JCM
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Effectiveness of an Integrated Real-Time PCR Method for Detection of the *Mycobacterium tuberculosis* Complex in Smear-Negative Extrapulmonary Samples in an Area of Low Tuberculosis Prevalence

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Early extrapulmonary tuberculosis (EPTB) diagnosis is particularly difficult. Among 108 smear-negative extrapulmonary samples showing a positive culture for *Mycobacterium tuberculosis* complex (43 body fluids and 65 nonliquid specimens), 63 (58.3%) were positive with the Xpert MTB/RIF assay (GX). GX sensitivity was quite low for samples from sterile locations (especially for pleural fluids: 26.9%) but high for some nonliquid samples, like abscess aspirates (76.5%). In summary, GX may be a useful tool to be considered for EPTB diagnosis.

Tuberculosis (TB) is still one of the main causes of morbidity and mortality worldwide. Each year there are almost nine million new cases and two million deaths (20). Pulmonary TB is the main form of the disease. However, due to certain social and epidemiological factors, such as coinfection with HIV, disease patterns have changed in recent years (5a, 7, 19) and the frequency of disseminated and extrapulmonary tuberculosis (EPTB) has risen, even in industrialized areas. The incidence of exclusive EPTB in our setting (Catalonia, Spain) in 2009 was 26.1%, and 12.2% of all TB cases affected both pulmonary and extrapulmonary sites (6). The absence of some typical TB symptoms hinders the clinical diagnosis of EPTB and may mislead physicians to suspect other diseases. Extrapulmonary specimens have a very low bacterial load, so the sensitivity of direct microscopy examination for detecting MTBC is low. This is particularly true of pleural TB, for which *Mycobacterium tuberculosis* complex (MTBC) detection is achieved by smear microscopy in fewer than 5% of cases (11, 16).

GeneXpert (Cepheid, Sunnyvale, CA) is an automated, integrated, real-time PCR system which has recently been developed for rapid detection of MTBC and rifampin (RIF) resistance. The Xpert MTB/RIF assay (GX) has been evaluated in detail, and several studies have demonstrated its utility for direct detection in pulmonary specimens (2, 3, 8, 12). The effectiveness of GX for diagnosing extrapulmonary TB has not been conclusively demonstrated, especially in countries with low or medium TB prevalence, since the system was initially validated only for respiratory specimens and most published studies include low numbers of extrapulmonary samples (1, 5, 9, 18).

The aim of the present study was to evaluate the feasibility of GX for the detection of MTBC in extrapulmonary smear-negative specimens, in a Western country with a low prevalence of TB and EPTB. The study also classified the different sources of these specimens in order to test the hypothesis that the sensitivity of GX may vary considerably according to the origin of the sample.

A total of 149 smear-negative samples (one sample per patient) collected from July 1999 to May 2011 in Costa Ponent (Catalonia, Spain) were included in the study. On arrival at the mycobacterial laboratory, nonsterile clinical samples were pretreated using the *N*-acetyl-L-cysteine–NaOH digestion-decontamination method with a final volume of 2 ml (10). Sterile fluid specimens were directly processed, and biopsy specimens were disaggregated with a mortar and then resuspended with saline solution (2 ml). Afterwards, 1 ml of the specimens was frozen at −80°C. The remaining volume was processed as follows: (i) microscopic examination for acid-fast organisms (auramine-rhodamine and Ziehl-Neelsen stains) and (ii) mycobacterial culture using 0.2 ml of Lowenstein-Jensen medium and 0.5 ml of Bactec MGIT 960 medium (Becton Dickinson, Towson, MD) as solid and liquid media, respectively. Positive cultures were confirmed as MTBC by the use of DNA probes (Accuprobe; GenoProbe Inc., San Diego, CA). Among the 149 specimens studied, 108 specimens had a positive culture of MTBC: (i) 43 liquid specimens (37 sterile fluids, 3 gastric aspirates, and 3 urine specimens) and (ii) 65 nonliquid specimens (34 lymph nodes, 17 abscess aspirates, 12 tissue samples, and 2 stool specimens). In addition, 41 clinical samples with a negative mycobacterial culture from patients without TB were also studied: (i) 21 sterile fluids, five gastric aspirates, and one urine sample and (ii) four lymph nodes, two abscess aspirates, and eight tissue samples (Table 1). A GX assay was performed in April-May 2011. The portion (1 ml) of the specimens kept frozen (see above) was thawed and used for the GX assay, performed according to the manufacturer's protocol for pulmonary samples.

