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Detection and Identification of *Mycobacterium* spp. in Clinical Specimens by Combining the Roche Cobas Amplicor *Mycobacterium tuberculosis* Assay with *Mycobacterium* Genus Detection and Nucleic Acid Sequencing[▽]

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We have recently developed a PCR assay for detection of *Mycobacterium* spp. at the genus level based on the Cobas Amplicor platform. The sensitivities for smear-positive and smear-negative specimens were found to be 100% and 47.9%, respectively. The specificity was 97.7%, the positive predictive value 84.6%, and the negative predictive value 93.1%. In a follow-up study, we have systematically evaluated the *Mycobacterium* genus assay in parallel with the Cobas Amplicor *Mycobacterium tuberculosis* assay on 2,169 clinical specimens, including respiratory and nonrespiratory specimens. Based on the genus assay, nontuberculous mycobacteria were readily detected and identified to the species level by PCR-mediated sequencing. In addition, our data point to a limited specificity of the Cobas Amplicor *M. tuberculosis* assay.

Nontuberculous mycobacteria (NTM) are frequently associated with human disease. Infections with NTM are increasingly observed in immunocompromised patients (10, 16), although patients without underlying medical conditions are also affected. Diseases caused by NTM include lung disease (e.g., *Mycobacterium abscessus*, *Mycobacterium kansasii*), cutaneous ulcers (e.g., *Mycobacterium marinum*), disseminated infections (e.g., *Mycobacterium genavense*), lymphadenitis (e.g., *Mycobacterium avium*), and joint infections (e.g., *Mycobacterium haemophilum*) (for a review, see references 5 and 16). Rapid and reliable laboratory detection of NTM is crucial for clinical management and proper antibiotic therapy. Molecular genetic assays for the detection of NTM in clinical specimens are only infrequently implemented in routine diagnostics. With a view to developing an assay that is capable of detecting a large number of nontuberculous mycobacteria in clinical specimens, we have recently developed a semiautomated PCR-based assay for NTM on the basis of the Roche Cobas Amplicor platform (8). The sensitivity of the genus assay was 100% for smear-positive specimens and 47.9% for smear-negative specimens. The specificity of the genus assay was 97.7%, the positive predictive value (PPV) 84.6%, and the negative predictive value 93.1% (8). These values are comparable to those published for the Cobas Amplicor *Mycobacterium tuberculosis* test (11; reviewed in reference 9). We have now extensively evaluated the *Mycobacterium* genus assay under routine laboratory conditions.

MATERIALS AND METHODS

Decontamination of specimens, microscopy, and culture. Clinical specimens were decontaminated using the sodium hydroxide method for samples from sterile sites and the *N*-acetyl-L-cysteine-sodium hydroxide method for respiratory samples (6). Auramine-rhodamine fluorochrome staining was used for microscopic examination; positive microscopy results were confirmed using Ziehl-Neelsen staining (6). For the recovery of mycobacteria from culture, standard media were inoculated (7H11 plates and BBL MGIT [Becton, Dickinson and Company]) and maintained for 7 weeks at 37°C. Mycobacteria were identified by 16S rRNA gene sequence analysis as described previously (7).

Clinical specimens. Over a period of 12 months (April 2008 to March 2009), the Cobas Amplicor *M. tuberculosis* and *Mycobacterium* genus assays were performed in parallel on all specimens for which molecular detection of *M. tuberculosis* or NTM was requested. These included respiratory as well as nonrespiratory specimens.

DNA extraction. DNA was extracted from decontaminated samples (0.5 ml) by using the respiratory specimen preparation kit (Roche Diagnostics, Switzerland) according to the instructions for the Cobas Amplicor *M. tuberculosis* test (Roche Diagnostics, 2007) (12).

Detection of mycobacterial DNA. The Cobas Amplicor *M. tuberculosis* test was performed according to the manufacturer's instructions (Roche Diagnostics, Switzerland), and the *Mycobacterium* genus test was performed as described previously (8).

