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Early Diagnosis of Extrapulmonary Tuberculosis by a New Procedure Combining Broth Culture and PCR▽

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The diagnosis of extrapulmonary tuberculosis is difficult because of the paucibacillary nature of these infections. We developed a culture-enhanced PCR assay combining a preliminary step of broth culture in BacT/Alert MP bottles with the subsequent detection of *Mycobacterium tuberculosis* using the GenoType Mycobacteria Direct test. First, the procedure was applied to 10-fold-diluted suspensions of *M. tuberculosis* prepared in vitro. These experiments showed that a 15-day incubation time was required to detect bacilli in the suspension, with the lowest inoculum size yielding a single colony on Lowenstein-Jensen slants. The efficacy of culture-enhanced PCR at day 15 was subsequently evaluated with 225 nonrespiratory specimens from 189 patients with suspected tuberculosis. All these specimens were smear negative, and 31 (13.8%) from 27 patients were culture positive. The result of culture-enhanced PCR at day 15 was consistent with final culture results in all specimens tested. Compared to culture results, the sensitivity, specificity, positive predictive value, and negative predictive value were 100%. Four patients with a negative culture and a negative PCR result were diagnosed as having tuberculosis on the basis of histological findings or therapeutic response. When using a positive diagnosis of tuberculosis as a gold standard, the sensitivity, specificity, positive predictive value, and negative predictive value were 88.6%, 100%, 100%, and 97.9%, respectively. These results indicate that culture-enhanced PCR is a highly sensitive and specific method for the early detection of *M. tuberculosis* in extrapulmonary specimens.

Tuberculosis (TB) is one of the major causes of infection worldwide. Extrapulmonary forms have been increasingly reported, accounting for 20 to 50% of all cases of TB in recent studies (6, 8, 12). Extrapulmonary TB (ETB) remains a challenging diagnosis for both clinicians and microbiologists (7, 8). Signs and symptoms are most often nonspecific. Obtaining material for culture often requires invasive procedures that cannot be easily repeated. Because of the paucibacillary nature of extrapulmonary specimens and the irregular distribution of bacilli that tend to clump together, the sensitivity of smear microscopy is very low. Cases of ETB are more often culture negative than cases of pulmonary TB, and when culture is positive, growth on solid medium may require as long as 8 weeks. Moreover, histopathological findings are not always conclusive, in particular for tuberculous lymphadenopathy (20).

As a result of these diagnostic difficulties, the institution of appropriate therapy is often delayed in patients with ETB, resulting in increased morbidity and mortality, whereas patients without TB may receive unnecessary presumptive treatment for several weeks (11).

The utility of nucleic acid amplification tests (NAATs) in the setting of TB has been extensively evaluated, with the intended goal of enabling the clinician to make a more rapid and accurate diagnosis (2, 3, 13, 14, 17). Unlike with pulmonary TB, there is no clear recommendation for the use of NAATs in the setting of ETB. Three recent meta-analyses synthesizing the results of nearly 140 studies have examined the current evidence on the performance of these tests for the diagnosis of TB lymphadenitis, pleuritis, and meningitis (3, 13, 14). According to these studies, NAATs have high specificity and positive predictive value but low and variable sensitivity and negative predictive value in all forms of ETB. Whereas a positive result strongly suggests TB, a negative result does not exclude TB with certainty. Thus, the current evidence suggests that NAATs cannot replace conventional tests such as microscopy and culture and that they should be interpreted in conjunction with these tests and clinical data (15, 17). In clinical practice, the results of NAATs do not weigh significantly on decision making in suspected ETB, and antituberculous treatment given presumptively is rarely discontinued until final culture results are available (21). Further work is therefore needed to develop new methods with enhanced sensitivity while maintaining high specificity.

