furthermore, GT-CM, which provides only two MAC-related probe patterns, one *M. avium* specific and one *M. intracellulare* specific, misidentified as *M. intracellulare* all the MAC members other than *M. avium*, including, in adjunct to the newly defined species, also a nonnegligible number of “orphan” strains whose taxonomic status is at present uncertain but which are clearly not *M. intracellulare* (5).

Other discrepant results concerned the probes targeting *M. fortuitum*. In fact, both LiPA and GT-CM probes cross-reacted with *Mycobacterium conceptionense*, *Mycobacterium senegalense*, *Mycobacterium wolinskyi*, and *Mycobacterium neworleansense*; other species were incorrectly identified by one of the two systems only: *Mycobacterium boenickei*, *Mycobacterium*

*houstonense*, *Mycobacterium setense*, *Mycobacterium porcinum*, and *Mycobacterium parafortuitum* by GT-CM and *Mycobacterium alvei*, *Mycobacterium goodii*, *Mycobacterium mageritense*, *Mycobacterium septicum*, and *Mycobacterium thermoresistibile* by LiPA.

The probes aiming at the *Mycobacterium chelonae*-*Mycobacterium abscessus* group hybridized also with *Mycobacterium bolletii* and *Mycobacterium massiliense*, both in LiPA and in GT-CM. The recent proposal of degrading the latter two species to the status of subspecies of *M. abscessus* (11) may well explain these results.

The probe specific for *Mycobacterium marinum* and *Mycobacterium ulcerans*, which is present both in LiPA and in GT-CM, reacted also with other mycobacterial species, isolated so far from fishes only: “*Mycobacterium seriolae*” cross-hybridized with both systems and *Mycobacterium shottsii* (20) and *Mycobacterium pseudoshottsii* (21) with LiPA only (19). Interest-

TABLE 2. Comparison of the LiPA specificities declared by the manufacturer and those observed in this study

| Probe | Mycobacteria for which the probe was found to be specific                                    |   |
|-------|--|---|
|       | Declared by the manufacturer   | Additionally observed in this study   |
| MTB   | <i>M. tuberculosis</i> complex   |   |
| MKA-1 | <i>M. kansasii</i> group i   |   |
| MKA-2 | <i>M. kansasii</i> group ii  |   |
| MKA-3 | <i>M. kansasii</i> groups iii, iv, v; <i>M. gastri</i>                                       |   |
| MXE   | <i>M. xenopi</i>   |   |
| MGO   | <i>M. gordonae</i> , ( <i>M. simiae</i> )  |   |
| MGV   | <i>M. genavense</i>  |   |
| MSI   | <i>M. simiae</i>   | “ <i>M. sherrisii</i> ”   |
| MMU   | <i>M. marinum</i> , <i>M. ulcerans</i>   | <i>M. pseudoshottsii</i> , <sup>a</sup> “ <i>M. seriolae</i> ,” <i>M. shottsii</i> <sup>a</sup>   |
| MCE   | <i>M. celatum</i>  |   |
| MAIS  | <i>M. avium</i> complex, <i>M. scrofulaceum</i> , <i>M. malmoense</i> , <i>M. hemophilum</i> | <i>M. arosiense</i> , <i>M. heidelbergense</i> , <i>M. mantenii</i> , <i>M. nebraskense</i> , “ <i>M. paraffinicum</i> ,” <i>M. parascrofulaceum</i>  |
| MAV   | <i>M. avium</i>  |   |
| MIN-1 | <i>M. intracellulare</i>   |   |
| MIN-2 | <i>M. chimaera</i> <sup>b</sup>  | <i>M. nebraskense</i> , “ <i>M. paraffinicum</i> ”  |
| MSC   | <i>M. scrofulaceum</i>   | <i>M. parascrofulaceum</i>  |
| MML   | <i>M. malmoense</i>  |   |
| MHP   | <i>M. hemophilum</i>   |   |
| MCH-1 | <i>M. chelonae</i> , <i>M. abscessus</i> , ( <i>M. xenopi</i> )                              | <i>M. massiliense</i>   |
| MCH-2 | <i>M. chelonae</i> , <i>M. abscessus</i>   | <i>M. massiliense</i>   |
| MCH-3 | <i>M. chelonae</i>   |   |
| MFO   | <i>M. fortuitum</i> , <i>M. peregrinum</i> , <i>M. smegmatis</i>                             | <i>M. alvei</i> , <i>M. conceptionense</i> , <i>M. goodii</i> , <i>M. mageritense</i> , <i>M. neworleansense</i> , <i>M. senegalense</i> , <i>M. septicum</i> , <i>M. thermoresistibile</i> , <i>M. wolinskyi</i> |
| MSM   | <i>M. smegmatis</i>  |   |

<sup>a</sup> *M. pseudoshottsii* and *M. shottsii* were not included in the test panel; the respective cross-reactions have been reported by others (19).

