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Sequence Variant for Internal Transcribed Spacer Region of *Mycobacterium abscessus*

During the implementation of a PCR-based assay to differentiate between *Mycobacterium abscessus* and *M. chelonae* using the 16S to 23S internal transcribed spacer region (1), a sequence variant of *M. abscessus* was discovered. The sequence variant did not bind the *M. abscessus* specific probe, resulting in no or low fluorescence detected. The base change was found at position 39 of the amplicon (GenBank accession no. DQ177308). The base change was confirmed by sequencing with four additional patient isolates. A total of 6 isolates out of 129 tested from 3 March 2005 to 21 July 2005 had low fluorescence detected for a frequency of 4.65%.

The PCR assay has since been improved by redesigning the *M. abscessus* probe (Nanogen, Inc., Bothell, WA) to incorporate proprietary modified bases for more-efficient hybridization characteristics. The probe modification included incorporation of a super G (proprietary modified base G) at the site of the polymorphism as well as a super A (proprietary modified base A) further downstream (Table 1). The improved assay allowed for a detectable level of fluorescence for all isolates. The fluorescence detected in the FAM (6-carboxyfluorescein) channel on the SmartCycler II (Cepheid, Inc., Sunnyvale, CA) increased from an average of 41.7 ± 36 (range of 0 to 100) fluorescence units to 180 ± 22 (range of 150 to 200) fluorescence units.

The assay with the modified probe performed with 100% sensitivity after testing 22 *M. abscessus* isolates, 6 of which were sequence variants, and 100% specificity after testing 10 *M. chelonae* isolates. All samples tested were proven by 16S rRNA gene sequencing to be among the *M. chelonae-M. abscessus* complex, as previously reported (1).

TABLE 1. New MGB Eclipse probe showing modification from the old probe

Probe	Probe sequence
New probe	...MGB-quencher 5′AGTAGGG*ATCTGTA*GTGG3′ FAM[a]
Original probe	.....MGB-quencher 5′AGTAGGCATCTGTAGTGG3′ FAM

[a] G*, super G; A*, super A.

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REFERENCE

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Haleina Neal
ARUP Laboratories
Salt Lake City, Utah

Joann L. Cloud*
June I. Pounder
Sam R. Page
ARUP Institute for Clinical and Experimental Pathology
500 Chipeta Way
Salt Lake City, Utah 84108

Gail L. Woods
University of Arkansas Medical Sciences
Little Rock, Arkansas

*Phone: (801) 583-2787, ext. 2439
Fax: (801) 584-5109
E-mail: cloudjl@aruplab.com