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# Detection and Identification of Mycobacteria by DNA Amplification and Oligonucleotide-Specific Capture Plate Hybridization

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**We have developed an easy and rapid detection and identification system for the diagnosis of mycobacterial diseases. The system is based on selective amplification by PCR of mycobacteria with primers based on the genes coding for 16S rRNA. During PCR, a label (digoxigenin-11-dUTP) is incorporated in the amplicon. After amplification, the amplicon is hybridized in streptavidin-coated microtiter plates prepared with biotinylated species-specific oligonucleotides (oligonucleotide-specific capture plate hybridization [OSCPH]). One oligonucleotide specific for the genus *Mycobacterium* and seven species-specific (*Mycobacterium tuberculosis*, *M. avium*, *M. intracellulare*, *M. scrofulaceum*, *M. xenopi*, *M. genavense*, and *M. chelonae*) oligonucleotides were designed as capturing probes. After specific hybridization, an enzyme immunoassay reveals the specifically bound complexes and thus permits identification of the mycobacterium. A total of 70 mycobacterial strains were tested. For 69 strains, results concordant with conventional identification were obtained. One *M. chelonae* strain was negative with the *M. chelonae* probe and was later reidentified as *M. fortuitum*. Moreover, for 15 clinical samples suspected of harboring nontuberculous mycobacteria, OSCPH was able to confirm all culture results and could identify one *M. genavense* infection for which standard culture results were negative. PCR-OSCPH is easily applicable and much faster than culture. It could become a valuable alternative approach for the diagnosis of mycobacterial infections.**

Mycobacteria are important pathogens involved in different diseases in humans. Most important is *Mycobacterium tuberculosis*, with which an estimated 8 million people worldwide are newly infected each year, resulting in approximately 3 million deaths annually (32). In part because of the pandemic of human immunodeficiency virus, the decline of the incidence of *M. tuberculosis* infections has slowed down and, in fact, in many parts of the world their incidence as well as multidrug resistance is rising again. Nontuberculous mycobacteria (NTM) are also increasing in importance (12, 14, 23). In a recent report on human immunodeficiency virus-negative patients from Switzerland, from 1983 to 1988, the incidence of tuberculosis declined from 16.2 to 13.2 per 100,000 inhabitants while the incidence of mycobacteriosis increased from 0.4 to 0.9 per 100,000 inhabitants (9). In England, a shift occurred between 1970, when 1 NTM was isolated for every 60 *M. tuberculosis* strains, and 1992, when this ratio rose to 1 NTM for every 6 *M. tuberculosis* isolates (33). This has made a rapid identification of mycobacteria extremely important. Classical methods for identification are based on morphological, biochemical, and growth properties (15). More recently, hybridization techniques for identifying cultures of some clinically important NTM have become commercially available (20, 24). Most of these methods are lengthy, labor-intensive, or expensive and often possible in only a few reference centers. The advent of the PCR has been a breakthrough in the diagnosis of mycobacterial infections, but methods are complicated, limited to one mycobacterial species, and restricted to research laboratories. A direct isothermic amplification technique is now commercially available but only for the detection of *M. tuberculosis* (16).

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We report here an assay system for the identification of mycobacterial species with a simple sample preparation technique, DNA amplification by PCR, and identification to the species level in a microtiter plate enzyme-linked immunoassay. The simplicity of this system makes it applicable in the clinical laboratory.

## MATERIALS AND METHODS

**Principle of OSCPH.** In oligonucleotide-specific capture plate hybridization (OSCPH), the target for amplification is the 16S rRNA gene (16S rDNA). By the use of genus-specific primers, a broad range of mycobacteria can be detected (1a, 6). During PCR, the amplicon is labelled by incorporation of digoxigenin-11-dUTP (DIG-11-dUTP). Streptavidin-coated microtiter plates are prepared by preincubation with biotinylated species-specific oligonucleotides. After cycling, the amplified DIG-11-dUTP product is captured specifically by the species-specific probes. Detection of this complex is done by classical enzyme-linked immunoassay (Fig. 1).

