AUDIT, RESEARCH AND GUIDELINE UPDATE

Rapid molecular detection of tuberculosis and rifampicin drug resistance: retrospective analysis of a national UK molecular service over the last decade

N Seoudi,1,2 S L Mitchell,1,3 T J Brown,1,3 F Dashti,2 A K Amin,2 F A Drobniewski1,2,3

ABSTRACT

Background Fast and reliable detection of Mycobacterium tuberculosis complex (MTBC) and drug resistance is crucial in establishing effective treatment and enforcing timely public health measures.

Methods The authors analysed the performance of a national UK molecular diagnostic service over a decade, based on the use of a line probe assay (Innolipa, LiPA) compared with conventional liquid and solid cultures with rapid molecular identification and culture-based drug resistance testing.

Findings Data were available for 7836 consecutive patient samples using LiPA and the reference microbiological technique (conventional liquid and solid cultures with rapid molecular identification and culture-based drug resistance testing). For all sputum specimens (n = 3382) the sensitivity, specificity, positive predictive value, negative predictive value and accuracy for MTBC detection were 93.4%, 85.6%, 92.7%, 86.9% and 90.7%; the equivalent values for smear-positive sputum specimens (n = 2606) were 94.7%, 80.9%, 93.9%, 83.3% and 91.3%. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy for detection of rifampicin resistance in all sputum samples (n = 1667) were 92.1%, 99.3%, 89.4%, 99.5% and 98.9% respectively; the equivalent values for smear-positive sputum specimens (n = 1477) were 93.3%, 99.3%, 87.5%, 99.6% and 99%. Between January 2006 and December 2008, LiPA saved 25.3 and 32.2 days for TB diagnosis and rifampicin resistance of smear-positive samples, respectively.

Interpretation A molecular diagnostic service, using a non-automated line probe assay approach, provides a rapid and reliable national service for diagnosing MTBC and rifampicin resistance.

INTRODUCTION

The rapid identification of multidrug resistant tuberculosis (MDRTB) (ie, tuberculosis (TB) isolates resistant to at least isoniazid and rifampicin) reduces the time for the instigation of appropriate treatment, helps to reduce the spread of drug-resistant TB and may improve survival.1–4

Conventional drug susceptibility testing (DST) can take 1–2 weeks once a positive culture has been obtained. In recent years, the use of assays for the genetic detection of mutations that confer resistance have been developed and evaluated. Among these, line probe assays (LPAs) are viewed as a rapid, very specific and sensitive tool for the detection of multidrug-resistant TB.5 Moreover, the WHO have recently endorsed a policy of the use of LPAs for the rapid screening of patients at risk of MDRTB6 and in 2010 recommended the use of the GeneXpert, XpertMTB/RIF following a successful multicentre analysis.7

In 1998, national and population-based services were proposed for the diagnosis of TB and rifampicin resistance directly from smear-positive patient specimens,8 and these were adopted in the UK and in other countries using LPAs. The UK Health Protection Agency National Mycobacterium Reference Laboratory (HPA NMRL) implemented this national molecular TB diagnostic service (branded as ‘Fastrack’) in 1999–2000 with promising initial results.5 Rapid identification of rifampicin resistance and MDRTB with automated liquid culture is performed at the same time; specimens identified as MDRTB have any resulting cultures analysed for all first-line and second-line drugs simultaneously.

Using a different LPA, excellent sensitivity, specificity, positive predictive value (PPV) and negative predictive (NPV) were obtained when used directly on 536 smear-positive sputum specimens in South Africa,9 demonstrating that these techniques worked well in low and middle income countries.

The study evaluates the accuracy of results obtained for TB diagnosis and rifampicin detection compared with rapid culture-based methods in the context of a routine, non-trial national molecular diagnostic service. Sensitivity, specificity, PPV and NPV, analysis of discrepant results and turnaround times were obtained and compared with our previously published data to underline a decade of experience of these assays.

This study complements other published studies on the automated GeneXpert system and its XpertMTB/RIF assay7 by providing extensive evidence for an alternative system of diagnosis supporting WHO policy statements on the use of both LPAs and XpertMTB/RIF, and demonstrating the value of a national operational service.