ETB accounted for 35% of cases of TB documented in our hospital in 2002, and this rate increased to 53% in 2005. In an attempt to shorten the time necessary for diagnosing ETB, we developed a procedure consisting of a preliminary step of broth culture followed by the detection of *Mycobacterium tuberculosis* using the GenoType Mycobacteria Direct (GTMD) assay (Hain Lifescience, Nehren, Germany). This test is a commercially available genetic assay designed to detect *M. tuberculosis* complex and four atypical *Mycobacterium* species directly from respiratory specimens (5, 19). The procedure combining broth culture and the GTMD test will be referred to as culture-enhanced PCR in the present study.

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In the first part of the study, an in vitro simulation of *M. tuberculosis*-containing fluid or tissue was used to determine the optimal time of broth culture for detecting the smallest inoculum of bacilli with the GTMD test. In the second part of the study, the procedure was used to detect *M. tuberculosis* in nonrespiratory clinical specimens from patients with suspected ETB, and the results were compared to those of conventional microbiological methods, histopathological findings, and therapeutic response.

(Part of this study was presented at the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy in 2006 [San Francisco, CA].)

MATERIALS AND METHODS

Evaluation of the culture-enhanced PCR procedure with in vitro-prepared *M. tuberculosis* suspensions. (i) Preparation of *M. tuberculosis* suspensions. A clinical isolate of *M. tuberculosis* identified in our laboratory was used for the experiments. Colonies cultured on a Lowenstein-Jensen slant were dispersed in sterile water in order to obtain a suspension containing approximately 5×10^7 CFU/ml. This suspension was subjected to 10-fold serial dilutions in sterile water (up to 10^{-8}). Five hundred microliters of each of the nine suspensions (including the stock suspension) was mixed into 500 μl of ground lymph nodes previously shown to be free of bacteria. Thus, 18 suspensions containing 10-fold-variable numbers of *M. tuberculosis* bacteria with or without lymph node tissue were obtained, and each of these suspensions was subsequently subjected to (i) microscopic examination, (ii) quantitative culture on Lowenstein-Jensen slants, (iii) broth culture in BacT/Alert MP bottles (bioMérieux, Durham, NC), and (iv) culture-enhanced PCR assay.

(ii) Microscopic examination. Smears were prepared with 100 μl of suspension, stained with auramine-fluorochrome, and examined for the presence of fluorescent bacilli.

(iii) Culture on solid medium. Four hundred microliters of each suspension was inoculated onto two Lowenstein-Jensen slants (200 μl/slant). The slants were incubated at 37°C and examined weekly for growth until found positive or for 12 weeks. To determine the inoculum sizes, the number of colonies on the slants that showed a nonconfluent growth was counted and the number of CFU/ml was calculated for each suspension.

(iv) Broth culture and PCR assay. Seven BacT/Alert MP bottles were inoculated with each of the 18 suspensions (500 μl/bottle). All bottles were incubated in the BacT/Alert instrument (bioMérieux, Lyon, France) at 37°C without shaking. For each suspension, one of the seven bottles was left in the instrument until a positive result or for 8 weeks in order to determine the time to positivity in broth culture. The six other bottles were removed from the instrument for PCR analysis at various times before positive detection. To this purpose, an aliquot of 5 ml was removed from these six bottles at days 0 (before incubation), 2, 4, 6, 8, and 15, respectively, and the bottles were reloaded in the BacT/Alert instrument until flagged as positive or for 8 weeks.

The 5-ml aliquots were centrifuged, and the pellet was resuspended in 1 ml of sterile water. Five hundred microliters was used for PCR assay using the GTMD test according to the manufacturer's recommendations (5). The remaining 500 μl was frozen at −20°C until used for retesting when the first PCR result was negative.

Evaluation of the culture-enhanced PCR procedure with clinical extrapulmonary specimens. Specimen processing and analysis were carried out as follows. Nonrespiratory specimens from patients with suspected ETB and without antituberculous therapy that were sent to our laboratory from January 2006 through December 2007 were evaluated by the culture-enhanced PCR procedure. From January 2006 to May 2006, all specimens were subjected to two PCR assays after an 8-day broth culture and a 15-day broth culture, respectively. From June 2006 to December 2007, the index of clinical suspicion of TB was evaluated and classified as moderate or strong by the clinician in charge of the patient. Specimens from patients for whom suspicion was strong were subsequently processed in order to perform two PCR assays, after an 8-day and a 15-day broth culture, respectively, whereas those from patients for whom suspicion was moderate were subjected to a single PCR assay after a 15-day culture.

