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Is Real-Time PCR Better than Conventional PCR for *Mycobacterium tuberculosis* Complex Detection in Clinical Samples?

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Cobas Amplicor MTB and later Cobas TaqMan MTB were used to test a very large series of consecutive specimens received for tuberculosis diagnosis. Performance parameters were estimated and compared overall and for separate specimen categories. Both systems showed excellent specificity, and that of TaqMan was the higher. The sensitivities were similar but satisfactory only with respiratory specimens and smear-positive samples.

The molecular methods for the diagnosis of tuberculosis (TB) directly with clinical specimens, in use since the early 1990s, remain far from replacing microscopy and culture. Their major limitation is poor sensitivity with paucibacillary specimens such as microscopy-negative and extrapulmonary samples (16). Several generations of tests, based on different technologies, have been introduced in the past 15 years. Major progress has been made in improving specificity, which is nearly 100% with the presently available methods. One of the first commercialized amplification methods was the Amplicor MTB PCR (Amplicor; Roche Diagnostics, Basel, Switzerland), which is based on the amplification of a 584-bp region of the 16S rRNA gene common to all mycobacteria (18). A few years ago, the same company developed the new Cobas TaqMan MTB PCR (TaqMan) system, which relies on real-time PCR (RT-PCR) (21).

The objective of this investigation was a retrospective comparison of the diagnostic performance of the two kits when used for the routine molecular diagnosis of TB with large numbers of clinical specimens of both pulmonary and extrapulmonary origin. The study was performed in a country with a low TB prevalence by using a database recording the laboratory results of all of the specimens received in a reference center between December 2004 and April 2010 with a request for nucleic acid amplification for the diagnosis of TB. The Amplicor system was in use until May 2008, when it was replaced with the TaqMan system. A total of 13,510 specimens (from 9,789 patients) were analyzed, 7,443 with Amplicor and 6,067 with TaqMan; the compositions of the two groups of samples are reported in Tables 1 and 2. The proportion of nonrespiratory samples was 36.2% in the first group and 28.5% in the second.

Specimen preparation was the same for both PCR systems. Samples from normally nonsterile body sites were decontaminated with *N*-acetyl-L-cysteine–NaOH (1% final concentration) and concentrated by centrifugation (6). Samples from normally sterile sites were homogenized (when needed) before resuspension in saline and concentration by centrifugation. On all of the sediments, acid-fast microscopy (auramine-rhodamine staining), culture in both solid (Lowenstein-Jensen) and liquid (MGIT; BD Biosciences, Sparks MD) media, and nucleic acid extraction and analysis were performed as recommended by the producer (18). The mycobacteria grown in culture were identified using commercial line probe assays (GenoType Mycobacterium; Hain Lifescience, Nehren, Germany) or by 16S rRNA gene sequencing.

For statistical analysis, the results of culture and identification were adopted as a surrogate gold standard for the determination of specificity, sensitivity, and positive and negative likelihood ratios (LR) of the amplification result. Comparisons of categorical variables were done with Fisher's exact test or the chi-square test with Yates' correction (two tailed), according to the magnitude of the values. A P value of <0.05 was considered statistically significant.

There were 4,751 (63%) respiratory specimens in the Amplicor group and 4,340 (71.5%) in the TaqMan system; the proportions of smear-positive specimens were 3.7% and 2.7%, respectively. Statistical parameters divided for specimen categories are summarized in Table 3.

Amplicor detected specific DNA in 346 out of 458 samples that were culture positive for the *Mycobacterium tuberculosis* complex (MTC), while it scored negative with 93 specimens that were positive for nontuberculous mycobacteria (NTM) by culture. With TaqMan, amplification was positive for 191 out of 247 samples yielding MTC by culture and negative for 119 samples that yielded NTM. Thirty culture-negative samples scored positive with Amplicor, and four scored positive with TaqMan.

The overall sensitivity was 75.5% for Amplicor and 77.3% for TaqMan—a nonsignificant difference ($P = 0.64$). The specificity of both methods was high, but that of the TaqMan system (99.9%) was significantly higher ($P < 0.001$) than that of the Amplicor system (99.5%). The proportion of missed amplifications because of the presence of inhibitors was significantly lower with TaqMan, at 3.0% versus 4.1% ($P < 0.001$).

As expected, the sensitivity of both systems, was significantly higher with the pulmonary specimens than with the extrapulmonary ones and with the smear-positive ones than with the smear-negative ones.

