ORIGINAL ARTICLE

Examination of specimens for mycobacteria in clinical laboratories in 21 countries: a 10-year review of the UK National Quality Assessment Scheme for Mycobacteria Culture

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ABSTRACT

Results from clinical diagnostic microbiology laboratories taking part in the UK National Quality Assessment Service (UK NEQAS) scheme for Mycobacteria Culture between 1993 and 2003 were evaluated and assessed to determine whether the perceived increase in the use of rapid methods is improving time-to-positive reporting of results. Four simulated sputum specimens containing mycobacteria in mixed cultures with normal commensal organisms were distributed three times a year. Participating laboratories were required to report on the presence of 'mycobacteria' and on the time required to obtain a positive result. The overall level of performance with the mycobacteria culture external quality assessment specimens remained consistently high, with an average success rate of 94% over 10 years. The mean time-to-positive decreased from 24 to 17 days during the previous 8 years. A survey questionnaire, circulated in 2002, addressed the use of continuous automated mycobacterial liquid culture (CAMLiC) and molecular methods. The increase in the use of rapid culture methods for the detection of *Mycobacterium tuberculosis* has resulted in an overall reduction in time-to-positive data reported by participants, and has provided an indication of participants' ability to meet the 21-day target recommended by the CDC for the detection and identification of *M. tuberculosis*.

Keywords CAMLiC, detection, external quality assessment, molecular methods, mycobacteria, tuberculosis

INTRODUCTION

The UK National External Quality Assessment (EQA) Service for Microbiology (UK NEQAS) is a well-established comprehensive quality assurance organisation for clinical diagnostic microbiology laboratories in the UK and other countries [1,2]. As part of this service, simulated clinical specimens are prepared in the organising laboratory (Quality Assurance Laboratory (QAL), London, UK) and despatched to participating laboratories, which then examine these specimens and report their results to the QAL. The service covers a wide repertoire of microbiological tests, including culture for mycobacteria. Four simulated sputum specimens are distributed to participating laboratories three times a year. Participants are required to report only on the presence of 'mycobacteria' to obtain a fully correct score, as many laboratories lack the expertise to identify these bacteria to species level, and referral to a reference centre is the preferred option [3–6]. Results are recorded for participants reporting to the species level, but scoring is unchanged. Participants are also asked to report the time required to obtain a positive result. Participants are provided with an analysis following each distribution of specimens, showing both individual and overall performance of participants within their own country, and within all countries.

MATERIALS AND METHODS

In total, 355 laboratories currently participate in the *Mycobacterium* culture scheme, of which 186 are in the UK and 169 are in other countries, namely, Austria, Belgium, Iceland, Ireland,

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Finland, Gambia, Greece, Hong Kong, Israel, Italy, Macau, The Netherlands, Norway, Portugal, Slovenia, South Africa, Sweden, Switzerland, Tanzania and Turkey. In 1993 there were 246 participants, of which 209 were in the UK and 37 were in other countries. Strains of *Mycobacterium* were provided by the *Mycobacterium* Reference Laboratories (MRLs) in Cardiff, Newcastle and Dulwich, UK, and included isolates from recent infections and isolates referred for confirmatory testing. Resistant strains of *Mycobacterium tuberculosis* were not used, in the interests of health and safety. The *Mycobacterium* spp. distributed were *M. avium-intracellulare* (*n* = 1), *M. bovis* (*n* = 1), *M. chelonei* (*n* = 1), *M. fortuitum* (*n* = 1), *M. kansaii* (*n* = 3), *M. malmoense* (*n* = 1) and *M. tuberculosis* (*n* = 101). The simulated sputum specimens, which consisted of freeze-dried cultures of mycobacteria with commensal organisms, were distributed to all participants. Specimens that contained commensal flora were included occasionally in order to challenge the efficacy of decontamination procedures. The quality of the specimens was checked before and after distribution to participants by the QAL and at least one reference laboratory. Between 35 and 40 (no less than 10%) vials selected randomly were processed for culture to ensure viability and to exclude contamination. The lyophilised matrix was considered unsuitable for the preparation of smears for screening for atypical acid-fast bacteria, as the quality of the resulting smears was variable (a separate scheme is organised for detection of atypical acid-fast bacteria). Within each distribution, five sets of specimens from selected participants were returned to the QAL, where they were examined to exclude deterioration of the specimen between preparation and receipt by the participants. Specimens were labelled and packed in accordance with UK and, where appropriate, international regulations for transport and postage of infectious materials.