Upon receipt, specimens were kept at +4°C prior to being processed. Tissue specimens were sliced and homogenized with sterile water in a mortar under sterile conditions, and 100 μl of these homogenized tissues or fluid specimens was inoculated onto two blood agar plates (bioMérieux) which were incubated overnight at 37°C under aerobic and anaerobic conditions. Subsequent decontamination with *N*-acetylcysteine was performed only when growth of bacteria or fungi was observed on blood agar. Specimens were then centrifuged at $3,300 \times g$ for 20 min (cerebrospinal fluid and bone marrow excepted), and the pellet was resuspended in 1 or 1.5 ml of sterile water according to whether a single PCR assay or two PCR assays were scheduled. A 100-μl portion was used to prepare a smear for staining with auramine-rhodamine and microscopic examination for the presence of fluorescent bacilli. Another 400-μl portion was used for inoculation onto two Lowenstein-Jensen slants (200 μl per slant) that were incubated at 37°C and examined weekly for growth for 12 weeks. The remaining 500-μl or 1-ml portion was inoculated into one or two BacT/Alert MP bottles (500 μl per bottle) that were incubated at 37°C without shaking and monitored for growth for up to 8 weeks. A 2.5-ml aliquot was removed from each bottle after 8 days and 15 days of incubation, respectively, or only after 15 days when a single bottle had been inoculated. The bottles were then reloaded and left in the instrument until positive detection or for a total period of 8 weeks. However, cultures that had already been detected as positive by the instrument before day 8 or day 15 were not subjected to PCR assay. The 2.5-ml aliquot removed was centrifuged and resuspended in 500 μl of sterile water. The 500-μl suspension was used for PCR with the GTMD test according to the manufacturer's recommendations as previously described.

Diagnosis of TB and analysis of results. The criteria for a positive diagnosis of TB were as follows: documented TB with positive culture and/or positive histology or probable TB based on clinical grounds and improvement after a trial of antituberculous therapy. The sensitivity, specificity, positive predictive value, and negative predictive value of our method were calculated in comparison with culture results only and, separately, in comparison with a combination of culture, histology, and response to presumptive treatment as a reference standard.

RESULTS

In vitro experiments with *M. tuberculosis* suspensions. Inoculum sizes of each suspension were evaluated by culture on solid medium. The stock suspension and the 10^{-1} to 10^{-7} suspensions grew *M. tuberculosis* on Lowenstein-Jensen slants, with inoculum sizes ranging from 5×10^7 CFU/ml to 5 CFU/ml (Table 1). Microscopic examination was positive only for inocula of $\geq 5 \times 10^3$ CFU/ml. Suspensions that were positive on Lowenstein-Jensen slants were all detected as positive by the BacT/Alert automated instrument. The time to positivity of broth cultures ranged from 3 days to 32 days according to the inoculum size. Times to positivity of a given suspension did not differ significantly for the bottles that were removed at various times for PCR analysis and the bottle that was left in the instrument. For the 10^{-8} suspension, culture on Lowenstein-Jensen slants remained negative after 12 weeks and broth culture was not detected as positive by the BacT/Alert instrument after 8 weeks. Therefore, this 10^{-8} suspension was considered to be free of *M. tuberculosis*.

