JCM
Journals.ASM.org

Evaluation of Cobas TaqMan MTB for Direct Detection of the *Mycobacterium tuberculosis* Complex in Comparison with Cobas Amplicor MTB

Guido V. Bloemberg,[a] Antje Voit,[a] Claudia Ritter,[a,b] Vanessa Deggim,[a] Erik C. Böttger[a,b]

Institut für Medizinische Mikrobiologie[a] and Nationales Zentrum für Mykobakterien,[b] Zürich, Switzerland

The Roche Cobas Amplicor MTB assay, recently replaced by the Roche Cobas TaqMan MTB assay, was one of the first commercially available assays for detection of the *Mycobacterium tuberculosis* complex based on nucleic acid amplification. We reported previously on the limited specificity of the Cobas Amplicor MTB assay, in particular for positive samples with an optical density at 660 nm (OD_{660}) of <2.0. Using a selected set of respiratory samples, which were scored as false positive by the Cobas Amplicor test, we demonstrate here that the specificity of the Cobas TaqMan assay is significantly improved. In addition, our study of a set of 133 clinical samples revealed that the Cobas TaqMan MTB assay showed significantly less PCR inhibition than the Cobas Amplicor test. An overall concordance of 98.2% was observed between the two assays. In a subsequent prospective study, we evaluated the performance of the Roche Cobas TaqMan MTB assay on 1,143 clinical specimens, including respiratory ($n = 838$) and nonrespiratory ($n = 305$) specimens. Using culture as the gold standard, we found a sensitivity of 88.4% and a specificity of 98.8% for the 838 respiratory specimens, compared to a sensitivity of 63.6% and a specificity of 94.6% for the 305 nonrespiratory specimens. We conclude that the Cobas TaqMan MTB assay is a significantly improved tool for the direct detection of *M. tuberculosis* DNA in clinical specimens.

Direct detection of *Mycobacterium tuberculosis* complex DNA from clinical specimens has become an important part of diagnostic mycobacteriology. PCR-based assays detect DNA that can originate from living or dead cells. The clinical interpretation of *M. tuberculosis* complex DNA detection is dependent on the patient's clinical data and complementary laboratory data, such as smear microscopy and culture. Several commercial tests have been introduced during the past few years, for example, the Cobas Amplicor *M. tuberculosis* (MTB) PCR test (Roche Diagnostics, Rotkreuz, Switzerland), the Gen-Probe Amplified *M. tuberculosis* direct (MTD) test (Gen-Probe, Inc., San Diego, CA), and the BD ProbeTec direct system (Becton Dickinson, Sparks, MD).

The Cobas Amplicor test is based on amplification of a 584-bp 5′ part of the 16S rRNA gene (1, 2) using biotinylated primers, a capture probe, and photometric staining for quantification (3, 4). The Cobas Amplicor test was extensively evaluated for various clinical specimens and demonstrated high sensitivity and specificity, in particular for smear-positive samples (5). We reported previously on a substantial rate of false-positive samples, in particular when the Cobas Amplicor MTB test showed results with optical density at 660 nm (OD_{660}) values of >0.35 and <2.0 (6). The observed false-positive results were demonstrated to be due to cross-reactivity of the capture probe with closely related species, such as nontuberculous mycobacterial species and *Corynebacterium* spp. (6).

Recently, Roche Diagnostics (Rotkreuz, Switzerland) replaced the Cobas Amplicor MTB test with the Cobas TaqMan MTB test. The Cobas TaqMan MTB test is a real-time PCR assay that amplifies part of the 16S rRNA gene with the use of a TaqMan probe for the detection of *Mycobacterium tuberculosis* complex DNA in clinical specimens (7). Here, we evaluated the Cobas TaqMan MTB assay and compared its performance with that of the Cobas Amplicor MTB assay. In a prospective study, we analyzed the performance of the Cobas TaqMan MTB assay for routine mycobacteriology laboratory use during a 6-month period in which 1,143 specimens were submitted for MTB PCR testing.

MATERIALS AND METHODS

Patient population. The Institute of Medical Microbiology (IMM) serves the 850-bed tertiary University Hospital of Zurich and smaller surrounding hospitals. The patients included adults and children.