GX detected DNA of MTBC in 63 of the 108 clinical extrapulmonary specimens with MTBC-positive cultures (58.3%). As expected in smear-negative samples, most of the semiquantitative results given by the GX report were "Low" ($n = 27$) or "Very Low" ($n = 34$) and only two samples had a "Medium" bacterial load. As for the rifampin susceptibility result, GX did not detect any *rpoB* mutation, corroborating the results obtained with the conventional drug susceptibility test of the strains corresponding to these specimens (all susceptible).

Unlike respiratory specimens, the group of extrapulmonary

Received 23 November 2011 **Accepted** 27 November 2011
Published ahead of print 7 December 2011
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doi:10.1128/JCM.06467-11

TABLE 1 Results of Xpert MTB/RIF according to the source and MTBC culture of the samples

Clinical specimen	No. of specimens with[a]:					Total no. of specimens	Sensitivity	Specificity
	Positive MTBC culture		Negative MTBC culture					
	GX^+	GX^-	GX^+	GX^-	GX IND			
Sterile fluids								
Pleural fluid	7	19	0	5	0	31	40.5%	100%
Cerebrospinal fluid	2	0	0	12	0	14		
Joint fluid	5	2	0	0	0	7		
Ascitic fluid	0	1	0	2	0	3		
Pericardial fluid	1	0	0	2	0	3		
Nonsterile fluids								
Gastric aspirate	2	1	0	3	2[b]	8	66.7%	100%
Urine	2	1	0	1	0	4		
Lymph nodes	24	10	0	4	0	38	70.6%	100%
Abscess aspirates								
Knee abscess	1	0	0	0	0	1	76.5%	100%
Cervical abscess	3	2	0	1	0	6		
Skin abscess	3	1	0	0	0	4		
Osteitis pus	4	1	0	0	0	5		
Empyema	2	0	0	1	0	3		
Tissues								
Pelvic biopsy	0	0	0	1	0	1	41.7%	100%
Testicular biopsy	1	0	0	0	0	1		
Colon biopsy	0	0	0	2	0	2		
Vertebral disc biopsy	0	0	0	1	0	1		
Spondylodiscitis puncture	0	0	0	1	0	1		
Pericardial biopsy	0	0	0	1	0	1		
Bone biopsy	1	2	0	0	0	3		
Bone marrow biopsy	0	0	0	1	0	1		
Synovial biopsy	0	2	0	0	0	2		
Mediastinal tissue	0	1	0	0	0	1		
Skin biopsy	1	1	0	1	0	3		
Larynx biopsy	0	1	0	0	0	1		
Costal cartilage biopsy	2	0	0	0	0	2		
Stool	2	0	0	0	0	2	100%	100%
Total	63	45	0	39	2	149	58.3%	100%

[a] MTBC, *Mycobacterium tuberculosis* complex; GX, Xpert MTB/RIF; GX IND, Xpert MTB/RIF indeterminate result.
[b] The two invalid GX results were not included in the calculation of the specificity.

