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Automated Extraction and Amplification for Direct Detection of *Mycobacterium tuberculosis* Complex in Various Clinical Samples[▽]

With the incidence of culture-positive tuberculosis (TB) cases at 25.3 per 100,000 and a 25% rate of TB/HIV coinfection, the TB incidence in French Guiana is the highest of all French regions (3, 5). In this context, there is an urgent need for simple, automated systems for molecular diagnosis of TB that can be adapted to small laboratories. Introduction of a nuclear amplification test in a routine clinical laboratory is an additional expense, and its cost-effectiveness and clinical utility need to be evaluated and seem to be optimized when used for diagnosis in patients with an intermediate-to-high likelihood of TB (8, 14).

With these objectives, our study aimed to evaluate a molecular assay developed for the detection of *Mycobacterium tuberculosis* complex (MTC) on the robotic workstation easyMAG/easyQ (EMEQ; bioMérieux, France), in comparison with culture, the gold standard. Following extraction based on the method described by Boom et al. (1), nucleic acid sequence-based amplification (NASBA) with real-time molecular beacon detection was performed on the extract (9, 12).

The specificity of the EMEQ assay was initially evaluated with 33 mycobacterial isolates (16 *M. tuberculosis*, 2 *Mycobacterium bovis*, and 15 nontuberculous mycobacterium isolates). Subsequently, detection of MTC was performed on request by specialized physicians during a 1-year prospective study of hospitalized patients (90 clinical specimens split into 25 respiratory and 65 nonrespiratory samples). Samples were processed for acid-fast bacillus (AFB) smear microscopy and cultured, and isolates were identified using biochemical tests in conjunction with restriction fragment length polymorphism of the *hsp65* gene (7) and the GenoType mycobacterium assay (Hain Lifescience GmbH, Germany).

A total of 52 specimens were culture positive, and 44 were identified as MTC and detected by EMEQ. The 8 remaining culture-positive specimens were atypical mycobacteria (6 *Mycobacterium avium* and 2 *Mycobacterium fortuitum* specimens) and found to be negative with EMEQ. No inhibition in the specimens included was detected. Out of 38 specimens that did not grow, 35 were found to be negative with EMEQ (Table 1). Analysis of culture-negative but EMEQ-positive samples resolved 2 out of 3 discrepancies, as follows: (i) for a gastric aspirate specimen, a second aspirate specimen received the same day cultured positive for *M. tuberculosis*, and (ii) for a urine specimen, another urinary specimen collected 1 month later cultured positive for *M. tuberculosis* using the Bactec MGIT 960 liquid culture system in another laboratory. Nonetheless, for the single remaining specimen of ascitic fluid, which tested positive by the EMEQ assay in 2 different runs, no further samples could be collected. The patient who provided the remaining specimen, whose ascites was first clinically labeled as being of pancreatic origin, remained

“out of sight” despite subsequent calls for specimen collection.

In spite of the reported lack of sensitivity of molecular methods for nonrespiratory and paucibacillary specimens (2, 11, 13), the sensitivity in our study was 100% (Table 2); it was affected by neither the type of specimen nor the AFB counts (9 AFB-negative specimens were EMEQ positive). No false-negative result was observed. The specificity was 100% for laboratory isolates as well as respiratory clinical specimens and 97.1% for nonrespiratory specimens.

The negative predictive value (NPV) of a diagnostic test for tuberculosis is of great importance in deciding isolation release (6, 14), but predictive values have to be adjusted to the prevalence of the infection in the population tested (4). With a positive predictive value of 97.9% and a NPV of 100% (Table 2), EMEQ appears to be useful as a diagnostic test and to rule out TB in the group studied for intermediate-to-high clinical suspicion.

In addition, with 160 min needed for automatic extraction and amplification, incorporation of an internal control enabling the detection of samples inhibitors without duplicate testing of a seeded tube (10), and risk of contamination being minimized by amplification in closed tubes, the EMEQ assay seems to be a reliable method for use in routine clinical laboratories. These preliminary results will need to be confirmed by further studies.

TABLE 1. Results of AFB staining, culture, identification of MTC, and EMEQ for clinical specimens tested^a

Type of specimen	No. of specimens	No. of specimens testing positive by:			
		AFB staining	Culture	MTC identification	EMEQ
Respiratory					
Sputum	18	11	15	12	12
Bronchoalveolar lavage fluid	6	2	3	3	3
Bronchial aspirate	1	0	1	1	1
Nonrespiratory					
Gastric aspirate	29	22	24	24	25
Biopsy	15	6	4	2	2
Bone marrow	5	1	2	0	0
Pleural fluid	4	1	2	2	2
Urine	3	0	0	0	1
Blood	3	1	1	0	0
Abscess fluid	2	0	0	0	0
Nodule aspirate	1	0	0	0	0
Cerebrospinal fluid	1	0	0	0	0
Ascites	1	0	0	0	1
Culture supernatant	1	1	0	0	0
Total	90	45	52	44	47

^a Before resolution of discrepancies.

TABLE 2. Correlation of EMEQ and MTC by culture^a

Type of specimen	No. of specimens with indicated results (EMEQ/MTC by culture)				Sensitivity/specificity (%)	Predictive value (%)	
	Positive/positive	Positive/negative	Negative/positive	Negative/negative		Positive	Negative
Respiratory	16	0	0	9	100/100	100	100
Nonrespiratory	30	1	0	34	100/97.1	96.8	100
Total	46	1	0	43	100/97.7	97.9	100

^a After resolution of discrepancies.

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