**Strains and clinical specimens.** Mycobacterial reference strains ($n$ = 12), strains belonging to the different serovars (1 to 20) of *M. avium-M. intracellulare* ($n$ = 20) (19), and 38 well-characterized strains isolated in our institute were used for testing (Table 1). In addition, 15 clinical specimens (3 sputum, 8 biopsy, and 2 pus samples, 1 blood sample, and 1 peritoneal effusion sample) from patients with clinically suspected infection by NTM (AIDS patients and patients with previous NTM isolation) were investigated in parallel by PCR-OSCPH and culture. Identification was done by conventional methods (15).

**PCR.** For PCR, a colony of each strain was dissolved in 0.5 ml of phosphate-buffered saline–1% Tween 20 (PBST) and boiled for 10 min. After a short spin down, 2 to 5 μl of the supernatant was used in the PCR. Preparation of samples as well as primers based on 16S rDNA and PCR conditions were essentially as described previously (1a, 6–8). In short, after a simple preparation protocol (8), PCR (35 cycles) was done with *Mycobacterium* genus-specific primers (g2R plus rM582R) (1a, 6). During PCR, a label was incorporated in the amplified product by replacing the concentration of the deoxynucleoside triphosphates (dNTPs) in the PCR buffer by 25 μM (each) dATP, dCTP, and dGTP; 22 μM dTTP; and 2.5 μM DIG-11-dUTP (Boehringer GmbH, Mannheim, Germany).

**Specific probes.** Several probes were designed on the basis of sequence information for 16S rDNAs of more than 40 different mycobacteria (1a, 21, 25, 26) and sequences obtained in our laboratory. A *Mycobacterium* genus-specific probe was constructed to check for the presence of mycobacteria. All species-specific probes were designed (software, OLIGO and OLIGOSCAN) in such a way that they had at least two base pair differences with other species and that selective hybridization was possible for all probes at the same temperature (Table 2).

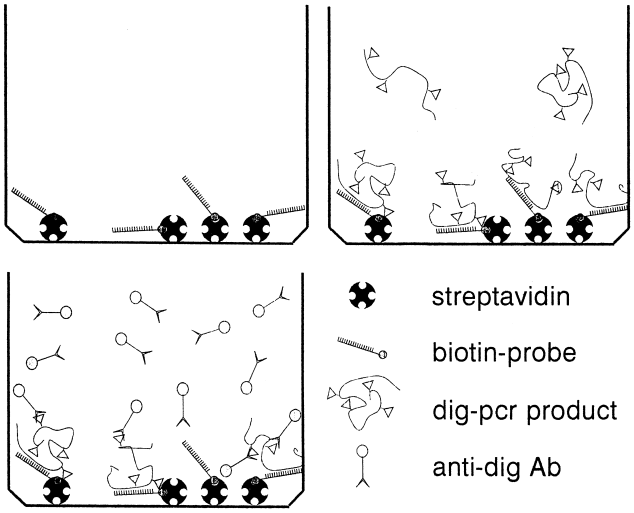


FIG. 1. Principle of the OSCPH assay. Ab, antibody.

Probes for *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. scrofulaceum*, *M. chelonae*, *M. genavense*, and *M. xenopi* were designed. The primers and probes used are presented in Table 3. In order to minimize steric hindrance, the 5′-biotin-end-labelling of the oligonucleotides was performed with a 16-atom mixed-polarity spacer arm (EUROGENTECH, Seraing, Belgium).

**OSCPH.** Streptavidin-coated microtiter plates were kindly provided by Boehringer GmbH. The plates were prepared with the capturing probes by incubation with 50 μl of the different biotinylated probes, diluted to 20 pmol/ml in PBST, for 1 h at 37°C. Each probe was placed in a different horizontal row. After a washing with PBST, the plates were dried at 37°C for 1 h and stored at 4°C (prepared plates were stable at 4°C for at least 2 months).

