Comparative evaluation of PCR and commercial DNA probes for detection and identification to species level of Mycobacterium avium and Mycobacterium intracellulare.

A. Devallois, Mathieu Picardeau, K. S. Goh, C. Sola, V. Vincent, Nalin Rastogi

Comparative evaluation of PCR and commercial DNA probes for detection and identification to species level of Mycobacterium avium and Mycobacterium intracellulare.

A Devallois, M Picardeau, K S Goh, C Sola, V Vincent and N Rastogi

***J. Clin. Microbiol.* 1996, 34(11):2756.**

JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1996, p. 2756–2759 Vol. 34, No. 11
0095-1137/96/$04.00+0

Comparative Evaluation of PCR and Commercial DNA Probes for Detection and Identification to Species Level of *Mycobacterium avium* and *Mycobacterium intracellulare*

ANNE DEVALLOIS,[1] MATHIEU PICARDEAU,[2] KHYE SENG GOH,[1] CHRISTOPHE SOLA,[1] VÉRONIQUE VINCENT,[2] AND NALIN RASTOGI[1]*

Unité de la Tuberculose et des Mycobactéries, Institut Pasteur, 97165 Pointe à Pitre Cedex, Guadeloupe, French West Indies,[1] and Centre National de Référence des Mycobactéries, Institut Pasteur, 75724 Paris Cedex 15, France[2]

Received 12 March 1996/Returned for modification 13 May 1996/Accepted 19 August 1996

Selective amplification of a 187-bp fragment within the DT6 sequence using the AV6 and AV7 primers for *Mycobacterium avium* and of a 666-bp fragment within the DT1 sequence of *Mycobacterium intracellulare* using the IN38 and IN41 primers was performed for 69 clinical isolates identified as *M. avium* complex by conventional methods. The results were compared in parallel with results with commercial *M. avium* and *M. intracellulare* probes. A positive response to either of the two PCRs or *M. avium-M. intracellulare* AccuProbes constituted positive detection as *M. avium* complex; this cumulative detection limit was 94.2% for PCR, compared with 90% for AccuProbe. Concordance, on the other hand, was considered an identical species identification using either DT1 PCR and the *M. intracellulare* probe or DT6 PCR and the *M. avium* probe. In this investigation, 90% of isolates gave concordant results. We conclude that DT6 and DT1 PCRs are inexpensive and at least equally sensitive, in-house options to the AccuProbe system for species identification of *M. avium* and *M. intracellulare*.

Mycobacterium avium complex (MAC) is one of the major opportunistic pathogens affecting mortality, morbidity, and quality of life of patients infected with AIDS (20, 21). Slow growth and lengthy biochemical testing for identifying MAC clinical isolates pose all of the usual problems associated with laboratory diagnosis of tuberculosis. MAC comprises two genetically distinct but difficult to discriminate species: *M. avium*, which predominates (87 to 98% of isolates) in AIDS patients, and *Mycobacterium intracellulare*, which is more frequent among non-AIDS patients (8). Conventional cultural and biochemical tests give little information to separate these two closely related and nearly undistinguishable species in a clinical microbiology setting (13).

For the above reasons, development of rapid identification methods using molecular probes and/or nucleic acid amplification for MAC isolates would be particularly helpful in a clinical laboratory. Moreover, identifying clinical isolates as *M. avium* and *M. intracellulare* would have both clinical and epidemiological implications. DNA probes able to identify species within the members of MAC include both in-house methods (22, 25) and commercial tests (10, 31) (Gen-Probe Rapid Diagnostic System and AccuProbe Culture Identification Test from Gen-Probe Inc., San Diego, Calif.).

The only commercialized probe today is the AccuProbe system, which is based on detection of rRNA. Instead of the ^{125}I-labelled probes used by the earlier Gen-Probe system, AccuProbe uses a chemiluminescent, acridinium ester-labelled probe detected with a luminometer with a sensitivity of ≥95% (14, 17, 33, 34). However, this method remains expensive for routine analysis and can be performed only after subculturing bacilli from clinical specimens, which takes between 2 and 3 additional weeks. The development of simple, in-house nucleic acid amplification methodology permitting direct detection and species identification of MAC organisms in clinical specimens would save both precious time and money.

