JOURNAL OF CLINICAL MICROBIOLOGY, May 2011, p. 1772–1776 Vol. 49, No. 5
0095-1137/11/$12.00 doi:10.1128/JCM.02157-10

Comparison of the Xpert MTB/RIF Test with an IS*6110*-TaqMan Real-Time PCR Assay for Direct Detection of *Mycobacterium tuberculosis* in Respiratory and Nonrespiratory Specimens▽

Sylvie Armand,[1] Pascale Vanhuls,[1] Guy Delcroix,[1] René Courcol,[1,2,3,4,5] and Nadine Lemaître[1,2,3,4,5]*

CHU Lille, F-59000 Lille, France[1]; Université Lille Nord de France, F-59000 Lille, France[2]; UDSL, F-59000 Lille, France[3]; INSERM U1019, F-59021 Lille, France[4]; and Centre National de la Recherche Scientifique UMR8204, F-59021 Lille, France[5]

Received 25 October 2010/Returned for modification 4 February 2011/Accepted 3 March 2011

The sensitivities of the Xpert MTB/RIF test and an in-house IS*6110*-based real-time PCR using TaqMan probes (IS*6110*-TaqMan assay) for the detection of *Mycobacterium tuberculosis* complex (MTBC) DNA were compared by use of 117 clinical specimens (97 culture positive and 20 culture negative for MTBC) that were frozen in sediment. The 97 clinical specimens included 60 respiratory and 37 nonrespiratory specimens distributed into 36 smear-positive and 61 smear-negative specimens. Among the 97 culture-positive specimens, 4 had rifampin-resistant isolates. Both methods were highly specific and exhibited excellent sensitivity (100%) with smear-positive specimens. The sensitivity of the Xpert MTB/RIF test with the whole smear-negative specimens was more reduced than that of the IS*6110*-TaqMan assay (48 *versus* 69%, $P = 0.005$). Both methods exhibited similar sensitivities with smear-negative respiratory specimens, but the Xpert MTB/RIF test had lower sensitivity with smear-negative nonrespiratory specimens than the IS*6110*-TaqMan assay (37 versus 71%, $P = 0.013$). Finally, the sensitivities of the Xpert MTB/RIF test and the IS*6110*-TaqMan assay were 79% and 84%, respectively, with respiratory specimens and 53% and 78%, respectively ($P = 0.013$), with nonrespiratory specimens. The Xpert MTB/RIF test correctly detected the rifampin resistance in smear-positive specimens but not in the one smear-negative specimen. The Xpert MTB/RIF test is a simple rapid method well adapted to a routine laboratory that appeared to be as sensitive as the IS*6110*-TaqMan assay with respiratory specimens but less sensitive with paucibacillary specimens, such as smear-negative nonrespiratory specimens.

Nucleic acid amplification assays (NAAAs) are commonly used in routine laboratories from industrialized countries for quick and specific detection of *Mycobacterium tuberculosis* complex (MTBC) in clinical specimens. Over the years, a significant improvement of PCR technologies has been achieved with the development of real-time PCR testing platforms. The main advantages of real-time PCR are a shortened turnaround time; automation of the procedure, which reduces hands-on time; and a decrease in the risk of cross-contamination (6). Recently, the GeneXpert system (Cepheid, Sunnyvale, CA), a real-time PCR that simultaneously detects both MTBC and rifampin resistance, was developed (1, 3, 9). In contrast to some real-time PCR instruments, the Xpert MTB/RIF is an on-demand assay described as a simple method that can be performed by personnel with minimal training and can provide results within 2 h (1, 3, 9). Recent studies (3, 9, 15, 16) reported a high sensitivity and specificity of the Xpert MTB/RIF test with respiratory specimens collected from patients living in countries with a high and a low prevalence of tuberculosis (TB). The detection of rifampin resistance, as a surrogate for multidrug-resistant TB (MDR-TB), directly from smear-positive respiratory specimens from patients having a high risk of MDR-TB has recently been recommended by the World Health Organization (23). Thus, the Xpert MTB/RIF test may improve the management of TB even in areas with low rates of TB and MDR-TB incidence, as in most of the industrialized countries (24).