The amplified products were heated at 94°C for 2 min, and 8 μl was immediately transferred to 480 μl of ice-cold hybridization buffer (3× SSC [20× SSC is 3 M NaCl and 0.3 M sodium citrate, pH 7.0], 0.05% sodium dodecyl sulfate [SDS], and 2% polyethylene glycol 6000). Of this mix, 60 μl was loaded in each well of a vertical lane (lanes 1 to 12) and incubated at 50°C (water bath) for 120 min. The plates were then washed five times with 0.5× SSC–0.05% SDS. The detection method and reagents were essentially as described by the manufacturer (DIG luminescent detection kit; Boehringer Mannheim Biochemica GmbH, Mannheim, Germany). The wells were rinsed once with washing buffer (0.1 M maleic acid, 0.15 M NaCl [pH 7.5], 0.3% Tween 20) and incubated with 100 μl of anti-DIG Fab conjugated with alkaline phosphatase diluted 1/2,500 in buffer 2 (0.1 M maleic acid, 0.15 M NaCl [pH 7.5], 1.0% blocking reagent). The plates were kept at 37°C for 45 min. After four washings with washing buffer and one with NE buffer (2% *N*-ethylaminoethanol, pH 10.2), 100 μl of substrate (2 mg of *para*-nitro-phenol-phosphate per ml of NE buffer) was added and color was allowed to develop for 90 min (or overnight if necessary). The optical densities (ODs) at 405 nm were read in an automatic reader.

## RESULTS

**Sensitivity.** To determine the sensitivity of the combined PCR-OSCPH assay, a dilution series ($10^{-1}$ to $10^{-8}$ of an *M. avium* culture containing $5.2 \times 10^9$ bacteria per ml) was made in PBST. After 35 cycles, visual inspection of an ethidium bromide-stained gel revealed a positive signal up to the $10^{-5}$ dilution (faint band), but the $10^{-6}$ dilution was negative (Fig. 2). In the OSCPH assay, however, the $10^{-5}$ dilution still gave maximal ODs, and a positive signal (OD, 0.800; overnight color development) was obtained even with the $10^{-7}$ dilution, which theoretically contained 2.6 bacteria (Fig. 2). When amplicons of culture suspensions were tested, the OD for positive samples normally varied between 1.5 and the maximum value (overnight color development). Negative samples had ODs below 0.2. As a cutoff, an OD of 0.3 was taken arbitrarily.

**Specificity.** No amplification was obtained with several different nonmycobacterial strains (1a, 6). When strains of 20 different mycobacterial species were tested (Table 1), the results with the *Mycobacterium* genus-specific probe were always positive.

The species-specific probes were positive exclusively with the correct species. All strains belonging to the *M. tuberculosis* complex (*M. tuberculosis* [$n = 8$], *M. africanum* [$n = 2$], *M. bovis* [$n = 1$], and *M. bovis* BCG [$n = 1$]) reacted strongly with probe Tb3.