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 (8). Auramine-rhodamine fluorochrome staining was used for microscopic examination; positive microscopy results were confirmed using Ziehl-Neelsen staining (8). For the recovery of mycobacteria from culture, standard media were inoculated (7H11 plates and BBL MGIT [Becton, Dickinson and Company, Allschwill, Switzerland]) and maintained for 7 weeks at 37°C. Mycobacteria were identified by 16S rRNA gene sequence analysis as described previously (2).

PCR detection of *Mycobacterium tuberculosis*. The Cobas TaqMan MTB test was developed primarily for liquefied, decontaminated, and concentrated samples from human respiratory patients. In addition to respiratory specimens, we also tested nonrespiratory specimens, such as biopsy specimens and urine, puncture fluid, and cerebrospinal fluid (CSF) samples. For this study, we used clinical samples submitted to the routine mycobacteriology laboratory for molecular detection of *M. tuberculosis*. The Cobas Amplicor MTB and Cobas TaqMan MTB assays (Roche Diagnostics, Rotkreuz, Switzerland) were performed according to the manufacturer's instructions (4, 7). DNA was extracted from decon-

Received 17 January 2013 Returned for modification 27 February 2013
Accepted 17 April 2013
Published ahead of print 24 April 2013
Address correspondence to Guido V. Bloemberg, bloemberg@imm.uzh.ch.

doi:10.1128/JCM.00142-13

TABLE 1 Analysis of selected clinical specimens with a low-positive value in the Cobas Amplicor MTB test

Sample no.	Specimen type	MTB Cobas Amplicor result		Results with:			*M. tuberculosis* detection in other patient samples	Comment
		OD_{660} value[a]	16S rRNA gene sequence[b]	MTB Cobas TaqMan	Smear	Culture[c]		
1	Respiratory	0.368	*Corynebacterium pseudodiphtheriticum*	Negative	Negative	*Mycobacterium kansasii*	Negative	False positive
2	Respiratory	0.432	Low-quality sequence	Negative	Negative	ND[d]	Negative	False positive
3	Respiratory	0.519	Low-quality sequence	Negative	Negative	Negative	Negative	False positive
4	Respiratory	0.537	*Corynebacterium pseudodiphtheriticum*	Negative	Negative	Negative	Negative	False positive
5	Respiratory	0.542	*Corynebacterium durum*	Negative	Negative	Negative	Negative	False positive
6	Respiratory	0.624	*Corynebacterium durum*	Negative	Negative	Negative	Negative	False positive
7	Respiratory	0.637	Low-quality sequence	Negative	Negative	Negative	Negative	False positive
8	Respiratory	0.664	*Mycobacterium chelonae/abscessus* complex	Negative	Negative	*Mycobacterium chelonae*	Negative	False positive
9	Respiratory	0.71	Low-quality sequence	Negative	Negative	Negative	Negative	False positive
10	Respiratory	0.846	Low-quality sequence	Negative	Negative	Negative	Negative	False positive
11	Respiratory	1.33	*Corynebacterium propinquum*	Negative	ND	ND	Negative	False positive

[a] OD_{660} values of >0.350 are scored positive for *M. tuberculosis* detection.
[b] Sequencing was performed on PCR products obtained by amplification of nucleic acids from the clinical samples.
[c] Identification by 16S rRNA gene sequence analysis, followed by *rpoB* or *hsp65* sequence analysis.
[d] ND, not determined.

taminated samples (0.5 ml) by using a respiratory specimen preparation kit (Roche Diagnostics) (4). According to the manufacturer, the Cobas Amplicor MTB 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* if 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 (4). For the Cobas TaqMan assay, a sample was interpreted as positive when a crossing point (CP) was registered at <45 (7).

Amplification, DNA purification, and sequencing of positive samples and cultures. Samples that were scored positive in the Cobas Amplicor MTB assay with OD_{660} values of ≥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 (5′-CAC ATG CAA GTC GAA CGG AAA GG-3′) and KY75 (5′-GCC CGT ATC GCC CGC ACG CTC ACA-3′) or primers 283 and 264 were used in separate PCRs as described previously (9, 10). PCR products were sequenced using the primer Mbakt-14 (5′-GRG RTA CTCGAG TGG CGA AC-3′) (11). If unsatisfactory PCR or sequencing results were obtained, reamplification was done with primers KY18 and 259 or primers 283 and 259 (2, 9). Amplicons with CPs of >45 or maximum curve values of <0.5 by the Cobas TaqMan MTB assay were purified using a PCR purification kit (Qiagen, Hombrechtikon, Switzerland) and subjected to sequencing using the primer Mbakt-14.

