Guidelines for Tuberculosis Control in New Zealand
2010
Chapter 11: Mycobacteriology: Laboratory Methods and Standards
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Summary

**Mycobacteria**

Mycobacteria are aerobic bacilli. Their cell walls have a high lipid content, which includes waxes with characteristic mycolic acids with long, branched chains. Their resistance to decolourisation by acid is termed ‘acid fastness’. Isolation of species of mycobacteria other than *Mycobacterium tuberculosis* (TB) from a patient may reflect colonisation, contamination or clinical infection whereas isolation of TB always indicates clinical infection.

**Diagnostic testing for tuberculosis**

**Specimen collection and transport**

All relevant clinical details need to be written on the request form. Request forms need to clearly state all the tests required on the specimen (eg, routine culture, cytology).

Sterile body fluids should be collected aseptically to avoid contamination with commensal flora. Volumes should be sufficient for culture and NAAT testing if required. For both NAAT testing and culture, the chances of recovering TB are higher when larger volumes are used.

**Sputum specimens**

An early morning specimen from deep productive cough is preferred. Samples produced later in the day are acceptable.

Send three consecutive specimens collected at 8–24 hour intervals.

**Specimen processing**

Specimens from non-sterile sites require decontamination to kill contaminating commensal flora and maximise chances of recovering mycobacteria. Tissues or aseptically collected body fluids do not usually require the digestion and decontamination procedures that are used for contaminated specimens.

**Smear microscopy and staining**

The detection of AFB in a stained smear is the quickest procedure to confirm the diagnosis of mycobacterial infection.

For uniform reporting of smear results, it is recommended that an internationally standardised reporting system be used (eg, the Centers for Disease Control (CDC)).

The predictive value of a positive smear result for tuberculosis (TB), rather than infection with non-tuberculous mycobacteria (NTM), depends on the pre-test probability of TB in that patient.

**Culture methods**

Liquid (broth) media reduce the time to detect the growth of mycobacteria by about seven days, and all mycobacterial cultures must include a liquid culture medium.

At least one solid medium must also be used for each specimen cultured for mycobacteria.
Mycobacterial identification
Nucleic-acid identification systems allow same-day identification of referred cultures of *M. tuberculosis* complex. Probes are specific mycobacterial DNA or RNA sequences that detect complementary target sequences through nucleic acid hybridisation. Immunochromatographic tests are also available that detect antigens specific to TB. The sensitivity and specificity of both commercial probe assays and antigen tests are close to 100% when used on isolates obtained from broth culture.

Nucleic acid amplification tests (NAATs)
Most (about 95%) smear-positive specimens have positive NAAT results, but only about 50% of smear-negative specimens are NAAT-positive. Because of their cost, as well as sensitivity and specificity issues, NAATs should be reserved for situations where a rapid result will have a significant bearing on management decisions. The routine use of NAATs for all respiratory specimens is not justified.

Interferon-gamma release assays
Interferon-gamma release assays (IGRAs) involve incubation of TB-specific antigens with lymphocytes in blood samples from patients with known or suspected TB infection (either latent or active). If the lymphocytes recognise the antigens, they produce interferon-γ which is then measured by enzyme immunoassay. Commercially available IGRAs have comparable sensitivity to tuberculin skin tests (TSTs) but have greater specificity, particularly in patients who have been previously vaccinated with BCG.

Molecular typing (fingerprinting) of *Mycobacterium tuberculosis*
The routine typing of *M. tuberculosis* isolates in New Zealand began in July 2002 in order to do the following:
- support epidemiological information on the likely source and spread of *M. tuberculosis* in New Zealand
- identify TB outbreaks
- identify false-positive cultures due to cross-contamination of specimens during collection, processing or culture
- identify mislabelled specimens
- assist contact investigations and management of contacts.

Drug susceptibility testing (DST)
All initial isolates from patients with culture-proven TB should have DST performed against first line drugs. Rapid DST using molecular methods may be indicated for patients at high risk of multi-drug resistant TB (MDR-TB) or for infected patients who are critically unwell.

Other laboratory issues
Both Australia and the United States have developed ‘level of service’ guidelines for the delivery of a diagnostic laboratory mycobacteriology service.

Levels of service: recommendations
Level I service – microscopy only.
Level II service – microscopy and culture: a broth medium included in all mycobacterial cultures.
Level III service – microscopy, culture, identification to species level, and susceptibility testing.

**Laboratory safety**
TB is hazardous to laboratory workers. Stringent safety precautions are required at all stages in the processing of samples and handling of cultures. All sputum specimens should be handled as if they contain TB.

**Cross-contamination and false-positive cultures**
False-positive cultures due to cross contamination are not uncommon. Laboratory and clinical staff should be aware of this possibility.
Clinicians should have a high index of suspicion of contamination as an explanation for unexpected culture results. They should contact the clinical microbiologist to discuss positive TB cultures that do not align with the clinical scenario.

**Internal quality control**
Quality standards for the laboratory diagnosis of TB should cover all aspects of the service, from the labelling and transportation of samples to the laboratory through to the issuing of reports and collation of data.

**Air flow and biological safety cabinet performance**
Mycobacteriology laboratories require regular checks and maintenance of laboratory airflow systems and biological safety cabinets to minimise risk to staff through faulty equipment or air-handling.

**Reporting guidelines**
Laboratories should review their turnaround times for reporting smear, culture or identification results to ensure they are meeting the reporting guidelines.

**External quality control (proficiency testing)**
In addition to normal internal quality control protocols, laboratories should take part in a quality control programme covering smear testing and processing (eg, the Royal College of Pathologists of Australasia programme).
Level III laboratories should participate in the College of American Pathologists’ programme, which covers identification and susceptibility testing.
Introduction

Clinical mycobacteriology laboratories play a pivotal role in the control of Mycobacterium tuberculosis (TB) by ensuring that TB is isolated; identified; and tested against appropriate drugs in a timely manner.1–6 Laboratories must maintain close communication with both clinicians and public health services responsible for TB control (for example, medical officers of health and the Institute of Environmental Science and Research (ESR)).

This chapter provides an overview of the laboratory diagnosis of TB, and updates the issues relating to quality, performance and safety in mycobacteriology laboratories. Although many mycobacterial species other than TB are accepted as true human pathogens, the focus of this chapter is TB.7
1 Classification of Mycobacteria

1.1 Description of mycobacteria

Mycobacteria are aerobic, slightly curved or straight bacilli (ie, rod shaped), 0.2–0.6 by 1.0–10 µm in size. The cell walls of mycobacteria have high lipid content due to the presence of characteristic mycolic acids with long, branched chains.

Although the unusual composition of the mycobacterial cell wall means that mycobacteria are not readily stained by the Gram stain method, they are, however, considered gram-positive. Special staining methods must be used to promote the uptake of dye and, once stained, mycobacteria are not easily decolourised; that is, they retain the stain even when washed with acid-alcohol solutions. Their resistance to decolourisation is termed ‘acid fastness’, hence the term ‘acid-fast bacilli’ (AFB).