According to the manufacturer, the Cobas Amplicor *M. tuberculosis* test is considered valid if the optical density at 660 nm (OD_{660}) of the positive control is >2.0 and the OD_{660} of the negative control is <0.25. A specimen is scored positive for *M. tuberculosis* when the OD_{660} is ≥0.35. A sample is scored negative for *M. tuberculosis* if the OD_{660} of the sample is <0.35 and the OD_{660} of the internal inhibition control is ≥0.35 (12).

The *Mycobacterium* genus test adapted to the Cobas Amplicor platform was performed as described previously by using a genus-specific capture probe (5′-TTTCACGAACAACGCGACAA-3′) coupled to magnetic beads (8). For the *Mycobacterium* genus assay, a result was considered valid if the OD_{660} of the positive control was ≥2.0 and the OD_{660} of the negative control was <0.5. A specimen was considered positive for mycobacteria if the OD_{660} was ≥0.5 and at least 2-fold higher than the background (negative control). A sample was considered negative if the OD_{660} of the sample was <0.5 and the OD_{660} of the internal control was ≥0.35. Specimens were scored positive for NTM if the OD_{660} was ≥0.50 for the genus assay and the Cobas Amplicor *M. tuberculosis* result was negative (OD_{660}, <0.35).

Amplification, DNA purification, and sequencing of positive samples and cultures. Samples that were scored positive by the genus assay or for which the OD_{660} by the Amplicor *M. tuberculosis* assay was ≥0.35 and <2.0 were subjected to PCR-mediated 16S rRNA gene sequence analysis. For gene amplification, the Cobas Amplicor pan-*Mycobacterium* primers KY18 and KY75 or primers 283 and 264 were used in separate PCRs as described previously (8). PCR products were sequenced using primer Mbakt-14 (3) and were analyzed using SmartGene IDNS software and databases (SmartGene, Zug, Switzerland). If unsatisfactory PCR or sequencing results were obtained, reamplification was done with primers

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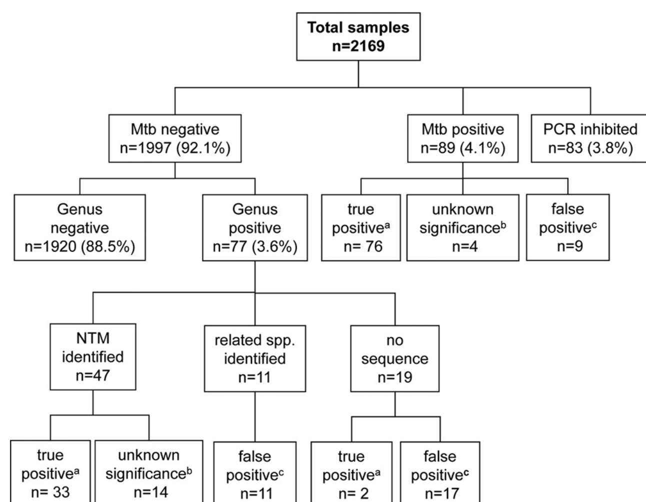

FIG. 1. Clinical specimens tested by the Cobas Amplicor *M. tuberculosis* assay and the *Mycobacterium* genus assay. Mtb, *M. tuberculosis*. A true-positive result ([a]) was confirmed by at least two other positive results, e.g., by smear, culture, or another sample from the same patient. A result of unknown significance ([b]) was obtained by sequence identification, but no other positive result was obtained. A result was considered false positive ([c]) when no sequence was obtained or when a related species was identified and no other positive result was obtained.

KY18 and 259 or with primers 283 and 259. Homology analysis and species identification were carried out as described previously (2, 3, 7).

Sequencing of 16S rRNA genes from cultures was performed using primers 283 and 264 for PCR amplification and primer Mbakt-14 for sequencing (3, 8). In case of *M. chelonae* complex identification, sequence analysis of *rpoB* (1) was used for species assignment, e.g., to *M. massiliense*, *M. bolletii*, *M. abscessus*, or *M. chelonae.* In case of *M. kansasii/gastri* identification, sequence analysis of *hsp65* was used for differentiation (13).

