Cross-Reactivity of Genetic Probe for Detection of 
Mycobacterium tuberculosis with Newly Described 
Species Mycobacterium celatum

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An acridinium ester-labeled DNA probe (AccuProbe; Gen-Probe Inc., San Diego, Calif.) for the identification 
of the Mycobacterium tuberculosis complex gave discrepant results with the newly described species M. 
celatum. Examination of 20 strains of M. celatum showed that 8 were positive with the probe; the remaining 12 
were negative.

Prompt patient care for the treatment of Mycobacterium 
tuberculosis requires rapid identification of tubercle bacilli. 
For definitive diagnosis, the bacilli must be isolated and 
cultured. Conventional methods are laborious, time-con-
suming, and subject to error. Tuberculosis control efforts are 

further complicated by the emergence of drug resistance. The Centers for Disease Control and Prevention has advised 
using the latest technologies available for the rapid process-
ing and identification of M. tuberculosis from clinical spec-
imens (4, 8). Genetic probes that confirm the identity of members of the M. tuberculosis complex isolated in culture 
are in widespread use and allow recognition of this organism weeks before identification by conventional biochemical reactions (3). In actual laboratory use, the M. tuberculosis 
complex probe (AccuProbe; Gen-Probe Inc., San Diego, Calif.) has proven to be highly sensitive and specific (5). Discrepant reactions are rare but have been reported for M. terrae and for an unidentified species (6, 10).

Recently, we described a new species of mycobacteria, 
which we named M. celatum (1, 9). This species was initially 
recognized by biochemical reactions to be similar to M. 
avium but presented a mycolic acid pattern that was M. 
xenopi like (2, 7). M. celatum was further differentiated into 
types 1 and 2. Conventional biochemical identification meth-
ods were not able to separate the two types. Differentiation 
was made by restriction fragment length polymorphism 
(RFLP) analysis of the amplified sequence of the Hsp65 
gene, multilocus enzyme electrophoresis, and 16S rRNA 
sequence analysis (1). Both types were deposited with the 
American Type Culture Collection as ATCC 511371 and 
ATCC 51130, representative of types 1 and 2, respectively. 
The purpose of this correspondence is to report the cross-
reactivity between M. celatum type 1 and the genetic probe 
for the M. tuberculosis complex and the subsequent ability 
of the probe to differentiate the M. celatum types. The 
frequency of isolation of M. celatum was determined.

Initial mycobacterial isolation procedures were used, and 
the isolates were processed by the clinical laboratory by 
testing for M. tuberculosis with the M. tuberculosis complex 
genetic probe (AccuProbe) according to the instructions of 
the manufacturer (Gen-Probe). Target RNA from a sono-
cated lysate of a mycobacterial cell suspension was hybrid-
ized to the acridinium ester-labeled DNA probe (Accu-
Probe). The amount of chemiluminescence emitted from 
hydrolysis of the DNA-RNA complex was estimated with a 
luminometer and quantified as relative light units (RLUs). 
Results were interpreted according to the criteria of the 
manufacturer. The expected values were >30,000 RLUs for 
a positive isolate and <30,000 RLUs for a negative isolate.

The sources of the 20 M. celatum strains, biochemical 
characteristics, varied geographic distribution, RFLPs, mul-
tilocus enzyme electrophoresis results, and partial 16S 
rRNA sequence have been described elsewhere (1). In brief, 
RFLP analysis grouped the strains into two types. Eight 
strains were type 1, with seven DNA bands, and 12 were 
type 2, with six bands, indicative of a single restriction site 
difference. Analysis of 14 enzymes by multilocus enzyme 
electrophoresis clustered the strains into two different 
groups. Each group contained the same strains as the two 
types demonstrated by RFLP analysis. Additionally, 16S 
rRNA sequencing indicated a 10-base difference between the 
M. celatum types. Because a cutoff point for the differenti-
ation of species of mycobacteria has not been established for 
16S rRNA sequence differences and because we were unable 
to recognize the two different types phenotypically, we 
defined the types together under a single designation, i.e., 
M. celatum.

To determine the clinical occurrence of M. celatum 
strains, a retrospective study for the last 5 years was 
conducted by the Veterans Affairs reference laboratory in 
West Haven, Conn. M. celatum was identified in this anal-
ysis by tabulating the number of isolates showing the follow-
ing characteristics: inability to grow at 45°C, a positive 3-day 
arylsulfatase reaction, resistance to 2 μg of rifabutin per ml, 
and a negative reaction with the M. avium complex nucleic 
acid probe. These isolates had been identified as "nonpho-
tochromogen, unable to identify."

