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Strains of *Mycobacterium terrae* Complex Which React with DNA Probes for *M. tuberculosis* Complex

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Following a recent report that two isolates of *Mycobacterium terrae* complex had given positive reactions with *M. tuberculosis* complex DNA probes, a joint study was undertaken to determine the extent of these findings in the clinical culture collection holdings of two state health laboratories. A total of 117 *M. terrae* complex strains (identified by standard biochemical methods) were subjected to *M. tuberculosis* complex probe testing with the two then-available kits (from Syngene, Inc., and Gen-Probe, Inc.). In addition to the two original isolates first reported, two further *M. terrae* complex isolates were found to react with the *M. tuberculosis* complex probes. Two modifications of the Accuprobe (Gen-Probe, Inc.) test method were evaluated. Extension of the selection time to 8 min was the most convenient modification and rendered the *M. terrae* complex isolates negative when tested with the Accuprobe *M. tuberculosis* complex probe. However, the effects of increased selection time on the overall sensitivity of the *M. tuberculosis* complex probe require further study.

In 1991, Lim et al., from the Microbial Diseases Laboratory, California Department of Health Services, reported that two strains of mycobacteria other than *Mycobacterium tuberculosis* were SNAP (Syngene, Inc., San Diego, Calif.) *M. tuberculosis* complex probe positive but Gen-Probe (Gen-Probe, Inc., San Diego, Calif.) *M. tuberculosis* complex probe negative (3). Subsequently, multiple single-colony pickings of each strain were sent to the Division of Consolidated Laboratory Services, Commonwealth of Virginia, to be tested with the nonisotopic Accuprobe (Gen-Probe, Inc.) *M. tuberculosis* complex probe. All pickings of both strains were Accuprobe *M. tuberculosis* complex probe positive. By biochemical testing, the first strain had been identified as *M. terrae* complex and the second had been identified as *M. avium* complex. However, by using high-performance liquid chromatography (4), the Mycobacteriology Laboratory at the Centers for Disease Control identified both strains as *M. terrae* complex. A joint study was undertaken by the California and Virginia laboratories to attempt to determine the extent of the problem of *M. terrae* complex reaction with *M. tuberculosis* complex probes. One *M. terrae* strain (ATCC 25267) and 114 strains from patients (Virginia, $n = 81$; California, $n = 33$) were included in the study. All had been identified by standard biochemical testing procedures (2) as *M. terrae* complex. One of the strains had been previously identified to the species level within the complex as *M. nonchromogenicum* (4). All strains were subcultured onto Lowenstein-Jensen Slants (Hardy Media, Santa Maria, Calif.; PML Microbiologicals, Tualatin, Oreg.; BBL Microbiology Systems, Cockeysville, Md.) and incubated at 30°C for approximately 3 weeks.

Each strain was tested by both the Accuprobe and SNAP *M. tuberculosis* complex probes in accordance with the manufacturers' directions. The SNAP probe kit utilizes a solid matrix (nylon membrane) for hybridization; the Accuprobe uses a liquid. The hybridization temperature for the SNAP procedure is 49°C (47 to 50°C); that for the Accuprobe is 60 ± 1°C. In the SNAP test, the hybridized probe is detected by alkaline phosphatase enzyme activity. Development of a blue-to-purple color indicates a positive test result. The Accuprobe system uses a chemiluminescent label, and the hybridized probe is measured by a luminometer. A positive test is ≥30,000 relative light units (RLU) when read on a Leader I luminometer.

The original two probe-positive strains and any strains found to be *M. tuberculosis* complex probe positive by both SNAP and Accuprobe in the joint study were each sequentially single colony picked five times from isolated colonies on Middlebrook 7H10 agar (prepared in house from Difco products). Each colony was first lightly straight line streaked on a portion of a Lowenstein-Jensen slant. The needle was then used to streak a 7H10 plate for isolation. The straight-line inoculation of the Lowenstein-Jensen slant was cross-streaked for growth with a sterile loop. The media were incubated for growth at 25°C between pickings. The fifth picking from each positive strain was again tested with both SNAP and Accuprobe *M. tuberculosis* complex probes and fully identified by standard biochemical tests (2).

To challenge the strength of the hybridization, the fifth sequential single-colony picks of the *M. terrae* complex strains found to be probe positive were tested by varying the temperature of hybridization. The hybridization temperatures used for SNAP tests were 49 (standard), 54, 57, and 59°C. The hybridization temperatures used for the Accuprobe were 60 (standard), 65, 70, 73, and 80°C.