<sup>b</sup> In the package insert, *M. chimaera* is improperly named *M. intracellulare* MAC-A.

TABLE 3. Comparison of the GT-CM and GT-AS specificities declared by the manufacturer and those observed in this study<sup>a</sup>

| Probe             | Mycobacteria for which the probe was found to be specific                               |   |
|-------------------|---|---|
|                   | Declared by the manufacturer  | Additionally observed in this study   |
| CM 2-(10)         | Gram-positive bacillus, high G+C content  |   |
| CM 2-3-(10)       | <i>Mycobacterium</i> spp.   |   |
| CM 2-3-4          | <i>M. avium</i>   |   |
| CM 2-3-5-10       | <i>M. chelonae</i> , <i>M. immunogenum</i>  |   |
| CM 2-3-5-6-10     | <i>M. abscessus</i> , <i>M. immunogenum</i>   | <i>M. bolletii</i> , <i>M. massiliense</i>  |
| CM 2-3-7-14       | <i>M. fortuitum</i>   | <i>M. boenickei</i> , <i>M. farcinogenes</i> , <i>M. houstonense</i> , <i>M. neworleansense</i> ,<br><i>M. parafortuitum</i> , <i>M. porcinum</i> , <i>M. senegalense</i> , <i>M. setense</i><br><i>M. wolinskyi</i> , <i>M. conceptionense</i> |
| CM 2-3-7          | <i>M. fortuitum</i> , <i>M. mageritense</i>   |   |
| CM 2-3-8-10       | <i>M. gordonae</i>  |   |
| CM 2-3-9          | <i>M. intracellulare</i>  | <i>M. arosiense</i> , <i>M. chimaera</i> , <i>M. colombiense</i> , <i>M. mantenii</i> , <i>M. saskatchewanense</i> , MAC <sup>b</sup>   |
| CM 2-3-9-10       | <i>M. scrofulaceum</i> , <i>M. parascrofulaceum</i> ,<br>“ <i>M. paraffinicum</i> ”     | <i>M. alsinense</i>   |
| CM 2-3-9-10-11    | <i>M. interjectum</i>   |   |
| CM 2-3-(9)-10-12  | <i>M. kansasii</i>  | <i>M. gastri</i>  |
| CM 2-3-(9)-10-13  | <i>M. malmoense</i> , <i>M. hemophilum</i> , <i>M. palustre</i> , <i>M. nebraskense</i> |   |
| CM 2-3-10-15      | <i>M. marinum</i> , <i>M. ulcerans</i>  | “ <i>M. seriolae</i> ”  |
| CM 2-3-10-16      | <i>M. tuberculosis</i> complex  | <i>M. riyadhense</i> , “ <i>M. simulans</i> ”   |
| CM 2-3-14         | <i>M. peregrinum</i> , <i>M. alvei</i> , <i>M. septicum</i>                             |   |
| CM 2-3-17         | <i>M. xenopi</i>  |   |
| AS 2-3-12         | <i>Mycobacterium</i> spp.   |   |
| AS 2-3-4-6        | <i>M. simiae</i>  | “ <i>M. sherrisii</i> ”   |
| AS 2-3-5-12       | <i>M. mucogenicum</i>   | <i>M. aubagnense</i> , <i>M. llatzerense</i> , <i>M. phocaicum</i> , “ <i>M. ratisbonense</i> ”   |
| AS 2-3-5-6-14     | <i>M. goodii</i>  |   |
| AS 2-3-6-12-14    | <i>M. celatum</i> types ii, iii   |   |
| AS 2-3-6-14       | <i>M. smegmatis</i>   |   |
| AS 2-3-6-16-17    | <i>M. genavense</i> , <i>M. triplex</i>   |   |
| AS 2-3-6-17       | <i>M. lentiflavum</i>   |   |
| AS 2-3-7-9        | <i>M. heckeshornense</i>  |   |
| AS 2-3-8-12       | <i>M. szulgai</i> , <i>M. intermedium</i>   |   |
| AS 2-3-8-16       | <i>M. phlei</i>   |   |
| AS 2-3-9-12       | <i>M. hemophilum</i> , <i>M. nebraskense</i>  |   |
| AS 2-3-9-10-12    | <i>M. kansasii</i>  |   |
| AS 2-3-9-10-12-13 | <i>M. kansasii</i>  |   |
| AS 2-3-10-12      | <i>M. kansasii</i>  |   |
| AS 2-3-10-12-13   | <i>M. kansasii</i>  |   |
| AS 2-3-11-12      | <i>M. ulcerans</i>  | “ <i>M. seriolae</i> ”  |
| AS 2-3-12-13      | <i>M. gastri</i>  |   |
| AS 2-3-12-15      | <i>M. asiaticum</i>   |   |
| AS 2-3-12-16      | <i>M. shimoidei</i>   |   |

<sup>a</sup> *M. duvalii* did not react with any of the GT-CM probes; it therefore behaved as an organism not in the *Mycobacterium* or high-G+C-content Gram-positive-bacillus group.