samples is quite heterogeneous, which explains the great variability obtained in the sensitivity values. Thus, among the 63 samples with a positive GX result, partial sensitivities differed according to the categories of the specimens: 40.5% in sterile fluids, 66.7% in nonsterile fluids, 70.6% in lymph nodes, 41.7% in tissue samples, 76.5% in abscess aspirates, and 100% (two of two) in stool material (Table 1). The reason why abscess aspirates and lymph node specimens show GX sensitivities comparable to those of pulmonary samples is probably their similarity regarding the inoculum size and physical properties. The low GX positivity in the biopsy samples may be due in part to the low bacterial load and to the texture of the specimens, which are often very resistant to breakup. Within the group of sterile fluids (which showed poorer sensitivity results), marked differences were found according to the origin of the samples. Only 7 of 26 (26.9%) of pleural fluids were GX positive, which meant a significantly lower sensitivity than in the other sterile fluids (72.7%; $P = 0.02$): five of seven joint fluids containing MTBC were detected with GX, as well as both CSF samples; the sole pericardial fluid sample was GX positive, and the sole ascitic fluid sample was GX negative. The low detection ability in some sterile specimens, such as pleural fluids, could be explained by the low bacterial load contained in these samples. Techniques based on nucleic acid amplification have recently been considered for pleural TB diagnosis, in order to improve sensitivity and specificity (13, 14, 17). The lower efficiency obtained in these pleural effusion specimens with GX (though better than the sensitivity achieved by other methods) highlights the assay's limited detection ability in very-low-yield samples. On the other hand, no differences in GX positivity were observed with regard to the anatomical site from which the samples were obtained either within the group of nonsterile liquid samples (gastric aspirate and urine samples) or within the group of nonliquid spec-

imens (lymph nodes, abscess aspirates, and tissue and stool specimens).

All MTBC culture-positive samples included in the study contained enough bacterial load to show growth in at least one of the culture media in less than 6 weeks. Nevertheless, in light of previous reports (12), an association between the bacterial load (represented by the days required for growth) and the qualitative and semiquantitative results of the GX was found. The median time to growth in liquid medium for Medium/Low results was 17 days, compared with 20.5 for samples showing Very Low results, indicating a statistically significant difference ($P = 0.001$). When the time to growth was analyzed with regard to the qualitative GX detection (positive/negative), the results were similar. In the broth medium, the median time required for growth for the MTBC specimens detected with GX was significantly lower (19 days compared to 23.5 days) than in the GX-negative samples ($P < 0.001$). These results suggest that the negative predictive value of the GX may decrease when dealing with specimens with a very low bacterial load.

The GX showed good specificity: among the 41 specimens with negative mycobacterial culture, GX offered an interpretable result (negative) in 39 samples. Two samples (gastric aspirates) gave an invalid result. The most plausible reason for these invalid assays is that these reactions were inhibited by the low pH in the gastric specimens, since they had been directly processed with GX (without decontamination or buffering).

To summarize, the Xpert MTB/RIF technique has demonstrated a substantial capacity for the diagnosis of EPTB mostly from nonsterile fluids (gastric aspirates and urine samples), lymph nodes, or abscess aspirate specimens. Even though its sensitivity decreases notably when the source of the sample is a sterile location such as a pleural effusion or a biopsy specimen, the technique still offers higher sensitivity for direct detection of MTBC than most conventional techniques. Therefore, GX may be a potentially useful additional tool in cases of EPTB not detected by microscopy, when clinical suspicion is high (4), and when performance of the test may be cost-effective.

ACKNOWLEDGMENTS

This study was supported by Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III—FEDER, Spanish Network for the Research in Infectious Diseases (REIPI RD06/0008). R. Moure received a grant from the Institut d'Investigació Biomèdica de Bellvitge (IDIBELL).

We are grateful to Cepheid and Izasa S.A. for providing us with the Xpert MTB/RIF reagents. We also thank J. M. Caldito for technical assistance.