Information supplied to participants

Documentation accompanied each distribution, informing participants of the type of specimen, the examination required, and the hazards associated with the specimens. Participants were asked to return results within 10 weeks to the QAL. Following assessment and scoring of the results at the QAL, a summary of performance was reported to participants.

Scoring scheme

The scoring scheme used to assess results was the same in principle as that used in other schemes [7]. A score of '2' was allocated for a completely correct result (i.e., correct reporting to species level, or correct reporting to the genus level with referral of the isolate to a reference laboratory for confirmation), as well as for a negative result for specimens containing commensal flora only. A partially correct result was allocated a score of '1' (i.e., correct reporting to the genus level, but failure to refer strains for full identification). A score of '0' was allocated for an incorrect result (e.g., a false-negative result), and a score of '−1' was allocated for the reporting of unexpected pathogens, including false-positive results.

Questionnaire

A questionnaire requesting information on the methods used by participating laboratories, focusing on liquid culture systems and molecular methods, was distributed in 2002.

RESULTS

Results obtained by participating laboratories with specimens distributed

The percentages of laboratories obtaining fully correct results with specimens containing mycobacteria were 82–97% for *M. tuberculosis* (Fig. 1) and 82–96% for mycobacteria other than *M. tuberculosis* (MOTT) (Fig. 2). With three successive specimens containing *M. kansasii*, the percentages of participants reporting the correct species were 8% in 1995, 11% in 1998, and 19% in 2002. For other species, the percentages were: *M. fortuitum* in 1995, 6%; *M. chelonae* in 1999, 6%; *M. avium-intracellulare* in 2002, 24%; and *M. bovis* in 2003, 18%. Of those laboratories that attempted identification to the species level, the percentages reporting an incorrect species were ≤1% for all three specimens containing *M. kansasii*, 3% for *M. fortuitum*, 4% for *M. chelonii*, 2% for *M. avium-intracellulare*, and 11% for *M. bovis*. During the distribution period, between 2% and 10% of laboratories had false-positive results for specimens with commensal flora only.

Time-to-positive reporting

Laboratories were first requested to report the time required to provide a positive report in 1994. Data concerning the number of weeks elapsed were collected with each specimen. More recently, participants were requested to report time required to
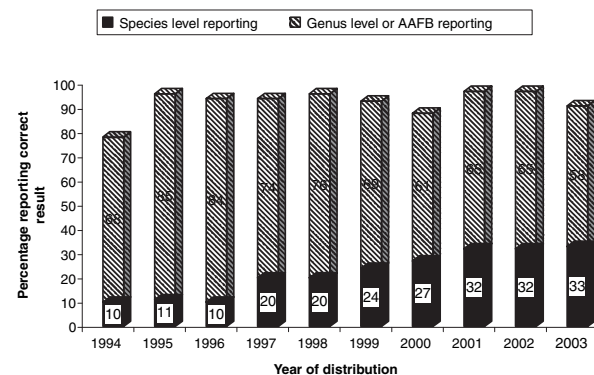