Sequences were analyzed using SmartGene IDNS software and databases (SmartGene, Zug, Switzerland). Homology analysis and species identification were carried out as described previously (9, 12, 13). Sequencing of 16S rRNA genes from cultures was performed using the primers 283 and 264 for PCR amplification and the primer Mbakt-14 for sequencing (10). In the case of *Mycobacterium chelonae* complex identification, sequence analysis of *rpoB* (14) was used for species assignment, e.g., to *M. massiliense*, *M. bolletii*, *M. abscessus*, or *M. chelonae*. In the case of *M. kansasii*/*M. gastri* identification, sequence analysis of *hsp65* was used for differentiation (15).

RESULTS

Retrospective testing of clinical specimens categorized as false positive by the Cobas Amplicor MTB assay. We studied 11 clinical respiratory specimens (collected from 11 patients in 2009) which were scored positive by the Cobas Amplicor assay (OD_{660}s, >0.350 and <1.5) (Table 1). As determined by 16S rRNA gene sequencing, five of these samples showed the presence of *Corynebacterium* spp., one sample showed the presence of the *M. chelonae* complex, and the remaining 5 samples produced DNA sequences of low quality, which did not allow species identification. On the basis of 16S rRNA gene sequencing and homology analysis, microscopy, cultural recovery, and clinical data, these samples were categorized as false positive by the Cobas Amplicor assay. All samples were scored negative by the Cobas TaqMan MTB test (Table 1).

Comparative analysis of the Cobas Amplicor MTB and Cobas TaqMan MTB assays. The 133 clinical specimens received in the routine diagnostic laboratory for testing for the presence of the *Mycobacterium tuberculosis* complex were subjected in parallel to the Cobas Amplicor and Cobas TaqMan assays (Table 2). Clinical samples (n = 133) consisted of 100 respiratory specimens (sputum, n = 52; tracheal bronchial aspirate, n = 24; and bronchial alveolar lavage fluid, n = 24) and 33 nonrespiratory specimens (biopsy, n = 10; urine, n = 8; pleura aspirate, n = 7; ascites, n = 2;

TABLE 2 Comparative analysis of Cobas Amplicor and Cobas TaqMan MTB based on parallel testing of clinical samples (n = 112)[a]

Result by Cobas TaqMan MTB test	Result by COBAS Amplicor MTB test					
	Positive	Negative	Total	Agreement	Ratio	%
Positive	8	0	8	Positive	8/10	80.0
Negative	2[b]	102	104	Negative	102/102	100
Total	10	102	112[c]	Discrepant	2/112	1.8
				Total	110/112	98.2

[a] Clinical samples (n = 112) consisted of 82 respiratory specimens (sputum, n = 43; bronchial alveolar lavage fluid, n = 20; and tracheal bronchial aspirate, n = 19) and 30 nonrespiratory specimens (urine, n = 8; pleura aspirate, n = 7; biopsy, n = 7; ascites, n = 2; cerebrospinal fluid [CSF], n = 2; ear swab, n = 1; aspirate, n = 1; catheter drainage, n = 1; and deep wound swab, n = 1). The eight samples that were positive in both the Cobas Amplicor and Cobas TaqMan assays were respiratory (n = 6), deep wound swab (n = 1), and CSF (n = 1) specimens. The two false-positive samples in the Cobas Amplicor assay were both respiratory specimens.
[b] False positives by Cobas Amplicor MTB.
[c] A total of 112 samples not inhibited for PCR were eligible for this analysis.

TABLE 3 Evaluation of the Cobas TaqMan MTB test performance using respiratory and nonrespiratory clinical specimens ($n = 1,109$) with culture as the gold standard

Specimen type	MTB PCR results	MTB culture results		Total no. of specimens	Sensitivity (%)	Specificity (%)
		No. of specimens positive	No. of specimens negative			
Respiratory and nonrespiratory ($n = 1,109$)	Positive	75	23[a]	98		
	Negative	16	995	1,011		
	Total	91	1018	1,109	82.4	97.7
Respiratory ($n = 829$)	Positive	61	9[a]	70		
	Negative	8	751	759		
	Total	69	760	829	88.4	98.8
Nonrespiratory ($n = 280$)	Positive	14	14[a]			
	Negative	8	244			
	Total	22	258		63.6	94.6

[a] These specimens were all from patients who had a clinical history of tuberculosis (see Table 4). Accordingly, the specificity of Cobas TaqMan MTB is to be calculated as 100%.

cerebrospinal fluid, $n = 2$; ear swab, $n = 1$; aspirate, $n = 1$; catheter drainage, $n = 1$; and deep wound swab, $n = 1$).