Molecular methods such as 16S rRNA sequencing have been particularly helpful to clarify the rather confusing taxonomy within the *M. avium-M. intracellulare* complex (9, 15, 24), but unfortunately, this methodology remains relatively cumbersome, with application limited to reference laboratories. Although amplification of conserved mycobacterial sequences followed by either hybridization with species-specific probes to variable regions within the amplified target (2, 12) or a restriction enzyme analysis (26) has been reported, only few targets specific for *M. avium* or for *M. intracellulare* have been described (4).

Thierry et al. (29) recently described DT6 and DT1 sequences which provide a completely independent identification system for the detection of *M. avium* and *M. intracellulare*. PCR based on the amplification of DT1 and DT6 sequences, reportedly absent from all other mycobacterial species studies (29), has already been used successfully for MAC reference strains and clinical isolates (27–29); however, PCR results were not compared with the full spectrum of commercially available probes. The aim of the present study was both to corroborate previously reported findings and to investigate simultaneous detection and species identification by DT1-DT6 amplification compared with those by the *M. avium* and *M. intracellulare* probes of the AccuProbe system, considered a current "gold standard" (14, 17, 33, 34).

MATERIALS AND METHODS

Origin and identification of isolates. All 69 strains (29 Caribbean and 40 European isolates) (Table 1) used in this study were grown as fresh Löwenstein-Jensen slants at 37°C. The Caribbean strains, from patients residing in Guadeloupe and Martinique, were isolated at the Institut Pasteur of Guadeloupe from clinical specimens, and all the assays (PCR and AccuProbe) were performed locally. The European isolates, addressed to the National Reference Center for Mycobacteria, Paris, France, were studied at the Institut Pasteur, Paris. The majority of strains were isolated from blood, except that strains Gpe 1 and 2 and Av 17 were isolated from sputum, Gpe 3 was isolated from pleural liquid, and

* Corresponding author. Mailing address: Unité de la Tuberculose et des Mycobactéries, Institut Pasteur, Morne Jolivière B.P. 484, 97165 Pointe à Pitre Cedex, Guadeloupe, French West Indies. Phone: (590) 893-881. Fax: (590) 893-880.

TABLE 1. Comparison of PCR and AccuProbe results[a] for 69 Caribbean (Gpe) and European (Av) isolates

Strain(s)	PCR result			AccuProbe result[b]		
	DT1	DT6	Species identification	*M. avium*	*M. intracellulare*	Species identification
Gpe 1–3, 6, 8–10	−	+	*M. avium*	+	−	*M. avium*
Gpe 12–14	−	+	*M. avium*	+	−	*M. avium*
Gpe 15	−	−	Untypeable	−	−	Untypeable
Gpe 16–18, 20	−	+	*M. avium*	+	−	*M. avium*
Gpe 21	−	−	Untypeable	−	−	Untypeable
Gpe 23	−	+	*M. avium*	+	−	*M. avium*
Gpe 24	+	−	*M. intracellulare*	−	+	*M. intracellulare*
Gpe 25–29	−	+	*M. avium*	+	−	*M. avium*
Av 1–14	−	+	*M. avium*	+	−	*M. avium*
Av 15[c]	+	−	*M. intracellulare*	−	+	*M. intracellulare*
Av 16–34	−	+	*M. avium*	+	−	*M. avium*
Av 36–39	−	+	*M. avium*	+	−	*M. avium*
Av 41	−	+	*M. avium*	+	−	*M. avium*
Gpe 4	−	+	*M. avium*	−	−	Untypeable
Gpe 5,[d] 11	−	−	Untypeable	−	+	*M. intracellulare*[e]
Gpe 7, 19	+	−	*M. intracellulare*[e]	−	−	Untypeable
Gpe 22[f]	+	+	*M. avium-M. intracellulare*	−	−	Untypeable
Av 40	−	+	*M. avium*	−	−	Untypeable

[a] The top part of the table shows results for 62 isolates which gave concordant results by the PCR method and the AccuProbe system, whereas the bottom part illustrates discrepant data for 7 isolates.

[b] The MAC probe was used only if both the *M. avium* and *M. intracellulare* probes gave negative results; isolates Gpe 4, 7, 19, 15, 21, and 22 were MAC probe positive, whereas isolates Gpe 5 and 11 and Av 40 were MAC probe negative.

[c] As no *M. intracellulare* strains were found among European isolates arbitrarily selected for this study, a previously confirmed *M. intracellulare* strain from our own culture collection (Av 15) served as an internal control.

[d] This was the only isolate with a false-negative DT1 PCR result, as Southern hybridization confirmed the presence of the DT1 fragment.