Fig. 1. Mean percentage of laboratories with fully correct results in successive EQA specimens containing *Mycobacterium tuberculosis*. Total percentage fully correct is the mean performance measured over all specimens distributed within the same year. AAFB, atypical acid-fast bacteria.
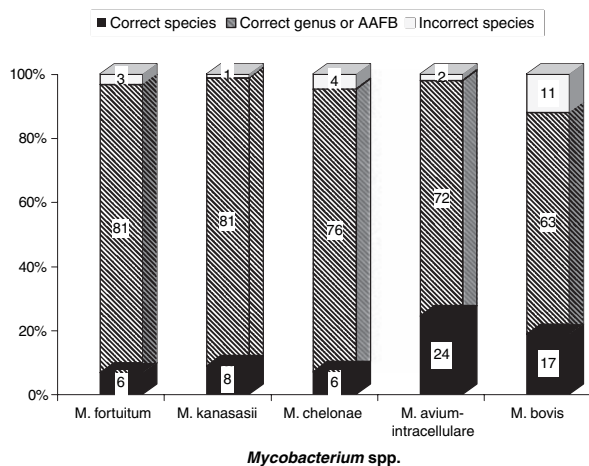
Fig. 2. Percentage of laboratories with completely correct results in four successive specimens containing mycobacteria other than *Mycobacterium tuberculosis* (MOTT) and one containing *Mycobacterium bovis*. When the number of specimens containing an MOTT strain was more than one, performance is expressed as the mean: for *M. fortuitum*, $n = 1$; for *M. kansasii*, $n = 3$; for *M. chelonae*, $n = 1$; for *M. avium-intracellulare*, $n = 2$; and for *M. bovis*, $n = 1$. AAFB, atypical acid-fast bacteria.

provide a positive result in days rather than weeks, reflecting the general trend towards faster reporting; an analysis is shown in Table 1.

Questionnaire

A questionnaire requesting details of the methods used was distributed in February 2002 to 351 participating laboratories; 264 were returned, a response rate of 77%. A further five laboratories returned the questionnaire, indicating that they were no longer performing culture for *Mycobacterium* on clinical specimens. Reasons for this change

in procedure, where indicated, included refurbishment of containment level 3 facilities and recent withdrawal of a *Mycobacterium* culture service.

Number of specimens examined annually

The numbers of specimens processed by respondents per year are shown in Table 2. A significant proportion of respondents (22%) did not state the number of specimens examined annually. Of the remainder, 90% examined ≤5000 specimens/year (median, 2000). Of the 10% of respondents examining >5000 specimens/year, seven examined >10 000 specimens.

Participants were asked to state the types of clinical specimens examined for mycobacteria. All 264 respondents examined sputum and bronchoalveolar lavage specimens for mycobacteria, 99% examined urine specimens, 94% examined cerebrospinal fluid, 50% examined blood cultures, 91% examined tissue, 97% examined pus and other aspirated fluids, and 11% examined specimens categorised as 'other'. Reports included in the 'other specimen' category were received from 21 laboratories examining faeces for mycobacteria, six examining wound swabs and five examining water and environmental samples.

Methods used for detection or identification of mycobacteria

Overall, 80 (30%) respondents used direct amplification test methods on clinical specimens. In-house amplification tests were performed by seven laboratories; 33 laboratories used Gen-Probe MTD (Gen-Probe, San Diego, CA, USA) and 28 used AMPLICOR MTB (Roche Molecular

Table 1. Time-to-positive reporting

	Cumulative percentage of participants and modal period required to report a positive result		
Days to positive report[a]	1995	1998	2002
14	15	46	56
21	55	65	83
28	87	76	93
> 28[b]	96	96	97
Mode[c]	28 days	21 days	16 days

[a]Time required by participants for a positive report, for all specimens examined. The data displayed are the mean time-to-positive report periods accumulated for all specimens in the same year.
[b]The maximum number of positive (correct) reports submitted and averaged over all specimens distributed in the same year.
[c]Until 2002, participants were asked to report time-to-positive reporting in terms of weeks rather than days.

Table 2. Number of specimens examined for mycobacteria per year by the participating laboratories

No. of participating laboratories handling the indicated no. of specimens	No. of specimens/year
58	< 1000
69	1000–2000
37	2001–3000
17	3001–4000
4	4001–5000
8	5001–6000
2	6001–7000
0	7001–8000
3	8001–9000
2	9001–10 000
7	> 10 000
57	Not stated

Systems, Pleasanton, CA, USA). A further 12 laboratories used other methods: two used the LiPA Mycobacteria kit (Innogenetics, Gent, Belgium); four used the ProbTec ET system (Beckton Dickinson, Franklin Lakes, NJ, USA); one used Mycoprep (Abbott Laboratories, Abbott Park, IL, USA); and five used the LCx system (Abbot Laboratories). Eight respondents used more than one method.