So far, the Xpert MTB/RIF assay has not been compared with preexisting real-time PCR assays. Here, we report the first comparison of the sensitivity of this novel commercial NAAA with that of an in-house IS*6110* real-time PCR using TaqMan probes (IS*6110*-TaqMan assay) routinely used in our laboratory since 2004 (13). The sensitivities of both assays were mainly determined with paucibacillary specimens (smear negative) culture positive for MTBC because it is well documented that in-house or commercial NAAAs exhibit excellent specificity and high sensitivity with smear-positive specimens but reduced sensitivity with smear-negative specimens, especially nonrespiratory specimens (5, 8, 12, 17, 18, 19, 21). Because CHU Lille, is located in a low-prevalence area for MDR-TB, the accuracy of the Xpert MTB/RIF test was mainly evaluated for the detection of MTBC in specimens that had a rifampin-susceptible isolate.

MATERIALS AND METHODS

Specimen processing. A total of 97 clinical specimens culture positive for MTBC (93 rifampin-susceptible and 4 rifampin-resistant isolates), frozen in sediment, and preserved in archives were included. These specimens were isolated from 95 patients hospitalized at CHU Lille, and all were received for routine mycobacterial microscopy and cultivation in the laboratory between January 2009 and June 2010, except for two specimens with rifampin-resistant

* Corresponding author. Mailing address: Laboratoire de Bactériologie-Hygiène, Centre de Biologie Pathologie, CHRU de Lille, Bvd. du Professeur Jules Leclercq, 59037 Lille cedex, France. Phone: (33) 3 20 44 54 80. Fax: (33) 3 20 44 48 95. E-mail: nadine.lemaitre@chru-lille.fr.

▽ Published ahead of print on 16 March 2011.

isolates that were received in 2005 and 2006. The clinical specimens included 60 respiratory and 37 nonrespiratory specimens. The 60 respiratory specimens (33 sputum, 13 bronchial aspirate, 11 gastric aspirate, and 3 bronchoalveolar lavage [BAL] fluid specimens) were distributed among 28 smear-positive and 32 smear-negative specimens, respectively. Among the 37 nonrespiratory specimens (18 lymph node tissue, 7 pleural fluid, 6 bone tissue, and 5 abscess specimens and 1 urine specimen), 8 and 29 were smear positive and smear negative, respectively. In addition, 20 clinical specimens (10 respiratory and 10 nonrespiratory) culture negative for MTBC sent to our laboratory between January 2009 and June 2010 were included as negative controls.

All these specimens have been processed by the following standard methods (11). Briefly, after decontamination with 2% sodium hydroxide–*N*-acetyl-L-cysteine and centrifugation at 3,000 × *g* for 20 min, the sediments of specimens considered contaminated (respiratory, urine, wound drainage, or abscess specimens) were stained with auramine-rhodamine fluorochrome and inoculated into both one Löwenstein-Jensen slant and one BacT/Alert MP culture bottle (bioMérieux, Marcy l'Etoile, France). Nonrespiratory specimens from closed and normally sterile sites were not decontaminated prior to smear preparation and culture but were concentrated by centrifugation at 3,000 × *g* for 20 min. After inoculation, the remainder of the sediment was systematically frozen at −80°C and preserved in archives for further analysis, if necessary.

Real-time PCR procedures. Frozen sediments were thawed and were divided into two equal portions, one for the Xpert MTB/RIF test and the other one for the IS*6110*-TaqMan assay. NAAAs were applied in parallel in a blind manner by one laboratory technician, and the results from the two NAAAs were compared by a second person. The Xpert MTB/RIF test was done according to the instructions in the package insert. Briefly, 500 μl of sediment was mixed with 1.5 ml sample treatment buffer, shaken vigorously, and incubated for 15 min at room temperature before it was transferred to the Xpert MTB/RIF cartridge. For the IS*6110*-TaqMan assay, pretreatment of sediment was done with three cycles of freezing at −80°C and boiling for 1 min and addition of proteinase K. Then, DNA extraction was done using the MagNA pure LC automated system (Roche Diagnostics, Mannheim, Germany) with MagNA pure LC DNA isolation kit III (bacteria, fungi) reagents, as recommended by the manufacturer. Real-time PCR was performed on an ABI 7500 TaqMan system (Applied Biosystems, Warrington, United Kingdom) to amplify and detect the IS*6110* multicopy elements, as previously described (13). Each sample was tested in duplicate and coamplified with an exogenous internal positive control (Applied Biosystems) for detecting endogenous PCR inhibitors.