Growth rates for mycobacteria are slow compared to most other bacteria (16–18 hours to undergo one cycle of replication compared to 20 minutes for most bacteria).

1.2 Classification

1.2.1 Mycobacterium tuberculosis complex (MTB complex)

The Mycobacterium genus is divided into the *M. tuberculosis* complex and ‘non-tuberculous mycobacteria’. The *M. tuberculosis* complex includes *M. tuberculosis*, *M. bovis* (*M. bovis* subsp. *bovis*, *M. bovis* subsp *caprae* and *M. bovis* BCG), *M. microti*, *M. canettii*, *M. africanum* and *M. pinnipedii*.  

*M. bovis* is the name given to the bovine tubercle bacillus in 1896. The bacillus Calmette-Guérin (BCG) is derived from *M. bovis* that has attenuated pathogenicity and is used as a vaccine against TB. *M. africanum* and *M. microti* occupy positions along the phenotypic continuum between *M. tuberculosis* and *M. bovis*. *M. pinnipedii* is associated with granulomatous lesions in pinnipeds (seals, seal lions, etc). *M. canettii* is probably the source species of the *M. tuberculosis* complex and is associated with lymphadenitis and pulmonary disease in Africa.

1.2.2 Mycobacterium other than MTB complex

A variety of terms have been used to describe the rest of the *Mycobacterium* genus, including mycobacteria other than tuberculosis (MOTT), environmental mycobacteria, atypical mycobacteria (ATM), and non-tuberculous mycobacteria (NTM). The term ATM was first used because when these organisms were grouped together they were not typical of *M. tuberculosis*. However, the term NTM is now preferred and is used in this chapter to refer to these species as a group.
2 Diagnostic Testing for Tuberculosis

Several publications cover the topic of diagnostic testing for TB.\textsuperscript{1–6}

2.1 Smear and culture testing

2.1.1 Specimen collection

- Relevant clinical details must be written on the request form as well as a clear statement regarding the tests requested (e.g., routine culture, cytology).
- Sterile, leak-proof disposable plastic containers must be used to send specimens to the laboratory.
- Containers must be clearly labelled with the patient’s name, the specimen type, and the time and date of collection.
- Specimens should be collected aseptically in order to minimise contamination with commensal flora.
- Sufficient material must be collected for all the tests required. Do not use fixatives or preservatives for culture specimens.
- Swabs are not recommended for the isolation of mycobacteria.

2.1.2 Specimen transport to laboratory

- Specimens should be transported to the laboratory in as short a time as possible to avoid overgrowth by contaminating commensal flora.
- Specimens should be refrigerated if transport is delayed.
- Although sputum specimens can be stored for up to seven days at 4°C without a decrease in the viability of *M. tuberculosis* or a decrease in the sensitivity of smear results, delays in transport to the laboratory should be avoided if possible.

2.1.3 Sputum specimens

Early morning specimens from deep productive cough are preferred, although samples produced later in the day are acceptable. Three consecutive specimens should be collected at 8–24 hour intervals. For additional comments, see Chapter 2.

Specimens should not be pooled. Pooling specimens that have been collected over several days delays the turnaround time and increases the chance of overgrowth by contaminating respiratory flora.

While a patient is on treatment for TB, specimens should be sent for smear and culture so the efficacy of treatment can be followed. Patients, who are still producing sputum that is smear-positive during treatment, should have specimens sent for quantitative smear reporting and culture approximately every four weeks (see also Chapter 3).
2.1.4 Other respiratory specimens
Bronchial washings, lavages and induced sputum specimens should be sent in separate sterile containers. For information on the role of sputum induction in the diagnosis of TB, see Chapter 2.

2.1.5 Early morning urines
The entire volume of early morning urine should be collected into a clean container. The minimum volume required is 40 ml. Send one specimen on three consecutive mornings.

Unacceptable specimens for mycobacterial culture include pooled urine from catheters; urine from 24-hour collect specimens, and volumes less than 40 ml. Indications for early morning urine tests are discussed in Chapter 2.

2.1.6 Tissues, curettings, bone and aspirates
Tissues, curettings, bone and aspirates should be collected into sterile containers. The request for mycobacterial culture should be clearly noted on the request form. If histopathology is also required, the specimens should first be processed for microbiology and then sent on to histopathology. The biopsy must not be sent in formalin.

2.1.7 Blood and bone marrow
Specimens of blood and bone marrow must be inoculated immediately into the mycobacterial blood culture system used by the receiving laboratory. This must be done at the bedside. The minimum volume of blood for culture is 5 mL for adults and 1 mL for children.

2.1.8 Wound swabs
The rate of recovery of mycobacteria from swabs is poor. For this reason, swabs are only acceptable if a biopsy or aspirate cannot be obtained. Under these circumstances, the swab should be placed in a transport medium before transporting to the laboratory.

2.1.9 Gastric aspirate (lavage)
If possible, Gastric aspirate (lavage) specimens should be processed within four hours of collection. When transportation is expected to be delayed, the specimen should be collected into 10% sodium carbonate. Early morning specimens should be sent on three consecutive days. The role of gastric aspirate testing is discussed in Chapter 2.
2.1.10 Processing and decontamination

Sputum specimens

Sputum specimens contain oropharyngeal flora, which, unless eliminated, will overgrow TB cultures. Optimal recovery of mycobacteria from clinical specimens requires special laboratory decontamination procedures designed to eliminate contaminating bacteria while releasing mycobacteria trapped in organic material (mucus, cells, serum and other proteinaceous material).¹

Most laboratories decontaminate and liquefy sputum samples using sodium hydroxide which also serves as a mucolytic agent. Other decontamination / mucolytic agents include dithiothreitol (sputolysin) and N acetyl-L-cysteine (NALC). The stronger the decontamination agent used, the higher its temperature and the longer it is in contact with the specimen, the greater the killing action will be on both contaminants and mycobacteria.

Following decontamination, the decontaminant must be neutralised and the sample centrifuged to concentrate the mycobacteria.

All decontaminating methods are to some extent toxic to mycobacteria. The best yield of mycobacteria from cultures will be obtained when the mildest decontamination procedure is used that sufficiently reduces contamination. However, even under optimal conditions decontamination kills all but 10–20% of mycobacteria in a specimen.¹ Decontamination is generally considered to be inadequate for sputum specimens when rates of contamination with oropharyngeal flora are greater than 5%.

Practice point

During processing there are many opportunities for generating splashes and aerosols that can lead to cross-contamination of specimens. All handling should be done using techniques that minimise the risk of cross-contamination.