RESULTS

Over a period of 12 months, 2,169 clinical samples were tested for the presence of mycobacterial DNA in a prospective study design (Fig. 1). A negative PCR result in both assays was obtained for 1,920 (88.5%) of the specimens. Inhibition of the PCR, as indicated by a negative internal control as used in the *M. tuberculosis* assay, occurred for 83 (3.8%) of the specimens. Eighty-nine (4.1%) samples tested positive by the Cobas Amplicor *M. tuberculosis* assay, indicating the presence of *M. tuberculosis*. Seventy-seven (3.6%) samples tested positive by the *Mycobacterium* genus assay and negative by the Cobas Amplicor *M. tuberculosis* assay, indicating the presence of NTM.

The 77 samples positive by the *Mycobacterium* genus assay and negative by the *M. tuberculosis* assay included 60 respiratory specimens, 8 bronchoalveolar lavage samples, 3 biopsy specimens, 2 wound specimens, 1 cerebrospinal fluid sample, and 3 urine samples; 22 of the 77 genus assay-positive samples were smear positive. For 47 of the 77 samples (61%), 16S rRNA gene sequence analysis of the amplicon resulted in the identification of an NTM species (Table 1). NTM species detected by the genus assay included *M. chelonae* complex (n = 12), *M. gordonae* (n = 4), *M. fortuitum* (n = 2), *M. intracellulare* (n = 7), *M. avium* (n = 7), *M. kansasii/gastri* (n = 4), *M. celatum* (n = 1), M. *mucogenicum/phocaicum* (n = 1), *M. aubagnense* (n = 1), *M. frederiksbergense* (n = 2), and *M. terrae* (n = 1). For 5 samples, the 16S rRNA gene sequence obtained had no match of significant homology in the database to allow for species assignment, resulting in assignment to genus level; these sequences were reported as *Mycobacterium* spp. For 26 of the 47 specimens with assignment of the PCR amplicon to the NTM species level, an NTM was recovered by culture, and the 16S rRNA gene sequence of the NTM was identical to the DNA sequence obtained by molecular analysis of the clinical specimen (Table 1).

For 30 samples that were positive by the *Mycobacterium* genus assay, sequence analysis of the PCR product did not result in NTM species assignment (Table 2); for 2 of these samples, NTM were recovered by culture. For 11/28 samples, sequencing of the PCR product revealed species of closely related genera, e.g., *Corynebacterium* (n = 10) and *Gordonia* (n = 1) (Table 2). For 17/28 samples, no readable 16S rRNA gene sequence was obtained, even though two different PCR protocols were used.

To determine the relevance of a positive *Mycobacterium* genus PCR result, we scored positive samples as true positives if either (i) the sample was smear positive, (ii) the corresponding NTM was recovered by culture, or (iii) the corresponding NTM was recovered from other samples from the patient. According to these criteria, 35 of the 77 genus assay-positive samples (45%) were regarded as true positive (Fig. 1). For another 14 samples (18%), sequencing of the PCR product revealed NTM, but none of the criteria defined above was fulfilled; we considered these results to be of unknown significance. In 28 samples (36%), we failed to identify an NTM by sequence analysis of the PCR product, and we considered these genus PCR results to be false positive. The majority of true-positive samples showed OD_{660} values of >2.0 (32/35); in comparison, the majority of samples that were scored as false positive had OD_{660} values of <2.0 (22/28).