Statistical analysis. The results of NAAAs were compared to those of culture for MTBC (i.e., the "gold standard" of TB diagnosis). Because the analysis was done using known mycobacterial positive and negative specimens, all of these were considered true positives (Tps) and true negatives (Tns), respectively. The NAAA results were classified as Tp, false negative (Fn), Tn, and false positive (Fp). Sensitivity and specificity were calculated by [Tp/(Tp + Fn)] × 100 and [Tn/(Tn + Fp)] × 100, respectively. Statistical comparison for categorical variables was made using chi-square and McNemar tests. Comparison of variables not normally distributed was done with the Kruskal-Wallis rank-sum test. A P value of ≤0.05 was considered significant.

RESULTS

Inconclusive results obtained with both real-time PCR assays. Nine (9%) of the 97 culture-positive clinical specimens subjected to both assays had inconclusive results. All of these were smear-negative specimens. Four smear-negative nonrespiratory specimens (two lymph node tissue specimens, one bone tissue specimen, and one abscess specimen) analyzed by the Xpert MTB/RIF test were classified "invalid" (presence of inhibitors) or "no result" (aborted test). Five specimens with inconclusive results were found with the IS*6110*-TaqMan assay, including four smear-negative respiratory specimens (one sputum, one bronchial aspirate, one gastric aspirate, and one BAL fluid specimen) and one smear-negative nonrespiratory specimen (from an abscess), which corresponded to a signal detected in only one of the duplicates. The absence of inconclusive results was not due to the presence of inhibitors with the IS*6110*-TaqMan assay. Because there was not enough material to repeat the analysis, these inconclusive results were excluded from the analysis. The remaining 88 MTBC culture-positive clinical specimens included 36 smear-positive and 52 smear-negative specimens that were distributed among 56 respiratory and 32 nonrespiratory specimens (Table 1).

TABLE 1. Xpert MTB/RIF test and IS*6110*-TaqMan assay correlation for the 88 MTBC culture-positive clinical specimens

Specimen (total no.)	No. of specimens with indicated results by Xpert MTB/RIF test/IS*6110*-TaqMan assay[a]			
	Pos/pos	Neg/neg	Pos/neg	Neg/pos
Smear-positive specimens (36)				
Respiratory (28)	28	0	0	0
Sputum (20)	20	0	0	0
Bronchial aspirate (4)	4	0	0	0
Gastric aspirate (4)	4	0	0	0
Nonrespiratory (8)	8	0	0	0
Lymph node (5)	5	0	0	0
Abscess (2)	2	0	0	0
Pleural fluid (1)	1	0	0	0
Smear-negative specimens (52)				
Respiratory (28)	15	8	1	4
Sputum (12)	5	6	0	1
Bronchial aspirate (8)	5	0	1	2
Gastric aspirate (6)	3	2	0	1
BAL fluid (2)	2	0	0	0
Nonrespiratory (24)	9	7	0	8
Lymph node (11)	3	5	0	3
Bone (5)	3	1	0	1
Pleural fluid (6)	2	1	0	3
Abscesss (1)	1	0	0	0
Urine (1)	0	0	0	1

[a] Pos, positive result; Neg, negative result.

Detection of MTBC in respiratory and nonrespiratory specimens. Sixty specimens (all of the 36 smear-positive specimens and 24 smear-negative specimens) were Xpert MTB/RIF and IS*6110*-TaqMan assay positive, and 15 (29%) of the 52 smear-negative specimens were negative with both methods (Table 1). Thirteen smear-negative specimens had discrepant results. One was positive with the Xpert MTB/RIF test and negative with the IS*6110*-TaqMan assay, and 12 were negative with the Xpert MTB/RIF test and positive with the IS*6110*-TaqMan assay. The median threshold cycle (C_T) values of the IS*6110*-TaqMan assay obtained from these 12 specimens (40 cycles; range, 36 to 43 cycles) were higher than those from the 24 smear-negative specimens found to be positive with both methods (37 cycles; range, 30 to 42 cycles) ($P = 0.001$). Moreover, these 12 specimens required much longer incubation times for positive growth (median time, 35 days; range, 21 to 42 days) than the 24 smear-negative specimens found to be positive with both methods (median time, 22 days; range, 13 to 42 days) ($P = 0.001$).