2.1.11 Other specimens

Non-sterile specimens other than sputum also require decontamination to eliminate contaminating bacterial flora¹ whereas ‘sterile specimens’ such as tissues and aseptically collected body fluids do not usually require decontamination. Body fluids infected with TB usually contain only few mycobacteria and should be concentrated by centrifugation to maximise recovery. Tissues can be ground and inoculated directly to both solid and liquid media.
2.2 Smear staining and microscopy

The detection of AFB in a stained smear is the quickest and easiest procedure to provide preliminary laboratory confirmation of the diagnosis of mycobacterial infection. All specimens, except urine and cerebrospinal fluid (where the volume provided for analysis is low) should have a stained smear read to detect the presence of AFB. Smears of urine are rarely positive and are usually not cost-effective. For cerebrospinal fluid (CSF) it is important to culture as large a volume as possible. For low volume samples, culture should be performed in preference to stained smears. The preferred minimum volume of CSF for culture is 2–3 mL.

Smears should be fixed by placing the prepared slides on a heated surface. The use of heat to fix smears does not kill mycobacteria and all slides must be handled as if infectious.

Three staining techniques are commonly used to detect AFB: two carbolfuchsin-based stains (Ziehl-Neelsen and Kinyoun) and fluorochrome stains. The classic Ziehl-Neelsen stain involves heating the slide during staining for better penetration of the dye. The Kinyoun acid-fast stain is a similar method but without heat where dye penetration is aided by using a higher concentration of phenol in the stain. Both carbolfuchsin methods stain the mycobacterial cells red against a methylene blue counter-stain. Both are examined under oil immersion at 1000 times magnification. At least 300 fields should be examined a slide is reported as ‘negative’.

A fluorochrome stain (auramine O or auramine-rhodamine) is the screening method recommended for laboratories with a fluorescent (ultraviolet) microscope. These stains bind to mycobacterial cell walls and fluoresce under ultraviolet illumination, so that mycobacteria appear bright yellow against a dark background. For this reason fluorochrome stained smears can be read at lower magnification, and in less time than carbolfuchsin based smears. Fluorescence microscopy is more sensitive than conventional microscopy and has similar specificity. If necessary, positive fluorochrome stains can also be ‘over-stained’ with a carbolfuchsin dye in order to further confirm the presence of AFB.

If too few AFB are present to call a smear ‘positive’ (see Table 11.1), another smear should be made from the same specimen (if possible) and a repeat specimen requested. For a sputum specimen to be smear-positive, it must contain approximately $10^5$ AFB/ml. Positive cultures can be expected when the sputum specimen contains 10–100 AFB/ml.

Acid-fast smears have high specificity but some other organisms may also stain acid-fast, including Nocardia species, Rhodococcus species, and Legionella micdadei as well as cysts from Cryptosporidium, Isospora, Cyclospora and Microsporidium spores.

Direct microscopy for AFB remains the most rapid and economical means of detecting infectious cases of pulmonary TB. The predictive value of a positive smear for TB as opposed to infection with NTM, will depend on host factors such as age, immune competence and underlying disease.
2.2.1 Reporting smear results

The number of fields that need to be examined and the number of AFB seen in a microscopic field will vary depending on the type of stain and the magnification being used. For standardised reporting of smear results, it is recommended that either the reporting system of the Centers for Disease Control and Prevention (CDC)\textsuperscript{11} or the reporting system of the International Union against Tuberculosis and Lung Disease / World Health Organization be used.\textsuperscript{12} The wording of reports and the corresponding number of AFB present in the smear for both systems are summarised in Table 2.1. It is also recommended that laboratories report, the numerical result in brackets alongside the 1+ to 4+ result (eg, 2+ AFB seen (1–9 AFB/10 fields)).

Laboratories using carbol-fuchsin-stained smears simply report as indicated in Table 2.1. Laboratories using a fluorochrome stain need to convert the number of AFB seen to the corresponding number seen on a carbol-fuchsin-stained smear.

**Table 2.1:** Acid-fast smear evaluation and reporting

<table>
<thead>
<tr>
<th>Report</th>
<th>AFB seen by staining method and magnification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carbol-fuchsin stain x 1000</td>
</tr>
<tr>
<td></td>
<td>x 250</td>
</tr>
<tr>
<td><strong>CDC</strong></td>
<td></td>
</tr>
<tr>
<td>No AFB seen</td>
<td>0</td>
</tr>
<tr>
<td>Doubtful; repeat</td>
<td>1–2/300 F (3 sweeps)†</td>
</tr>
<tr>
<td>1+</td>
<td>1–9/100 F (1 sweep)</td>
</tr>
<tr>
<td>2+</td>
<td>1–9/10 F</td>
</tr>
<tr>
<td>3+</td>
<td>1–9/F</td>
</tr>
<tr>
<td>4+</td>
<td>&gt; 9/F</td>
</tr>
<tr>
<td><strong>IUATLD/WHO</strong></td>
<td></td>
</tr>
<tr>
<td>No AFB in at least 100 fields</td>
<td>0/negative</td>
</tr>
<tr>
<td>Actual AFB count</td>
<td>1 to 9 AFB in 100 fields††</td>
</tr>
<tr>
<td>+</td>
<td>10 to 99 AFB in 100 fields</td>
</tr>
<tr>
<td>++</td>
<td>1 to 10 AFB per field in at least 50 fields</td>
</tr>
<tr>
<td>+++</td>
<td>&gt;10 AFB per field in at least 20 fields</td>
</tr>
</tbody>
</table>

Notes: AFB = acid-fast bacilli; CDC = Centers for Disease Control and Prevention; F = microscope fields; IUATLD = International Union against Tuberculosis and Lung Disease; WHO = World Health Organization.

† In all cases, one full sweep refers to scanning the full length (2 cm) of a smear 1 cm wide by 2 cm long.

†† A finding of 1–3 bacilli in 100 fields does not correlate well with culture positivity.

Source: Adapted from Kent and Kubican (1985).\textsuperscript{11}
2.3 Culture methods

A variety of media are available to use for recovering mycobacteria, including solid and liquid (broth) media.1,3

Several liquid media are available for recovering and sub-culturing mycobacteria. Liquid media reduce the time to detect the growth of mycobacteria by about seven days and have been a significant advance in the laboratory diagnosis of TB. Liquid media also recover more isolates than solid media and also detect mixed mycobacterial cultures more frequently. All mycobacterial cultures must include a liquid-culture medium.1–3

2.3.1 Commercial broth-based culture systems

Early broth-based systems detected mycobacterial growth using radioisotope labelled carbon dioxide. The classic example of such a system is the BACTEC 460TB system. Some laboratories still use this system although it has the disadvantage of requiring repeated sampling of vials using syringes.