Serovars 1 to 20 of *M. avium-M. intracellulare* (19) were

TABLE 1. Strains used in this study

| Species | Strain(s)[a] |
|---|---|
| *M. avium* | ATCC 25291, CIPT 140310005, ITG 1983, ITG 4178, ITG 4179, ITG 5887 (sv1), ITG 5872 (sv2), ITG 5983 (sv3), ITG 5874 (sv4), ITG 5903 (sv5), ITG 5904 (sv6), ITG 5882 (sv8), ITG 5927 (sv9), ITG 5984 (sv10), ITG 5897 (sv11) |
| *M. bovis*, *M. bovis* BCG | ITG 8424, ITG 8434 |
| *M. chelonae* | ITG 7794, ITG 7971 |
| *M. abscessus* | ATCC 19977, ITG 7701, ITG 4994 |
| *M. fortuitum* | ITG 4306 |
| *M. genavense* | ITG 8777, ITG 92.202, ITG 8859, ITG 92.742 |
| *M. gordonae* | ATCC 14470 |
| *M. haemophilum* | ITG 776 |
| *M. intracellulare* | ITG 5908 (sv7), ITG 5915 (sv12), ITG 5913 (sv13), ITG 5917 (sv14), ITG 5929 (sv15), ITG 5880 (sv16), ITG 5921 (sv17), ITG 5920 (sv18), ITG 5922 (sv19), ITG 5924 (sv20) |
| *M. kansasii* | ATCC 12478, ITG 5432 |
| *M. marinum* | ATCC 927, ITG 7732 |
| *M. malmoense* | ITG 4842 |
| *M. paratuberculosis* | ATCC 19698, ITG 2887 |
| *M. scrofulaceum* | ATCC 19981, CIPT 140220031, ITG 4988, ITG 8792, ITG 8794 |
| *M. simiae* | ITG 4484, ITG 4485 |
| *M. smegmatis* | ATCC 607 |
| *M. szulgai* | CIPT 140240003 |
| *M. terrae* | CIPT 140320001 |
| *M. tuberculosis*, *M. africanum* | H37Ra, ITG 4928, ITG 6732, ITG 6733, ITG 6734, ITG 6735, ITG 6828, ITG 6862, ITG 6720, ITG 8644 |
| *M. ulcerans* | ITG 1837 |
| *M. xenopi* | ITG 4986, ITG 9728, ITG 9730 |

[a] ATCC, American Type Culture Collection, Rockville, Md.; CIPT, Collection Institut Pasteur, Paris, France; ITG, Institute of Tropical Medicine, Antwerp, Belgium; sv, serovar (according to reference 19; strains kindly provided by A. H. Kolk).

TABLE 2. Alignment of the 16S rDNA nucleotide sequences of different mycobacterial species and comparison with the probe for *M. avium*[a]

| Probe or species | Nucleotide sequence[b] | Position[c] |
|---|---|---|
| Av probe | CCTCAAGACGCATGTCTTCT | |
| *M. asiaticum* | ..... ..... ..... | 0 |
| *M. aurum* | ..... ..... ..... | 0 |
| *M. avium* | TAGGA ---------- GGTGG | 177 |
| *M. bovis* | TAGGA --A-GG--T---------G- GGTGG | 180 |
| *M. chitae* | ..... ..... ..... | 0 |
| *M. cookii* | ..... ..... ..... | 0 |
| *M. fallax* | ..... ..... ..... | 0 |
| *M. flavescens* | ..... ..... ..... | 0 |
| *M. fortuitum* | ..... ..... ..... | 0 |
| *M. gadium* | ..... ..... ..... | 0 |
| *M. gastri* | ..... ..... ..... | 0 |
| *M. genavense* | ..... ..... ..... | 0 |
| *M. gordonae* | ..... ..... ..... | 0 |
| *M. haemophilum* | ..... ..... ..... | 0 |
| *M. intermedium* | ..... ..... ..... | 0 |
| *M. intracellulare* | | |
| 1 | TAGGA ---TT--G----------TA GGTGG | 178 |
| 2 | TAGGA ---TT----------T- GGTGG | 178 |
| *M. kansasii* | ..... ..... ..... | 0 |
| *M. leprae* | TAGGA -T-----G----------G- GGTGG | 192 |
| *M. malmoense* | ..... ..... ..... | 0 |
| *M. marinum* | ..... ..... ..... | 0 |
| *M. nonchromogenicum* | ..... ..... ..... | 0 |
| *M. paratuberculosis* | TAGGA ---------- GGTGG | 177 |
| *M. scrofulaceum* | ..... ..... ..... | 0 |
| *M. senegalense* | ..... ..... ..... | 0 |
| *M. simiae* | ..... ..... ..... | 0 |
| *M. smegmatis* | ..... ..... ..... | 0 |
| *M. szulgai* | ..... ..... ..... | 0 |
| *M. terrae* | ..... ..... ..... | 0 |
| *M. triviale* | ..... ..... ..... | 0 |
| *M. tuberculosis* | TAGGA --A-GG--T---------G- GGTGG | 141 |
| *M. vaccae* | ..... ..... ..... | 0 |
| *M. xenopi* | ..... ..... ..... | 0 |