Eighty-nine (4.1%) samples were positive by the Cobas Amplicor *M. tuberculosis* test. All samples with OD_{660} values of ≥2.0 (n = 61 [69%]) were either smear positive or positive for *M. tuberculosis* by culture. For samples with OD_{660} values of <2.0 (n = 28 [31%]), we analyzed the specificity of the Cobas Amplicor *M. tuberculosis* assay by sequencing the PCR products obtained with the primers used in the Cobas Amplicor system and the PCR product obtained with more-specific primers for *Mycobacterium*, in order to prevent preferential amplification of related genera due to larger DNA amounts (Table 3). A sample was scored as true positive if either (i) the sequence analysis of the 16S rRNA gene PCR product obtained directly from the clinical sample revealed *M. tuberculosis* complex, (ii) the sample was smear positive, or (iii) *M. tuberculosis* was cultured from the corresponding sample or from other samples of the patient. A sample was scored negative for *M. tuberculosis* if the sample and at least two additional samples from the patient were negative in all tests. Otherwise, the positive PCR result of the sample was considered to be of unknown significance.

Investigation of the 28 samples positive by the Cobas Amplicor *M. tuberculosis* assay with OD_{660} values of <2.0 (Table 3) showed that 11/28 were culture positive for *M. tuberculosis*; for 9 of these, sequence analysis of the PCR product obtained by amplification of nucleic acid extracted from the clinical

TABLE 1. Analysis of specimens positive by the *Mycobacterium* genus PCR, where sequence analysis of the PCR product resulted in the identification of NTM ($n = 47$)

Specimen[a]	OD_{660} by:		Identification by 16S rRNA gene sequence[b]	Result by:		Interpretation
	Genus assay	*M. tuberculosis* assay		Smear	Culture[c]	
Respiratory	>4.00	<0.35	*M. chelonae* complex	Positive	*M. abscessus*	True positive
Respiratory	>4.00	<0.35	*M. chelonae* complex	Positive	*M. chelonae* complex	True positive
Respiratory	>4.00	<0.35	*M. gordonae*	Positive	*M. gordonae*	True positive
Respiratory	>4.00	<0.35	*M. chelonae* complex	Positive	*M. abscessus*	True positive
Respiratory	>4.00	<0.35	*M. chelonae* complex	Positive	*M. abscessus*	True positive
Respiratory	>4.00	<0.35	*M. fortuitum*	Positive	*M. fortuitum*	True positive
BAL	>4.00	<0.35	*M. intracellulare*	Positive	*M. intracellulare*	True positive
Respiratory	>4.00	<0.35	*M. intracellulare*	Positive	*M. intracellulare*	True positive
Respiratory	3.95	<0.35	*M. chelonae* complex	Positive	*M. abscessus*	True positive
Respiratory	3.78	<0.35	*M. chelonae* complex	Positive	*M. abscessus*	True positive
Wound	3.76	<0.35	*M. intracellulare*	Positive	*M. intracellulare*	True positive
Respiratory	3.64	<0.35	*M. gordonae*	Positive	*M. gordonae*	True positive
Wound	3.64	<0.35	*M. intracellulare*	Positive	*M. intracellulare*	True positive
Respiratory	3.47	<0.35	*M. avium*	Positive	*M. avium*	True positive
Respiratory	3.46	<0.35	*M. kansasii/gastri*	Positive	*M. kansasii*	True positive
Respiratory	3.25	<0.35	*M. avium*	Positive	*M. avium*	True positive
Respiratory	3.01	<0.35	*M. avium*	Positive	*M. avium*	True positive
Respiratory	2.95	<0.35	*M. intracellulare*	Positive	*M. intracellulare*	True positive
BAL	2.27	<0.35	*M. kansasii/gastri*	Positive	*M. kansasii*	True positive
Respiratory	>4.00	<0.35	*M. intracellulare*	Negative	*M. intracellulare*	True positive
Respiratory	>4.00	<0.35	*M. kansasii/gastri*	Negative	*M. kansasii*	True positive
Respiratory	3.95	<0.35	*M. avium*	Negative	*M. avium*	True positive
Respiratory	3.95	<0.35	*M. avium*	Negative	*M. avium*	True positive
Respiratory	3.78	<0.35	*M. chelonae* complex	Negative	*M. abscessus*	True positive
Respiratory	3.07	<0.35	*M. gordonae*	Negative	*M. gordonae*	True positive
Respiratory	0.53	<0.35	*M. chelonae* complex	Negative	*M. bolletii*	True positive
Respiratory	>4.00	<0.35	*M. chelonae* complex	Positive	Negative	True positive
Respiratory	>4.00	<0.35	*M. chelonae* complex	Positive	Negative	True positive
Respiratory	3.47	<0.35	*M. chelonae* complex	Negative	Negative	True positive[d]
BAL	2.76	<0.35	*M. celatum*	Negative	Negative	True positive[d]
Respiratory	2.62	<0.35	*M. gordonae*	Negative	Negative	True positive[d]
Respiratory	2.35	<0.35	*M. intracellulare*	Not done	Negative	True positive[d]
Respiratory	1.65	<0.35	*M. avium*	Not done	Negative	True positive[d]
Respiratory	3.65	<0.35	*M. aubagnense*	Negative	Negative	Unknown significance
Respiratory	3.34	<0.35	*M. avium*	Negative	Negative	Unknown significance
Respiratory	3.21	<0.35	*Mycobacterium* sp.	Negative	Negative	Unknown significance
Respiratory	2.82	<0.35	*M. frederiksbergense*	Negative	Negative	Unknown significance
Respiratory	2.73	<0.35	*Mycobacterium* sp.	Negative	Negative	Unknown significance
Respiratory	2.17	<0.35	*M. kansasii/gastri*	Negative	Negative	Unknown significance
Respiratory	0.89	<0.35	*M. mucogenicum*	Negative	Negative	Unknown significance
Respiratory	0.77	<0.35	*M. fortuitum*	Negative	Negative	Unknown significance
Respiratory	0.68	<0.35	*Mycobacterium* sp.	Negative	Negative	Unknown significance
Respiratory	0.54	<0.35	*Mycobacterium* sp.	Negative	Negative	Unknown significance
Respiratory	0.52	<0.35	*M. chelonae* complex	Negative	Negative	Unknown significance
Respiratory	0.52	<0.35	*M. frederiksbergense*	Negative	Negative	Unknown significance
BAL	0.51	<0.35	*M. terrae*	Negative	Negative	Unknown significance
CSF	0.50	<0.35	*Mycobacterium* sp.	Not done	Not done	Unknown significance