Twenty-one of the 133 samples (respiratory, $n = 18$; and biopsy, $n = 3$) showed inhibition of the PCRs (i) by both assays ($n = 6$), (ii) by the Cobas Amplicor assay only ($n = 12$), or (iii) by the Cobas TaqMan assay only ($n = 3$). One tracheal bronchial aspirate showed inhibition of the PCR by the Cobas Amplicor assay but was scored positive by the Cobas TaqMan assay. Sequencing of the amplicon generated by the Cobas TaqMan assay revealed the presence of *M. tuberculosis* complex DNA. The sample was smear negative but culture positive for *M. tuberculosis*, confirming the positive result from the Cobas TaqMan assay. The remaining clinical samples that showed inhibition ($n = 20$) were all scored negative for *M. tuberculosis* by culture.

The 112 noninhibited specimens (82 respiratory and 30 nonrespiratory) were eligible for analysis of concordance between the Cobas Amplicor and the Cobas TaqMan assays (Table 2). Two sputum specimens, which were scored positive for *M. tuberculosis* by the Cobas Amplicor assay (OD_{660}s, 0.597 and 0.643, respectively) and negative by the Cobas TaqMan assay, were evaluated in further detail. Sequence analysis of the PCR amplicon showed the presence of *Mycobacterium massiliense* in one sample and *Corynebacterium pseudodiphtheriticum* in the second sample; these samples were smear and *M. tuberculosis* culture negative. From these two patients, a total of 5 samples were evaluated, none of which was positive by culture or PCR for *M. tuberculosis*. Evaluation of the patients' clinical histories did not reveal any sign of *M. tuberculosis* infection. As a result, both samples were judged to be false positive by the Cobas Amplicor assay. Eight samples were positive in both the Cobas Amplicor and the Cobas TaqMan assays; all had OD_{660} values of >2.0 in the Cobas Amplicor assay. The 8 *M. tuberculosis*-positive samples included 6 respiratory, 1 deep wound swab, and 1 CSF sample. The overall agreement between the Cobas Amplicor and Cobas TaqMan assay results ($n = 112$) was 98.2%, with a positive agreement of 80% and a negative agreement of 100% (Table 2).

Prospective evaluation of the Cobas TaqMan MTB assay in the routine mycobacteriology laboratory. Over a period of 6 months, 1,143 respiratory and nonrespiratory clinical samples submitted to the routine mycobacteriology laboratory were tested for the presence of *M. tuberculosis* DNA in a prospective study design. Inhibition of the PCR, as indicated by a negative internal control as used in the *M. tuberculosis* assay, occurred for 34 (3.0%) of the specimens. None of the PCR-inhibited samples was smear positive and/or *M. tuberculosis* culture positive. A negative PCR result was obtained for 1,011 (88.5%) of the specimens. Ninety-eight (8.5%) samples tested positive by the Cobas TaqMan *M. tuberculosis* assay. Seventy-five of the 98 specimens were positive for *M. tuberculosis* with MTB PCR and culture, and 52 of these specimens were smear microscopy positive. Twenty-three of the 98 MTB PCR-positive specimens were *M. tuberculosis* culture negative, of which 7 were smear microscopy positive (Tables 3 and 4). Evaluation of the patients' clinical histories showed that all 23 culture-negative specimens were finally categorized as true positives for the detection of *M. tuberculosis* DNA (Table 4). The 23 specimens were collected from 13 patients who had a clinical history of tuberculosis. For 12 of the 13 patients, at least one positive *M. tuberculosis* culture was grown from another sample. For 1 of the 13 patients (Table 4, specimen no. 20), no positive culture was registered. However, this patient had undergone a *Mycobacterium bovis* BCG instillation for bladder carcinoma treatment and showed clinical symptoms of disseminated tuberculosis.