Most modern mycobacteriology laboratories use automated, continuously monitored systems to culture mycobacteria in liquid media. These systems use specialised vials/tubes into which processed specimens are inoculated. Several mechanisms are used to detect the growth of mycobacteria. For example, the MGIT 960 system detects bacterial growth using a fluorescence-quenching based oxygen sensor within each tube. As mycobacteria multiply within the tube, oxygen is consumed and fluorescence is detected by the system.8 The contents of the tube can then be stained to look for the presence of mycobacteria and tested for the presence of TB using molecular probe-based assays or TB antigen tests. Examples of different continuously automated systems include the BACTEC MGIT 960 (BD), the BACTEC 9000 MB (BD), the MB/BacT Alert 3D (Biomerieux).

2.3.2 Solid media

Solid media may be egg-based or agar-based. Egg-based media, of which Löwenstein-Jensen (LJ) is the most commonly used, support the growth of *M. tuberculosis* well and have a long shelf life.1 Agar-based media have the advantage of being transparent, allowing earlier detection of mycobacterial colonies (Middlebrooks agar). They are more expensive than LJ, have a shorter shelf life1 and are not commonly used in New Zealand.

Solid media should be inspected regularly and kept for eight weeks. Cultures from smear-positive specimens that have no growth at eight weeks should be kept for a further six to eight weeks. Detection of colonies on solid media offers several advantages over growth in broth. Colonial morphology may provide clues for identification and mixed cultures can be detected.

At least one solid medium must be used for each mycobacterial culture.1–3
2.4 Mycobacterial identification

2.4.1 Nucleic acid probes

Nucleic-acid probes allow same-day identification of *M. tuberculosis* recovered from liquid culture medium. Specific probes are commercially available for several mycobacterial species including: *M. tuberculosis* complex; *M. avium* complex; *M. kansasii* and *M. gordonae* (AccuProbe, Gen-Probe Inc, San Diego, California). These probes produce a fluorescent signal on binding to their specific target nucleic acid sequence.

Although the specificity of nucleic acid probes is generally close to 100%, the uncommon NTM, *M. celatum* can cross-react with the *M. tuberculosis* probe if present. The sensitivity of the Accuprobe varies according to the species targeted. For example, the sensitivity of the M-TB AccuProbe is 100% whereas the sensitivity of the *M. avium* complex AccuProbe is 95–97%.13 Despite the high sensitivity and specificity of these tests, as with all laboratory tests, additional testing should be performed if the results do not correlate with clinical or epidemiological risk factors.

Two ‘line probe; assays have been developed; LiPA Mycobacterial kit, (Innogenetics) and GenoType® Mycobacteria (Hain Lifescience). These kits can be used to identify isolates recovered from either solid or liquid media. In contrast to the AccuProbe, line probe assays allow testing for several mycobacterial species at once because more than one probe can be immobilised onto a plastic membrane strip. Line-probe assays have the advantage of allowing several species to be identified from a single PCR reaction and thus do not require pre-selection of the appropriate probe.

2.4.2 Antigen tests

Antigen testing using a commercially available immunochromatographic kit can be used to identify MTB complex in positive broth cultures. Such assays use the *Mycobacterium tuberculosis* protein 64 as a target antigen (MPT-64). This is an antigen specific to MTB-complex which is secreted during bacterial growth. The sensitivity and specificity of these assays compared to the Accuprobe molecular assay are approximately 97% and 100% respectively. The kit requires a 15-minute assay time, is easy to perform and is relatively cheap.14

2.5 Nucleic acid amplification tests

Nucleic acid amplification tests (NAATs) utilise a variety of molecular techniques to amplify target DNA sequences and thus detect and identify mycobacteria in specimens or cultures. Both commercial and ‘in-house’, amplification assays can usually provide results within a single working day. Despite the faster turnaround time for NAATs compared to culture methods, culture should always be performed alongside NAATs for two reasons:

1) culture methods tend to be more sensitive and
2) culture of *M. tuberculosis* allows susceptibility testing to be performed.5,6
If there is insufficient specimen to process for both culture and NAAT, culture must be performed in preference.

Laboratories undertaking NAATs for mycobacteria must participate in quality assurance programmes. Positive and negative control tubes should be included with each NAAT reaction to rule out inhibition of amplification and cross-contamination respectively.

NAATs are a useful adjunct to culture methods but because of their cost and suboptimal sensitivity, NAATs should be reserved for clinical situations where an expedited positive result will have an impact on management decisions. (See also the section ‘Recommendations for NAAT testing’, below.)

2.5.1 Sensitivity and specificity

The analytical sensitivity of NAATs for TB can be as low as 10 mycobacteria, as judged by serial dilutions of a suspension of known colony count. Many ‘in-house’ PCR assays target the insertion sequence IS6110. Therefore, both *M. tuberculosis* and *M. bovis* are detected by such assays. Additional molecular targets, such as the region of difference (RD) gene can be utilised to distinguish species within the *M. tuberculosis* complex. Rare strains of *M. tuberculosis* lacking IS6110 have been reported from China and Vietnam. Strains such as these will be missed in this assay, but none has yet been encountered in New Zealand. Around 82–100% of smear-positive specimens have positive NAAT results but only about 50% of smear-negative specimens are NAAT positive. Thus a negative NAAT test on a sputum specimen should not be used to rule out TB, particularly when the patient is smear negative.

When amplicons (the products of NAAT reactions) are sequenced to confirm identity, the specificity of NAATs for TB is 100%. However, as with all PCR assays contamination with exogenous target DNA can cause false-positive results. Specimens that contain inhibitors of *Taq* polymerase (the enzyme used in the PCR reaction) cannot be assayed by PCR.

2.5.2 Applicable specimens

NAATs are an alternative way to make the diagnosis of TB:

- as an adjunct to culture, where speed of diagnosis is desired (eg, TB meningitis)
- where fresh tissue is not available (eg, retrospective analysis of fixed tissues that are histologically suggestive of TB); however, formalin fixation does affect the molecular arrangement of DNA and may lead to a decrease in the efficacy of amplification – the sensitivity of the assay decreases, the longer the tissue has been fixed.

NAATs generally have low sensitivity when used on pauci-bacillary specimens such as pleural and peritoneal fluids. For this reason it is preferred that all the fluid is cultured. Fine-needle biopsy may be suitable for NAAT testing, but if the specimen comprises only the washings from a biopsy needle (after histology and other culture aliquots have been taken) the likelihood of detecting TB is extremely low.
Sputum samples should be sent for culture and may not be accepted for PCR by all laboratories although the Cepheid GeneXpert® platform can be used directly on sputum specimens (see below).

2.5.3 How to request

The laboratory may decline to assay inadequate or inappropriate material. Pertinent clinical details including CSF results in suspected meningitis must accompany the request. Ideally, the need for PCR should be discussed with a clinical microbiologist. Results should be available within three working days after the specimen is received in the laboratory.

Auckland, Waikato, Wellington and Christchurch Hospital laboratories offer PCR testing for appropriate clinical specimens.