Reference strains run in the genetic probe assay included 
M. tuberculosis ATCC 25177 as a positive control and M. 
intracellulare ATCC 13950 as a negative control. The RLUs 
for the positive control were 651,558, over 200 times the 
RLUs for the negative control. The eight strains of M. 
celatum type 1 had RLUs (average ± standard deviation) of 
314,662 ± 68,882; values ranged from 203,175 to 394,467.

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**M. tuberculosis**

**M. celatum (ATCC 51131)**

| 50 | TACACATGCAAAGTCGAAAGGAAAGGGCTTCTTCGATGATGCC |
| 100 | GAACGGGGGAGTATAACAGAGGGTGTGGATCTCGCCACTTCCGGGATCTAAGCT |

**M. celatum (ATCC 51130)**

| 150 | TGGGAAAATCTCGGCTCTAAATCCGGAATAGGACACAGGAGATGCGATCATCTTGGT |

| 200 | GTGGAAAAACTTTTACCGGATGGGAGGATAGGGCCGCGCCCTATACGTTTTG |

| 250 | TGTTGGGTGATGCCTACCCAGCCGCGGCTATACGTTTTT |

| 300 | GTTGCAGGCGCCACACTGGGACTGAGATACGGCCCAGACTCCTACGGGAGGC |

| 350 | AGCAGTGGGGAAATATTCACAGATTGCGCAATGGCGCAAGCCTGTAGACGAGCAGCC |

| 400 | CTTGGGGGATGACGGCCTTCGGGTTGTAAACCTCTTCACCATCGACGAA |

| 450 | GCTGGGTCTTTCTCGGCTATGACGAGTAGGATGAGGAGAAGAGCACCAGGCCCAA |

FIG. 1. Alignment of partial 16S rRNA gene sequences for *M. tuberculosis*, *M. celatum* type 1 (ATCC 51131\(^{\dagger}\)), and *M. celatum* type 2 (ATCC 51130). Boxed letters indicate the lack of a consensus among the three sequences.

The 12 strains of *M. celatum* type 2 had (average RLU ± standard deviation) of 4,225 ± 1,783; values ranged from 2,288 to 8,152.

Since *M. celatum* type 1 reacted with the *M. tuberculosis* probe and *M. celatum* type 2 did not, we compared 16S rRNA sequences for the two types and for *M. tuberculosis* (Fig. 1). The homologous sequence alignment shown in Fig. 1 is for 450 bases of the partial 16S rRNA gene sequence. A comparison of the remaining 1,110 nucleotides between *M. celatum* types 1 and 2 and *M. tuberculosis* did not reveal any differences (1). Genetic probes are short oligonucleotide sequences; therefore, *M. tuberculosis* and *M. celatum* type 1 were expected to contain a limited, homologous sequence region. Furthermore, *M. celatum* type 2 was expected to demonstrate some oligonucleotide variability in this region. Bases found at positions 131, 132, 135, and 148 of the sequence meet this criterion. As expected, this region is within the target sequence region for the commercially available, proprietary probe (Gen-Probe) for the *M. tuberculosis* complex (2a, 10).

Since a false-positive result could result in an incorrect diagnosis and inappropriate treatment for tuberculosis, we attempted to define the occurrence of this species in the clinical laboratory. The rate of isolation of *M. celatum* was determined by examination of 13,530 laboratory isolates over a period of 5 years. The retrospective analysis revealed 24 isolates of *M. celatum* from 17 different patients (Table 1). The rate of isolation of both types of *M. celatum* was only 0.1%. Previous studies had shown the occurrence of *M. celatum* type 1 to be approximately 40% of the total number
of *M. celatum* isolates examined (1). Therefore, the rate of isolation of *M. celatum* type 1 is 0.05%.

In summary, *M. celatum* is a rarely encountered species that may be responsible for discrepant reactions with genetic probes for the *M. tuberculosis* complex. However, probes remain highly sensitive and specific for use in the clinical laboratory as a means for rapidly identifying isolates of the *M. tuberculosis* complex.

**REFERENCES**


**TABLE 1.** Five-year, retrospective examination of the rate of isolation of *M. celatum*

<table>
<thead>
<tr>
<th>Yr</th>
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* Isolates were submitted for reference studies as clinically significant to the Veterans Affairs reference laboratory in West Haven, Conn.