[e] Contrary to DT6-positive isolates, DNA from DT1-positive isolates did not hybridize with IS*1245*.

[f] This isolate showed a mixed serovar (1+20) and both DT1 and DT6 fragments as confirmed by Southern hybridization; it may represent a mixed culture.

strains Gpe 4, 11, 19, 23, and 24 and Av 6, 7, and 21 were isolated from gastric washings. Strain identification was performed on the basis of biochemical and cultural characteristics, including mycolic acid analysis (5). Serotyping was determined by thin-layer chromatography of peptidoglycolipids (32, 33).

Preparation of genomic DNA. For PCR tests, the bacterial DNA was prepared either by suspending the organisms in 100 μl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8) containing 1% (wt/vol) Triton and heating them at 100°C for 30 min or by using Chelex-100 (Bio-Rad, Richmond, Calif.) essentially as previously described (16). In the latter case, a loop of bacteria from Löwenstein-Jensen slants was scraped, suspended in 300 μl of a suspension of 10% (wt/wt) Chelex-100 (containing 0.1% [wt/vol] sodium dodecyl sulfate, 1% [vol/vol] Nonidet P-40, and 1% [vol/vol] Tween 20), incubated for 20 min at 95°C, and centrifuged (5 min, 15,000 × *g*), and the DNA from the supernatant was extracted with phenol-chloroform and precipitated with ethanol. The preparation of DNA by either of the two methods gave identical PCR results.

The AccuProbe system. The AccuProbe test is based on nucleic acid hybridization for the identification of *M. avium* or *M. intracellulare* from cultures and uses acridinium ester-labelled, single-stranded DNA probes that are complementary to the rRNA of target organisms (10). One loopful of bacteria from fresh Löwenstein-Jensen slants was lysed by sonication in a tube containing 100 μl each of lysis and hybridization reagents for 15 min; this was followed by incubation of 100 μl of lysate with lyophilized DNA probe at 60°C for 15 min. The contents were mixed well after the addition of 300 μl of selection reagent, incubated further at 60°C for 5 min, and kept at room temperature for at least 5 min, and the results were expressed as photometric light units with an AccuLDR photometer or relative light units with a Leader luminometer. A positive reaction was above the cutoff value of 900 photometric light units or 30,000 relative light units with a repeat range of 600 to 899 photometric light units or 20,000 to 29,999 relative light units. Parallel positive controls included *M. avium* ATCC 25291 or *M. intracellulare* ATCC 13950 in agreement with the probes tested. *Mycobacterium tuberculosis* ATCC 25177 tested negative with all the three probes used.

PCR assays. The PCR method used was essentially similar to that described by Thierry et al. (29); AV6 and AV7 primers (5′-ATGGCCGGGAGACGATCTA TGCCGGCGTAC-3′ and 5′-CGTTCGATCGCAGTTTGTGCAGCGCGTAC A-3′, respectively) directed the amplification of a 187-bp fragment within the DT6 sequence, whereas the IN38 and IN41 primers (5′-GAACGCCCGTTGG CTGGCCATTCACGAAGGAG-3′ and 5′-GCGCAACACGGTCGGACAGG CCTTCCTCGA-3′) directed the amplification of a 666-bp fragment within the DT1 sequence. Briefly, amplification reactions were performed in 50-μl mixtures containing 50 mM Tris-HCl (pH 8.5), 2 mM $MgCl_2$, 100 μg of bovine serum albumin per ml, 100 pmol of each primer, a 200 μM concentration of each of the four deoxyribonucleoside triphosphates (dATP, dGTP, dTTP, and dCTP), 2 ng of template DNA, and 2 U of *Thermus aquaticus* DNA polymerase (Gibco-BRL Life Technologies, Cergy-Pontoise, France). The amplification mixture was overlaid with 50 μl of mineral oil and was subjected to 30 cycles of amplification (Perkin-Elmer Corp., Norwalk, Conn.) as follows. Samples were incubated at 94°C for 1 min to denature the DNA, 60°C for 1 min to anneal the primers, and 72°C for 1 min to extend the annealed primers. Each amplification experiment included a negative control sample without DNA and a positive control sample with 2 ng of *M. avium* ATCC 25291 (serotype 2) for DT6 primers and 2 ng of *M. intracellulare* (serotype 23) for the DT1 primers, as the latter serotype is known to react uniquely with the DT1 probe (29). Fifty percent of the amplification reaction mixture was analyzed by electrophoresis on a 3% NuSieve–agarose gel (3:1; FMC BioProducts, Rockland, Maine) by using the 100-bp ladder (Pharmacia Biotech, Uppsala, Sweden) as a marker. Gels were stained with ethidium bromide and photographed on a UV transilluminator.

