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Multicenter Evaluation of a Pathogenic *Mycobacterium* Screening Probe

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The introduction of nucleic acid amplification assays into the clinical laboratory has reduced the time needed to diagnose diseases caused by members of the *Mycobacterium tuberculosis* complex (MTBC). However, several mycobacterial species other than those of the MTBC are known to cause disease, especially in immunocompromised individuals. A screening assay has been developed for the detection of the major pathogenic mycobacterial species. The assay utilizes pan-genus primers to amplify mycobacterial DNA and a screening probe (KY493) that detects all major pathogenic mycobacteria. A multicenter European study was conducted to assess the performance of the screening probe in the clinical laboratory. The screening probe was evaluated against individual probes specific for *M. tuberculosis*, *M. avium*, and *M. intracellulare*, a genus-specific probe with broader species coverage, and culture. The screening probe had a sensitivity equivalent to that of the species-specific probes; all specimens positive with any of the species-specific probes were also positive with the screening probes. Compared to culture, the sensitivity of the screening probe was 89% (154 of 173) for all culture-positive specimens tested. This value was 89.6% for the genus-specific probe. The screening probe was more specific than the genus-specific probe. Specificity was 93.9% (661 of 704) compared to culture results alone. The comparable specificity value for the genus-specific probe was 84.8%. When clinical data were taken into consideration, the sensitivity of the screening assay was similar to that of culture (81% versus 76.2%) but the positive predictive value of the test was lower (76.2% versus 100% for culture). However, the screening probe was more sensitive than smear and may be a useful tool in the rapid diagnosis of mycobacterial disease.

Several species of mycobacteria, such as members of the *Mycobacterium tuberculosis* complex and *M. leprae*, are major human pathogens. Other mycobacterial species, such as *M. avium* and *M. intracellulare*, may be clinically significant, especially in immunocompromised individuals. The microscopic examination of specimen smears for acid-fast bacilli (AFB) is a rapid method of screening for the presence of mycobacteria in clinical samples. However, its sensitivity is usually low (1), and culture is required for species identification. Nucleic acid amplification techniques such as PCR coupled with hybridization to a genus-specific probe (5) provide a sensitive screening method that can detect the presence of mycobacterial DNA sooner than culture (2). However, mycobacteria are ubiquitous organisms that can be found in soil and the water supply. The presence of these environmental mycobacteria can give rise to false-positive results with a genus-specific probe. To circumvent this problem, a screening assay that detects all of the major pathogenic mycobacteria but not most environmental mycobacteria has been developed. In this assay, mycobacterial DNA are amplified by PCR with pan-genus primers (6). Amplification products are then hybridized to a screening probe (KY493). The KY493 screening probe detects as little as 10 ng of DNA (equivalent of three bacteria) from the major pathogenic mycobacteria, such as members of the *M. tuberculosis* complex and nontuberculous mycobacteria frequently associated with disease, such as *M. leprae*, *M. avium*, *M. intracellulare*, *M. kansasii*, *M. xenopi*, and *M. malmoense*. Specimens positive by this probe can be further evaluated by hybridizing the amplicons to species-specific probes.

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A multicenter study was conducted to evaluate the utility of KY493 as a screening tool in the clinical setting. A total of 893 respiratory specimens from 443 patients were collected between July 1997 and April 1998 at four study sites (Hopital Cantonal Universitaire de Geneve, Geneva, Switzerland; Inst. Laboratoriumsdiagnostik, Zentralkrankenhaus, Gauting, Germany; North Manchester General Hospital, Manchester,

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TABLE 1. Performance of the KY493 screening probe and KY166 genus-specific probe relative to culture in the detection of mycobacteria in respiratory specimens

Probe, specimen type, and test result	No. of specimens with the following culture result:		Sensitivity (%)	Specificity (%)
	Positive	Negative		
KY493				
All specimens			89.0	93.9
Positive	154	43		
Negative	19	661		
Smear-positive specimens			95.2	100.0
Positive	119	0		
Negative	6	3		
Smear-negative specimens			72.9	93.9
Positive	35	43		
Negative	13	658		
KY166				
All specimens			89.6	84.8
Positive	155	107		
Negative	18	597		
Smear-positive specimens			96.0	99.9
Positive	120	2[a]		
Negative	5	1		
Smear-negative specimens			72.9	85.0
Positive	35	105		
Negative	13	596		

[a] Corrected specificity. One specimen was smear and KY166 positive due to bronchoscope contamination.