When their performance with different clinical specimens is considered, both methods reached a sensitivity of >90% with sputum samples; both had a sensitivity of <50% with cerebrospi-

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TABLE 1 Comparison of Amplicor and culture results[a]

Material(s)	True positives, MTC^+/PCR^+	False positives, MTC^-/PCR^+	True negatives		False negatives, MTC^+/PCR^-	Cases not included				Total no. (%)
			MTC^-/PCR^-	NTM^+/PCR^-		$MTC^+/PCRi$	$MTC^-/PCRi$	Cont/PCRi	Cont/PCR^-	
Biopsy specimen	37	7	355	4	15	3	55	0	3	479 (6.4)
Bronchial lavage fluid	53	9	2,870	52	34	0	64	1	11	3,094 (41.6)
Cavitary fluid[b]	5	1	398	1	1	0	16	0	0	422 (5.7)
Gastric aspirate	13	2	138	1	9	1	7	0	3	174 (2.3)
CSF[c]	2	2	346	0	3	0	8	0	1	362 (4.9)
Pleural fluid	4	3	825	1	13	0	19	0	1	866 (11.6)
Pus	13	1	134	2	9	3	2	0	0	164 (2.2)
Sputum	207	5	1,156	32	21	7	41	1	13	1,483 (19.9)
Urine	5	0	281	0	6	4	70	0	2	368 (4.9)
Other	7	0	20	0	1	2	1	0	0	31 (0.4)
All	346	30	6,523	93	112	20	283	2	34	7,443

[a] PCR^+, PCR positive; PCR^-, PCR negative; PCRi, PCR inhibited; MTC^+, culture positive for MTC; MTC^-, culture negative for MTC; NTM^+, culture positive for NTM and negative for MTC; Cont, culture contaminated.
[b] Peritoneal and synovial.
[c] CSF, cerebrospinal fluid.

nal and pleural fluid samples. Urine samples showed a disturbingly high frequency of inhibition, more so with the Amplicor system. The specificity of both methods was excellent with all of the types of samples examined and ranged from 98.1 to 100% with Amplicor and from 99.1 to 100% with TaqMan.

The overall sensitivity of microscopy was 51.6% compared to culture and 66.8% compared to nucleic acid amplification. Several studies have already assessed the performance of the Cobas Amplicor system (2, 7–9, 12–15, 17, 20, 22). Most are biased by specimen selection or by spectrum composition, so much so as to bar a proper meta-analysis. On the other hand, the system is almost superseded and is reported here for the sake of comparison with its successor.

Only a few studies, in contrast, have so far assessed the TaqMan system (3–5, 10, 21). In general, their sensitivity estimates are higher than ours, perhaps because they dealt with a much higher proportion of smear-positive samples (>12% versus our 2.7%). In the only report concerning nonrespiratory specimens (3), a sensitivity as high as 78% was obtained; the sharp difference from our estimate here (64%) remains inexplicable. Two of the studies above compared Amplicor with TaqMan; one of them (5), with a limited sample, reported 100% sensitivity for both systems, while the other reported 20% greater sensitivity with TaqMan than with Cobas (10). In contrast, we were not able to detect any difference; it is noteworthy, however, that while the TaqMan sensitivity reported by Kim et al. is similar to ours (77.3% versus 79.1%), Amplicor was much more sensitive in our study (75.5% versus 58.3%).

The present study, based on the PCR results of about 13,500 consecutive clinical samples, unexpectedly demonstrates that a shift from a conventional to an RT-PCR assay system (both produced by the same company and using the same sample size and extraction procedure) did not produce any benefit in terms of sensitivity. In contrast, a significant improvement in specificity and a decrease in the number of invalid results were achieved. The LR furthermore revealed a clear improvement in posttest probabilities for results of TaqMan in comparison with those of Amplicor.

TABLE 2 Comparison of TaqMan and culture results[a]

Material(s)	True positives, MTC^+/RTP^+	False positives, MTC^-/RTP^+	True negatives		False negatives, MTC^+/RTP^-	Cases not included				Total no. (%)
			MTC^-/RTP^-	NTM^+/RTP^-		$MTC^+/RTPi$	$MTC^-/RTPi$	Cont/RTPi	Cont/RTP^-	
Biopsy specimen	18	2	208	5	3	2	50	0	0	288 (4.7)
Bronchial lavage fluid	35	2	2,644	64	16	0	68	0	8	2,837 (46.8)
Cavitary fluid[b]	1	0	153	0	1	0	1	0	0	156 (2.6)
Gastric aspirate	6	0	83	0	8	2	1	0	0	100 (1.6)
CSF[c]	1	0	228	0	3	0	0	0	0	232 (3.8)
Pleural fluid	1	0	646	0	10	0	4	0	1	662 (10.9)
Pus	10	0	63	4	3	0	1	1	0	82 (1.3)
Sputum	114	0	1,200	44	12	3	26	0	4	1,403 (23.1)
Urine	4	0	243	0	0	0	23	0	0	270 (4.4)
Other	1	0	34	2	0	0	0	0	0	37 (0.6)
All	191	4	5,502	119	56	7	174	1	13	6,067

[a] RTP^+, RT-PCR positive; RTP^-, RT-PCR negative; RTPi, RT-PCR inhibited; MTC^+, culture positive for MTC; MTC^-, culture negative for MTC; NTM^+, culture positive for NTM and negative for MTC; Cont, culture contaminated.
[b] Peritoneal and synovial.
[c] CSF, cerebrospinal fluid.