[a] BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid.
[b] Sequencing was performed on PCR products obtained by amplification of nucleic acids extracted from the clinical sample.
[c] Identification by 16S rRNA sequence analysis, followed when necessary by sequencing and homology analysis of *rpoB* (in the case of *M. chelonae* complex) or *hsp65* (in the case of *M. kansasii/gastri*).
[d] A corresponding NTM species was recovered by culture in another patient sample or samples.

samples revealed a 16S RNA sequence characteristic of *M. tuberculosis*. Seventeen of 28 samples did not show growth of *M. tuberculosis* in culture. Sequence analysis of the PCR products obtained from these 17 clinical samples revealed the presence of *M. tuberculosis* in 4. For 10 specimens, sequence analysis revealed an NTM ($n = 2$) or a species of a closely related genus, such as *Corynebacterium* spp. ($n = 7$) and a *Dietzia* sp. ($n = 1$). For 5 samples positive by the *M. tuberculosis* assay, no readable sequence could be obtained (Table 3). Altogether, 76 of the 89 samples (85.4%) that were positive by the Cobas Amplicor *M. tuberculosis* assay were considered true positive. For 4 specimens (4.5%), the positive result was considered to be of unknown significance, and for 9 samples (10.1%), the Cobas Amplicor *M. tuberculosis* result was considered false positive. For each patient with a positive result of unknown significance or a false-positive result, the clinical history was evaluated. No history of *Mycobacterium* infections was found for any of these patients.