2.5.4 Commercially available NAATs validated for use on respiratory specimens

Available assays for TB include:
- Gen-Probe Amplified *M. tuberculosis* Direct Test (MTD II)
- Becton Dickinson ProbeTec Direct (SDA) system
- Roche Cobas® Amplicor® *M. tuberculosis* test
- Roche Cobas® TagMan® MTB Test
- Loop-mediated isothermal amplification (LAMP), Eiken Chemical Co Ltd
- Hain Lifescience Genotype® Mycobacterium Direct (RNA)
- Cepheid Xpert MTB/RIF assay.

2.5.5 Recommendations for nucleic-acid amplification tests

The routine use of NAATs for respiratory specimens is not justified. These tests should be used only when diagnostic, therapeutic or infection control issues require a rapid result. Requests for NAAT testing for TB should be discussed with the clinical microbiologist.

Settings in which NAATs should be considered include the following:
- smear-positive cultures of clinical and public health importance (eg, where a non-tuberculous mycobacterium is likely and a major public health investigation could be prevented by rapid diagnosis)
- respiratory smear-negative specimens in someone with a high probability of TB, when there are significant risks to starting TB treatment inappropriately
- non-respiratory specimens where prompt management decisions are necessary
- immuno-compromised patients at high risk of TB, where delay in diagnosis may compromise the prognosis or make empirical treatment of other conditions necessary
• when culture is not possible (eg, paraffin embedded tissue)
• when a patient who has been treated for TB previously presents with signs and symptoms of TB and a rapid diagnosis of infection and testing for rifampicin resistance is essential.

NAATs should not be considered for:
• smear-negative specimens with low probability of TB
• smear-positive patients with high probability of TB but of no public health concern
• paucibacillary non-respiratory specimens (eg, pleural fluid; pleural biopsy is the preferred specimen)
• testing for cure, because it may be falsely positive (mycobacterial DNA may persist for a time despite the organisms being killed by treatment).

2.6 Immunological tests for tuberculosis infection (see also Chapter 8)

The traditional test for latent TB infection is the tuberculin skin test (TST). Problems with the TST include the need for return visits, subjectivity in reading results and cross-reactivity with the BCG vaccine. Several immunological methods to diagnose TB infection have been evaluated as alternatives to the TST.

Immunological tests for TB measure some aspect of the immune response to TB (humoral or cellular) in order to infer the presence of TB infection. TB infection may be latent or active. Unlike culture and NAATs, immunological tests do not differentiate between active and latent TB infection.

2.6.1 Interferon gamma release assays – underlying principle

Interferon gamma release assays (IGRAs) involve incubation of peripheral blood lymphocytes with mycobacterial antigens. The underlying principle of IGRAs is that specific lymphocytes that have been previously exposed to mycobacterial antigens will release interferon-γ (IFN-γ) on re-exposure. IFN-γ is then measured using either an enzyme immunoassay or elispot technique. The currently available commercial assays use antigens that are present in TB but absent from BCG (for example; CFP-10, ESAT-6 and TB7.7). These antigens are encoded by genes located within the region of difference 1 (RD1) of the *M. tuberculosis* genome. This is a region of the genome that is absent in BCG and most NTM. Only a few other NTM contain the genes for these antigens (*M. kansasii*, *M. marinum* and *M. szulgai*).
2.6.2 Interferon gamma release assays – performance characteristics (see also Chapter 8)

Two commercial IGRA s are available: The QuantiFERON-TB Gold assay (QFN) (Cellestis Limited, Carnegie, Victoria, Australia) and the T.SPOT TB assay (Oxford Immunotec, Oxford, United Kingdom). The QFN assay is available in two formats; a 24-well culture plate and an ‘in-tube’ method. The ‘in-tube’ method is used by all labs in NZ offering IGRA (see Appendix 1). The QFN assay uses an EIA method to measure the amount of IFN-γ released whereas the T. SPOT TB assay uses an enzyme-linked immunospot assay to quantify the number of cells producing IFN-γ per unit volume.

Commercially available IGRA s are more specific than the TST, particularly among BCG vaccinated populations. Furthermore when culture proven TB is used as a gold standard, IGRA also appear to have similar sensitivity to the TST (approximately 80%).

However, despite the sensitivity of IGRA for active TB being similar to the TST, the sensitivity of IGRA for latent TB infection (LTBI) is more difficult to assess because no reliable gold standard exists. It is possible, for example, that some discordant results observed between the TST and IGRA s represent remote, cleared infection rather than true latent TB. Ideally, large prospective studies are needed to determine rates of progression to active TB among patients with discordant TST/IGRA results.

Several recent reviews have compared the performance of IGRA in comparison to the TST in a variety of clinical settings. Studies using either or both of the two commercially available IGRA s have evaluated their performance in the diagnosis of both LTBI and active tuberculosis. The utility of IGRA s in specific groups has also been examined: including patients with HIV, health care workers, injecting drug users, patients with inflammatory arthropathies, contacts of smear-positive cases, and paediatric populations.

More recently several studies have looked at the cost-effectiveness of using the IGRA instead of the Mantoux in various settings. In high-income countries such as Canada and Germany, IGRA s have been shown to be more cost effective when used to test TST-positive patients.

2.6.3 Interpretation of IGRA results (see also Appendix 2)

- A positive result suggests TB infection, but will not differentiate between LTBI and active TB.
- A negative result should not be used to definitively exclude TB in someone who has clinical features of TB because the sensitivity of IGRA for active TB is only approximately 80%.
- An indeterminate result may indicate immune-compromise or reflect poor processing (for example inadequate mixing of tubes after inoculation for the QFN in-tube test).
2.6.4 Serological tests

Serological tests for TB do not correlate well with the presence of active or latent TB infection. For this reason they are not recommended for use in the diagnosis of infection (either latent or active) in New Zealand.
3 Molecular Typing (DNA fingerprinting) of *Mycobacterium tuberculosis*

The routine typing of *M. tuberculosis* isolates in New Zealand started in July 2002. Applications for molecular typing for TB control were proposed in 2003. The applications are:
- detection of cross-contamination of clinical specimens and isolates
- differentiation between relapse and exogenous re-infection
- identification of outbreaks
- reinforcing (or disproving) epidemiological links
- evaluating contact investigation and management
- providing a basis for the study of TB epidemiology.

All isolates of *M. tuberculosis* look similar on culture plates. Earlier typing methods relied on antibiotic susceptibility profiles, an unusual biochemical reaction, or susceptibility to viruses capable of infecting *M. tuberculosis*. The latter two are not used in contemporary mycobacteriology. Comparing susceptibility profiles is of limited value because the majority of isolates in New Zealand (88% in 2008) are fully susceptible.36

The only adequate way to show the uniqueness, or otherwise, of an isolate of *M. tuberculosis* is to use molecular typing methods.