[a] A consensus table of matches was made (using software OLIGOSCAN) with a minimum match length of 7 bp as an analysis parameter.
[b] Dashes indicate homologous sequence; dots indicate no homology.
[c] Position on the 16S rDNA.

tested. Strains belonging to serovars 1 to 6 and 8 to 11 were identified as *M. avium* (positive signal only with probe Av), and strains belonging to serovars 7 and 12 to 20 were identified as *M. intracellulare* (Intra probe positive). *M. paratuberculosis* was also positive with probe Av because on the 16S rDNA level the two species have identical sequences, and thus no differentiation between these species is possible (2).

The five *M. scrofulaceum* strains tested were all positive with the Scrof probe. Recently, some taxonomical changes have been introduced for the species *M. chelonae*. The former *M. chelonae* subsp. *chelonae* and *M. chelonae* subsp. *abscessus* have been transferred to *M. chelonae* and *M. abscessus*, respectively. Probe Chel was constructed to identify both species. Four of the five *M. chelonae-M. abscessus* strains were positive with the Chel probe. The fifth strain was positive with the genus-specific probe but negative with all species-specific probes, including the Chel probe. The three strains of *M. xenopi* tested were positive with the Xen probe. Four strains identified as *M. genavense* (4, 13) were all positive with the Gena probe.

All *M. fortuitum*, *M. gordonae*, *M. kansasii*, *M. marinum*, *M. malmoense*, *M. simiae*, *M. smegmatis*, *M. szulgai*, *M. terrae*, and *M. ulcerans* strains were negative with all species-specific probes.

**Clinical specimens.** Fifteen samples were selected because of a clinical suspicion of NTM infection. These specimens were tested in parallel by conventional culture and identification and by PCR-OSCPH. Results are summarized in Table 4. The

TABLE 3. Oligonucleotides (primers and probes) used in PCR and OSCPH

| Primer or probe[a] | Position[b] | Sequence[c] | $T_m$ (°C)[d] |
|---|---|---|---|
| Primers | | | |
| g2R | 50 | GAGAATTCGTGCTTAACACATGCAAGTCG | |
| rM582R | 582[e] | ATGGATCCGTGAGATTTCACGAACAACGC | |
| Probes | | | |
| MycG | 295[e] | GGCCGGACACCCTCTC | 50.0 |
| Av | 177 | CCTCAAGACGCATGTCTTCT | 46.7 |
| Chel | 172 | GGACCACACACTTCATGG | 45.2 |
| Gena | 170 | CACGGAACGCATGTTTTG | 43.9 |
| Intra | 167 | ACCGGATAGGACCTTTAGG | 46.0 |
| Scrof | 447 | GGCTCACTTTGTGGGTTG | 45.2 |
| Tb3 | 196 | TTGTGGTGGAAAGCGCTTTA | 44.6 |
| Xen | 182 | CATTCTGCGCATGTGGG | 44.3 |

[a] MycG, general probe for mycobacteria; the other probes are specific for the following species: Av, *M. avium*; Chel, *M. chelonae-M. abscessus*; Gena, *M. genavense*; Intra, *M. intracellulare*; Scrof, *M. scrofulaceum*; Tb3, *M. tuberculosis*; and Xen, *M. xenopi.*
[b] Position of 5′ nucleotide in *M. bovis* (28).
[c] Cloning sites are underlined.
[d] $T_m$, melting temperature (calculated with software OLIGO 3) in 50 mM $Na^+$.
[e] Reverse oligonucleotide.