United Kingdom; and Hopital Saint-Louis, Paris, France). All specimens were collected as part of routine mycobacterial testing in each laboratory. Both archival and prospective specimens were used. Each specimen was analyzed by microscopic examination (smear), culture, and PCR. Clinical data were obtained from each center except Manchester and analyzed using specific criteria to allow confirmation of a diagnosis of tuberculosis or atypical mycobacterial infection.

Respiratory samples (sputa, gastric aspirates, bronchial washes, and aspirates) were liquefied and decontaminated by the *N*-acetyl-cysteine–NaOH method as recommended by the Centers for Disease Control and Prevention (3). Aliquots of the resultant sediments were analyzed by microscopic examination for acid-fast organisms after staining with auramine and cultured in solid (Lowenstein-Jensen) and liquid (MGIT or 12B) media. A 100-μl aliquot of each sediment was processed for PCR with reagents from the sample preparation kit of the Amplicor MTB assay (Roche Diagnostics, Somerville, N.J.).

Mycobacterial DNA was amplified using reagents from the Amplicor MTB assay. An internal control template was included in each amplification reaction to monitor the presence of inhibitory substance (4). Amplification products were hybridized to each of five probes immobilized separately in the wells of microtiter plates. The following probes were used: a genus-specific probe (KY166), a screening probe (KY493), an *M. tuberculosis* probe (KY172-T3), an *M. avium* probe (KY167), and an *M. intracellulare* probe (KY169). Hybridization products were detected colorimetrically as described previously (6).

Specimens found to be inhibitory were retested, either undiluted or diluted 10-fold. Positive cases were identified by either of the following criteria: (i) culture was positive for *M. tuberculosis* or other mycobacteria, and (ii) patients were strongly suspected of having tuberculosis (TB) (i.e., patients were from areas of endemicity and presented with clinical symptoms of TB which improved with antituberculous therapy). Patients under treatment at the time of the study were excluded from the analysis (23 in Gauting, 2 in Paris, and 1 in Geneva).

A total of 893 specimens were tested. The overall percentage of culture-positive specimens was 19.6% (Paris, 4.6%; Manchester, 10%; Geneva, 24.4%; and Gauting, 40.1%). There were 175 culture-positive specimens, of which 130 (74.3%) were smear positive. Of the culture-positive specimens, 156 were positive for *M. tuberculosis* complex and 19 were positive for atypical mycobacteria: 11 were positive for *M. avium*, 2 were positive for *M. intracellulare*, 2 were positive for *M. malmoense*, 2 were positive for *M. xenopi*, 1 was positive for *M. kansasii*, and 1 was positive for *M. peregrinum*.

At initial testing, 73 (8.2%) of the 893 specimens were inhibitory (i.e., had negative internal control amplification and negative results for all five probes). Repeat testing, with samples either undiluted or diluted 1/10, was performed on these specimens. Dilution of some of the specimens prior to repeat testing likely did not affect the PCR sensitivity in this study. None of the specimens that gave negative results after repeat testing at a 1/10 dilution were culture positive. Only 16 (1.8% of the total) specimens remained negative after repeat testing. These were excluded from the final analysis.

The combined results from all four sites showed that the screening probe, KY493, was more specific than the genus-specific probe, KY166 (Table 1). The two probes had identical sensitivities. When the use of probe KY493 was compared to culture, the overall sensitivity and specificity were 89 and 93.9%, respectively. Negative predictive values (NPV) and positive predictive values (PPV) were 97.3 and 78.2%, respectively. The overall sensitivity and specificity of probe KY166 were 89.6 and 84.8%, respectively. NPV and PPV were 97.1 and 59.6%, respectively. Results from the individual sites differed somewhat in detail, but in each case the specificity of KY493 was higher than that of KY166 (data not shown).