3.1 Restriction fragment length polymorphism

Restriction fragment length polymorphism (RFLP) is a method for fingerprinting isolates of *M. tuberculosis*.37 Most strains of *M. tuberculosis* contain 6–20 copies of a particular insertion sequence called IS6110. The location and number of copies of IS6110 varies between strains. After cutting up the genome with restriction enzymes and separating different-sized fragments by gel electrophoresis, the fragments are detected using a fluorescent labelled ‘probe’ for IS6110 (that is a fluorescent labelled complementary DNA sequence for IS6110). Strains of TB are characterised by the position and number of bands, called the strain’s ‘fingerprint’. Strains with identical or closely related fingerprints are considered ‘clonal’ while isolates with unique patterns are considered unrelated. Patients who are found to share the same clonal strain by RFLP are likely to be linked epidemiologically and to share a common chain of transmission. Strains with no or low copies of IS6110 require alternative typing methods to test their relatedness.
3.2 Spoligotyping and variable number tandem repeat – mycobacterial interspersed repetitive units typing (VNTR-MIRU typing)

Alternative typing methods include ‘spacer oligonucleotide typing’ (spoligotyping) and ‘variable-number tandem repeat typing’ (VNTR). Spoligotyping is a PCR-based technique that detects the presence or absence of spacers in the direct-repeat locus of *M. tuberculosis*. VNTR utilises genetic elements called ‘mycobacterial interspersed repetitive units’ (MIRU). MIRU-VNTR genotyping involves PCR amplification of at least 12 target MIRU loci followed by determination of the size of each of the PCR products. Each PCR product is assigned a numerical value based on its size, so that for 12 loci MIRU, a 12 digit profile is obtained. The size of each product is determined by the number of ‘repeats’ at each locus. The numerical profile generated for any given isolate can be used to make comparisons with MIRU profiles obtained from isolates processed at other laboratories. A recent large comparison of IS6110, spoligotyping and MIRU-VNTR for typing isolates with low copy numbers of IS6110 showed that for these isolates, MIRU-VNTR had resolution surpassing both other methods. MIRU-VNTR is in common use in Australia.

3.3 Molecular typing of *M. tuberculosis* in New Zealand

Until recently, RFLP was the method used for the genotyping of all strains nationally. However, although, electronic storage of all RFLP profiles did allow for comparison between isolates over time, as the database increased in size, analysis of strain relatedness became more time consuming and technically difficult. In addition, unlike VNTR-MIRU profiles, RFLP profiles are not directly comparable between laboratories. Because of these issues a recent review of the molecular typing process has led to a change from routine use of RFLP to VNTR-MIRU.

Genotyping has general applications in New Zealand. For example, typing information confirmed transmission of a single strain of *M. tuberculosis* in an Auckland school and community outbreak, as it did in a large chain of transmission within an Auckland church group. Typing has also been of pivotal importance in determining the duration and extent of a prolonged outbreak in the North Island. Thus, establishing a link between patients in geographically diverse places would not have been possible without routine typing of TB in New Zealand.

A review of universal genotyping of TB isolates after the first five years (July 2002 to June 2007) was recently undertaken. Over this period, 1411 culture-confirmed cases of TB were notified and 1368 (97%) isolates were matched with the notification. Over one-third of the typed cases could be assigned to a cluster. There were 472 cluster cases distributed between 130 different clusters. The mean number of cases per cluster group was 3.6 and the median 2.0. Four clusters had more than 20 associated cases, comprising 22, 23, 27 and 55 cases respectively. Cluster cases were more likely to be Māori or of Pacific ethnicity and less likely to be Asian.
4 Drug Susceptibility Testing (DST)

4.1 When to perform DST

Multi-drug resistant TB is becoming increasingly common worldwide (MDR-TB, defined as resistance to both rifampicin and isoniazid). All initial isolates from patients with culture-proven TB must have DST performed against “first line drugs” (isoniazid, rifampicin, pyrazinamide and ethambutol +/- streptomycin).2,44,45

The need for repeat DST to first line agents on isolates recovered from patients during TB treatment depends on the patient’s clinical progress, and the risk of resistance developing. Patients who have received a ‘partial regimen’ at any stage of their treatment are at increased risk of developing resistance to first line drugs (see also Chapter 3).

4.2 Phenotypic DST methods

All phenotypic DST methods for M. tuberculosis use specific ‘critical concentrations’ (or ‘breakpoints’) for each anti-tuberculous drug. Although the ‘agar proportion’ method using solid agar is the traditional method for performing DST, most laboratories now use broth-based DST using either the BACTEC 460 or the MGIT960 system. Critical concentrations for the MGIT 960 system have recently been published by the WHO.46 These concentrations represent the lowest drug concentration that inhibits 95% of ‘wild TB strains’ (ie, strains that have never been exposed to the drugs), without inhibiting growth of resistant strains.

An isolate is considered to be ‘resistant’ to a drug when growth in the presence of a critical drug concentration exceeds growth of the same isolate diluted 1:100 in drug-free media.

The BACTEC 460 or the MGIT960 systems can be used to test rifampicin, isoniazid, ethambutol and streptomycin, however DST for pyrazinamide using the MGIT 960 is technically difficult and consequently, many labs use the “Wayne’s test” to test for pyrazinamide resistance. Pyrazinamidase activity is required to convert pyrazinamide into its active form.8 Therefore, failure to detect pyrazinamidase activity by the Wayne’s test indicates pyrazinamide resistance. M. bovis is intrinsically resistant to pyrazinamide. M. tuberculosis complex isolates resistant only to pyrazinamide should be suspected of being M. bovis. They should have appropriate biochemical tests or molecular tests performed to fully speciate the isolate.

DST to isoniazid should be performed using two critical concentrations: low and high level. Isolates resistant to the low-level critical concentration but susceptible to the high-level critical concentration should be reported as having low level resistance to isoniazid.47
4.3 Rapid DST methods

Rapid DST methods use molecular techniques to detect resistance, usually within 1–2 days of a positive TB culture. The Cepheid GeneXpert system is also validated to detect rifampicin resistance directly from respiratory specimens within several hours.

Rifampicin resistance occurs due to mutations in a particular ‘hotspot’ of the rpoB gene. ‘In-house’ PCR of this region, followed by sequencing can identify these mutations. Rifampicin resistance usually occurs in combination with isoniazid resistance (85–95% of the time) and so identification of mutations in the rpoB gene usually indicates that the isolate is also resistant to isoniazid. Commercial assays that detect mutations in the rpoB gene include:

- Hain Lifescience Genotype® Mycobacterium Direct (RNA)
- Cepheid Xpert MTB/RIF assay.

Patients with disseminated disease due to TB and patients at high risk of having multi-drug resistant TB (MDR-TB) in particular, may benefit from rapid DST. Patients at high risk of MDR-TB include patients with relapsed TB after previous treatment and patients from areas of high MDR-TB prevalence.