TABLE 2. Analysis of specimens positive by the *Mycobacterium* genus PCR, where sequence analysis of the PCR product did not result in NTM species assignment ($n = 30$)

Specimen[a]	OD_{660} by:		Identification by 16S rRNA gene sequence[b]	Result by:		Interpretation
	Genus assay	Cobas Amplicor *M. tuberculosis* test		Smear	Culture	
Biopsy	2.63	<0.35	No sequence	Negative	*M. intracellulare*	True positive
Respiratory	0.99	<0.35	No sequence	Positive	*M. avium*	True positive
Respiratory	3.64	<0.35	*Corynebacterium* sp.	Negative	Negative	False positive
Urine	3.14	<0.35	*C. genitalium*	Not done	Negative	False positive
Respiratory	2.67	<0.35	*Corynebacterium* sp.	Negative	Negative	False positive
Urine	2.53	<0.35	*C. amycolatum*	Not done	Negative	False positive
BAL	1.56	<0.35	*C. pseudogenitalium*	Negative	Negative	False positive
Biopsy	1.47	<0.35	*C. afermentans*	Negative	Negative	False positive
Respiratory	0.88	<0.35	*C. propinquum*	Negative	Negative	False positive
BAL	0.63	<0.35	*Gordonia* sp.	Negative	Negative	False positive
Respiratory	0.58	<0.35	*C. propinquum*	Negative	Negative	False positive
Respiratory	0.51	<0.35	*C. argentoratense*	Negative	Negative	False positive
Respiratory	0.50	<0.35	*C. propinquum*	Negative	Negative	False positive
Respiratory	3.95	<0.35	No sequence	Negative	Negative	False positive
Respiratory	2.04	<0.35	No sequence	Negative	Negative	False positive
Respiratory	1.64	<0.35	No sequence	Negative	Negative	False positive
Respiratory	1.29	<0.35	No sequence	Negative	Negative	False positive
Respiratory	1.09	<0.35	No sequence	Negative	Not done	False positive
Respiratory	0.91	<0.35	No sequence	Negative	Not done	False positive
Respiratory	0.89	<0.35	No sequence	Negative	Negative	False positive
Respiratory	0.89	<0.35	No sequence	Negative	Negative	False positive
Respiratory	0.74	<0.35	No sequence	Negative	Negative	False positive
BAL	0.68	<0.35	No sequence	Negative	Negative	False positive
Respiratory	0.61	<0.35	No sequence	Negative	Negative	False positive
BAL	0.58	<0.35	No sequence	Negative	Negative	False positive
Biopsy	0.55	<0.35	No sequence	Negative	Negative	False positive
Urine	0.53	<0.35	No sequence	Not done	Negative	False positive
Respiratory	0.53	<0.35	No sequence	Negative	Negative	False positive
Respiratory	0.52	<0.35	No sequence	Negative	Negative	False positive
Respiratory	0.50	<0.35	No sequence	Negative	Negative	False positive

[a] BAL, bronchoalveolar lavage.
[b] Sequencing was performed on PCR products obtained by amplification of nucleic acids extracted from the clinical sample. "No sequence" means that no sequence was obtained from the amplification product.