<sup>b</sup> All the strains included in the MAC but not belonging to any of the species described so far within this complex were identified by GT-CM as *M. intracellulare*.

ingly, GT-AS, whose probes can differentiate *M. marinum* from *M. ulcerans*, misidentified “*M. seriolae*” as *M. ulcerans*.

“*Mycobacterium sherrisii*” cross-hybridized, in LiPA and in GT-AS, with the probes specific for *Mycobacterium simiae*, a species to which it is closely related.

Two recently described species, *Mycobacterium parascrofulaceum* and *Mycobacterium alsinense*, were misidentified as *Mycobacterium scrofulaceum*, the former by LiPA and the latter by GT-CM.

The probe pattern of GT-AS regarded by the manufacturer as specific for *Mycobacterium mucogenicum* also recognized *Mycobacterium aubagnense*, *Mycobacterium llatzerense*, *Mycobacterium phocaicum*, and “*Mycobacterium ratisbonense*.”

The identification errors which may have serious consequences for the patient are the ones involving species belonging to the *Mycobacterium tuberculosis* complex; fortunately, none of the systems investigated in this study

misidentified members of this complex as NTM. Three NTM species were, in contrast, incorrectly assigned to the *M. tuberculosis* complex: *Mycobacterium holsaticum* by Accu-Probe and *Mycobacterium riyadhense* (34) and “*Mycobacterium simulans*” (31) by GT-CM.

A number of mycobacterial species were surprisingly not recognized as members of the genus *Mycobacterium* by GT-CM. *Mycobacterium elephantis*, “*Mycobacterium engbaekii*,” *Mycobacterium frederiksbergense*, *Mycobacterium hassiacum*, *Mycobacterium hodleri*, *Mycobacterium pulveris*, *Mycobacterium moriokaense*, and *Mycobacterium sphagni* hybridized in fact with the probe deemed as specific for Gram-positive bacilli with high guanine-plus-cytosine content, while *Mycobacterium duvalii* was not even recognized by this probe.

Only a small number of NTM species isolated in clinical laboratories have medical relevance (9); nevertheless, correct identification is essential for epidemiological investigations

and, when an NTM plays a pathogenic role, for correct diagnosis and for treatment of the patient.

In the industrialized world, the highest level of mycobacteriological diagnostics is entrusted to reference centers (2), and the quality of the results issued by such laboratories should represent the state of the art. For what concerns the identification of NTM, the accuracy should go beyond the results obtained with commercially available DNA probes. We intend, with this report, to provide useful hints for selection of the cases in which in-depth investigation is recommended.

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**List of mycobacteria tested (and not tested) in the study**