Continuous automated or semi-automated mycobacterial culture (CAMLiC or SAMLiC) methods were used by 184 (70%) respondents. BACTEC 460 TB (Becton Dickinson) was used by 34 respondents, BACTEC 9000 MB by 19 respondents and BACTEC MGIT 960 by 65 respondents. One respondent used ESP II (Trek Diagnostics, Cleveland, OH, USA), 35 respondents used MB/BacT (Organon Teknika/bioMérieux, Durham, NC, USA), and 35 used BacTALERT3D (Organon Teknika).

Overall, 230 (87%) of the 264 respondents used conventional culture for the isolation of mycobacteria. Of these, 80 used conventional culture alone, while 150 used both conventional culture and an automated method. An automated method alone was used by 34 respondents.

Level of identification

The number of respondents referring all suspected mycobacteria to a reference laboratory without attempting identification in-house was 172 (65%); 36 respondents identified *M. tuberculosis* only and referred all other species to a reference laboratory for further identification; 40 identified all species of mycobacteria in-house. A further four respondents identified some, but not all, species of mycobacteria in-house and referred the remainder to a reference laboratory for identification. There was no correlation between the number of specimens examined and the level of identification performed. The number of specimens processed by laboratories performing identification to the species level ranged from 350/year to > 12 000/year.

Molecular methods used to identify mycobacterial isolates

Of the 52 (20%) laboratories that employed molecular detection methods to identify suspected mycobacteria, 41 used Accuprobe (Gen-Probe),

seven used AMPLICOR MTB, and eight used an in-house assay/PCR. Other methods were specified by 27 respondents, including LCx used by 11, Innolipa (Innogenetics) used by eight, ProbTec used by two, and Mycoprep used by one. More than one method was used by 12 respondents.

Methods used for susceptibility testing of mycobacteria

Susceptibility testing for mycobacteria was performed in-house by 61 (23%) of the 264 respondents; the other laboratories indicated that mycobacteria were referred to a reference laboratory for testing. Solid media were used for susceptibility testing by 18 respondents, 41 used an automated method, two used Etest (AB Biodisk, Solna, Sweden), and ten used more than one method. Among the laboratories using an automated method, BACTEC 460 TB was used by 56%, BACTEC MGIT 960 was used by 34%, BacTALERT3D was used by 12%, and MB/BacT was used by one.

DISCUSSION

The overall standard of performance with the EQA specimens was high, with an average success rate of 94% during the previous 10 years. The percentage of participants reporting identification of mycobacteria to the species level doubled from 15% in 1993 to 32% in 2003. This may reflect the fact that non-UK laboratories, which may be expert or reference laboratories, have joined the scheme more recently. The reporting of pathogens from negative EQA specimens is a recognised occurrence for simulated specimens from other distribution types [7], but in the case of mycobacteria culture it is difficult to see how such reports are generated. The highest percentage of laboratories reporting a false-positive result (8%) concerned a specimen distributed in 1999. Of the 23 laboratories claiming to isolate mycobacteria from this specimen, two also reported a false-negative result for a positive specimen in the same distribution, indicating that a transposition error probably occurred. In a study of false-positive mycobacteria cultures by Breese *et al.* [8], a review of the literature revealed a median false-positive rate of 3.1% (interquartile range 2.2–10%), as detected by DNA fingerprinting. False-positive results can be caused by clerical

error, contamination of clinical equipment, mis-identification and laboratory cross-contamination [8,9]. Proficiency testing programme samples are a known source of cross-contamination because of the high bacterial load of many of these specimens [8]. Breese *et al.* [8] demonstrated how simple changes in administrative and procedural processes in their laboratory reduced the rate of false-positive results from 4% to 0%.

Accurate laboratory diagnosis is essential to ensure effective clinical management of infected patients [10–14]. The performance of participating laboratories with MOTT has improved over time. In the case of specimens containing MOTT, *M. tuberculosis* was the species most often reported incorrectly. Identification of MOTT has become increasingly relevant in recent years, as the incidence of these bacteria has increased, especially in association with patients positive for human immunodeficiency virus or suffering from AIDS.

