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Evaluation of Nonradioactive DNA Probes for Identification of Mycobacteria

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Commercial chemiluminescent DNA probes (Accuprobe; Gen-Probe, San Diego, Calif.) for the identification of *Mycobacterium tuberculosis* (MTB) complex, *M. avium* complex (MAC), *M. gordonae*, and *M. kansasii* were evaluated with 134 clinical isolates. These included 36 MTB complex, 40 MAC, 27 *M. gordonae*, 9 *M. kansasii*, and 22 *Mycobacterium* spp. The specificity was 100% for the four probes. The sensitivity was 100% for the MTB complex and *M. gordonae* probes and 95.2% for the MAC probe. Five of the nine *M. kansasii* isolates tested were not detected with the probe.

Identification of mycobacteria by conventional biochemical tests is time-consuming and laborious. The radiometric BACTEC TB system (Becton Dickinson, Grenoble, France) can rapidly differentiate members of the *Mycobacterium tuberculosis* (MTB) complex from other mycobacteria by testing the inhibition of growth in the presence of *p*-nitro-α-acetylamino-β-hydroxypropiophenone (12). More recently, ^{125}I-labeled DNA probes for the MTB complex, *M. avium*, and *M. intracellulare* (Gen-Probe, San Diego, Calif.) have been developed (3, 4, 9, 10, 14). However, both methods require radioisotope facilities, resulting in the limitation of their practical use in many clinical laboratories. Recently, acridinium ester-labeled DNA probes (Accuprobe; Gen-Probe) have been made available as an alternative to DNA probes labeled with ^{125}I (5). In solution, hybridization occurs first and is followed by the addition of a selection reagent which hydrolyzes the acridinium ester on the free probe (1). The purpose of the study described here was to assess the sensitivity and specificity of these commercially available chemiluminescent DNA probes by comparison with conventional methods and to evaluate their applicability for routine use in the clinical laboratory.

One hundred thirty-four mycobacterial isolates were tested from July 1991 through December 1991 (Table 1). These included 36 MTB complex, 40 *M. avium* complex (MAC), 9 *M. kansasii*, 27 *M. gordonae*, and 22 *M.* spp. All strains were recovered from clinical specimens. Duplicate strains from the same patient were eliminated. All isolates were identified by using conventional methods which included acid-fast Ziehl-Neelsen staining, growth rate studies, and pigment production; biochemical testing included arylsulfatase, semiquantitative catalase, Tween 80 and urea hydrolysis, nitrate reduction, and niacin testing. Further analysis of strains was performed by determination of mycolic acid patterns (2) and serotype by thin-layer chromatography (2).

Accuprobe culture identification tests for MTB complex, MAC, *M. gordonae*, and *M. kansasii* were performed as described by the manufacturer. The assay was performed on growth of various ages, but not more than 1 month old, from Lowenstein-Jensen medium. Briefly, one loopful of 1 μl of bacteria was sonicated in tubes containing glass beads and lysing reagents in a sonication bath (Branson 1200) for 15 min. It was subsequently heated at 95°C for 10 min. Then, 100 μl of the lysates was incubated for 15 min at 60°C in a water bath with the lyophilized DNA probe. Next, 300 μl of a selection reagent was added. The tube was mixed well, further incubated at 60°C for 5 min, and kept at room temperature for 5 min. The assay results were read on a luminometer (Leader 50; Gen-Probe). Results were expressed as relative light units (RLU). Samples producing signals greater than or equal to 30,000 RLU were considered positive. The whole procedure can be completed within 1 h.

Among the 134 strains included in the study (Table 1), 90 were first biochemically identified before Accuprobe testing, whereas the 44 remaining strains were submitted to hybridization at once and then were identified biochemically.

Within the first set of already identified strains, 23 and 57 isolates were studied with the four probes or with their homologous probes, respectively. In addition, 10 *M. terrae* strains were tested with the Accuprobe MTB complex only. The RLU for positive results were between 150,000 and 700,000. The RLU for negative results were between 1,500 and 5,000 except for those for the *M. kansasii* probe (range, 3,000 to 7,000 RLU). The specificity was 100% for the four probes tested because no false-positive reactions were detected. All isolates of the MTB complex and *M. gordonae* were correctly hybridized. Of the 34 isolates biochemically identified as members of MAC, one strain was not recognized (17,000 RLU). Determination of mycolic acids and serotype confirmed that this strain was *M. avium* serotype 4. Furthermore, this strain was identified as MAC complex with another available DNA probe (Syngene, San Diego, Calif.). After repeat testing it was hybridized (400,000 RLU) with the Accuprobe MAC. The failure of the first test could be explained by an insufficient density of the test suspension. One strain identified as *M. simiae* was positive with the MAC probe. Determination of mycolic acids enabled us to reveal a mixed culture of *M. simiae* and MAC. Mixed mycobacterial infections involving these two species have previously been reported in patients with AIDS and are difficult to diagnose by conventional methods (7, 13).