MATERIALS AND METHODS

Clinical specimens

A total of 8501 consecutive primary specimens were referred to the UK HPA NMRL at the request of National Health Service (NHS) physicians over a 7-year period (January 2003–December 2009) for molecular ‘Fastrack’ analysis. A single specimen from each patient was analysed in any one year. The reference assay was conventional culture-based diagnosis and rifampicin resistance (see below).
Out of the 7836 samples with both molecular and comparative culture-based results, 4420 were respiratory samples, including 3382 sputum samples, 733 bronchoalveolar lavage (BAL) samples, 269 pleural fluid aspirates and 36 other respiratory samples such as lung biopsy; 5406 samples were non-respiratory, including 1638 cerebrospinal fluid (CSF) samples.

**Microscopy, culture and susceptibility testing**

All samples were decontaminated and processed according to the NMRL standard operating procedure, which has previously been described.\(^5\) NaOH/N acetyl-L-cysteine (NaOH/NALC) (6 ml) was added to each sample in a 50 ml Falcon tube. The sample was allowed to be incubated with the NaOH/NALC for 30 min with periodic gentle mixing. Subsequently, each sample was diluted (1:40 dilution) with phosphate buffer (pH 6.8) followed by centrifugation at 5000 \( \times \) g for 30 min. The supernatant was discarded and 2 ml of sterile phosphate buffer was added to resuspend the pellet. Half of the suspension was used for the molecular assay, 0.25 ml of each decontaminated sample was cultured on Lowenstein-Jensen (LJ) slopes and 0.5 ml was inoculated into mycobacterial growth indicator tubes (MGIT) (Becton, Dickinson and Company, New Jersey, USA). Suitable samples for microscopy examination were investigated by fluorescent auramine phenol staining according to the NMRL standard operating procedure.

First-line DST was carried out on all culture-positive MTBC isolates using the resistance ratio method on LJ slopes\(^10\) and including rifampicin, isoniazid, ethambutol and pyrazinamide. Second-line (reserve) DST was performed using the MGIT system. The isolated microorganism was identified by using GenoType-Series molecular assay (Hain Lifescience GmbH, Nehren, Germany).

**LiPA**

DNA was extracted from 1 ml of each decontaminated sample and a commercial and validated LiPA assay (Innolipa, LiPA; Innogenetics, Ghent, Belgium) was performed for all samples included in the analyses (7836 specimens) according to the manufacturer’s instructions and as previously described.\(^5\)

**Standardisation and blinding**

Staff performing molecular analyses were blind to the culture-based reference methods. Bacteriological staff usually had no prior experience of molecular diagnostic assays but were appropriately trained to perform the LiPA over a 1-week period using written standard operating procedures. A PhD grade molecular scientist oversaw the service. Culture-based internal and external quality assurance and proficiency systems were in place throughout and were introduced in 2003 for molecular assays.

**Statistical analysis**

Data were entered into a Microsoft Excel spreadsheet (Microsoft Corporation, Washington, USA) and analysed by Prism (GraphPad, USA). The accuracy of the LiPA assay for detecting MTRBC and rifampicin resistance was compared with the accepted standards of culture (ie, one MGIT plus one LJ slope), and phenotypic DST.\(^11\)\(^12\) Sensitivity, specificity, PPV, NPV and diagnostic accuracy were calculated as follows: sensitivity (%)= (true positive/true positive + false negative) \( \times \) 100; specificity (%)= (true negative/false positive + true negative) \( \times \) 100; PPV = (true positive/true positive + false positive) \( \times \) 100; NPV = (true negative/false negative + true negative) \( \times \) 100; and diagnostic accuracy (%) = (true positive + true negative/total number) \( \times \) 100. An increase in sensitivity indicated a decrease in false-negative cases, while an increase in specificity indicated a decrease in false-positive cases. PPV is defined as the probability that a person has the disease and their test was positive, while NPV is defined as the probability that a person does not have the disease and their test was negative. CIs were calculated using the method of Vollset.\(^13\) The sensitivity, specificity, PPV and NPV were compared (using the \( \chi^2 \) test) with our previously published data.\(^5\) Statistical significance was determined using a p value \( \leq 0.05\).

Detailed turnaround times were calculated for a recent 3-year period (2006–8). The time to diagnosis was calculated from the date of receiving the sample in the NMRL to the date of issuing the results to the clinician. The time to diagnosis was based on direct LiPA testing of patient specimens compared with the time taken for culture growth and identification of the isolated mycobacterium. The NMRL use GenoType-Series molecular assays for rapid identification of mycobacterial cultures (performed daily, Monday–Friday).