4.4 DST to ‘second line drugs’

DST to second line drugs should be performed on all isolates of MDR-TB. Critical concentrations for second line agents and testing methods for the MGIT 960 have been published by the World Health Organization (WHO) and in the literature.

Currently LabPlus, Auckland Hospital is the only laboratory in New Zealand that performs second line DST. The second line drugs that can be tested in New Zealand include amikacin, capreomycin, ofloxacin, ethionamide, rifabutin, and streptomycin (ofloxacin is generally considered a proxy for moxifloxacin and other fluoroquinolones).

Unfortunately, reliable testing methods for other second line drugs such as cycloserine and PAS have not been developed for the MGIT 960.

Isolates of MDR-TB that are also resistant to fluoroquinolones and one or more second line injectable drugs (such as amikacin, kanamycin and capreomycin) are defined as ‘extensively drug resistant TB’ (X-DR TB).

4.5 Quality control for first and second line DST

Because a diagnosis of MDR TB or X-DR TB has profound implications for the patient and for public health investigations, laboratories that perform DST for TB must have rigorous quality controls in place to ensure that results are reliable. Results that are unusual or unexpected should be carefully cross-checked to eliminate the possibility of contamination with NTM or another organism before a final report is issued. All broth cultures used for DST must be routinely sub-cultured onto blood agar once growth is detected in order to rule out the possibility that the culture is contaminated with bacteria other than TB.
5 Other Laboratory Issues

5.1 Levels of service

The Centers for Disease Control and prevention (CDC), American Thoracic Society (ATS), and the College of American Pathologists (CAP) have developed a classification system that defines levels of service capability for laboratories within the USA that process clinical specimens for mycobacteria. Levels of service are defined according to specimen workload, personnel expertise and cost-effectiveness.9

In Australia, the National Tuberculosis Advisory Committee has published *Guidelines for Australian Mycobacteriology Laboratories (2006).*50 These guidelines provide a framework for maintaining high-quality laboratory testing for TB. This framework is based on the premise that mycobacteriology laboratories must process adequate numbers of clinical specimens in order to maintain proficiency.

5.1.1 USA Guidelines

The three levels of service (I, II, III) are summarised in Table 5.1.

**Level I service: microscopy only**

Level I laboratories collect and transport specimens to referral laboratories for culture, identification and susceptibility testing. These laboratories can examine direct smears. When preparing direct smears, the laboratory should concentrate the sputum before preparing the stained film and render the mycobacteria non-viable before staining. To maintain proficiency at Level 1 status, a laboratory must prepare at least 15 specimens per week and examine them for AFB.

**Level II service: microscopy and culture**

Level II laboratories, in addition to level I services, culture and identify *M. tuberculosis* complex. The laboratory may also perform susceptibility testing. A Level II laboratory must process and culture at least 20 specimens per week.

**Level III service: microscopy, culture, identification to species level, and susceptibility testing**

Level III laboratories, in addition to performing Level I and II services, identify mycobacterial species and perform susceptibility testing when indicated.

Three laboratories offer services for identification and susceptibility testing for mycobacteria in New Zealand (Auckland, Waikato and Wellington Hospital laboratories). A reference service for identification and susceptibility testing should be supported by specialised clinical advice.
### Table 5.1: Levels of service

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Acid-fast smears</td>
<td>Yes</td>
</tr>
<tr>
<td>Culture</td>
<td>No</td>
</tr>
<tr>
<td>Identification of <em>Mycobacterium tuberculosis</em> complex</td>
<td>No</td>
</tr>
<tr>
<td>Identification of all mycobacteria</td>
<td>No</td>
</tr>
<tr>
<td>Drug susceptibility of <em>Mycobacterium tuberculosis</em> complex</td>
<td>No</td>
</tr>
<tr>
<td>Drug susceptibility of non-tuberculous mycobacteria</td>
<td>No</td>
</tr>
</tbody>
</table>

#### 5.1.2 Australian guidelines (see Table 5.2)

The Australian guidelines are divided into three areas:
- Laboratories performing smear microscopy (CDC/ATS/CAP level I).
- Laboratories performing mycobacterial culture (CDC/ATS/CAP levels II and III).
- Laboratories performing susceptibility tests (CDC/ATS/CAP levels II and III).

The guidelines reaffirm and reiterate the biosafety requirements for Australian mycobacteriology laboratories as outlined in the Australian/New Zealand Standard *Safety in Laboratories. Part 3: Microbiological aspects and containment facilities* (A/NZS 2243.3:2002).

Any laboratory in New Zealand performing mycobacterial staining or culture should be IANZ accredited to the Australian/New Zealand Standard in full.

### Table 5.2: Biosafety and quality assurance recommendations

<table>
<thead>
<tr>
<th>Laboratory capability</th>
<th>Physical containment level</th>
<th>Quality assurance recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear only</td>
<td>PC2</td>
<td>Yes, Royal College of Pathologists of Australasia smear Quality Assurance Programme (QAP)</td>
</tr>
<tr>
<td>Culture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 &lt; 5000 cultures per year</td>
<td>PC2</td>
<td>Yes, Royal College of Pathologists of Australasia smear and culture QAP or equivalent</td>
</tr>
<tr>
<td>2 ≥ 5000 cultures per year</td>
<td>PC3</td>
<td></td>
</tr>
<tr>
<td>3 Knowingly handling MDRTB</td>
<td>PC3</td>
<td></td>
</tr>
<tr>
<td>Susceptibility testing</td>
<td>PC3</td>
<td>Yes, as above and other external programmes which include DST such as the Centers for Disease Control and Prevention QAP or World Health Organization QAP</td>
</tr>
</tbody>
</table>
5.2 Timely reporting of results

Improvements in technology are of little use if the results do not reach their destination promptly. Positive smears should be reported by telephone to the clinician at once, and followed up immediately with a hard copy. Reference laboratories have a particular responsibility to keep their referring laboratories informed at all stages of identification and susceptibility testing. Preferably this should be done by fax or email. If email is used, there must be a system so the messages are cleared daily. Culture and identification results should be reported within 14 days for smear-positive specimens. Susceptibility results should be reported within 15–30 days of specimen collection for smear-positive specimens.\textsuperscript{52}

5.3 Laboratory safety

TB is hazardous to laboratory workers. Stringent safety precautions are required at all stages in the processing of samples and handling of cultures. All sputum specimens should be handled as if they contain TB.

The Australian/New Zealand Standard \textit{Safety in Laboratories Part 3: Microbiological aspects and containment facilities} sets out requirements, responsibilities and general guidelines relating to safety in laboratories and containment of micro-organisms.\textsuperscript{51}

The standard covers the following areas:

- organisation and responsibility
- degree of hazard from micro-organisms
- classification of laboratories, practices and procedures
- laboratory spills
- general precautions and special equipment
- work areas
- laboratory cleaning
- waste disposal
- transport of infectious and other biological materials.