The GTMD test was positive at day 0 (before incubation) only for smear-positive suspensions (Table 1). For smear-negative, culture-positive suspensions, the test detected *M. tuberculosis* after a time of incubation ranging from 2 days to 15 days as the inoculum size decreased from 5×10^2 CFU/ml to 5 CFU/ml (Table 1). *M. tuberculosis* was detected in the suspension with the smallest inoculum, which yielded a single colony on the Lowenstein-Jensen slant, after a 15-day broth culture only. PCR at day 8 was positive for the 10^{-6} suspension, which yielded 10 colonies on the Lowenstein-Jensen slant, whereas PCR at day 2, 4, or 6 failed to detect *M. tuberculosis* in that suspension. For a given inoculum size, smear, culture, and PCR results were similar for suspensions prepared with or without lymph node tissue (data not shown).

These in vitro experiments indicated that PCR at day 8 performed better than PCR at day 2, 4, or 6 for detecting *M.*

TABLE 1. Comparison of smear, culture, and culture-enhanced PCR results with in vitro-prepared *M. tuberculosis* suspensions[a]

Dilution	Inoculum size (CFU/ml)[b]	Smear result	Result for culture on Lowenstein-Jensen slant (no. of colonies)	Broth culture		Culture-enhanced PCR result according to incubation period (days)[d]:					
				Positivity	Time to detection (days)[c]	0	2	4	6	8	15
Stock	5×10^7	+	+ (confluent)	Yes	3	+	+	NP	NP	NP	NP
10^{-1}	5×10^6	+	+ (confluent)	Yes	5	+	+	+	NP	NP	NP
10^{-2}	5×10^5	+	+ (confluent)	Yes	8	+	+	+	+	NP	NP
10^{-3}	5×10^4	+	+ (confluent)	Yes	12	+	+	+	+	+	NP
10^{-4}	5×10^3	+[e]	+ (confluent)	Yes	17	+	+	+	+	+	+
10^{-5}	5×10^2	−	+ (80)	Yes	20	−	+	+	+	+	+
10^{-6}	50	−	+ (10)	Yes	26	−	−	−	−	+	+
10^{-7}	5	−	+ (1)	Yes	32	−	−	−	−	−	+
10^{-8}	0	−	−	No	−	−	−	−	−	−	−

[a] +, positive; −, negative; NP, not performed.
[b] The inoculum size was calculated from the number of colonies on Lowenstein-Jensen slants.
[c] Time to detection for the BacT/Alert MP bottle that was left in the instrument.
[d] PCR was not performed after the bottle was detected as positive by the instrument.
[e] Only 10 bacilli were visible on the smear.

tuberculosis in paucibacillary suspensions. However, a 15-day incubation time was required to obtain a positive PCR result for the suspension with the smallest inoculum size. As a result, we subsequently applied the GTMD test to broth cultures of our clinical specimens at day 8 and day 15.

Evaluation of the procedure with clinical specimens. From January 2006 to May 2006, the efficacy of the procedure consisting of two PCR assays at day 8 and day 15 was evaluated with 24 specimens from 22 patients with suspected ETB. These specimens included 16 tissue biopsy samples (seven lymph node biopsy, six bone biopsy, two digestive biopsy, and one skin biopsy sample) and seven samples of organic fluids or pus (three of pleural fluids, one of ascitic fluid, one of cerebrospinal fluid, and one of urine and one brain abscess sample). Of these 24 specimens, all were smear negative and seven (29%) originating from five patients were culture positive for *M. tuberculosis* on Lowenstein-Jensen slants and in liquid medium (Table 2). The mean time for growth on Lowenstein-Jensen slants was 34 days (range, 26 to 45 days), and the mean time to detection in BacT/Alert bottles was 25 days (range, 15 to 44 days). All culture-negative specimens were PCR negative. Of the seven culture-positive specimens, six were PCR positive at day 8 and all were PCR positive at day 15 (Table 2). Thus, the overall rate of day 8 PCR-positive specimens was 6/24 (25%). Given the cost and feasibility of the test, PCR analysis was subsequently applied to 8-day cultures only for specimens from patients for whom there was a strong suspicion of TB.

Thus, from June 2006 to December 2007, 201 further specimens were studied, including 19 specimens from 19 patients for whom there was a strong clinical suspicion of ETB. Overall, 225 nonrespiratory specimens from 189 patients were investigated from January 2006 through December 2007. The specimens comprised 141 tissue biopsy specimens, 73 fluid specimens, and 11 purulent exudates (Table 3).