In stratifying clinical specimens by their acid-fast smear status and anatomical sites using culture as the gold standard, both real-time PCR assays exhibited a sensitivity of 100% for smear-positive specimens, independent of the site where the clinical specimens were obtained (Table 2). However, the Xpert MTB/RIF test's sensitivity for smear-negative specimens decreased to 48% in comparison with the results of the IS*6110*-TaqMan assay (69%, $P = 0.005$). There was no significant difference in sensitivities between the Xpert MTB/RIF test and

TABLE 2. Sensitivities of Xpert MTB/RIF test and IS*6110*-TaqMan assay and median time to detection of MTBC in liquid culture

Specimen	Sensitivity (%)		*P* value	Median time (days) to detection of MTBC
	Xpert MTB/RIF	IS*6110*-TaqMan		
Smear positive	100	100	NS[a]	13 (6–28[b])
Respiratory	100	100	NS	13 (6–28)
Nonrespiratory	100	100	NS	15.5 (11–21)
Smear negative	48	69	0.005	28 (13–42)
Respiratory	57	68	NS	23 (13–37)
Nonrespiratory	37	71	0.013	32 (13–42)
All	69	81	0.005	21 (6–42)

[a] NS, not significant.
[b] Data in parentheses are minimum and maximum values (i.e., range).

the IS*6110*-TaqMan assay for detection of MTBC in smear-negative respiratory specimens (57% versus 68%). In contrast, the Xpert MTB/RIF test gave poorer results for smear-negative nonrespiratory specimens than the IS*6110*-TaqMan assay (37% versus 71%, $P = 0.013$). Finally, the Xpert MTB/RIF test and the IS*6110*-TaqMan assay had similar sensitivities with respiratory specimens of 79% (44/56) and 84% (47/56), respectively, but the Xpert MTB/RIF test had a lower sensitivity (17/32, 53%) with nonrespiratory specimens than the IS*6110*-TaqMan assay (25/32, 78%) ($P = 0.013$). It could be pointed out that the decrease of sensitivity of both real-time PCRs, especially the Xpert MTB/RIF test, was associated with a progressive increase of the median time required for the detection of a positive result with the BacT/Alert instrument. Indeed, this time ranged from 13 days for smear-positive specimens to 28 days for smear-negative specimens ($P < 0.0001$) and even reached 32 days for smear-negative nonrespiratory specimens. The Xpert MTB/RIF test correctly detected the rifampin resistance in all three smear-positive respiratory specimens but not in the one smear-negative pleural fluid specimen which was found not to be positive for MTBC. With regard to specificity, all 20 specimens culture negative for MTBC were negative with both methods. Consequently, for the overall panel of specimens, positive predictive values were 100% for both methods and negative predictive values were 42% and 55% for the Xpert MTB/RIF test and the IS*6110*-TaqMan assay, respectively.

Feasibility and cost of Xpert MTB/RIF test in routine laboratory. Compared to the in-house IS*6110*-TaqMan assay used routinely in our laboratory, the Xpert MTB/RIF method is an easier real-time PCR because bacterial lysis, DNA extraction, and amplification are automated and integrated in an individual cartridge (Table 3). Thus, the only manual step is the liquefaction and inactivation of the specimen with the sample treatment reagent. Consequently, the total hands-on time is less than 3 min/sample (Table 3). In contrast to the Xpert MTB/RIF test, the IS*6110*-TaqMan assay runs in batch mode. By using a fully automated DNA extraction system, the procedure is also simplified since the laboratory technician only inoculates samples in a 32-well MagNa pure LC cartridge. During the extraction procedure, the PCR master mix is manually prepared in a 96-well TaqMan plate, and at the end of the DNA extraction process, the samples are manually inoculated in the plate. No other manual manipulations are necessary, after the reaction 96-well plate is placed in the ABI 7500 system. The duration of the run is 1 h 40 min, regardless of the number of specimens tested. Finally, the total hands-on time was ~15 min for 8 samples, i.e., ~2 min/sample, whereas that of the Xpert MTB/RIF test was ~3 min/sample. However, the total time to a result is longer (~2 h 45 min) for the IS*6110*-TaqMan assay than the Xpert MTB/RIF test. About 4,000 clinical specimens for mycobacterial cultivation are sent to us each year, and we receive ~5 requests for NAAAs for direct detection of MTBC per day. The Xpert MTB/RIF method appeared to be well adapted to these flow conditions by using two MTB/RIF test platforms of analysis. However, the cost of the Xpert MTB/RIF method was almost five times that of the IS*6110*-TaqMan assay.