Results from the questionnaire concerning methods indicated that most respondents processed ≤2000 specimens/year for mycobacteria culture. Some duplication in reporting numbers of specimens processed may have occurred when the respondent was a reference laboratory that included referred samples. It is clear that most respondent laboratories do not use direct amplification test methods for the detection of mycobacteria in clinical samples but, rather, commercial assays. The performance characteristics of the available commercial assays include high specificity, but the sensitivity is lower than that of the reference method of liquid culture combined with solid culture. Therefore, at present, direct amplification test methods cannot be used to replace conventional methods for the diagnosis and management of tuberculosis [6,10,11,15,16].

Mycobacteria grow fastest in liquid culture, and automated liquid culture has been shown to be more sensitive than conventional solid culture in many studies [10,17,18]. In 1993, the CDC recommended that a broth culture system should be used for primary recovery of mycobacteria [16]. A large proportion (70%) of respondents in the methods survey indicated that they used a SAMLiC or CAMLiC method for isolation of mycobacteria. CDC recommendations concerning culture go further, stating that a solid culture medium should be inoculated in addition to liquid culture. Solid culture media, although taking longer to detect mycobacteria, have been shown to have a contamination rate lower than that of liquid culture systems [10,17,18], and are useful in the identification of many MOTT, in particular the pigmented species, thereby obviating the need for expensive molecular typing. Only 57% of respondents used both conventional culture and an automated liquid culture system, thus failing to optimise the laboratory diagnosis of mycobacteria in accordance with CDC recommendations. Although 30% of respondents used conventional culture alone, the questionnaire did not ask respondents to differentiate between culture on solid and in liquid media; it is therefore not possible to comment on whether culture on solid media alone is leading to unnecessary delays in the reporting of positive cultures. Most (85%) respondents referred isolates of mycobacteria to a reference laboratory for confirmation of identity or for typing, and 80% referred isolates for susceptibility testing. According to expert opinion, this is the preferred option, and several countries have implemented a national system for fast-tracking high-priority specimens to promote the rapid diagnosis of infectious pulmonary tuberculosis [3,5,11,16,19].

Increasingly, nucleic acid probes are being used for the identification of mycobacteria from positive cultures [10,11,13–16]. The methods survey indicated that only 20% of respondents used molecular techniques to identify isolates of mycobacteria. Although PCR and other nucleic acid amplification techniques are rapid, specific and relatively sensitive, there is a cost implication, and a certain degree of expertise is required in the performance of these tests [6,16]. In this connection, Moström *et al.* [20] have highlighted the need for training and education in molecular biology.

Optimal therapy for the treatment of mycobacterial infection is dependent on the delivery of the results of susceptibility tests to clinicians as quickly as possible, and CDC recommendations state that susceptibility results should be available within an average of 28 days. Susceptibility testing of mycobacteria requires considerable expertise, available normally only at expert reference laboratories. This is reflected in the results of the methods survey, which indicated that only 23% of respondents performed in-house susceptibility testing, while the remainder referred isolates to a reference

laboratory. Most (72%) of the respondents who performed susceptibility testing used an automated method, indicating the potential for timely delivery of results.

Overall, the results from the UK NEQAS scheme indicate that at least those laboratories that participated in the study are in a good position to meet the challenge of the CDC recommendation that isolation and identification of mycobacteria should take place within 21 days. However, these EQA specimens are more likely to be representative of smear test-positive specimens, containing a bacterial load of $\geq 10^3$ organisms/mL, and therefore do not challenge the performance of laboratories with specimens containing low numbers of mycobacteria. The increased use of liquid culture systems has almost certainly facilitated the early detection of positive cultures. The rising incidence of tuberculosis and the impact of MOTT in vulnerable groups have emphasised the vital role of the laboratory in the diagnosis of this disease. In facing these challenges, laboratories must adopt procedures that allow the rapid and effective detection of mycobacteria, but they must also accept the limitations of the new approaches and the necessity for robust and routine quality assurance.

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