Microscopic examination was negative in all cases. Of the 225 specimens, 24 (10.7%) grew *M. tuberculosis* on Lowenstein-Jensen slants and 31 (13.8%) from 27 patients grew *M. tuberculosis* in broth cultures. The rates of positive broth cultures according to the types of specimens are given in Table 3. None of the specimens was culture positive for nontuberculous mycobacteria. The mean time for growth on Lowenstein-Jensen slants was 35 days (range, 21 to 60 days), and the mean time to detection by the BacT/Alert instrument was 23 days (range, 10 to 45 days). Overall, *M. tuberculosis* was detected by the GTMD test at either day 15 or, when performed, day 8 in the 31 culture-positive specimens (Table 4). PCR assays were negative in the 194 culture-negative specimens. Thus, compared to culture results, the sensitivity, specificity, positive pre-

TABLE 2. Culture-enhanced PCR results for five patients with smear-negative, culture-positive specimens during the period January 2006 to May 2006[a]

Patient	Specimen	Smear result	Histology result	Time for growth on Lowenstein-Jensen slant (days)	Time to detection in BacT/Alert MP bottles (days)	Culture-enhanced PCR result	
						Day 8	Day 15
1	Cerebrospinal fluid	−	NP	27	21	+	+
2	Ascitic fluid	−	NP	45	28	+	+
3	Cervical lymph node	−	+[b]	25	15	+	+
	Cervical lymph node	−	+	33	22	+	+
4	Vertebral disk	−	+	26	21	+	+
	Vertebral disk	−	+	41	44	−	+
5	Vertebral disk	−	+	39	27	+	+

[a] +, positive; −, negative; NP, not performed.
[b] Positive histology was defined as granuloma with caseation and necrosis.

TABLE 3. Results of broth culture for 225 nonrespiratory specimens

Type of specimen (no.)	No. (%) of positive cultures with medium:	
	Lowenstein-Jensen slant	Broth
Tissue specimens (141)	17 (12)	22 (15.6)
Lymph node (53)	9 (17)	14 (26.4)
Bone (37)	6 (16.2)	6 (16.2)
Intestinal (23)	0	0
Cerebral (6)	0	0
Liver (4)	0	0
Other (18)	2[a] (11.1)	2[a] (11.1)
Organic fluids (73)	5 (6.8)	7 (9.6)
Bone marrow (16)	0	0
Cerebrospinal fluid (13)	2 (15.4)	2 (15.4)
Ascitic fluid (25)	1 (4)	3 (12)
Pleural fluid (9)	1 (11.1)	1 (11.1)
Peritoneal fluid (7)	1 (14.3)	1 (14.3)
Articular fluid (2)	0	0
Urine (1)	0	0
Purulent exudates (11)	2 (18.2)	2 (18.2)

[a] One peritoneal and one mastoid specimen.

dictive value, and negative predictive value of the procedure were 100%. A negative internal amplification control of the GTMD test indicating the presence of inhibitors was observed in 9 (4%) of the 225 specimens, including six tissue specimens (three bone specimens, one lymph node sample, one parotid gland sample, and one intestinal biopsy sample) and three fluid samples (two ascitic fluid samples and one bone marrow sample).

In addition to the 27 patients with culture-documented TB, four patients were diagnosed as having TB despite negative cultures (Table 5). ETB was documented by a positive histology showing typical granuloma with caseation and necrosis in three patients. It is noteworthy that, in two of these three patients, the specimen sent for histopathological examination was different from the specimen sent to the microbiology laboratory (vertebra D9 versus vertebra D8 in one patient and peritoneal biopsy sample versus ascitic fluid sample in the other patient). A further culture-negative patient was considered to have meningeal TB on the basis of clinical factors and a good response to antituberculous treatment. Thus, using the combination of culture, histopathological findings, and response to treatment as a reference standard, 31 (16.4%) of the 189 patients included were diagnosed as having ETB, and 35 (15.6%) of the 225 specimens studied were collected from these patients (Table 4). The four patients who were diagnosed as having ETB despite a negative culture were PCR negative. When using a positive diagnosis of TB as a gold standard, the sensitivity, specificity, positive predictive value, and negative predictive value of the procedure were 88.6%, 100%, 100%, and 97.9%, respectively.