A perfect agreement between PCR results using DT1- and DT6-derived primers with the results obtained by Southern hybridization analysis with entire DT1 and DT6 probes has been shown previously (29). We, however, reconfirmed the lack of false-negative PCR tests for isolates that were *M. avium* and/or *M. intracellulare* AccuProbe positive, by applying previously described Southern hybridization methodology using DT1 and DT6 probes or IS*1245* (11, 29).

RESULTS AND DISCUSSION

Selective amplification of an expected 187-bp fragment was observed with DT6 primers, whereas the DT1 primers selectively amplified a 666-bp fragment. The DT1 and DT6 amplification for all the isolates was compared with the AccuProbe data in parallel, and the results obtained are summarized in Table 1.

A positive response to either of the two PCRs or *M. avium-M. intracellulare* probes constituted positive detection as MAC, whereas concordance was considered an identical species identification by either DT1 PCR and the *M. intracellulare* probe or DT6 PCR and the *M. avium* probe. The cumulative detection limit was 94.2% for PCR, compared with 90% for AccuProbe, while 90% of isolates gave concordant results with both methods. When the ability of a single test to detect either

M. avium or *M. intracellulare* was assessed, DT6 PCR alone was able to detect 61 of 69 isolates, compared with 58 of 69 isolates with the *M. avium* AccuProbe.

Direct comparison of our PCR results with the MAC AccuProbe was not performed because of the reported inability of this probe to detect all MAC isolates, including some *M. avium* and/or *M. intracellulare* isolates (10), and also because of marked genetic heterogeneity of *M. avium-M. intracellulare* AccuProbe-negative, MAC AccuProbe-positive isolates (24). The test was, however, performed on *M. avium-M. intracellulare* AccuProbe-negative samples in this investigation, and these results are indicated in footnote *b* of Table 1.

A total of 7 of 69 isolates gave discrepant results upon initial analysis of data. DT1-DT6 PCR was able to detect all the five AccuProbe-untypeable isolates; however, two isolates typed as *M. intracellulare* by AccuProbe remained untypeable by PCR (Table 1); the reconfirmation by Southern hybridization showed that one isolate was truly DT1-DT6-negative whereas one (isolate Gpe 5) effectively contained the DT1 fragment, giving a false-negative DT1 PCR result in the present study. Isolate Gpe 22 gave positive results for both DT1 and DT6 PCRs; it presented a mixed serotype, 1+20, harboring both the DT1 and DT6 fragments upon Southern hybridization, and could represent a mixed infection, which may be responsible for a false-negative AccuProbe test, as mentioned by the manufacturer (10). Both isolate Gpe 4 (MAC AccuProbe positive; *M. avium* and *M. intracellulare* probe negative) and isolate Av 40 (MAC AccuProbe negative; *M. avium* and *M. intracellulare* probe negative) were typed as *M. avium* by DT6 PCR (Table 1). Further identification of the isolates giving discrepant results was performed using the recently described IS*1245* probe, which is highly discriminatory for *M. avium* (11). The results confirmed that both Gpe 4 and Av 40 contained IS*1245* and were indeed *M. avium*. Of four isolates that were IS*1245* negative and consequently were not *M. avium* (Gpe 5, 7, 11, and 19), at least three (Gpe 5, 7, and 19) did contain DT1 fragments, as revealed upon Southern hybridization (Table 1), and may represent *M. intracellulare*. These resolved results showed that Gpe 11 was the only isolate among 69 strains studied which was AccuProbe positive but lacked the corresponding DT fragment.

It is noteworthy that 2 of the 62 concordant isolates (Gpe 15 and 21) were untypeable by both DT1 and DT6 PCRs as well as *M. avium* and *M. intracellulare* AccuProbes (although they were MAC AccuProbe positive) and did not probe positive with either IS*1245* or DT1-DT6 upon Southern hybridization, corroborating previous observations (11, 29).