**RESULTS**

Of the 8501 consecutive samples received by the NMRL for LiPA assay during the period between January 2003 and December 2009, comparison with bacteriological culture was possible for 7836 samples (culture could not be performed or was contaminated in 290 (3.4%) samples; PCR was not performed for 140 (1.6%) samples (see below); PCR was inhibited or not interpretable in 285 (3.4%) samples (including 50 specimens which also had contaminated cultures); hence these were excluded from the analysis) (figure 1).
Specimens were analysed by sample type and subdivided by microscopy result if known. Specimens were also analysed as ‘respiratory’ (sputum, BAL, pleural fluid and other respiratory samples such as lung biopsy) and ‘non-respiratory’ (CSF, lymph node biopsy, aspirate, pus, blood, pericardial fluid and others including urine and other biopsies) and subdivided by microscopy result if known (tables 1, 2 and supplementary online tables A1–A2).

The acid-fast bacilli smear microscopy was positive for 3656 of 7836 primary specimens (46.4%), negative for 3076 (39.5%) and unknown for 1124 (14.3%). These 1124 specimens that did not have microscopy performed routinely or did not have a microscopy result were included in the main analysis but excluded from the specific sub-analysis described in tables 1–2 and online supplementary tables A1–A2. A total of 3299 MTBC and 520 non-tuberculosis mycobacteria (NTM) were cultured in the 7-year study period.

LiPA
LiPA was not performed in 140 of 8501 (1.6%) specimens as a culture had been sent previously and the MTBC and rifampicin results were already known. For the 8361 processed samples, the results of LiPA analysis for MTBC were negative for 4745 (56.8%), positive for 3382 (39.8%) and 285 (3.4%) were inhibited or not interpretable. Of the 3331 PCR-positive cases, 4745 (56.8%), positive for 3331 (39.8%), and 285 (3.4%) were equivocal results.

The overall sensitivity, specificity, PPV, NPV and diagnostic accuracy of LiPA assay for MTBC detection were analysed using different sample types in relation to the standard culture-based technique as shown in table 1 and online supplementary table A1. Overall sensitivity, specificity, PPV, NPV and diagnostic accuracy of rifampicin resistance in comparison to the standard resistance ratio drug susceptibility method (phenotypic technique) as shown in table 2 and online supplementary table A2.

A single molecular test performed against liquid and solid culture for all specimens had an overall sensitivity of 87.8%, specificity of 91.1%, PPV of 87.7%, NPV of 90.8% and overall accuracy of 89.5% for MTBC detection (table 1). For all sputum specimens (n=3382) the sensitivity, specificity, PPV, NPV and diagnostic accuracy for rifampicin resistance in comparison to the standard resistance ratio drug susceptibility method (phenotypic technique) were as follows: 92.4%, 89.1%, 93.9%, 83.3% and 91.3% respectively. The equivalent figures for smear-positive specimens (n=2606) were 94.7%, 80.9%, 93.9%, 83.3% and 91.3% respectively. The results for non-smear-positive samples were good (with the exception of CSF), although with lower sensitivity, for example, BAL smear-positive sensitivity was 86.8%.

The DST data were available for 2306 specimens for both techniques (LiPA and the standard resistance ratio susceptibility method). A total of 136 (5.9%) specimens were reported as rifampicin resistant by LiPA and 2170 (94.1%) were sensitive. In contrast, 152 (5.7%) specimens were reported as rifampicin resistant using the standard resistance ratio susceptibility method and 2174 (94.9%) were sensitive.

The overall sensitivity, specificity, PPV, NPV and accuracy for the detection of rifampicin resistance for all specimens which became culture positive and for which comparative phenotypic DST data were available (n=2306), was as follows: 92.4%, 99.4%, 89.7%, 99.5% and 99.0%, respectively. Out of the 2306 samples analysed; the acid-fast bacilli smear microscopy was