Ninety-one specimens were *M. tuberculosis* culture positive, and 16 of them (sputum, $n = 4$; aspirate, $n = 3$; lymph node, $n = 3$; tracheal bronchial aspirate, $n = 2$; bronchial aspirate, $n = 2$; gastric fluid, $n = 1$; and ascites, $n = 1$) were negative by MTB PCR testing. None of these 16 specimens was smear microscopy positive.

Since all specimens were analyzed for culture, we used this parameter as the gold standard for the calculation of sensitivity and specificity. Overall, we determined a sensitivity of 82.4% and a specificity of 97.7% for the Cobas TaqMan MTB assay (Table 3).

The overall sensitivity of the Cobas TaqMan MTB assay for microscopy smear-positive samples was 100% and for smear-negative samples was 73.6%.

The 1,143 clinical specimens consisted of 838 respiratory samples (sputum, $n = 501$; tracheal bronchial aspirate, $n = 181$; bronchial alveolar lavage fluid, $n = 139$; bronchial aspirate, $n = 8$; and others, $n = 9$) and 305 nonrespiratory specimens (aspirate, $n = 102$; tissue, $n = 78$; urine, $n = 27$; cerebrospinal fluid (CSF), $n = 23$; biopsy, $n = 9$; wound swab, $n = 12$; lymph node, $n = 11$; gastric fluid, $n = 9$; bone marrow, $n = 9$; ascites, $n = 6$; abscess,

TABLE 4 Analysis of specimens positive by the Cobas TaqMan MTB test and negative for culture

Specimen no.	Specimen type	MTB PCR CP[a]	Assay results		Clinical history[b]	TB antibiotic therapy[c]
			Culture	Smear microscopy		
1	Tissue	28.29	Negative	Negative	Spondylitis TB	Yes
2	Lymph node	30.85	Negative	Positive	Previous miliary TB	Past therapy
3	Lymph node	30.85	Negative	Positive	Pulmonary TB	No
4	Sputum	32.8	Negative	Positive	Pulmonary TB	Yes
5	Abscess	33.42	Negative	Positive	Spondylitis TB	Yes
6	Sputum	33.5	Negative	Positive	Pulmonary TB	Yes
7	Sputum	34.01	Negative	Positive	Pulmonary TB	Yes
8	Sputum	36.07	Negative	Negative	Pulmonary TB	Yes
9	Sputum	36.39	Negative	Positive	Pulmonary TB	Yes
10	Gastric fluid	36.5	Negative	Negative	Pulmonary TB	No
11	Tissue	36.97	Negative	Negative	Spondylitis TB	yes
12	Tissue	37.76	Negative	Negative	Spondylitis TB	Yes
13	Tissue	38.4	Negative	Negative	Spondylitis TB	Yes
14	Sputum	38.56	Negative	Negative	Previous pulmonary TB	Past therapy
15	Tissue	38.9	Negative	Negative	Spondylitis TB	Yes
16	Sputum	39.09	Negative	Negative	Previous pulmonary TB	Past therapy
17	Aspirate	39.23	Negative	Negative	Pulmonary TB	Yes
18	Wound swab	40.01	Negative	Negative	Spondylitis TB	Yes
19	Lymph node	40.33	Negative	Negative	Previous pulmonary TB	Past therapy
20	Urine	40.81	Negative	Negative	BCG instillation in bladder	No
21	Sputum	40.85	Negative	Negative	Pulmonary TB	Yes
22	Sputum	41.69[d]	Negative	Negative	Previous pulmonary TB	Past therapy
23	Tissue	>45[d]	Negative	Negative	Spondylitis TB	Yes

[a] CP, crossing point.
[b] For all patients, except the patient with a BCG instillation in the bladder (specimen 20), *M. tuberculosis* was cultured from at least one other patient specimen.
[c] TB, tuberculosis.
[d] *M. tuberculosis* identification was confirmed by sequence analysis of the PCR amplicon.

$n = 5$; stool, $n = 5$; blood EDTA, $n = 4$; nonspecified, $n = 3$; bone, $n = 1$; and ejaculate, $n = 1$) (Table 5).

Nine of the 838 (1.1%) respiratory samples, which were all sputum specimens, were inhibited for PCR. Seven hundred fifty-nine of the 829 noninhibited specimens were MTB PCR negative, and 70/829 were MTB PCR positive. Using culture results as the gold standard, we found a sensitivity of 88.4% and a specificity of 98.8% for respiratory specimens (Table 3).