REFERENCES

1. **Armand S, Vanhuls P, Delcroix G, Courcol R, Lemaitre N.** 2011. Comparison of the Xpert MTB/RIF test with an IS6110-TaqMan real-time PCR assay for direct detection of *Mycobacterium tuberculosis* in respiratory and nonrespiratory specimens. J. Clin. Microbiol. **49**:1772–1776.
2. **Boehme CC, et al.** 2010. Rapid molecular detection of tuberculosis and rifampin resistance. N. Engl. J. Med. **363**:1005–1015.
3. **Boehme CC, et al.** 2011. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet **377**:1495–1505.
4. **Catanzaro A, et al.** 2000. The role of clinical suspicion in evaluating a new diagnostic test for active tuberculosis: results of a multicenter prospective trial. JAMA **283**:639–645.
5. **Causse M, Ruiz P, Gutierrez-Aroca JB, Casal M.** 2011. Comparison of two molecular methods for rapid diagnosis of extrapulmonary tuberculosis. J. Clin. Microbiol. **49**:3065–3067.

5a. **Fitzgerald DW, Sterling TR, Hass DW.** 2010. *Mycobacterium tuberculosis*, p 3129–3163. *In* Mandell GL, Bennett JE, Dolin R (ed), Mandell, Douglas, and Bennett's principles and practice of infectious diseases, 7th ed, vol 2. Churchill Livingstone, New York, NY.

6. **Generalitat de Catalunya.** 2009. Informe anual. Situació epidemiológica i tendencia de l'endèmia tuberculosa a Catalunya. Departament de Salut, Barcelona, Catalonia, Spain. http://www.gencat.cat/salut/depsalut/html/ca/dir2474/inf2009tuber.pdf.
7. **Golden MP, Vikram HR.** 2005. Extrapulmonary tuberculosis: an overview. Am. Fam. Physician **72**:1761–1768.
8. **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.
9. **Hillemann D, Rusch-Gerdes S, Boehme C, Richter E.** 2011. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. J. Clin. Microbiol. **49**:1202–1205.
10. **Kubica GP, Dye WE, Cohn ML, Middlebrook G.** 1963. Sputum digestion and decontamination with N-acetyl-L-cysteine-sodium hydroxide for culture of mycobacteria. Am. Rev. Respir. Dis. **87**:775–779.
11. **Light RW.** 1999. Useful tests on the pleural fluid in the management of patients with pleural effusions. Curr. Opin. Pulm. Med. **5**:245–249.
12. **Moure R, et al.** 2011. Rapid detection of *Mycobacterium tuberculosis* complex and rifampin resistance in smear-negative clinical samples by use of an integrated real-time PCR method. J. Clin. Microbiol. **49**:1137–1139.
13. **Nagesh BS, Sehgal S, Jindal SK, Arora SK.** 2001. Evaluation of polymerase chain reaction for detection of *Mycobacterium tuberculosis* in pleural fluid. Chest **119**:1737–1741.
14. **Pai M, Flores LL, Hubbard A, Riley LW, Colford JM, Jr.** 2004. Nucleic acid amplification tests in the diagnosis of tuberculous pleuritis: a systematic review and meta-analysis. BMC Infect. Dis. **4**:6.
15. Reference deleted.
16. **Seibert AF, Haynes J, Jr, Middleton R, Bass JB, Jr.** 1991. Tuberculous pleural effusion. Twenty-year experience. Chest **99**:883–886.
17. **Trajman A, et al.** 2008. Novel tests for diagnosing tuberculous pleural effusion: what works and what does not? Eur. Respir. J. **31**:1098–1106.
18. **Vadwai V, et al.** 2011. Xpert MTB/RIF: a new pillar in diagnosis of extrapulmonary tuberculosis? J. Clin. Microbiol. **49**:2540–2545.
19. **von Reyn CF, et al.** 2011. Disseminated tuberculosis in human immunodeficiency virus infection: ineffective immunity, polyclonal disease and high mortality. Int. J. Tuberc. Lung Dis. **15**:1087–1092.
20. **WHO.** 2011. Global tuberculosis control: 2011 report. World Health Organization, Geneva, Switzerland.