| Table 1 Results of LiPA compared with culture in detecting Mycobacterium tuberculosis complex (2003–2009) |
| Sample and AFB smear results (2003–2009) | Sensitivity (%) | CI | Specificity (%) | CI | PPV (%) | CI | NPV (%) | CI | Accuracy (%) | CI |
| All smear +ve | 90.9 to 93.0 | 89.1 to 94.9 | 77.5 to 81.8 | 92.4 to 94.7 | 2782/2970 (90.4) | 89.3 to 91.4 |
| Respiratory smear +ve | 94.7 to 91.5 | 160.4 to 93.4 | 90.4 to 92.6 | 91.6 to 93.2 | 2782/2970 (90.4) | 89.3 to 91.4 |
| Non-respiratory smear +ve | 87.9 to 88.7 | 328/373 (87.9) | 84.5 to 91.2 | 75.1 to 79.4 | 137/184 (75.3) | 69.4 to 80.9 |
| Sputum smear +ve | 94.7 to 91.5 | 1862/2089 (91.3) | 90.2 to 92.4 | 93.4 to 94.3 | 2970/3203 (92.4) | 91.7 to 93.5 |
| Sputum smear −ve | 90.9 to 94.4 | 198/200 (99.0) | 90.2 to 92.4 | 92.4 to 94.3 | 2970/3203 (92.4) | 91.7 to 93.5 |
| Non-respiratory smear +ve | 87.9 to 88.7 | 328/373 (87.9) | 84.5 to 91.2 | 75.1 to 79.4 | 137/184 (75.3) | 69.4 to 80.9 |
| Sputum smear −ve | 90.9 to 94.4 | 198/200 (99.0) | 90.2 to 92.4 | 92.4 to 94.3 | 2970/3203 (92.4) | 91.7 to 93.5 |
| All* | 93.1 to 93.9 | 75.8 to 78.3 | 90.6 to 91.7 | 78.4 to 83.4 | 3203/3636 (88.1) | 87.0 to 89.1 |
| Respiratory* | 91.5 to 92.6 | 75.7 to 73.7 | 93.4 to 94.3 | 89.1 to 91.4 | 2782/2970 (90.4) | 89.3 to 91.4 |
| Non-respiratory* | 88.1 to 89.4 | 70.8 to 75.0 | 92.6 to 93.5 | 88.1 to 91.4 | 2782/2970 (90.4) | 89.3 to 91.4 |

*Smear negative + smear positive + smear unknown.

/B0

Respiratory samples + non-respiratory samples + unclassified samples.

/C0

Smear negative, negative; +ve, positive.

/D0

A single molecular test performed against liquid and solid culture for all specimens had an overall sensitivity of 87.3%, specificity of 92.4%, PPV of 89.7%, NPV of 99.3% and overall accuracy of 91.1%, PPV of 87.7%, NPV of 90.8% and overall accuracy of 89.5% for MTBC detection (table 1). For all sputum specimens (n=3382) the sensitivity, specificity, PPV, NPV and diagnostic accuracy for rifampicin resistance in comparison to the standard resistance ratio drug susceptibility method (phenotypic technique) were as follows: 92.4%, 89.1%, 93.9%, 83.3% and 91.3% respectively. The equivalent figures for smear-positive specimens (n=2606) were 94.7%, 80.9%, 93.9%, 83.3% and 91.3% respectively. The results for non-smear-positive samples were good (with the exception of CSF), although with lower sensitivity, for example, BAL smear-positive sensitivity was 86.8%.
Discrepant results
In the period 2005—2009, there were 420 false-negative results for MTBC; these samples were found to be negative using 16S rDNA sequencing and rifampicin resistance (25.3 ± 19.4 and 32.2 ± 16.9 days saved, respectively) (table 3).

### Table 3: Days saved to reach earlier diagnosis by implementing the Innolipa rDNA sequencing assay

<table>
<thead>
<tr>
<th></th>
<th>All samples</th>
<th>Smear positive only</th>
<th>Smear negative only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days saved before culture results</td>
<td>35.85 ± 24.89</td>
<td>25.30 ± 19.36</td>
<td>47.57 ± 26.32</td>
</tr>
<tr>
<td>Days saved before rifampicin sensitivity results</td>
<td>31.99 ± 17.71</td>
<td>32.15 ± 16.90</td>
<td>31.85 ± 20.64</td>
</tr>
</tbody>
</table>

Tuberculosis assay in comparison to the standard culture and sensitivity techniques (2006—2009).
MDRTB are injectable drugs that are also resistant to a quinolone and one or more of the extensively drug-resistant TB (XDRTB) (ie, MDRTB isolates non-compliance, which can increase the likelihood of developing toxicity and the length of treatment often contribute to administered for a long time, and drugs can be toxic. Drug importance of operator experience in performing the LiPA assays.