This standard is being reviewed (2010).

5.4 Cross-contamination and false-positive cultures

A positive culture of \textit{M. tuberculosis} is usually considered to be definitive evidence for disease. However, false-positive cultures are not rare.\textsuperscript{53–59} Almost all studies that have evaluated more than 100 isolates have identified false-positive cultures, many of which were not recognised as such by laboratory and clinical personnel.\textsuperscript{54}
False-positive cultures can adversely affect patients, their contacts, hospitals and the public health system. Examples of these effects include psychological stress, social stigmatisation of patients and their families, unnecessary and costly medical treatment, eg, additional medical visits, chest X-rays, additional specimen collection and culturing, and adverse side effects resulting from unnecessary anti-TB treatment. Contact investigations lead to unnecessary Mantoux tests, chest X-rays, and many hours of wasted time.

The process of culturing mycobacteria is inherently prone to cross-contamination for several reasons, including the following:

- multiple steps are involved in processing mycobacterial cultures
- viability of *M. tuberculosis* for long periods in laboratory environments
- large number of mycobacteria present in some specimens.

The potential for error underscores the need to recognise false positive cultures promptly.

Two mechanisms can potentially result in contamination and laboratory error.

- Mislabelling or switching of specimens during handling.
- Instrument or reagent contamination, resulting in carry-over of mycobacteria from one sample to another. This may occur during initial processing, processing for susceptibility testing and by airborne contamination by aerosols in the biological safety cabinet.\textsuperscript{53–59}

Primary prevention of laboratory error requires use of standardised laboratory procedures designed to minimise the potential for errors.

Indicators of potential false-positive *M. tuberculosis* cultures include the following:

- all specimens but one from a patient are AFB smear-negative, and the single smear-positive specimen is *M. tuberculosis* culture-positive
- the patient’s signs, symptoms and clinical course are inconsistent with TB
- an *M. tuberculosis* culture-positive specimen, also likely to be strongly AFB smear-positive, was processed the same day as the suspected specimen
- the DNA fingerprint pattern of the suspected isolate is identical to that of the putative contaminating source isolate
- there are no known epidemiological links between the patient with the suspected isolate and the patient with the putative contaminating source isolate
- the duration of time for detection of growth in culture suspected of being contaminated was prolonged or only sparse colonies were detected on solid medium.

False-positive cultures may also occur following the use of contaminated clinical equipment, eg, bronchoscopes, or from the mislabelling of specimens when they are collected.
Practice points

Timely recognition and investigation of false-positive cultures of *M. tuberculosis* requires close co-operation and communication between clinicians, laboratories and public health. When culture results are inconsistent with the patient’s signs and symptoms or clinical course, the clinician must discuss the result with the laboratory and local public health.

If false-positive cultures and/or contamination are suspected, laboratory staff should notify the patient’s doctor and should have genotyping performed on the isolates from the putative source and the potentially contaminated specimen.

Laboratory staff should record the date and order of processing to enable easy identification of clusters of positive cultures. Simple procedural changes have been shown to decrease the rate of cross-contamination. These changes include:

- reducing the number of smear-positive specimens processed from a patient
- handling high-risk specimens, eg, proficiency test samples, separately
- having only one tube uncapped at a time in the biological safety cabinet
- using aliquots of buffer and other reagents and not larger multi-use volumes
- waiting after the specimen centrifugation step to allow time for aerosol settling in the test tube.

5.5 Quality control

Quality standards for the laboratory diagnosis of TB should cover all aspects of the service, from the transportation of samples to the laboratory to the issuing of reports and collation of data. This section provides guidelines that laboratories should follow. Not all of the following guidelines are directly covered by the International Accreditation New Zealand (IANZ) laboratory accreditation system.

5.5.1 Internal quality control

Contamination rate with commensal flora

If the method used to decontaminate specimens for mycobacterial culture is too harsh, mycobacteria in the specimens will be killed. If the process is too mild, cultures will become overgrown with contaminating commensal bacteria. Laboratories should monitor to ensure that essentially all smear-positive specimens grow a mycobacterium and that the bacterial and fungal contamination rate for sputum specimens is within the range generally considered to be acceptable (3–5%). If contamination rates lie outside of this range then a review of decontamination methodology may be required.

Cross-contamination

All smear-negative single isolate positive cases should have their DNA fingerprint profiles compared with concurrent isolates that the laboratory has recovered to ensure that the result is not due to laboratory cross-contamination or mislabelling of specimens.
Air-flow and biological safety cabinet performance

Regular maintenance and checks of the performance of the airflow system that serves the mycobacterial lab and the biological safety cabinet are required to ensure the safety of laboratory staff. An adequately performing biological safety cabinet is also required to reduce the risk of specimen cross-contamination.

Meeting reporting guidelines

Laboratories should review their turnaround times for reporting smear, culture or identification results in order to ensure they are meeting the reporting guidelines.

5.5.2 External quality control proficiency testing

In addition to normal internal laboratory controls, laboratories undertaking processing and smear examination should take part in a quality assurance programme (QAP) covering these procedures (eg, the Royal College of Pathologists of Australasia’s programme). Level III laboratories should participate in a programme that covers identification and susceptibility testing (eg, the College of American Pathologists’ programme).

The Australian Society for Microbiology Special Interest Group for Mycobacteria undertakes an annual survey, which also gives excellent coverage of identification and susceptibility testing. Recent surveys have included PCR and fingerprinting assessments. This activity is voluntary for participating laboratories.
Appendix 1: The QuantiFeron Gold In-tube Assay®

One millilitre of blood is collected into each of three specialised blood collection tubes:

- a ‘TB antigen tube’ that contain the three synthetic TB antigens (ESAT-6, CFP-10, TB7.7)
- a ‘nil control tube’ (measures the background concentration of IFN-γ in the patient’s blood)
- a ‘positive control tube’ that contains mitogen (a non-specific stimulator of IFN-γ release from lymphocytes).

The concentration of IFN-γ in each tube is measured after 16–24 hours incubation. The final test result is defined as the concentration of IFN-γ in the TB antigen tube minus the concentration of IFN-γ in the nil control tube (that is, background IFN-γ). The test manufacturer has set a positive result to be equal to or greater than 0.35 international units per mL (IU/mL).

The mitogen tube should stimulate release of IFN-γ from the lymphocytes of immunocompetent individuals. Patients with low concentrations of IFN-γ (ie less than 0.5 IU per mL) in the mitogen tube following incubation are considered to have an ‘indeterminate’ result.

Indeterminate results may occur either due to immune compromise or due to technical factors such as inadequate mixing of the mitogen tube following addition of blood. Repeat testing of patients with indeterminate results may yield either a positive or negative in a proportion of patients, particularly when technical factors are suspected as being the cause of an indeterminate result.
Appendix 2: Interpretation of the QuantiFERON Gold In-tube Assay®

Results interpretation guide
References