A second modification of the Accuprobe *M. tuberculosis* complex testing procedure was also tried on each probe-positive strain. While maintaining the recommended hybridization temperature, incubation with the selection reagent was increased from the standard 5 min to 8 min. The 8-min selection incubation is normally used with the *M. kansasii* probe.

Of the 114 *M. terrae* complex patient-derived strains tested, only one additional strain reacted with both the SNAP and Accuprobe *M. tuberculosis* complex probes, for a total of 3 positive strains from 116 patients. A fourth strain reacted with the Accuprobe only. The hybridization reaction

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with this strain was strong on the original culture (120,037 RLU) and the subculture (454,683 RLU). There was no evidence of a mixed culture. The identification was confirmed by the Centers for Disease Control. *M. terrae* complex strain ATCC 25267 was probe negative. All of the probe-positive strains were from sputum, and none were considered clinically significant.

The three strains that reacted with both probes upon initial testing continued to be *M. tuberculosis* complex SNAP and Accuprobe positive (range, 178,013 to 412,920 RLU) through the fifth sequential picking. The three strains retained the biochemical reactions of the original isolate. There were no signs of mixed cultures. These three strains were used for the subsequent extended time and temperature studies.

Increasing the temperature of the hybridization step to 70°C for the Accuprobe did result in RLU readings in the negative range for all three *M. terrae* complex strains. While the positive *M. tuberculosis* control remained positive through 73°C, the reactivity was diminished (95,932 RLU). Increasing the temperature of the SNAP hybridization step reduced the color intensity of the reaction at 59°C; two of the three *M. terrae* complex strains were negative, but the third strain was as positive (same intensity of color) as five of nine *M. tuberculosis* complex strains included as controls.

When the Accuprobe-positive strains were again tested by Accuprobe with the 8-min selection incubation, negative readings were obtained (range, 2,222 to 20,869 RLU). The RLU readings of the positive and negative control strains were not affected by the increased selection time. While additional numbers of patient-derived *M. tuberculosis* complex strains would need to be tested to determine the potential of false-negative results, the increase in selection time to eliminate cross-reactivity is certainly the most convenient modification to consider. However, further studies are needed to determine the effects of increased selection time on the overall performance of the *M. tuberculosis* complex probe before laboratories institute this change.

This study shows that the number of *M. terrae* complex strains which react with *M. tuberculosis* complex probes is not likely to be large and may vary somewhat geographically (3 of 35 California strains versus 1 of 81 Virginia strains were positive). The phenomenon, nonetheless, remains a serious concern. Should a laboratory probe and report on the basis of a broth isolation system without benefit of microscopic morphology, colony morphology, and growth rate and perform BACTEC drug susceptibility testing, the organisms tested would be reported not only as *M. tuberculosis* complex strains but also as multidrug-resistant *M. tuberculosis* complex strains (1).

Recognition of the occurrence of false-positive *M. tuberculosis* complex probe results should influence the criteria used to select isolates for probe tests. The microscopic morphology of *M. tuberculosis* complex isolates grown in BACTEC 12B broth medium (Becton-Dickinson, Sparks, Md.) exhibits characteristic "cording" that is strongly acid fast (5). If typical cording is not seen in a smear from 12B, the broth culture should not be tested with the *M. tuberculosis* complex probe, or if it is tested and found positive, the results should not be considered final until growth from a subculture onto a Middlebrook 7H10 agar plate can be observed for a possible mixed mycobacterial population that includes *M. tuberculosis*. The *M. terrae* complex strains which cross-reacted with the *M. tuberculosis* complex probes in our study did not have the classic smooth-domed appearance of *M. terrae* complex colonies on Middlebrook 7H10 agar (2). These strains formed rough, wrinkled colonies when viewed microscopically and could resemble *M. tuberculosis* colonies to an inexperienced observer. However, growth on subculture was microscopically observed much earlier (<4 days) than is usual for *M. tuberculosis*. On Lowenstein-Jensen medium, the growth was flat and "creamy" and would not be confused with the rough, "dry" morphology of *M. tuberculosis*. All probe-positive broth cultures should be subcultured to a Middlebrook plate to ensure that they are not mixed, and no probe-based identification should be considered final until sufficient growth is obtained on the plate to ensure by visual inspection that some *Mycobacterium* species not detectable by the probe(s) used has not been missed. This step will confirm that the colony morphology and growth rate of the organism are consistent with the identification obtained by the probe.

DNA probes remain the method of choice for early identification of the *M. tuberculosis* complex. Specific identification of members of the *M. tuberculosis* complex still is dependent upon biochemical and morphologic criteria. When DNA probe results are not consistent with morphologic findings or identification, the isolate should be sent to a reference laboratory, where analysis of discrepant results can be undertaken.

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