Scant biochemical differences between *M. avium* and *M. intracellulare* partially lie in the activities of catalase (18) and arylsulfatase (7, 30) and their ability to grow in the presence of sodium nitrite (23). High-performance liquid chromatography has been useful in the identification of *M. avium* or *M. intracellulare* (3); however, it is time-consuming and not readily available to most clinical laboratories. Serotyping based on the detection of glycopeptidolipid antigens (6, 32, 33, 35) is often used in reference laboratories as an important tool for epidemiologic investigations, but it is not generally available for routine clinical microbiology laboratories and is of limited use in patient management (9).

Considering the reported heterogeneity of MAC organisms, which may include taxonomically ill-defined species other than *M. avium* and *M. intracellulare* (1, 8, 19, 20, 24), we intentionally chose to concentrate on relatively more homogeneous *M. avium* and *M. intracellulare* typing methods. The present investigation was aimed at rapid identification to the species level of *M. avium* and *M. intracellulare*. We chose the PCR methodology based on DT1 and DT6 primers (27, 29), as they were reported to amplify species-specific sequences and may be applicable in the future in a clinical setting for direct detection and species identification of *M. avium* and *M. intracellulare* after validation on clinical specimens.

Of 69 isolates, however, 2 remained untypeable by both PCR and *M. avium-M. intracellulare* AccuProbe tests (Gpe 15 and 21; both MAC AccuProbe positive), which corroborated recent findings about the genetic variability of MAC organisms which also contain species other than *M. avium* and *M. intracellulare* (9, 24). In conclusion, our observations further extend the results of previous studies on DT1-DT6 PCR and/or AccuProbe tests (9, 24, 27, 29, 31, 33) on Caribbean and European clinical isolates. This study shows that compared with the *M. avium* AccuProbe system, DT6 PCR is an at least equally sensitive first-line test for *M. avium* detection in a European and/or Caribbean setting. On the other hand, because of the extremely low prevalence of *M. intracellulare* in these areas (four isolates in 3 years in the French Carribean isolates and none among the randomly chosen European isolates), the taxonomic status of DT1-containing clinical isolates will be further clarified by 16S rRNA sequencing (15), once a sufficient number of such isolates is collected.

ACKNOWLEDGMENTS

The Mycobacteria and TB Project at Guadeloupe received financial support through Project CORDET, Ministry of Overseas Departments and Territories, Paris, France, and Pharmacia S.A., St. Quentin-Yvelines, France. N.R. is grateful to the Fogarty International Center, National Institutes of Health, Bethesda, Md., for an AIDS-FIRCA award (TWOO533, awarded jointly with W. W. Barrow, Southern Research Institute, Birmingham, Ala.). A.D. was awarded a Ph.D. fellowship from the Ministry of Education and Research, Paris, France, and M.P. received a research fellowship from the Agence Nationale de Recherches sur le SIDA, Paris, France.

N.R. is grateful to Gen-Probe-France (Chatillon, France) for lending a Leader-50 luminometer and to J. L. Guesdon (Institut Pasteur, Paris, France) for helpful discussions.

REFERENCES

1. **Baess, I.** 1983. Deoxyribonucleic acid relationships between different serovars of *Mycobacterium avium*, *M. intracellulare*, and *M. scrofulaceum*. Acta Pathol. Microbiol. Scand. **91:**201–203.
2. **Böddinghaus, B., T. Rogall, T. Flohr, H. Blöcker, and E. C. Böttger.** 1990. Detection and identification of mycobacteria by amplification of rRNA. J. Clin. Microbiol. **28:**1751–1759.
3. **Butler, W. R., and J. O. Kilburn.** 1988. Identification of major slowly pathogenic mycobacteria and *Mycobacterium gordonae* by high-performance liquid chromatography of their mycolic acids. J. Clin. Microbiol. **26:**50–53.
4. **Crawford, J. T.** 1994. Development of rapid techniques for identification of *M. avium* infections. 11th Forum in Microbiology. Res. Microbiol. **145:**177–181.
5. **David, H., V. Levy-Frébault, and M.-F. Thorel.** 1989. Méthodes de laboratoire pour mycobactériologie clinique. Institut Pasteur, Paris.
6. **Denner, J. C., A. Y. Tsang, D. Chatterjee, and P. Brennan.** 1992. Comprehensive approach to identification of serovars of *Mycobacterium avium* complex. J. Clin. Microbiol. **30:**473–478.
7. **Falkinham, J. O., III.** 1990. Arylsulfatase activity of *Mycobacterium avium*, *M. intracellulare*, and *M. scrofulaceum*. Int. J. Syst. Bacteriol. **40:**66–70.
8. **Falkinham, J. O., III.** 1994. Epidemiology of *Mycobacterium avium* infections in the pre- and post-HIV era. Res. Microbiol. **145:**169–172.
9. **Frothingham, R., and K. H. Wilson.** 1994. Molecular phylogeny of the *Mycobacterium avium* complex demonstrates clinically meaningful divisions. J. Infect. Dis. **169:**305–312.
10. **Gen-Probe Inc.** 1992. AccuProbe, *Mycobacterium avium*, *Mycobacterium intracellulare* culture identification test. Manual for in vitro diagnostic use. Gen-Probe Inc., San Diego, Calif.
11. **Guerrero, C., C. Bernasconi, D. Burki, T. Bodmer, and A. Telenti.** 1995. A novel insertion sequence element from *Mycobacterium avium* IS*1245* is a specific target for analysis of strain relatedness. J. Clin. Microbiol. **33:**304–307.
12. **Hance, A. J., B. Grandchamp, V. Levy-Frébault, D. Lecossier, J. Rauzier, D. Bocart, and B. Gicquel.** 1989. Detection and identification of mycobacteria