DISCUSSION

We have recently developed a *Mycobacterium* genus assay based on the Cobas Amplicor platform (8). Here we report on a 12-month prospective evaluation for detection and identification of *Mycobacterium* spp. in clinical samples in which we combined the Cobas Amplicor *M. tuberculosis* test with the *Mycobacterium* genus assay. All clinical samples submitted for Cobas Amplicor *M. tuberculosis* testing were subjected to the *Mycobacterium* genus test in parallel. Direct 16S rRNA gene amplification of nucleic acids extracted from the clinical sample, followed by sequence analysis, facilitated rapid identification of the NTM at the species level for most of the samples positive by the genus assay (Table 1). The majority of NTM identified were found to represent well-established clinical pathogens, while we consider *M. frederiksbergense* (17), *M. aubagnense*, *M. terrae*, *M. gordonae*, and the unclassified *Mycobacterium* spp. to be of little clinical relevance, if any. Specimens with OD_{660} values of $\geq$2.0 by the *Mycobacterium* genus assay were frequently confirmed by other criteria for the presence of NTM, in contrast to specimens with OD_{660} values of <2.0. Such low OD_{660} values are probably due to cross-reactivity of the genus probe or very low numbers of NTM. Cross-reactivity of the genus probe with other closely related species was indicated by sequence analysis of the amplicon that resulted in the identification of closely related species, such as *Corynebacterium* and *Gordonia* spp. (Table 2). *In silico* homology analysis of the *Mycobacterium* genus probe shows 3 or 4 mismatches, which are predominantly positioned in the middle of the sequence, with the most closely related genera (Table 4). The temperature conditions of the Cobas Amplicor protocol cannot be changed. To increase the specificity of the *Mycobacterium* genus assay, the sequence of the PCR product must be analyzed before a positive result is reported. In 47 of the genus assay-positive samples, a nontuberculous mycobacterium was identified by sequence analysis; of these, 33 were true positive as judged by additional criteria. The other 14 samples were considered to be of unknown significance. On the basis of these results, patients were not treated. For each patient, we have evaluated the clinical history and have not found additional positive clinical samples or a history of *Mycobacterium* infections.

The Cobas Amplicor *M. tuberculosis* assay is commonly regarded as a reliable assay for the majority of specimens tested (4, 11, 15). However, 9 of 28 samples with OD_{660} values of <2.0 could not be confirmed as positive for *M. tuberculosis* by other criteria, such as smear positivity, sequence analysis of the

TABLE 3. Analysis of specimens positive by the Cobas Amplicor *M. tuberculosis* test with OD_{660} values of <2.0 ($n = 28$)

Specimen[a]	OD_{660} by the *M. tuberculosis* test	Identification by 16S RNA sequencing[b]	Result by:		Detection of *M. tuberculosis* in other patient sample(s)[d]	Interpretation
			Smear	Culture[c]		
Respiratory	0.44	*M. tuberculosis* complex	Positive	*M. tuberculosis*	ND	True positive
Biopsy	1.65	*M. tuberculosis* complex	Negative	*M. tuberculosis*	ND	True positive
BAL	1.26	*M. tuberculosis* complex	Negative	*M. tuberculosis*	ND	True positive
Respiratory	0.37	*M. tuberculosis* complex	Negative	*M. tuberculosis*	ND	True positive
Respiratory	1.52	*M. tuberculosis* complex	Negative	*M. tuberculosis*	ND	True positive
Biopsy	1.16	*M. tuberculosis* complex	Negative	*M. tuberculosis*	ND	True positive
Biopsy	0.39	No sequence	Negative	*M. tuberculosis*	ND	True positive
Biopsy	1.01	*M. tuberculosis* complex	Negative	*M. tuberculosis*	ND	True positive
Respiratory	0.74	*M. tuberculosis* complex	Negative	*M. tuberculosis*	ND	True positive
BAL	1.36	*M. tuberculosis* complex	Negative	*M. tuberculosis*	ND	True positive
Respiratory	0.59	No sequence	Negative	*M. tuberculosis*	ND	True positive
Biopsy	1.84	*M. tuberculosis* complex	Negative	Negative	Negative	True positive
Respiratory	1.64	*M. tuberculosis* complex	Negative	Negative	Negative	True positive
Stool	0.60	*M. tuberculosis* complex	Negative	Negative	Positive	True positive
Biopsy	0.83	*M. tuberculosis* complex	Negative	Negative	Positive	True positive
Respiratory	0.74	*C. propinquum*	Negative	Negative	No other samples	Unknown significance
Respiratory	0.46	*M. xenopi*	Negative	Negative	No other samples	Unknown significance
Respiratory	1.16	*C. pseudodiphtheriticum*, *C. durum*	Negative	Negative	Positive	Unknown significance
Respiratory	0.68	*C. propinquum*	Negative	Negative	No other samples	Unknown significance
Respiratory	0.96	*M. abscessus*	Positive	*M. abscessus*	Negative	False positive
Respiratory	0.62	*Dietzia* sp.	Negative	Negative	Negative	False positive
Respiratory	0.40	*C. propinquum*	Negative	Negative	Negative	False positive
Respiratory	0.76	*C. pseudodiphtheriticum*	Negative	Negative	Negative	False positive
Respiratory	1.68	No sequence	Negative	Negative	Negative	False positive
Respiratory	0.45	*C. durum*	Negative	Negative	Negative	False positive
BAL	1.38	No sequence	Negative	Negative	Negative	False positive
Respiratory	0.46	No sequence	Negative	Negative	Negative	False positive
Respiratory	0.42	*C. propinquum*	Negative	Negative	Negative	False positive