TABLE 5 Prospective analysis of nonrespiratory samples ($n = 305$) tested by Cobas MTB TaqMan PCR and culture

Specimen type	Total no. of samples	No. of samples with the indicated Cobas MTB TaqMan PCR and *M. tuberculosis* culture results				No. (%) of samples inhibited for PCR
		Negative and negative	Negative and positive	Positive and positive	Positive and negative	
Aspirate	102	93	3	4	1	1 (1.0)
Tissue	78	57		3	6	12 (15.4)
Urine	27	26			1	0
CSF	23	22				1 (4.3)
Biopsy	9	8		1		0
Wound swab	12	11			1	0
Lymph node	11		3	4	3	1 (9.1)
Gastric fluid	9	6	1		1	1 (11.1)
Bone marrow	9	5				4 (44.4)
Ascites	6	5	1			0
Abscess	5	1		2	1	1 (20.0)
Stool	5	2				3 (60.0)
Blood	4	3				1 (25)
Not specified	3	3				0
Bone	1	1				0
Ejaculate	1	1				0
Total	305	244	8	14	14	25 (8.2)

Twenty-five of the 305 (8.2%) nonrespiratory samples (tissue, $n = 12$; bone marrow, $n = 4$; stool, $n = 3$; abscess, $n = 1$; blood EDTA, $n = 1$; CSF, $n = 1$; lymph node, $n = 1$; gastric fluid, $n = 1$; and aspirate, $n = 1$) were inhibited for PCR (Table 5). Two hundred fifty-two of the 280 noninhibited specimens were MTB PCR negative, and 28/280 were MTB PCR positive. Using culture as the gold standard, we found a sensitivity of 63.8% and a specificity of 94.6% for nonrespiratory specimens (Table 3).

DISCUSSION

PCR-based tests allow rapid and reliable laboratory detection of *M. tuberculosis* DNA, which is crucial for early diagnosis and clinical management and for proper therapy. If there is a suspicion of tuberculosis on the basis of the clinical data, it is recommended that three clinical samples be submitted to the diagnostic laboratory for smear microscopy, culture, and, preferably, MTB PCR analyses. The Cobas Amplicor MTB test is commonly regarded as a reliable assay for the majority of specimens tested (16, 17, 18). However, we previously reported on a substantial rate of false-positive results for OD_{660} values of <2.0 (6).

The limited specificity was corroborated by an additional 11 specimens that were identified in our routine diagnostic laboratory in 2009 and categorized as false positives based on microscopy, cultures, results of other patients' specimens, and evaluation of the patients' clinical histories (Table 1). Roche recently replaced the Cobas Amplicor MTB test with the Cobas TaqMan MTB test. We initially studied the 11 selected patient specimens that were scored false positive with the Cobas Amplicor MTB test. A significantly increased specificity was found, as all 11 samples tested negative with the Cobas TaqMan assay (Table 1).

To comparatively analyze the Cobas Amplicor and Cobas TaqMan MTB tests, a set of 133 clinical samples randomly obtained from our routine diagnostic workflow, consisting of 82 respiratory and 30 nonrespiratory specimens, were tested in both assays. A 50% reduction in the number of specimens for which PCR was inhibited was observed using the Cobas TaqMan MTB test (9/133 inhibited) in comparison with the Cobas Amplicor MTB test (18/133 inhibited). Since samples were tested in parallel using the same nucleic acid extract, this can be explained only by differences in test conditions. Of note, one of these specimens tested positive by the Cobas TaqMan assay. Analysis of the results of the 112 eligible samples showed a concordance of 98.2% (Table 2). Two respiratory samples scored positive in the Cobas Amplicor MTB test and negative in the Cobas TaqMan MTB assay (Table 2). In-depth analyses of these samples combined with the patients' clinical histories categorized the two samples as false positives.