by amplification of mycobacterial DNA. Mol. Microbiol. **3:**843–849.
13. **Inderlied, C. B., C. A. Kemper, and L. E. M. Bermudez.** 1993. The *Mycobacterium avium* complex. Clin. Microbiol. Rev. **6:**266–310.
14. **Jonas, V., C. Knott, D. P. Henderson, S. J. Snead, and N. G. Warren.** 1992. Detection of *Mycobacterium avium* complex strains using acridinium ester-labeled probes, U-7, p. 166. *In* Abstracts of the 92nd General Meeting of the American Society for Microbiology 1992. American Society for Microbiology, Washington, D.C.
15. **Kirschner, P., A. Meier, and E. C. Böttger.** 1993. Genotypic identification and detection of mycobacteria, p. 173–190. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology. American Society for Microbiology, Washington, D.C.
16. **Lamballerie, X. D., C. Zandotti, C. Vignoli, C. Bollet, and P. de Micco.** 1992. A one-step microbial DNA extraction method using "Chelex 100" suitable for gene amplification. Res. Microbiol. **143:**785–790.
17. **Lebrun, L., F. Espinasse, J. Poveda, and V. Vincent-Levy-Frebault.** 1992. Evaluation of nonradioactive DNA probes for identification of mycobacteria. J. Clin. Microbiol. **30:**2476–2478.
18. **Mayer, B. K., and J. O. Falkinham III.** 1986. Catalase activity and heat inactivation for differentiation of *M. avium*, *M. intracellulare*, and *M. scrofulaceum*. Int. J. Syst. Bacteriol. **36:**207–212.
19. **McFadden, J. J., Z. M. Kunze, F. Portaels, V. Labrousse, and N. Rastogi.** 1992. Epidemiological and genetic markers, virulence factors and intracellular growth of *Mycobacterium avium* in AIDS. Res. Microbiol. **143:**423–430.
20. **Rastogi, N., W. W. Barrow, J. O. Falkinham III, C. O. Thoen, J. T. Crawford, B. T. Mangura, L. B. Reichman, L. B. Heifets, B. Dautzenberg, L. S. Young, L. E. M. Bermudez, C. D. Inderlied, A. E. Suzuki, J. M. Inamine, P. R. J. Gangadharam, M. V. Reddy, M. Denis, H. Shiratsuchi, J. L. Johnson, J. J. Ellner, J. T. Belisle, and P. J. Brennan.** 1994. 11th Forum in Microbiology, "Laboratory and clinical aspects of the *Mycobacterium avium* epidemic: contributing factors associated with variability of drug susceptibility and immune responsiveness, and the multifaceted nature of pathogenicity." Res. Microbiol. **145:**167–261.
21. **Rastogi, N., J. J. McFadden, M. L. Gougeon, L. Montagnier, F. M. Collins, C. R. Horsburgh, R. J. Coker, T. J. Hellyer, I. N. Brown, J. N. Weber, I. M. Orme, D. Chatterjee, J. D. A. Van Embden, D. Van Soolingen, P. M. Small, P. W. M. Hermans, S. E. Hoffner, G. Källenius, S. B. Svenson, R. S. Wallis, J. J. Ellner, H. Shiratsuchi, G. A. W. Rook, A. Vyakarnam, D. M. Yajko, L. S. Young, L. E. M. Bermudez, C. B. Inderlied, Z. M. Kunze, F. Portaels, and V. Labrousse.** 1992. 8th Forum in Microbiology, "Mycobacteria and AIDS: epidemiological and genetic markers, virulence factors and interactions with the immune system." Res. Microbiol. **143:**357–440.
22. **Saito, H., H. Tomioka, K. Sato, H. Tasaka, and D. J. Dawson.** 1990. Identification of various serovar strains of *Mycobacterium avium* complex by using DNA probes specific for *Mycobacterium avium* and *Mycobacterium intracellulare*. J. Clin. Microbiol. **28:**1694–1697.
