Gen-Probe Rapid Diagnostic System for the Mycobacterium avium Complex Does Not Distinguish between Mycobacterium avium and Mycobacterium paratuberculosis

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Three reference and 16 field strains of Mycobacterium paratuberculosis were tested with the Gen-Probe Mycobacterium avium complex DNA probe (Gen-Probe Inc., San Diego, Calif.). All reference strains and 12 of 16 field strains gave positive hybridization results with the probe. This study shows that the M. avium complex probe does not distinguish between M. avium and M. paratuberculosis and indicates heterogeneity in the 16S rRNA gene of M. paratuberculosis.

In 1987, Gen-Probe Inc., San Diego, Calif., introduced two commercial gene probe kits for the rapid diagnosis of mycobacteria: the Gen-Probe Rapid Diagnostic System for the Mycobacterium tuberculosis complex and the Gen-Probe Rapid Diagnostic System for the Mycobacterium avium complex. According to the manufacturer’s description (R. K. Enns), both systems were tested against a large variety of mycobacteria and other species of bacteria. The M. avium and Mycobacterium intracellulare DNA probes were tested against 92 other bacterial species belonging to 41 genera and against 30 other mycobacterial species without the probes cross-hybridizing with any of them. However, M. paratuberculosis was not included. This is highly surprising, since evidence exists suggesting that there is a close phenotypic relationship between M. avium and M. paratuberculosis (1, 12). That there also is a close genetic relationship has been demonstrated more recently (11, 18). There are currently eight published reports (6–8, 10, 13–15, 20) on the use of the Gen-Probe Rapid Diagnostic Systems, but none of them has included strains of M. paratuberculosis as test strains. The present note therefore describes the testing of the M. avium and M. intracellulare DNA probes against reference and field strains of M. paratuberculosis.

M. paratuberculosis ATCC 19698 and the British bovine vaccine strains 2E and 316F were used as reference strains. Fifteen field strains isolated from mesenteric lymph nodes and small intestines of slaughtered goats in Norway were primarily isolated on selective Dubos medium (16). In addition, one strain isolated from sheep on the Faroe Islands, Norway, previously shown to give a pattern similar to that of M. avium in gas chromatographic fatty acid analysis was tested (19). According to Chiodini et al. (4), mycobactin-dependent mycobacteria with rough colonies requiring at least 8 weeks of cultivation for visible growth are identified as M. paratuberculosis. Middlebrook 7H9 broth with 10% OADC (oleate, albumin, dextrose, catalase) enrichment (Difco) with 0.5% glycerol and 2% mycobactin prepared from Mycobacterium phlei was used for secondary culture. Strains were grown to a turbidity approximating that of a McFarland tube 1 and homogenized by vibration. The hybridization procedure was carried out according to the instructions of the manufacturer. Briefly, 100 μL of broth was incubated with lysing solution in an ultrasonic bath for 15 min at 50 to 60°C to release the rRNA. One ml of 125I-labeled probe solution was added and incubated at 72°C for 1 h. In order to adsorb hybridized probe, a separation suspension containing hydroxyapatite was added, and the mixture was incubated at 72°C for 5 min, followed by centrifugation (2,000 × g, 2 min), one wash, and counting in a gamma counter. Percent hybridization was calculated as follows: [(sample cpm − background cpm)/(total probe cpm − background cpm)] × 100. Strains were tested with both the M. avium probe and the M. intracellulare probe.

Hybridization results for reference strains of M. paratuberculosis with the M. avium and M. intracellulare probes are shown in Table 1. All reference strains hybridized with the M. avium probe, indicating homology in the probed region of the 16S rRNA. Of 16 field strains of M. paratuberculosis, 12 were also positive for hybridization with the M. avium probe. Negative hybridization results may have been caused by incomplete lysis of the bacteria by this procedure. However, after repeated testing with vigorous vibration with glass beads in addition to the lysis procedure, four strains remained negative for hybridization with the M. avium probe. All of the M. paratuberculosis strains were negative for hybridization with the M. intracellulare probe. Nevertheless, the present study shows that the Gen-Probe Rapid Diagnostic System for the M. avium complex does not distinguish between M. avium and M. paratuberculosis.

It may be questioned why four strains in the present study were negative for hybridization with the probe used. M. paratuberculosis is rather inactive biochemically (2, 9), and because there is variability in reactions between strains (4), biochemical reactions appear to be an unreliable basis for characterization of this bacterium. Biochemical tests are therefore not routinely performed on the bacterium. However, acid-fast staining of direct smears and tissue sections from one goat with a strain negative for hybridization with the probe revealed an abundance of acid-fast bacilli, a strong indication of infection with M. paratuberculosis. Two other

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probe-negative strains originated from herds notoriously infected with *M. paratuberculosis*. The fourth strain was a pigmented sheep strain from the Faroe Islands, where strongly pigmented strains of *M. paratuberculosis* from sheep occur (19).

*M. paratuberculosis* has been shown to be genetically very homogeneous, regardless of host and location (3, 21). However, a recently published report (5) shows that strain variability can be demonstrated both by restriction endonuclease analysis and by hybridization patterns with an *M. paratuberculosis*-specific probe. This strain variability may be consistent with the recently shown difference in pathogenicity between the classical bovine strain and the apparently specific Norwegian goat pathogenic strain of *M. paratuberculosis* (17). Our different hybridization results may indicate some genetic variation between different strains of *M. paratuberculosis*. The 16S rRNA can be regarded as an important phylogenetic and taxonomic indicator (22). Further investigation of the 16S rRNA gene of *M. paratuberculosis* and *M. avium* could be an additional approach to clarifying the relationship between these two organisms.

### REFERENCES