DISCUSSION

This report describes the first comparison of the sensitivity of the novel real-time PCR assay Xpert MTB/RIF assay with that of an in-house TaqMan real-time PCR targeting the IS*6110* multicopy element routinely used at the CHU Lille laboratory since 2004 (13). We focused the analysis on smear-negative specimens because most of the demands from clinicians at CHU Lille concern this type of specimen, as a result of the difficulty of detecting smear-negative TB. Furthermore, the sensitivities of NAAAs are still far from ideal when they are routinely applied to these specimens. The NAAAs were done with frozen specimens because at CHU Lille ~15% of these

TABLE 3. Main characteristics of Xpert MTB/RIF test and IS*6110*-TaqMan assay

Characteristic	Xpert MTB/RIF	IS*6110*-TaqMan
Work flow design	On demand	Batch mode
Sample format	Individual cartridge	Microplate, 96 wells
Sample preparation	Liquefaction and inactivation of sample (15 min/sample)	Three cycles of boiling-freezing and incubation with proteinase K (~20 min)
DNA extraction	Integrated in individual cartridge	Automated with MagNa pure LC system in batch mode (30 min[a])
Time to results	~2 h	~2 h 45 min[a]
Hands-on time	~3 min/sample	~15 min[a]
Cost	50 euros (~$65)/sample	11 euros (~$15)/sample[a]

[a] For 8 samples.

are requested by clinicians several days or weeks after smear and culture, when results of histology or other investigations are available and evoke a TB diagnosis. Moreover, the similar sensitivities of the Xpert MTB/RIF test with fresh and frozen specimens were reported in analytic studies (9). Since then, several studies have shown the high level of performance of the Xpert MTB/RIF test with frozen specimens, thus demonstrating the high degree of robustness of this test even with specimens stored frozen (9, 15, 16).

As would be expected, both methods were highly specific and exhibited excellent sensitivity for smear-positive specimens (respiratory and nonrespiratory specimens) but a reduced sensitivity for smear-negative specimens, a finding similar to the results of most previous studies (5, 12, 17, 18, 19, 21). While the levels of sensitivity of the in-house IS*6110*-TaqMan assay for smear-negative respiratory and nonrespiratory specimens were in the range of previously published values (8, 9, 12, 13, 15, 16), those of the Xpert MTB/RIF test were lower. As shown in Table 2, the low sensitivity of the Xpert MTB/RIF test with whole smear-negative specimens resulted from a more marked decrease in sensitivity with smear-negative nonrespiratory specimens than with smear-negative respiratory specimens. Because the same segment of *M. tuberculosis rpoB* was used to detect both MTBC and rifampin resistance, false-negative results for MTBC detection led to no characterization of rifampin resistance in the sole smear-negative nonrespiratory specimen. Finally, the Xpert MTB/RIF test was as sensitive as the in-house IS*6110*-TaqMan assay with respiratory specimens, but the sensitivity with nonrespiratory specimens was lower ($P = 0.013$), likely because there was a higher proportion of smear-negative specimens among the nonrespiratory specimens (24/32, 75%) than the respiratory specimens (28/56, 50%). In our study, the Xpert MTB/RIF test's sensitivity with smear-negative respiratory specimens (57%) was found to be lower than that (~72%) reported in recent studies performed with respiratory specimens (3, 9, 15, 16). Here, this result may be explained by the inclusion of gastric aspirates and BAL fluid among the smear-negative respiratory specimens, for which NAAAs are less sensitive than they are with sputum specimens and bronchial aspirates (21).