TABLE 2. Sensitivity of AFB smear and detection by KY493 following PCR relative to culture

Site[a]	Sensitivity (%)	
	Smear	KY493
Geneva	80.4	94
Gauting	88.8	96
Paris	50.0	67.5
Manchester	67.5	77.5
Total	74.3	89.0

[a] Culture positivity rates were 24.4, 40.1, 4.6, and 10% for the Geneva, Gauting, Paris, and Manchester sites, respectively.

TABLE 3. Comparison of the KY493 screening probe and culture in respiratory specimens relative to diagnosis and treatment for *M. tuberculosis* or atypical mycobacterial infection

Test and results	No. of patients with the following clinical outcome[a]:		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive	Negative				
Culture			76.2	100	100	94.9
Positive	48	0				
Negative	15	278				
PCR-KY493			81.0	93.8	76.2	95.6
Positive	51	16				
Negative	12	262				

[a] Combined results for the Gauting, Geneva, and Paris sites.

The screening assay had lower sensitivity and PPV with smear-negative, culture-positive specimens than with smear-positive, culture-positive specimens. However, sensitivity of the screening assay was higher than that of AFB smear (Table 2). This was true at all four sites but especially at the Paris site, where smear sensitivity was quite low. The lower smear sensitivity in Paris was perhaps due to an unusually low incidence of positive samples collected during the study period (4.6%) compared to what is normally seen (~10%). This fluctuation in the frequency of smear-positive samples demonstrates that samples were not selected and that some of the testing was performed on a diverse population rather than being targeted towards those suspected of having a mycobacterial infection. The observation that the KY493 PCR assay was nearly twice as sensitive as smear in this population demonstrates the robustness of this PCR assay and demonstrates that in an area with a low incidence of tuberculosis or other mycobacterial infections, the assay performed well.

The TB prevalence rates at the Geneva, Gauting, and Paris sites were 14.9, 23.6, and 7%, respectively. Two or more specimens were collected per patient, with up to eight samples tested at two sites (Geneva and Gauting). Using clinical indications of tuberculosis and atypical mycobacterial infection as criteria for analysis at the three sites with clinical data (Paris, Geneva, and Gauting), the overall sensitivity and specificity of KY493 in the patient population were 81 and 93.8%, respectively. NPV and PPV were 95.6 and 76.2%, respectively (Table 3). The overall sensitivity and specificity of culture were 76.2 and 100%, respectively, with NPV and PPV of 94.9 and 100%, respectively (Table 3).

The screening probe results from 16 patients were considered to be false positive. These 16 patients comprised 5 with lung carcinoma, 1 with leukemia, 4 with previous tuberculosis, 2 homeless patients (including 1 foreigner with two KY493-positive samples), and 4 patients lost to follow-up. The absence of positive culture for some of these patients could be due to the presence of environmental mycobacteria, the inability of the media to support growth of the bacteria, or sampling error.

Data obtained from this multicenter European study showed that the screening assay performed well in diagnosing mycobacterial infection in a clinical laboratory setting. The sensitivity of KY493 was identical to those of probes specific for *M. tuberculosis*, *M. avium*, and *M. intracellulare*. All samples positive with one of these probes were also positive with KY493 (data not shown). In addition, all but two of the mycobacteria isolated in this study for which probes were not available were detected by the screening probe. The two isolates not detected by KY493 (*M. xenopi* and *M. peregrinum*) were positive only after 45 days of culture in liquid media and were considered by the clinicians to be contaminants.

The main purpose of this study was to evaluate the potential for a new approach toward the rapid screening and diagnosis of mycobacterial infection and the effects that it may have on laboratory testing algorithms, workload, and ultimately patient care. The data presented here show that such a screening approach offers greater sensitivity than AFB smear. Furthermore, this approach has the potential for more rapid species identification when performed in a reflex testing mode by hybridizing positive amplicons to species-specific probes. The use of a reflex testing mode would significantly reduce the number of samples that have to be tested by species-specific probes. Automation of the reflex testing process would provide an even more efficient tool for the diagnosis of mycobacterial infections.

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