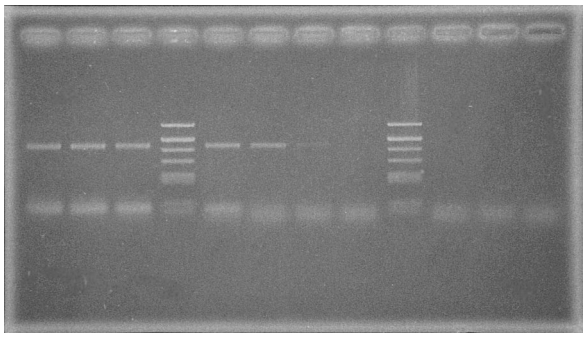

FIG. 2. PCR with a dilution series of an *M. avium* culture. The contents of the lanes and the corresponding OD readings in the OSCPH assay (given in parentheses) are as follows: lane 1, undiluted sample of $2.6 \times 10^7$ bacteria (2.689); lane 2, $10^{-1}$ dilution (2.792); lane 3, $10^{-2}$ dilution (2.532); lane 4, molecular weight marker; lane 5, $10^{-3}$ dilution (2.056); lane 6, $10^{-4}$ dilution (1.982); lane 7, $10^{-5}$ dilution (1.853); lane 8, $10^{-6}$ dilution (1.253); lane 9, molecular weight marker; lane 10, $10^{-7}$ dilution (0.805); lane 11, $10^{-8}$ dilution (0.114); lane 12, negative control (0.096). Visual inspection of the gel reveals positivity up to the $10^{-5}$ dilution (faint band).

organisms in all samples were correctly identified by the PCR-OSCPH method, both those in specimens which were microscopically strongly positive and those in paucibacillary specimens. Moreover, in one sample that did not produce an in vitro culture, *M. genavense* was identified. Subsequent isolation in nude mice confirmed the presence of *M. genavense* in the specimen.

## DISCUSSION

The rise in incidence of mycobacterial infections caused by NTM has made rapid identification more and more important for diagnosis and treatment. With traditional methods, 4 to 10 weeks (or more) usually elapse before results are available, and thus an easy and reliable assay is urgently needed. DNA amplification with genus-specific primers, based on 16S rDNA (1a, 5, 6, 25), spacer regions (11), or well-conserved genes (22, 29), is able to detect a broad range of mycobacteria. Within these amplified fragments, variable regions can often be recognized, offering the possibility of species determination. Identification to the species level has been performed by the use of dot blotting (10), restriction enzyme analysis (29, 30), or sequencing (1a, 18, 25). These methods, however, are complicated and sometimes difficult to interpret and often require preculturing of the mycobacteria. A combination of direct screening with PCR and detection by a reverse system of oligonucleotide hybridization offers the opportunity to identify the mycobacterium in the clinical sample within a very short time and without sophisticated technology. In OSCPH, multiple biotinylated sequence-specific oligonucleotides are coupled to a streptavidin-coated microtiter plate and act as capture probes. When the hybridization conditions are optimized, the amplified segment of the 16S rDNA, internally labelled with DIG-11-dUTP, is captured in a very specific way by the species-specific probe only. Incompletely matched amplicons (in this assay, the difference for species recognition was often limited to only two base pair mismatches) do not bind strongly and were removed by a washing. The bound complex is revealed by the use of anti-DIG Fab fragment, and a simple automated colorimetric readout provides the species determination.

Test conditions were altered repeatedly before the final protocol was established. For the streptavidin-coated plates, several commercially available brands, as well as homemade plates constructed according to different coating protocols, were compared. Large differences in reactivity (data not shown) were recorded, and finally Boehringer plates were chosen for the best sensitivity and specificity. The design of the different probes was facilitated by using available software (OLIGO and OLIGOSCAN). Special attention was paid to the facts that at least two base pair differences had to be present (preferentially in the middle of the probe) and that calculated dissociation temperatures were limited within 3°C. Different incubation protocols were tried as well as different incubation temperatures, resulting in a final protocol using very simple nonradioactive, nontoxic (no formamide), and readily available reagents. The price of DIG-11-dUTP is fairly high but could be considerably reduced by decreasing the concentration of the dNTPs. In this assay, about 1/10 of the usual concentration of dNTPs is used (25 μM), and DIG-11-dUTP is balanced with dTTP in a 1/10 ratio, without a loss in sensitivity (data not shown). Color development can be speeded up by raising this ratio, but at a higher cost. The performance and speed of this testing procedure could probably be increased considerably by using a chemiluminescence detection method.