In a prospective study design, we evaluated the Cobas MTB TaqMan assay for clinical samples ($n = 1{,}143$), respiratory and nonrespiratory, submitted to the routine mycobacteriology laboratory for *M. tuberculosis* PCR testing (Tables 3, 4, and 5). The results showed a 97.7% specificity for detection of *M. tuberculosis* using culture as the gold standard (Table 3). However, analysis of the data from all 13 patients with specimens ($n = 23$) that produced culture-negative and PCR-positive results showed a clinical history of tuberculosis; thus, the specificity of the Cobas TaqMan MTB assay is higher than 97.7%. The Cobas MTB TaqMan test was developed primarily for respiratory specimens. However, we also analyzed a substantial set of nonrespiratory specimens (Table 5), the results of which showed that this assay is also suitable for such samples (Table 3). With culture as the gold standard, the sensitivity for respiratory specimens (88.4%) was significantly higher than that for nonrespiratory samples (63.6%) (Table 3). Inhibition of PCR was more frequently observed for nonrespiratory samples (8.2%) than for respiratory samples (1.1%). Reduced sensitivity and increased PCR inhibition in nonrespiratory samples, such as stool, bone marrow, tissue, and blood, is presumably due to a diverse range of PCR inhibitors (19). In addition, unequal distribution of bacterial cells is more likely to occur in nonrespiratory specimens, such as tissue, than in respiratory samples. Overall, based on patients' clinical histories, the MTB PCR performed well in comparison to culture (for a detailed comparison on the basis of single-specimen analysis, see Table 3).

In general, the results found in this study are in line with those of recently published studies on the performance of the Cobas TaqMan assay (20, 21, 22, 23, 24, 25, 26). However, those studies were restricted mostly to respiratory samples, discrepant test results were not analyzed in detail, and sequence analysis of the amplicon was not used for validation of the test results.

In conclusion, the specificity of the Cobas TaqMan MTB test is significantly improved over that of the Cobas Amplicor MTB test, and the number of observed PCR inhibitions was reduced by 50% using the Cobas TaqMan assay. Another advantage of the Cobas TaqMan assay is the possibility of direct sequencing of the PCR amplicon following a relatively simple purification step. The sequence of the amplicon containing hypervariable regions A and B of the 16S rRNA gene (9) allows for the accurate identification of *M. tuberculosis* in the case of doubtful or borderline test results.

ACKNOWLEDGMENTS

This work was supported in part by the University of Zurich and the BAG (Federal Office of Public Health, Switzerland).

We declare no conflicts of interest.

REFERENCES

1. **Böddinghaus B, Rogall T, Flohr T, Blocker H, Böttger EC.** 1990. Detection and identification of mycobacteria by amplification of rRNA. J. Clin. Microbiol. **28:**1751–1759.
2. **Kirschner P, Rosenau J, Springer B, Teschner K, Feldmann K, Böttger EC.** 1996. Diagnosis of mycobacterial infections by nucleic acid amplification: 18-month prospective study. J. Clin. Microbiol. **34:**304–312.
3. **Tevere VJ, Hewitt PL, Dare A, Hocknell P, Keen A, Spadoro JP, Young KKY.** 1996. Detection of *Mycobacterium tuberculosis* by PCR amplification with pan-*Mycobacterium* primers and hybridization to an *M. tuberculosis* specific probe. J. Clin. Microbiol. **34:**918–923.
4. **Roche Diagnostics.** 2007. Cobas Amplicor *Mycobacterium tuberculosis* test: instruction manual. Roche Diagnostics, Mannheim, Germany.
5. **Piersimoni C, Scarparo C.** 2003. Relevance of commercial amplification methods for direct detection of *Mycobacterium tuberculosis* complex in clinical samples. J. Clin. Microbiol. **41:**5355–5365.
6. **Peter-Getzlaff S, Lüthy J, Voit A, Bloemberg GV, Böttger EC.** 2010. Detection and identification of *Mycobacterium* spp. in clinical specimens by combining Roche Cobas Amplicor *Mycobacterium tuberculosis* assay with *Mycobacterium* genus detection and nucleic acid sequencing. J. Clin. Microbiol. **48:**3943–3948.
7. **Roche Diagnostics.** 2009. Cobas TaqMan *Mycobacterium tuberculosis* test: instruction manual. Roche Diagnostics, Mannheim, Germany.
8. **Isenberg HD (ed).** 1992. Clinical microbiology procedures handbook, vol 1. ASM Press, Washington, DC.
9. **Kirschner P, Springer B, Vogel U, Meier A, Wrede A, Kiekenbeck M, Bange FC, Böttger EC.** 1993. Genotypic identification of mycobacteria by nucleic acid sequence determination: report of a 2-year experience in a clinical laboratory. J. Clin. Microbiol. **31:**2882–2889.
10. **Peter-Getzlaff S, Lüthy J, Böddinghaus B, Böttger EC, Springer B.** 2008. Development and evaluation of a molecular assay for detection of nontuberculous mycobacteria by use of the Cobas Amplicor platform. J. Clin. Microbiol. **46:**4023–4028.