**DISCUSSION**

We have had the ability to rapidly and accurately diagnose TB and rifampicin resistance in sputum microscopy smear-positive specimens since the 1990s. Patients with MDRTB are difficult to manage, treatment is costly and must be administered for a long time, and drugs can be toxic. Drug toxicity and the length of treatment often contribute to non-compliance, which can increase the likelihood of developing extensively drug-resistant TB (XDRTB) (ie, MDRTB isolates that are also resistant to a quinolone and one or more of the injectable drugs—amikacin, capreomycin and kanamycin).

Routine, specimens received at the NMRL are first cultured and then identified as MTBC using GenoType-Series molecular assay or DNA sequencing. The advantage of ‘Fastrack’ is that TB and MDRTB can be diagnosed within a day (permitting appropriate clinical, infection control and public health action, and improving patient outcomes) and the specimens are subjected to rapid automated culture. Specimens known to have MDRTB produce cultures that can then be analysed for all line and reserve drugs simultaneously.

The overall sensitivity, specificity, PPV and NPV for MTBC detection in all specimens using LiPA were 87.3%, 91.1%, 87.7% and 90.3%, respectively. These values are similar to those reported in our previous study at the beginning of the service, when overall sensitivity, specificity, PPV and NPV were 85.2%, 88.2%, 86.9% and 87.7%, respectively. As with other trials, sensitivity of PCR was higher in smear-positive respiratory samples (95.8%) compared with smear-negative respiratory samples (70.4%) and all non-respiratory samples (71.4%). The lower sensitivity of PCR in these samples may be explained by lower mycobacterial loads, suboptimal sample volumes, and irregular clumping of organisms in paucibacillary specimens when overall sensitivity, specificity, PPV and NPV were 85.2%, 88.2%, 86.9% and 87.7%, respectively. As with other trials, sensitivity of PCR was higher in smear-positive respiratory samples (95.8%) compared with smear-negative respiratory samples (70.4%) and all non-respiratory samples (71.4%).

This study is a large operational study of using LPAs in a non-trial context over a decade. It demonstrates the reality of an operational service and that non-automated LPAs using well-trained technical staff working to strict operating procedures achieves comparable sensitivity. At the NMRL, bacteriological staff performing this procedure usually had no prior experience but the service was designed to analyse performance with the chosen parameters in mind. It measures the real performance of a national service under non-trial conditions based on NHS referrals by clinicians. The relatively lower specificity for TB detection in smear-positive specimens that had not grown in culture may be because specimens were taken while patients were on TB therapy. There was some circumstantial evidence that therapy had been started before specimens were taken. The request forms for ‘Fastrack’ asked if any treatment had been administered and in over two-thirds of forms for this patient group, which can increase the likelihood of developing extensively drug-resistant TB (XDRTB) (ie, MDRTB isolates that are also resistant to a quinolone and one or more of the injectable drugs—amikacin, capreomycin and kanamycin). The principal weakness of the study is its retrospective nature but the service was designed to analyse performance with the chosen parameters in mind. It measures the real performance of a national service under non-trial conditions based on NHS referrals by clinicians. The relatively lower specificity for TB detection in smear-positive specimens that had not grown in culture may be because specimens were taken while patients were on TB therapy. There was some circumstantial evidence that therapy had been started before specimens were taken. The request forms for ‘Fastrack’ asked if any treatment had been administered and in over two-thirds of forms for this patient group.

**Table 4** Results of LiPA compared with culture in detecting Mycobacterium tuberculosis complex (2003–2009) in comparison with 1999–2002