All strains belonging to the *M. tuberculosis* complex were correctly identified by the Tb3 probe. However, because 16S rDNA is perfectly conserved, no differentiation between members of the complex can be made. For strains belonging to *M. avium-M. intracellulare*, the determination made by OSCPH correlated with what had been previously described (2, 19, 27). All *M. scrofulaceum* strains tested were correctly identified. The *M. genavense* strains, identified by sequencing (4), were all recognized easily. The use of the Chel probe initially gave problems when *M. chelonae-M. abscessus* strains were tested. Four strains reacted as expected with the Chel probe; however, the fifth strain was positive with the MycG probe but negative with the Chel probe. This strain (*M. abscessus* ITG 4994) was retested for biochemical and morphological characteristics and was reidentified as *M. fortuitum*, which is closely related to *M. chelonae* (17). No differentiation between *M. chelonae* and *M. abscessus* is possible.

Screening clinical specimens for the presence of *Mycobacterium* spp. by using the 16S rDNA as a target (6, 18) is feasible.

TABLE 4. Results for clinical samples tested in parallel by culture and PCR-OSCPH

| Origin | Ziehl result[a] | Identification by culture | OSCPH-positive probe |
|---|---|---|---|
| Sputum | 1+ | *M. avium* | Av |
| | 1+ | *M. avium* | Av |
| | 2+ | *M. tuberculosis* | Tb3 |
| Biopsy | | | |
| Lymph node | 3AFB/100f | *M. avium* | Av |
| Lymph node | 1+ | *M. intracellulare* | Intra |
| Lymph node | 1+ | *M. scrofulaceum* | Scrof |
| Lymph node | 1+ | Negative | Gena |
| Lymph node | 5AFB/100f | *M. chelonae* | Chel |
| Lymph node | Negative | *M. tuberculosis* | Tb3 |
| Lymph node | 5AFB/100f | *M. scrofulaceum* | Scrof |
| Liver | 2+ | *M. avium* | Av |
| Pus | 1+ | *M. avium* | Av |
| | 1+ | *M. chelonae* | Chel |
| Blood | 1+ | *M. avium* | Av |
| Peritoneal fluid | 2+ | *M. chelonae* | Chel |

[a] Clinical specimens were examined by microscopy after Ziehl-Neelsen staining, and the bacillary index was scored according to the scale of the American Thoracic Society (31).

Preliminary data for a small panel of clinical samples (Table 4) proved that PCR-OSCPH allowed direct and correct identification of the species (even in paucibacillary specimens) without the need for preculturing. Larger studies are needed to confirm these initial results.

The NTM most frequently identified in our laboratory belong to six different species (*M. avium*, *M. intracellulare*, *M. chelonae*, *M. xenopi*, *M. kansasii*, and *M. gordonae*) and represent 90% of the isolates received yearly (1). With one microtiter plate, 12 or 8 samples can be screened for 8 or 12 different mycobacterial species, respectively. In general, this is more than enough to cover most of the mycobacterial species isolated from clinical specimens. It is also possible to design specific probes within the 16S rDNA for almost all mycobacterial species (26). The panel screened can also be adapted to the prevalence of species encountered in a geographical area. For instance, in regions where *M. malmoense* is frequently isolated, a probe for this species can be introduced into the panel. Another possibility is to use several panels depending on the origin of the sample. In this way, sputum samples could be screened with a panel of probes different from that used for skin biopsies, in which, for example, probes for *M. leprae*, *M. marinum*, *M. ulcerans*, and *M. haemophilum* could be incorporated. If after intensive screening the assay produces a positive signal with only the *Mycobacterium* genus-specific probe, sequencing of the 16S rDNA can help in identifying the organism (3, 18).

In conclusion, we developed a method for easy and rapid identification of most of the clinically important mycobacteria. This system uses simple, currently available methodology and can be adapted to regional settings. Larger studies are in progress to validate the system in clinical laboratories.

## ACKNOWLEDGMENTS

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