TABLE 3 Sensitivities and specificities of the two amplification systems

Material(s) and system	No. of samples	% Prevalence	% Sensitivity	95% CI[d]	Positive LR	Negative LR	% Specificity
Biopsy specimen							
Amplicor	479	10.86	71.15	65–89	37.20	0.29	98.09
TaqMan	288	7.29	85.71	64–98	92.14	0.14	99.07
Bronchial lavage fluid							
Amplicor	3,094	2.81	60.92	51–71	198.39	0.39	99.69
TaqMan	2,837	1.80	68.63	56–81	929.90	0.31	99.93
Cavitary fluid[a]							
Amplicor	422	1.42	83.33	54–113	333.33	0.17	99.75
TaqMan	156	1.28	50.00	−19–119	∞	0.50	100.00
Gastric aspirate							
Amplicor	174	12.64	59.09	38–80	41.66	0.41	98.58
TaqMan	100	14.00	42.86	17–69	∞	0.57	100.00
CSF[b]							
Amplicor	362	1.38	40.00	17–103	69.60	0.60	99.43
TaqMan	232	1.72	25.00	−17–67	∞	0.75	100.00
Pleural fluid							
Amplicor	866	1.96	23.53	3–44	65.02	0.77	99.64
TaqMan	662	1.66	9.09	−7–26	∞	0.91	100.00
Pus							
Amplicor	164	15.37	59.09	40–78	80.95	0.41	99.27
TaqMan	82	15.85	76.92	54–100	∞	0.23	100.00
Sputum							
Amplicor	1,483	2.99	90.79	87–94	216.62	0.09	99.58
TaqMan	1,403	8.98	90.48	85–96	∞	0.10	100.00
Urine							
Amplicor	368	2.99	45.45	16–75	∞	0.55	100.00
TaqMan	270	1.48	100.00	100	∞	0.00	100.00
Other							
Amplicor	31	25.81	87.50	65–110	∞	0.13	100.00
TaqMan	37	2.70	100.00	100	∞	0.00	100.00
Respiratory[c]							
Amplicor	4,751	7.09	81.01	77–85	215.94	0.19	99.62
TaqMan	4,340	4.49	81.15	76–87	1,638.05	0.19	99.95
Nonrespiratory							
Amplicor	2,692	3.01	60.33	54–72	102.61	0.40	99.41
TaqMan	1,727	3.24	64.29	50–75	510.43	0.36	99.87
Smear positive							
Amplicor	274	81.02	98.65	97–100	4.44	0.02	77.78
TaqMan	163	84.05	100.00	100	∞	0.00	100.00
Smear negative							
Amplicor	7,169	3.25	53.22	47–60	140.77	0.47	99.62
TaqMan	5,904	1.90	49.11	40–58	1,375.25	0.51	99.96
All							
Amplicor	7,443	6.15	75.55	72–80	167.36	0.25	99.55
TaqMan	6,067	4.07	77.33	72–82	1,087.42	0.23	99.93

[a] Peritoneal and synovial.
[b] CSF, cerebrospinal fluid.
[c] Sputum, bronchial lavage fluid, and gastric aspirate.
[d] CI, confidence interval.

The lack of cross-reactivity of both system with 231 samples harboring NTM (belonging to 21 different species) leads us to believe that the real specificity is 100% and that the rare false-positive results are either attributable to contamination of the specimen or are true positives (a revision of clinical data was not performed in this study). If the latter is true, our sensitivities are underestimated.

Very recently, a number of publications have assessed the performance of Xpert TB/RIF, a fully automated amplification system implementing RT-PCR. The sensitivities with respiratory specimens, with the exception of a sensational 98% reported in the first assessment (1), were around 90%. Not surprisingly the lowest sensitivity was reported in the only published study (11) presenting a proportion of smear-positive samples (4.7%) similar to ours. A recent polycentric study of a large number of nonrespiratory specimens reported an overall sensitivity of 81% (19), which is clearly better than ours with TaqMan (64%); a separate analysis reveals comparable data for smear-positive specimens but a clearly worse sensitivity than that of Xpert with the smear-negative ones (70% versus 46%). Perhaps only with paucibacillary samples does the larger sample volume (500 μl) used by Xpert raise its sensitivity.

We therefore conclude that despite technical improvements, its sensitivity remains the Achilles' heel of DNA amplification for MTC detection. Most likely, the explanation for the suboptimal results obtained must be sought upstream of the amplification and detection steps. More sophisticated extraction procedures are needed; the lysis of the mycobacterial cell wall remains problematic.

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