The low accuracy of NAAAs for the diagnosis of smear-negative tuberculosis, especially nonrespiratory tuberculosis, is usually due to a low load of acid-fast bacilli in specimens (5, 17, 18). The paucibacillary nature of smear-negative specimens was attested to in our study by (i) the median time required for detection of a positive result with BacT/Alert, which significantly increased between smear-positive and smear-negative specimens, especially among smear-negative nonrespiratory specimens, and (ii) the delayed C_T values obtained with the IS*6110*-TaqMan assay from the 12 specimens with false-negative results with the Xpert MTB/RIF test. Moure et al. reported a similar inverse association between an extended median time to growth in liquid media for MTBC culture-positive specimens and negative results with the Xpert MTB/RIF test (16). Altogether, the data suggested that the mycobacterial load in specimens more critically affects the sensitivity of the Xpert MTB/RIF test than the IS*6110*-TaqMan assay.

The better sensitivity of our in-house IS*6110*-TaqMan assay could be attributed to the effect of the multicopy target IS*6110*, which is present at 10 to 15 copies in most genomes of MTBC (7, 22). Furthermore, the sensitivity of detection of a few target sequences by PCR is highly dependent on the efficiency of DNA extraction procedures (4, 14, 20), which should eliminate inhibiting substances and achieve optimal lysis. Freezing-thawing cycles performed prior to DNA extraction for the IS*6110*-TaqMan assay may increase the release of mycobacterial DNA and reduce the activity of PCR inhibitors. Although the multiple-step DNA purification process required with the automated MagNA Pure system, based on nucleic acid capture by magnetic silica particles, caused the IS*6110*-TaqMan assay to take more time than the Xpert MTB/RIF method, the IS*6110*-TaqMan assay could be more reliable for paucibacillary specimens (2, 20). With the Xpert MTB/RIF test, specimen processing has been designed to be simplified by use of a single nonprecise step that both liquefies and inactivates sputum (3). This single-step purification of DNA may limit complete mycobacterial cell lysis and therefore affect the rate of recovery of nucleic acids from paucibacillary specimens. This may explain why all tests with inconclusive results with the Xpert MTB/RIF test exclusively involved nonrespiratory specimens and suggests that these results were due to either the presence of inhibitors or aborted tests that could result from fluidics problems related to the viscosity of the specimens. Hilleman et al. have also recently reported a lower sensitivity of the Xpert MTB/RIF test with tissue specimens and suggested a preincubation step with proteinase K (10).

The Xpert MTB/RIF test is a simple method that is well adapted for routine use in a clinical laboratory and that proved to be very suitable for detection of MTBC in respiratory specimens and in the overall panel of smear-positive specimens (respiratory and nonrespiratory). However, the accuracy of the Xpert MTB/RIF test for the detection of MTBC in paucibacillary specimens, such as smear-negative nonrespiratory specimens, was found to be lower than that of the IS*6110*-TaqMan assay. Because of the high cost of the Xpert MTB/RIF test, further investigation is needed to evaluate whether the shorter turnaround time of this test than the real-time PCR assay using TaqMan probes has an effective impact on the management and implementation of infection control measures for TB in areas with low rates of TB and MDR-TB.

ACKNOWLEDGMENT

We acknowledge Florent Sebbane for critical review of the manuscript.

REFERENCES

1. **Blakemore, R., et al.** 2010. Evaluation of the analytical performance of the Xpert MTB/RIF assay. J. Clin. Microbiol. **48:**2495–2501.
2. **Böddinghaus, B., T. A. Wichelhaus, V. Brade, and T. Bittner.** 2001. Removal of PCR inhibitors by silica membranes: evaluating the Amplicor *Mycobacterium tuberculosis* kit. J. Clin. Microbiol. **39:**3750–3752.
3. **Boehme, C. C., et al.** 2010. Rapid molecular detection of tuberculosis and rifampin resistance. N. Engl. J. Med. **363:**1005–1015.
4. **Boom, R., et al.** 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. **28:**495–503.
5. **Daley, P., S. Thomas, and M. Pai.** 2007. Nucleic acid amplification tests for the diagnosis of tuberculous lymphadenitis: a systematic review. Int. J. Tuberc. Lung Dis. **11:**1166–1176.
6. **Espy, M. J., et al.** 2006. Real-time PCR in clinical microbiology: applications for routine laboratory testing. Clin. Microbiol. Rev. **19:**165–256.
7. **Flores, L. L., M. Pai, J. M. Colford, Jr., and L. W. Riley.** 2005. In-house nucleic acid amplification tests for the detection of *Mycobacterium tuberculosis* in sputum specimens: meta-analysis and meta-regression. BMC Microbiol. **5:**1–9.
8. **Greco, S., E. Girardi, A. Navarra, and C. Saltini.** 2006. Current evidence on

