Potential for Use of the Seegene Anyplex MTB/NTM Real-Time Detection Assay in a Regional Reference Laboratory

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Requests for direct molecular diagnosis of mycobacterial disease are increasingly warranted. The Anyplex MTB/NTM assay demonstrates sensitivities, specificities, and positive and negative predictive values of 1.00, 0.96, 0.93, and 1.00 for *Mycobacterium tuberculosis* complex (MTBC) and 1.00, 0.97, 0.75, and 1.00 for nontuberculous mycobacteria (NTM) detection, respectively, making it a suitable screening test for mycobacterial detection.

Despite the importance of accurate microbiological diagnosis of mycobacterial disease, conventional methods have limitations. Molecular detection and identification of the *Mycobacterium tuberculosis* complex (MTBC) and, increasingly important, nontuberculous mycobacteria (NTM) direct from clinical material offer rapid and sensitive results, with greater specificity and significantly reduced turnaround time compared with those of microscopy and culture, respectively (1, 2).

In-house nucleic acid amplification test (NAAT) publications abound, but there is a dearth of standardization (3–6). A number of Conformité Européenne (CE)-marked and/or FDA-approved commercial assays are now well documented in the literature (7–14). However, these tests generally do not allow for the detection of NTM, preclude MTBC detection without the concomitant detection of resistance determinants, require the use of assay-specific amplification platforms, and may necessitate subjective result interpretation. The Anyplex MTB/NTM real-time detection assay (Seegene) is an CE-marked test suitable for the direct detection and discrimination of both MTBC and NTM. It is validated for a wide range of sample types and can be run on a number of different amplification platforms. Result interpretation is automated and can be reported within 3.5 hours of sample receipt.

As part of a routine diagnostic service, direct molecular testing was performed on 110 samples (Table 1) submitted by physician request between 2008 and 2011 using the Genotype MTBDR*plus* (v1.0) assay (Hain Lifescience). DNA was extracted from 500 μl of a decontaminated respiratory sample or an untreated nonrespiratory specimen as described in the MTBDR*plus* (v1.0) assay kit insert (15). Each extraction included a positive and a negative extraction control. Concerns over the subjective interpretation of results, potential contamination through postamplification processing, and slow turnaround time prompted investigation into quantitative PCR (qPCR). The Anyplex MTB/NTM real-time detection assay was validated by testing these extracts retrospectively after storage (at −80°C).

Anyplex MTB/NTM testing used 5 μl of extract which was added to a 15-μl master mix containing 10 μl 2× Anyplex PCR master mix, 3 μl methoxypsoralen (8-MOP), and 2 μl 10× *M. tuberculosis*/NTM oligonucleotide mix. Amplification and detection were performed on a Rotor-Gene 3000 instrument for all sample extracts. MTBC detection targeted the IS*6110* and MPB64 genes, while NTM detection was based on amplification and detection of a section of the 16S rRNA gene. In addition to the in-

house extraction controls, each run included positive and negative amplification controls provided in the kit and an internal control in the master mix to detect inhibition. Result interpretation was performed automatically using the instrument's software according to threshold and cutoff values outlined by the manufacturer (16).

The Anyplex MTB/NTM assay demonstrated sensitivities, specificities, positive predictive values (PPVs), and negative predictive values (NPVs) of 0.86, 0.99, 0.96, and 0.95 and 1.00, 0.97, 0.77, and 1.00 for MTBC and NTM detection, respectively, when compared with culture (Table 2). The Anyplex MTB/NTM assay was considered suitable to replace the MTBDR*plus* (v1.0) test for

TABLE 1 Conventional and Anyplex MTB/NTM results by sample type

Sample type (no.)	PCR result	Smear+ culture+	Smear− culture+	Smear+ culture−	Smear− culture−
Retrospective pulmonary (54)[a]	+	33[b]	1[c]	2[d]	1[c]
	−	1[c]	1[c]	2	13
Retrospective extrapulmonary (56)[e]	+	0	1[c]	0	0
	−	0	2[c]	0	53
Prospective pulmonary (72)[f]	+	42[g]	5[c]	4[h]	2[c]
	−	0	0	7	12
Prospective extrapulmonary (33)[i]	+	0	0	0	0
	−	0	0	0	33

[a] Forty-four sputum and 10 bronchoalveolar lavage specimens.
[b] Twenty-three MTBC and 10 NTM.
[c] All MTBC.
[d] All NTM.
[e] Fifty-two CSF and 4 pleural fluid specimens.
[f] Fifty-nine sputum and 13 bronchoalveolar lavage specimens.
[g] Thirty-three MTBC and 9 NTM.
[h] One MTBC and 3 NTM.
[i] Thirty-two CSF and 1 knee fluid specimen.