TABLE 4. Comparison of culture-enhanced PCR results with broth culture results and diagnosis of TB[a]

Culture-enhanced PCR result	No. of specimens (total)			
	With broth culture result:		From patient type:	
	Positive (31)	Negative (194)	TB (35)	Non-TB (190)
Positive	31	0	31	0
Negative	0	194[a]	4	190

[a] TB was defined as a positive culture and/or positive histology and/or good response to therapy.

TABLE 5. Results for four patients with culture-negative and culture-enhanced PCR-negative ETB[a]

Patient	Specimen type	Culture result	PCR result at:		Diagnostic criterion for TB
			Day 8	Day 15	
1	Vertebra D8	−	−	−	Positive histology (vertebra D9)
2	Ascitic fluid	−	−	−	Positive histology (peritoneal biopsy)
3	Ileal biopsy	−	−	−	Positive histology (ileal biopsy)
4	Cerebrospinal fluid	−	−	−	Good response to antituberculous treatment

[a] −, negative.

The 19 patients with a strong suspicion of ETB were all diagnosed as having TB according to the above-mentioned criteria. PCR was positive at day 8 in only 10 (52.6%) of the 19 patients (Table 6). PCR was negative at day 8 and positive at day 15 in five further patients. The four remaining patients were PCR negative at both days 8 and 15 (Table 5). Culture in BacT/Alert MP bottles was positive for 15 (78.9%) of the 19 patients. The mean time to detection in broth culture for the 10 specimens with a positive PCR at day 8 was 15.7 days (range, 10 to 20 days). Eight of these 10 specimens were tissue specimens with a positive histology, and two were fluids for which histology was not available (Table 6).

DISCUSSION

The early diagnosis of ETB is challenging because of the paucibacillary nature of these infections, resulting in a very rarely positive smear microscopy finding and a long incubation time required for growth. This long delay without a positive or negative microbiological diagnosis could be shortened by the use of NAATs. However, these methods have relatively low sensitivity and negative predictive value when applied directly

TABLE 6. Results of culture-enhanced PCR at day 8 for 19 patients for whom there was a strong clinical suspicion of ETB, confirmed by culture, histology, and/or therapeutic response

PCR result at day 8	No. of patients with:				
	Broth culture result		Histology result		
	Positive	Negative	Positive[a]	Negative	Not performed
Positive	10	0	8	0	2
Negative	5	4	4	1	4

[a] A positive histology result was defined as granuloma with caseation and necrosis.

to extrapulmonary specimens (15). Thus, the development of procedures aimed at enhancing the sensitivity of NAATs is needed for the diagnosis of ETB. To this purpose, we designed a new procedure combining the GTMD assay with a preliminary culture step in order to amplify the amount of nucleic acid target and dilute inhibitors of amplification.

In the first part of the study, we evaluated our procedure with 10-fold-diluted *M. tuberculosis* suspensions mimicking paucibacillary clinical specimens. The suspensions were tested in the presence and in the absence of ground lymph node in order to examine the potential influence of PCR inhibitors. These experiments showed that a preliminary 8-day broth culture permitted PCR detection of *M. tuberculosis* in suspensions with a bacterial load of ≥50 CFU/ml, whereas a 15-day broth culture was required for detecting the lowest load of bacilli and for predicting final culture results with certainty. The latter result was confirmed on a series of 24 clinical specimens that were systematically subjected to culture-enhanced PCR at day 8 and day 15. This preliminary study of clinical specimens indicated that culture-enhanced PCR at day 8 was not positive in all patients with a positive culture. As a result, culture-enhanced PCR was subsequently evaluated by systematically performing the test at day 15 for all patients with suspected ETB whereas PCR was performed at day 8 only for a subgroup of patients for whom there was a strong clinical suspicion of ETB.