Discrepant reactions have recently been noted with the MTB complex probe and *M. terrae* isolates (8). Therefore, in addition to the 3 *M. terrae* strains studied with the four

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TABLE 1. Mycobacterial isolates included in the study

Organism	No. of strains with positive test/total no. of strains tested[a]					
	Previously identified mycobacteria tested with probes specific for:				Probing as preliminary identification[b]	Total of all strains tested
	MTB complex	MAC	*M. kansasii*	*M. gordonae*		
MTB complex	15/15[c]	0/5[d]	0/5[d]	0/5[d]	21/21[e]	36/36
MAC	0/2	33/34[f]	0/2	0/2	5/6[g]	38/40
M. kansasii	0/2	0/2	3/7	0/2	1/2	4/9
M. gordonae	0/2	0/2	0/2	12/12	15/15	27/27
M. xenopi	0/3	0/3	0/3	0/3		0/3
M. terrae	0/13	0/3	0/3	0/3		0/13
M. gastri	0/2	0/2	0/2	0/2		0/2
M. aurum	0/1	0/1	0/1	0/1		0/1
M. chelonei	0/1	0/1	0/1	0/1		0/1
M. fortuitum	0/1	0/1	0/1	0/1		0/1
M. simiae	0/1	1/1[h]	0/1	0/1		0/1

[a] Numbers indicate numbers of strains producing positive hybridization tests/total number of strains tested. Of the 134 strains tested, 90 were previously identified and 44 were hybridized to make a preliminary identification.
[b] These strains were first identified with Accuprobe and then confirmed by conventional methods.
[c] *M. tuberculosis*, $n = 6$; *M. africanum*, $n = 4$; *M. bovis*, $n = 2$; *M. bovis* BCG, $n = 3$.
[d] *M. tuberculosis*, $n = 2$; *M. africanum*, $n = 1$; *M. bovis*, $n = 1$; *M. bovis* BCG, $n = 1$.
[e] *M. tuberculosis*, $n = 20$; *M. bovis*, $n = 1$.
[f] One strain identified as *M. avium* serotype 4 hybridized positively only on second testing.
[g] The nonhybridizing strain was further identified as *M. intracellulare* serotype 7.
[h] Another strain turned out to be a mixed culture involving *M. simiae* and a MAC strain.

probes, we tested 10 other *M. terrae* strains with the MTB complex probe. No false-positive reactions were noted.

Of the seven isolates of *M. kansasii*, four were negative with Accuprobe. After repeat testing with the probe, none of these isolates could be hybridized. Photochromogenicity and nitrate-reducing capacity confirmed the identifications of these isolates.

In view of these encouraging results, we decided to first identify 44 isolates with Accuprobe (Table 1) and then to confirm the identifications by conventional methods. The choice of the probe used was based on clinical features, morphological aspects of the colony, pigmentation, and growth rate. This enabled us to correctly identify 42 strains. One strain of *M. kansasii* and one strain of MAC were not identified. This latter strain had a mycolic acid profile typical of MAC and was further identified as *M. intracellulare* serotype 7 and hybridized with the Syngene MAC probe. Among the 21 strains found to belong to the MTB complex with Accuprobe, one strain was confirmed as *M. bovis* by conventional methods. Therefore, attention must be given to dysgonic colonies for which complementary tests are needed, as noted by Heifets (6), in order to properly identify *M. bovis* strains.

The advantages of the technique described here are the simplicity and rapidity with which the identification of mycobacteria can be achieved. The specificity was 100% for the four probes tested. The sensitivities of the Accuprobe MTB complex and the Accuprobe *M. gordonae* were 100%. The sensitivity of the Accuprobe MAC was 95.2%. Other investigators have also described similar isolates of MAC that were negative with ^{125}I-labeled DNA probes (Gen-Probe) (8–11) or Accuprobe (5) but positive with the Syngene probe (8). Another limit of the probe technology is the identification of mixed cultures. Special care should be taken in the detection of organisms in such mixed cultures, especially in patients with AIDS.

No evaluation with the *M. kansasii* probe has been published. Of the nine *M. kansasii* strains tested in our study, five were not hybridized. So, these preliminary results show that the Accuprobe is not sensitive enough for routine use for identification of *M. kansasii*.

In conclusion, the nonradioactive nature and the extended shelf-life of the chemiluminescent probes described here offer the potential for widespread application in any clinical laboratory for the identification of MTB complex, MAC, and *M. gordonae* strains isolated from culture.

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