<table>
<thead>
<tr>
<th>Sample and AB smear results</th>
<th>All smear +ve</th>
<th>All smear -ve</th>
<th>Respiratory smear +ve</th>
<th>Respiratory smear -ve</th>
<th>Non-respiratory smear +ve</th>
<th>Non-respiratory smear -ve</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2003–2009</strong></td>
<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
<td>Positive Predictive Value (%)</td>
<td>Negative Predictive Value (%)</td>
<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
</tr>
<tr>
<td>All +ve</td>
<td>88.3 (84.2)</td>
<td>94.4</td>
<td>93.1</td>
<td>81.3</td>
<td>87.9 (82.4)</td>
<td>92.4</td>
</tr>
<tr>
<td>All -ve</td>
<td>78.0 (74.5)</td>
<td>82.4</td>
<td>79.5</td>
<td>65.4</td>
<td>76.0 (71.5)</td>
<td>79.6</td>
</tr>
<tr>
<td>Respiratory +ve</td>
<td>86.1 (82.1)</td>
<td>93.8</td>
<td>92.1</td>
<td>80.7</td>
<td>83.8 (78.3)</td>
<td>92.3</td>
</tr>
<tr>
<td>Respiratory -ve</td>
<td>72.9 (69.4)</td>
<td>79.5</td>
<td>76.4</td>
<td>63.4</td>
<td>74.4 (69.1)</td>
<td>78.7</td>
</tr>
<tr>
<td>Non-respiratory +ve</td>
<td>85.2 (81.2)</td>
<td>89.6</td>
<td>87.9</td>
<td>72.7</td>
<td>82.8 (77.3)</td>
<td>87.3</td>
</tr>
<tr>
<td>Non-respiratory -ve</td>
<td>72.4 (68.9)</td>
<td>78.3</td>
<td>74.9</td>
<td>61.5</td>
<td>75.0 (66.8)</td>
<td>78.0</td>
</tr>
</tbody>
</table>

*Statistically significant increase in the sensitivity of the LiPA testing of all smear negative samples by time (p<0.0005).
**There is a statistically significant increase in the sensitivity of the LiPA testing of smear negative respiratory samples by time (p<0.039).
***There is statistically significant increase in the sensitivity of the LiPA testing of smear negative non-respiratory samples by time (p=0.018).
group the free text section indicated that the patient was on treatment. It is well documented that PCR-based techniques would detect DNA for dead bacteria but these bacteria would not be cultivable. This study was not designed to measure the actual impact on clinical management but supports other studies that suggest earlier diagnosis of MDRTB is advantageous.\(^2\)\(^{20}\)\(^{\text{–}}\)\(^{22}\)

The recent study by Boehme et al.\(^1\) demonstrated a high sensitivity even for smear-negative sputum samples (72.5\% for single automated XpertMTB/RIF compared with culture) but we were able to show a comparable level of sensitivity for LPA, indicating that this remains a highly sensitive and specific tool in appropriate laboratories. The XpertMTB/RIF assay is simple to perform requiring limited training to achieve quality results but at a higher cost. LPA requires less proprietary equipment but a more appropriate PCR-suite infrastructure, longer training and greater operator expertise than XpertMTB/RIF. Having effective competitor assays will support cost reduction for XpertMTB/RIF, LPA and other assays under development.

As most parts of the world have a low prevalence of MDRTB this will lead to a low PPV. Globally, confirmation of positive XpertMTB/RIF (and/or LPA) results using another molecular method or with microbiological DST is needed to be in line with WHO policy recommendations. For maximum benefit, there should be rapid identification of TB cultures with phenotypic analysis of first-line and second-line DST once a molecular test has identified drug resistance mutations associated with rifampicin (and possibly isoniazid) resistance. This will significantly reduce the time between sputum collection and full susceptibility testing for MDRTB cases. Addressing timeliness in technological improvement should go in tandem with minimising organisational delay. Clinicians need to make prompt therapeutic changes following rapid DST.

Assays with higher sensitivities are needed to address paucibacillary samples from patients who are HIV positive (despite the significant improvement demonstrated for the XpertMTB/RIF assay in this patient group), children especially the very young who produce little or no sputum, and extrapulmonary material, particularly dilutional fluids such as CSF, ascites and pleural fluid.

**CONCLUSION**

A national molecular diagnostic service for TB and drug-resistant TB (using a non-automated LPA) provides a rapid and reliable national service for diagnosing MTBC and rifampicin resistance.

**Limitations of the study**

Ideally additional clinical information would have been useful. However, it was previously shown that smear-positive specimens from patients who remained persistently positive on treatment (arousing suspicion that the patient has drug-resistant *Mycobacterium tuberculosis* complex or was not adherent to therapy) were frequently culture positive on therapy as the bacilli were viable because of resistance or due to patients’ non-adherence to treatment.\(^23\) Therefore, not excluding patients currently on treatment might have reduced the specificity but not as much as might be expected.

**REFERENCES**