The rate of PCR inhibition in our study was 4%, which is lower than the rates usually reported for extrapulmonary specimens (1, 9). This finding may be explained by the dilution of PCR inhibitors resulting from the inoculation of specimens into BacT/Alert MP bottles. Of the 31 culture-positive specimens, all were paucibacillary as demonstrated by the negativity of smear microscopy and the time required to obtain a positive culture, namely, up to 60 days on solid medium and up to 45 days in BacT/Alert MP bottles. Moreover, seven specimens with a positive broth culture remained culture negative on solid medium after a 12-week incubation time. Compared with broth culture results, the sensitivity, specificity, positive predictive value, and negative predictive value of culture-enhanced PCR were 100%. Thus, a negative PCR result at day 15 was efficient in ruling out the presence of *M. tuberculosis* in the specimen tested several weeks before culture results were available. Our findings indicate that culture-enhanced PCR can provide an early microbiological result that may be used in conjunction with other diagnostic criteria for reevaluating treatment without waiting for final culture results. This procedure may be particularly important for fluid specimens, as no histological findings are available.

It is well known that culture cannot be considered a gold standard for evaluating the efficacy of a new diagnostic procedure for ETB because of a lack of sensitivity (10, 18). Therefore, we used the combination of culture, histological findings, and response to treatment as a reference standard. The sensitivity and negative predictive value of our procedure were 88.6% and 97.9%, respectively. Four patients who were diagnosed as having ETB on the basis of histological results ($n = 3$) or improvement after a trial of antituberculous therapy ($n = 1$) were PCR negative at day 15. The discrepancies between microbiological and histological results may be explained by the nonuniform distribution of bacilli in the aliquots apportioned for the individual tests. Moreover, microbiological and histopathological examinations were not performed on the same specimen type for two of the four patients. In contrast, culture and PCR were performed with the same aliquot, which explains the perfect correlation between these two methods. Thus, PCR results should always be compared with histological findings, when available, before the decision to initiate or discontinue antituberculous therapy. In our series, a single patient was eventually considered to have ETB on the basis of clinical suspicion despite negative culture, PCR, and histological results. Gamma interferon release assays, which have emerged as a specific method for the diagnosis of latent TB, may be useful for these few patients suspected of having ETB for whom all standard diagnostic criteria are negative (16).

From June 2006 onwards, PCR was performed at day 8 only for 19 patients for whom there was a strong clinical suspicion of ETB. A positive result was obtained for only 10 of these 19 patients who were diagnosed as having ETB. In contrast, histological findings, which are usually available within 8 days, were positive for 12 of the 13 biopsy specimens examined. These findings indicate that PCR at day 8 lacks sensitivity and should not be systematically performed. However, it may be useful for fluid specimens in patients with a high index of clinical suspicion since, in the absence of histological results, a positive PCR result may be the sole argument for deciding to initiate therapy without delay.

There are obvious limitations to the routine use of our procedure in the diagnosis of ETB. Because of the cost and feasibility of the test, the poorest countries that have the highest rates of TB cannot benefit from such technologies. The procedure is rather time-consuming and labor-intensive, and it should be performed by skilled technicians because of the risk of false-positive results due to contamination with amplicons. The screening of patients with suspected ETB by specialists may be useful to avoid the performance of costly unnecessary procedures (4, 21).

In conclusion, culture-enhanced PCR is a highly sensitive and specific method for the detection of *M. tuberculosis* in extrapulmonary specimens. A negative PCR result at day 15 predicted final culture results with certainty. Thus, PCR results may be used in conjunction with histological findings as a key factor to decide whether presumptive antituberculous treatment should be maintained or discontinued, thereby contributing to decreased costs and decreased potential toxicity related to prolonged unnecessary therapy.

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