23. **Sato, K., H. Tomioka, and H. Saito.** 1992. Differential susceptibilities of *Mycobacterium avium* and *Mycobacterium intracellulare* to sodium nitrite. J. Clin. Microbiol. **30:**2994–2995.
24. **Soini, H., E. Eerola, and M. K. Viljanen.** 1996. Genetic diversity among *Mycobacterium avium* complex AccuProbe-positive isolates. J. Clin. Microbiol. **34:**55–57.
25. **Sritharan, V., J. V. Iralu, and R. H. Barker, Jr.** 1995. Specificity of diagnostic PCR amplification for *M. avium* using the probe pMAV22. Mol. Cell. Probes **9:**71–74.
26. **Telenti, A., F. Marchesi, M. Balz, F. Bally, E. C. Böttger, and T. Bodmer.** 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J. Clin. Microbiol. **31:**175–178.
27. **Thierry, D., S. Baugé, J.-D. Poveda, V. Vincent, and J.-L. Guesdon.** 1993. Rapid identification of *Mycobacterium avium-intracellulare* complex strains: clinical practice evaluation of DT6 and DT1 probes. J. Infect. Dis. **168:**1337–1338.
28. **Thierry, D., P. Matsiota-Bernard, C. Nauciel, and J.-L. Guesdon.** 1994. Comparison of polymerase chain reaction and non-radioactive hybridization techniques for the identification of *Mycobacterium avium* strains. Mol. Cell. Probes **8:**469–471.
29. **Thierry, D., V. Vincent, F. Clément, and J.-L. Guesdon.** 1993. Isolation of specific DNA fragments of *Mycobacterium avium* and their possible use in diagnosis. J. Clin. Microbiol. **31:**1048–1054.
30. **Tomioka, H., H. Saito, K. Sato, and D. J. Dawson.** 1990. Arylsulfatase activity for differentiating *Mycobacterium avium* and *Mycobacterium intracellulare*. J. Clin. Microbiol. **28:**2104–2106.
31. **Tomioka, H., H. Saito, K. Sato, H. Tasaka, and D. J. Dawson.** 1993. Identification of *Mycobacterium avium* complex strains belonging to serovars 21-28 by three commercial DNA probe tests. Tubercle Lung Dis. **74:**91–95.
32. **Tsang, A. Y., I. Drupa, M. Goldberg, J. K. McClatchy, and P. J. Brennan.** 1983. Use of serology and thin-layer chromatography for the assembly of an authenticated collection of serovars within the *Mycobacterium avium-Mycobacterium intracellulare-Mycobacterium scrofulaceum* complex. Int. J. Syst. Bacteriol. **33:**285–292.
33. **Varnerot, A., F. Clément, and V. Vincent Lévy-Frébault.** 1992. Evaluation of nonradioactive DNA probes for the identification of *Mycobacterium avium* complex. Methods Mol. Cell. Biol. **3:**198–203.
34. **Viljanen, M. K., L. Olkkonen, and M.-L. Katila.** 1993. Conventional identification characteristics, mycolate and fatty acid composition, and clinical significance of MAIX AccuProbe-positive isolates of *Mycobacterium avium* complex. J. Clin. Microbiol. **31:**1376–1378.
35. **Wayne, L. G., R. C. Good, A. Tsang, R. Butler, D. Dawson, D. Groothuis, W. Gross, J. Hawkins, J. Kilburn, M. Kubin, K. H. Schröder, V. A. Silcox, C. Smith, M.-F. Thorel, C. Woodley, and M. A. Yakrus.** 1993. Serovar determination and molecular taxonomic correlation in *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Mycobacterium scrofulaceum*: a cooperative study of the International Working Group on Mycobacterial Taxonomy. Int. J. Syst. Bacteriol. **43:**482–489.