11. **Bosshard PP, Zbinden R, Abels S, Böddinghaus B, Altwegg M, Böttger EC.** 2006. 16S rRNA gene sequencing versus the API 20 NE system and the Vitek 2 ID-GNB card for identification of nonfermenting gram-negative bacteria in the clinical laboratory. J. Clin. Microbiol. **44:**1359–1366.
12. **Rogall T, Flohr T, Böttger EC.** 1990. Differentiation of *Mycobacterium* species by direct sequencing of amplified DNA. J. Gen. Microbiol. **136:** 1915–1920.
13. **Springer B, Stockman L, Teschner K, Roberts GD, Böttger EC.** 1996. Two-laboratory collaborative study on identification of mycobacteria: molecular versus phenotypic methods. J. Clin. Microbiol. **34:**296–303.
14. **Adékambi T, Colston P, Drancourt M.** 2003. *rpoB*-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. J. Clin. Microbiol. **41:**5699–5708.
15. **Telenti A, Marchesi F, Balz M, Bally F, Böttger EC, Bodmer T.** 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J. Clin. Microbiol. **31:**175–178.
16. **Eing BR, Becker A, Sohns A, Ringelmann R.** 1998. Comparison of Roche Cobas Amplicor *Mycobacterium tuberculosis* assay with in-house PCR and culture for detection of *M. tuberculosis*. J. Clin. Microbiol. **36:**2023–2029.
17. **Reischl U, Lehn N, Wolf H, Naumann L.** 1998. Clinical evaluation of the automated Cobas Amplicor MTB assay for testing respiratory and nonrespiratory specimens. J. Clin. Microbiol. **36:**2853–2860.
18. **Tortoli E, Tronci M, Tosi CP, Galli C, Lavinia F, Natili S, Goglio A.** 1999. Multicenter evaluation of two commercial amplification kits (Amplicor, Roche and LCx, Abbott) for direct detection of *Mycobacterium tuberculosis* in pulmonary and extrapulmonary specimens. Diagn. Microbiol. Infect. Dis. **33:**173–179.
19. **Hedman H, Radstrom P.** 2013. Overcoming inhibition in real-time diagnostic PCR, p 17–48. *In* Wilks M (ed), PCR detection of microbial pathogens, methods in molecular biology, vol 943, 2nd ed. Springer Verlag Science+Business Media LLC, New York, NY.
20. **Chandran SP, Kenneth J.** 2010. Evaluation of Cobas TaqMan real time PCR assay for the diagnosis of *Mycobacterium tuberculosis*. Indian J. Med. Res. **132:**100–102.
21. **Kim JH, Kim YJ, Ki CS, Kim JY, Lee NY.** 2011. Evaluation of Cobas TaqMan MTB PCR for detection of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **49:**173–176.
22. **Causse M, Ruiz P, Gutiérrez Aroca JB, Casal M.** 2011. Comparison of two molecular methods for rapid diagnosis of extrapulmonary tuberculosis. J. Clin. Microbiol. **49:**3065–3067.
23. **Cho SY, Kim MJ, Suh JT, Lee HJ.** 2011. Comparison of diagnostic performance of three real-time PCR kits for detecting *Mycobacterium* species. Yonsei Med. J. **52:**301–306.
24. **Yang YC, Lu PL, Huang SC, Jenh YS, Jou R, Chang TC.** 2011. Evaluation of the Cobas TaqMan MTB test for direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens. J. Clin. Microbiol. **49:**797–801.
25. **Tortoli E, Urbano P, Marcelli F, Simonetti MT, Cirillo MD.** 2012. Is real-time PCR better than conventional PCR for *Mycobacterium tuberculosis* complex detection in clinical samples? J. Clin. Microbiol. **50:**2810–2813.
26. **Linasmita P, Srisangkaew S, Wongsuk T, Bhongmakapat T, Watcharananan SP.** 2012. Evaluation of real-time polymerase chain reaction for detection of the 16S ribosomal RNA gene of *Mycobacterium tuberculosis* and the diagnosis of cervical tuberculous lymphadenitis in a country with a high tuberculosis incidence. Clin. Infect. Dis. **55:**313–321.