| Mycobacteria tested with LiPA, GenoType and AccuProbe <sup>a</sup> |                                 |                                   |                                   |
|--|---------------------------------|-----------------------------------|-----------------------------------|
| <i>M. abscessus</i>  | <u><i>M. colombiense</i></u>    | <i>M. intermedium</i>             | <i>M. poriferae</i>               |
| <u><i>M. africanum</i></u>   | <i>M. conceptionense</i>        | <u><i>M. intracellulare</i></u>   | <i>M. pulveris</i>                |
| <i>M. agri</i>   | <i>M. confluentis</i>           | <u><i>M. kansasii</i></u>         | " <i>M. ratisbonense</i> "        |
| <i>M. aichiense</i>  | <i>M. conspicuum</i>            | <i>M. kubicae</i>                 | <i>M. rhodesiae</i>               |
| <i>M. alvei</i>  | <i>M. cookii</i>                | <i>M. kumamotoense</i>            | <i>M. riyadhense</i>              |
| " <i>M. alsienae</i> "   | <i>M. cosmeticum</i>            | <i>M. lacus</i>                   | <u><i>M. saskatchewanense</i></u> |
| <u><i>M. arosiense</i></u>   | <i>M. diernhoferi</i>           | <i>M. llatzerense</i>             | <i>M. scrofulaceum</i>            |
| <i>M. arupense</i>   | <i>M. doricum</i>               | <i>M. lentiflavum</i>             | <i>M. senegalense</i>             |
| <i>M. asiaticum</i>  | <i>M. duvalii</i>               | <i>M. mageritense</i>             | <i>M. senuense</i>                |
| <i>M. aubagnense</i>   | <i>M. elephantis</i>            | <i>M. malmoense</i>               | <i>M. septicum</i>                |
| <i>M. austroafricanum</i>  | " <i>M. engbaekii</i> "         | <u><i>M. mantenii</i></u>         | " <i>M. seriolaie</i> "           |
| <u><i>M. avium</i></u>   | <i>M. fallax</i>                | <i>M. marinum</i>                 | <i>M. setense</i>                 |
| <u>MAC<sup>b</sup></u>   | <i>M. farcinogenes</i>          | <i>M. massiliense</i>             | " <i>M. sherrisii</i> "           |
| " <i>M. barrassiae</i> "   | <i>M. flavescens</i>            | <u><i>M. microti</i></u>          | <i>M. shimoidae</i>               |
| <i>M. boenickei</i>  | <i>M. florentinum</i>           | <i>M. monacense</i>               | <i>M. simiae</i>                  |
| <i>M. bohemicum</i>  | <i>M. fortuitum</i>             | <i>M. montefiorensense</i>        | " <i>M. simulans</i> "            |
| <i>M. bolletii</i>   | <i>M. frederiksbergense</i>     | <i>M. moriokaense</i>             | <i>M. smegmatis</i>               |
| <i>M. botniense</i>  | <i>M. gadium</i>                | <i>M. mucogenicum</i>             | <i>M. szulgai</i>                 |
| <u><i>M. bovis</i></u>   | <u><i>M. gastris</i></u>        | <i>M. murale</i>                  | <i>M. terrae</i>                  |
| <u><i>M. bovis</i> BCG</u>   | <i>M. genavense</i>             | <u><i>M. nebraskense</i></u>      | <i>M. thermoresistibile</i>       |
| <i>M. branderi</i>   | <i>M. gilvum</i>                | <i>M. neoaurum</i>                | <i>M. tokaiense</i>               |
| <i>M. brisbanense</i>  | <i>M. goodii</i>                | <i>M. neworleansense</i>          | <i>M. triplex</i>                 |
| <i>M. brumae</i>   | <u><i>M. gordonae</i></u>       | <i>M. nonchromogenicum</i>        | <i>M. triviale</i>                |
| <i>M. canariasense</i>   | <i>M. haemophilum</i>           | <i>M. noviomagense</i>            | <u><i>M. tuberculosis</i></u>     |
| " <i>M. canettii</i> "   | <i>M. hassiacum</i>             | <i>M. novocastrense</i>           | <i>M. tusciae</i>                 |
| <u><i>M. caprae</i></u>  | <i>M. heckeshornense</i>        | <i>M. obuense</i>                 | <i>M. ulcerans</i>                |
| <u><i>M. celatum</i> 1</u>   | <u><i>M. heidelbergense</i></u> | <i>M. palustre</i>                | <i>M. vaccae</i>                  |
| <u><i>M. celatum</i> 2</u>   | <i>M. hiberniae</i>             | <i>M. parascrofulaceum</i>        | <i>M. vanbaalenii</i>             |
| <u><i>M. celatum</i> 3</u>   | <i>M. hodleri</i>               | " <u><i>M. paraffinicum</i></u> " | <u><i>M. vulneris</i></u>         |
| <i>M. chelonae</i>   | <u><i>M. holsaticum</i></u>     | <i>M. parmense</i>                | <i>M. wolinskyi</i>               |
| <i>M. chitae</i>   | <i>M. houstonense</i>           | <i>M. peregrinum</i>              | <i>M. xenopi</i>                  |
| <u><i>M. chimaera</i></u>  | <i>M. immunogenum</i>           | <i>M. phlei</i>                   |                                   |
| <i>M. chlorophenolicum</i>   | <i>M. insubricum</i>            | <i>M. phocaicum</i>               |                                   |
| <i>M. chubuense</i>  | <i>M. interjectum</i>           | <i>M. porcinum</i>                |                                   |
| Mycobacteria tested with LiPA only                                 |                                 |                                   |                                   |
| <i>M. komossense</i>   | <i>M. pseudoshottsii</i>        | <i>M. shottsii</i>                | <i>M. sphagni</i>                 |
| Mycobacteria not tested  |                                 |                                   |                                   |
| <i>M. aromaticivorans</i>  | <i>M. leprae</i>                | <i>M. pinnipedii</i>              | <i>M. salmoniphilum</i>           |
| <i>M. aurum</i>  | <i>M. lepraemurium</i>          | <i>M. psychrotolerans</i>         | <i>M. seoulense</i>               |
| <i>M. crocinum</i>   | <i>M. madagascariense</i>       | <i>M. pyrenivorans</i>            | <i>M. stomatepiae</i>             |
| <i>M. fluoranthenvivorans</i>                                      | <i>M. pallens</i>               | <i>M. rufum</i>                   |                                   |
| <i>M. kyorinense</i>   | <i>M. parafortuitum</i>         | <i>M. rutilum</i>                 |                                   |

<sup>a</sup> Only underlined taxa were tested with AccuProbe.

<sup>b</sup> Strains of MAC not belonging to any of the species officially recognized within this complex.