diagnostic accuracy of commercially based nucleic acid amplification tests for the diagnosis of pulmonary tuberculosis. Thorax **61:**783–790.
9. **Helb, D., et al.** 2010. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. J. Clin. Microbiol. **48:**229–237.
10. **Hillemann, D., S. Ruesch-Gerdes, C. Boehme, and E. Richter.** 26 January 2011, posting date. Rapid molecular detection of extrapulmonary tuberculosis by automated GeneXpert® MTB/RIF system. J. Clin. Microbiol. doi: 10.1128/JCM.02268-10.
11. **Kubica, G. P., W. E. Dye, M. L. Cohn, and G. Middlebrook.** 1963. Sputum digestion and decontamination with N-acetyl-L-cysteine-sodium hydroxide for culture of mycobacteria. Am. Rev. Respir. Dis. **87:**775–779.
12. **Laraque, F., A. Griggs, M. Slopen, and S. S. Munsiff.** 2009. Performance of nucleic acid amplification tests for diagnosis of tuberculosis in a large urban setting. Clin. Infect. Dis. **49:**46–54.
13. **Lemaître, N., et al.** 2004. Comparison of the real-time PCR method and the Gen-Probe amplified *Mycobacterium tuberculosis* direct test for detection of *Mycobacterium tuberculosis* in pulmonary and nonpulmonary specimens. J. Clin. Microbiol. **42:**4307–4309.
14. **Mangiapan, G., et al.** 1996. Sequence capture-PCR improves detection of mycobacterial DNA in clinical specimens. J. Clin. Microbiol. **34:**1209–1215.
15. **Marlowe, E. M., et al.** 2 February 2011, posting date. Evaluation of the Cepheid Xpert MTB/RIF assay for the direct detection of *Mycobacterium tuberculosis* complex from respiratory specimens. J. Clin. Microbiol. doi: 10.1128/JCM.02214-10.
16. **Moure, R., et al.** 29 December 2010, posting date. Rapid detection of *Mycobacterium tuberculosis* complex and rifampin resistance in smear-negative clinical samples using an integrated real time PCR method. J. Clin. Microbiol. doi:10.1128/JCM.01831-10.
17. **Pai, M., et al.** 2003. Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: a systematic review and meta-analysis. Lancet Infect. Dis. **3:**633–643.
18. **Pai, M., L. L. Flores, A. Hubbard, L. W. Riley, and J. M. Colford, Jr.** 2004. Nucleic acid amplification tests in the diagnosis of tuberculous pleuritis: a systematic review and meta-analysis. BMC Infect. Dis. **4:**1–14.
19. **Piersimoni, C., and C. Scarparo.** 2003. Relevance of commercial amplification methods for direct detection of *Mycobacterium tuberculosis* complex in clinical samples. J. Clin. Microbiol. **41:**5355–5365.
20. **Santos, A., et al.** 2009. Comparison of methods of DNA extraction for real-time PCR in a model of pleural tuberculosis. APMIS **118:**60–65.
21. **Sarmiento, O. L., K. A. Weigle, J. Alexander, D. J. Weber, and W. C. Miller.** 2003. Assessment by meta-analysis of PCR for diagnosis of smear-negative pulmonary tuberculosis. J. Clin. Microbiol. **41:**3233–3240.
22. **Van Soolingen, D., P. W. Hermans, P. E. De Haas, D. R. Soll, and J. D. van Embden.** 1991. Occurrence and stability of insertion sequences in *Mycobacterium tuberculosis* complex strains: evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. J. Clin. Microbiol. **29:**2578–2586.
23. **World Health Organization.** 2008. The WHO/IUATLD Global Project on antituberculosis-drug resistance surveillance. Publication no. WHO/HTM/TB/2008.394. World Health Organization, Geneva, Switzerland.
24. **World Health Organization.** 2008. The WHO/IUATLD global tuberculosis control. Publication no. WHO/HTM/TB/2009.426. World Health Organization, Geneva, Switzerland.