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TABLE 2 Performance characteristics of molecular mycobacterial detection compared with those of culture

Assay name and type of detection	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
MTBDR*plus* (v1.0), prospective MTBC	0.79 (0.60–0.92)	0.99 (0.92–1.00)	0.96 (0.79–0.99)	0.92 (0.84–0.97)
Anyplex				
Retrospective MTBC	0.86 (0.68–0.96)	0.99 (0.92–1.00)	0.96 (0.80–0.99)	0.95 (0.87–0.98)
Retrospective NTM	1.00 (0.69–1.00)	0.97 (0.91–0.99)	0.77 (0.46–0.95)	1.00 (0.96–1.00)
Prospective MTBC	1.00	0.96[a] (0.89–1.00)	0.93[a] (0.83–1.00)	1.00
Prospective NTM	1.00	0.97[b] (0.92–1.00)	0.75[b] (0.44–1.00)	1.00

[a] All 3 MTBC culture-negative samples were from patients with a history of tuberculosis. One sample (smear positive) was from a patient for whom samples sent 2 months earlier and 3 months later were *M. tuberculosis* culture positive, one sample (smear negative) was from a patient for whom four specimens had been submitted in 1 week, with only one of the four samples being culture positive (not the one tested by PCR), and one sample (smear negative) was from a patient for whom a sample sent 3 weeks previously was *M. tuberculosis* and PCR positive.
[b] All were microscopy positive.

routine molecular diagnosis of mycobacterial infection based on assay agreement (accuracy, 96.4%; kappa = 0.897) and comparable retrospective performance (Table 2) with the added benefits of the real-time PCR format.

The prospective performance characteristics of the Anyplex MTB/NTM assay after its introduction as a routine diagnostic assay are shown in Table 2. One hundred five samples (Table 1) were extracted as previously described and amplified on the ABI 7500 fast PCR platform (Life Technologies) generating sensitivities, specificities, positive predictive values, and negative predictive values of 1.00, 0.96, 0.93, and 1.00 and 1.00, 0.97, 0.75, and 1.00 for MTBC and NTM detection, respectively.

Using culture as the gold standard, smear microscopy and the Anyplex MTB/NTM assay demonstrated overall sensitivities, specificities, positive predictive values, and negative predictive values (with 95% confidence intervals [CIs]) of 0.89 (0.78 to 1.00), 0.81 (0.68 to 0.94), 0.79 (0.65 to 0.93), and 0.90 (0.80 to 1.00) and 1.00, 0.90 (0.87 to 1.00), 0.94 (0.86 to 1.00), and 1.00, respectively. All culture-positive samples were correctly identified by the Anyplex MTB/NTM assay. Two samples that were culture and microscopy negative were detected by Anyplex MTBC, but these samples were from patients with a recent history of MTBC infection.

This work shows that the performance characteristics of the Anyplex MTB/NTM assay in a low-risk low-incidence region for tuberculosis (TB) were comparable with those for other commercial NAATs (2). It also supports a recent position statement that molecular testing for the detection of MTBC from respiratory specimens is superior to smear microscopy (17).

In spite of their proven clinical utility, conventional diagnostic methods suffer from several deficiencies, including subjective result interpretation and the requirement for highly trained staff for accurate reporting. Although culture is the current gold standard, results are frequently not available for more than 1 week and may take up to 12 weeks in some cases. While no diagnostic test is flawless, molecular techniques offer the potential for sensitive, specific, and timely diagnoses and differentiation of mycobacterial infection directly from patient specimens.

The reduced hands-on time and improved ease of result interpretation of the Anyplex MTB/NTM assay compared to those of the MTBDR*plus* (v1.0) assay facilitate accessibility to testing and therefore the availability of results within 1 working day, as suggested in the statement. The assay also satisfies a need for alternative commercial options for the direct molecular diagnosis of tuberculosis, which was identified by a Public Health England working party (17).

Another limitation of smear testing was highlighted during the study when a review of the enhanced TB surveillance database for patients tested during the study period showed that at least five patients who were NTM culture positive commenced treatment for tuberculosis, due in part to smear positivity, prior to isolate cultivation and identification. Rapid differentiation of MTBC and NTM using the Anyplex MTB/NTM assay for smear-positive samples may reduce the time, anxiety, patient morbidity, and health care expenditure associated with false-positive TB diagnoses. Whether there is an economic and/or clinical utility in using the assay for all specimens in a low-prevalence area should be investigated.

It is recognized that the absence of concomitant antibiotic resistance determinant detection in the Anyplex MTB/NTM assay could limit its utility. However, in a low-incidence area, where very few cases of TB and especially drug-resistant TB are seen, it may be more cost-effective to screen for TB in the first instance instead of routinely testing all samples for drug resistance markers. This work was undertaken before the release of an updated version of the Anyplex MTB/NTM assay, which allows isoniazid and rifampin resistance determinant detection in a nested real-time format. However, the preliminary data (unpublished) for the updated version are encouraging. An additional limitation to the study is that a comparison was not possible with the Hain MTBDR*plus* (v2.0) test due to a new extraction process being part of the later-version assay.

In conclusion, this study has shown that when selective PCR testing for TB is undertaken in a low-incidence area, the Seegene Anyplex MTB/NTM detection assay is suitable and convenient for the detection and differentiation of MTBC and NTM infections. The release of the Seegene Anyplex plus MTB/NTM and MDR-TB (multidrug-resistant TB) detection assays also offers the potential for further streamlining of rapid resistance detection in the same convenient format. Prospective performance statistics and analyses of enhanced TB surveillance data suggest that the economic and clinical utilities of replacing smear testing with PCR in similar low-incidence settings warrant further investigation.

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