[a] BAL, bronchoalveolar lavage.
[b] Sequencing was performed from PCR products directly obtained by amplification of nucleic acids extracted from the clinical sample. "No sequence" means that no sequence was obtained from the amplification product.
[c] Identification by 16S rRNA sequence analysis.
[d] ND, not determined.

amplicon, recovery from culture, or the presence of *M. tuberculosis* in additional samples from the patient. This resulted in a PPV of 89.9%, which is in accordance with the results of other studies (9). The detection in these samples of closely related bacterial species of the genera *Corynebacterium* and *Dietzia* suggests cross-reactivity of the Cobas Amplicor *M. tuberculosis* assay, resulting in false-positive test results. It has been noted previously that the Cobas Amplicor pan-*Mycobacterium* primers KY18 and KY75 are able to amplify the 16S rRNA genes of species closely related to *Mycobacterium*, such as *Corynebacterium*, *Nocardia*, and *Rhodococcus* species (14). Consequently, the corresponding genus assay values of these 9

TABLE 4. Homology analysis of the *Mycobacterium* genus probe with species of the most closely related genera

Bacterial species	GenBank accession no.[a]	DNA sequence[b]
Mycobacterium genus[c]		5′-TTTCACGAACAACGCGACAA-3′
Corynebacterium propinquum	AY244785	5′-......AG..G...T.....-3′
Corynebacterium durum	AF537593	5′-......AG..G........C-3′
Corynebacterium afermentans	X82054	5′-..A...A...G.........-3′
Corynebacterium pseudodiphtheriticum	AJ439343	5′-......AG..G...T.....-3′
Corynebacterium pseudotuberculosis	X81916	5′-......AG..G.........-3′
Corynebacterium tuberculostearicum	AJ438044	5′-......AG..G.........-3′
Corynebacterium amycolatum	FN668737	5′-......AG..G.........-3′
Corynebacterium argentoratense	AF537589	5′-......AG..G.........-3′
Nocardia pneumoniae	GQ853075	5′-......AG..G.........-3′
Gordonia bronchialis	CP001802	5′-......AG..G.........-3′
Tsukamurella pulmonis	AY741505	5′-......AG..G...T.....-3′
Dietzia maris	X79290	5′-......AG..G...T.....-3′
Rhodococcus equi	AY741716	5′-......AG..G.........-3′

[a] For the corresponding 16S rRNA gene sequence.
[b] Dots represent nucleotides identical to those in the *Mycobacterium* genus probe sequence.
[c] The *Mycobacterium* genus probe was published previously (7).

false-positive test results did not distinguish true- from false-positive results, probably due to the cross-reactivity of the genus probe, as discussed above.

We conclude that (i) a molecular detection assay for NTM, followed by nucleic acid sequence analysis allowing species assignment, is a valuable tool for the rapid detection of NTM in clinical specimens and (ii) sequence analysis of the amplicon is required when Cobas Amplicor *M. tuberculosis* results with OD_{660} values of <2.0 are obtained.

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We have no conflict of interest to disclose.

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