



UK Health
Security
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UK Standards for Microbiology Investigations

Investigation of specimens for *Mycobacterium* species



Acknowledgments

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Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	10/05.12.25
Issue number discarded	7.3
Insert issue number	7.4
Section(s) involved	Amendment
Whole document.	This is an administrative point change. The content of this UK SMI document has not changed. The last scientific and clinical review was conducted on 05/10/2020. Hyperlinks throughout document updated to Royal College of Pathologists website. Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms Partner organisation logos updated. Broken links to devolved administrations replaced. References to NICE accreditation removed. Scope and Purpose replaced with General and Scientific information to align with current UK SMI template. 'Public health responsibilities of diagnostic laboratories' section added.

Amendment number/date	9/5.10.20
Issue number discarded	7.2
Insert issue number	7.3
Anticipated next review date*	10.01.22
Section(s) involved	Amendment

Technical information: Concentration/centrifugation	Section updated to clarify the optional but recommended use of refrigerated centrifuges. The following sentence from the WHO guidance on recovery of mycobacteria has been added: “The rate at which mycobacteria sediment is critically dependent on time of centrifugation and relative centrifugal force applied to the specimen. A longer centrifugation time can offset a lower relative centrifugal force, but increased centrifugation time increases the temperature of the specimen, which leads to additional killing of mycobacteria (hence, a refrigerated centrifuge is highly recommended)” Reference: “Boujtita N. Concentration of Mycobacteria in Clinical Samples using the Thermo Scientific General Purpose Centrifuge with 8x50 mL Individually Sealed Rotor. 2011”, which was used previously have been removed and a new reference has been added to support the rational for the new sentence.
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Amendment number/date	8/28.02.18
Issue number discarded	7.1
Insert issue number	7.2
Anticipated next review date*	10.01.20
Section(s) involved	Amendment
Section 4.5.1.	In the section “Decontamination of specimens using 0.5N Sodium Hydroxide (NaOH 4% w/v) / modified Petroff method”, the correct final concentration equivalence of 4% NaOH has been updated to 1N.

Amendment number/date	7/22.01.18
Issue number discarded	7
Insert issue number	7.1
Anticipated next review date*	10.01.20
Section(s) involved	Amendment

Section 4.5.5.	The “incubation” column for automated liquid systems has been updated with information for users to follow manufacturer’s instructions.
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Amendment number/date	6/10.01.17
Issue number discarded	6.1
Insert issue number	7
Anticipated next review date*	10.01.20
Section(s) involved	Amendment
Whole document.	References updated. Section on Whole genome Sequencing and MALDI-TOF MS added. Flowchart updated to reflect the information on the table in subheading 4.5.5. Section 4.5.1 updated to reflect the decontamination procedures used in microbiology laboratories. Technical limitations section updated. Section 4.9 has been updated to mention the reference laboratories that samples could be referred to.
Safety considerations.	This section has been updated accordingly with information on the minimum requirement for when processing of samples in cabinets.
Reporting procedure.	This section has been updated accordingly.

*Reviews can be extended up to five years subject to resources available.

1 General information

[View general information](#) related to UK SMIs.

2 Scientific information

[View scientific information](#) related to UK SMIs.

3 Scope of document

Type of specimen

Sputum, gastric washing, sterile site body fluids (CSF, pleural fluids, ascites, joint fluid), urine, skin or tissue biopsies, bone, bone marrow, bronchoalveolar washings, blood and post-mortem specimens.

This UK SMI describes the detection and isolation of mycobacteria from a variety of clinical samples. The use of automated liquid culture systems, plus solid media, is recommended for greater recovery of mycobacteria. The combined application of both phenotypic and molecular technologies gives the most efficient approach to the laboratory diagnosis of tuberculous and non-tuberculous disease.

Management, prevention and control of tuberculosis (TB) are not covered by this UK SMI, but are described in Tuberculosis, the recently updated National Institute for Health and Clinical Excellence (NICE) guideline, NG33¹. This UK SMI complements the recommendations made in the guidance for the prevention and treatment of tuberculosis, issued by the Department of Health and the collaborative strategy for England 2015 to 2020, published by UK Health Security Agency and NHS England^{2,3}.

This UK SMI should be used in conjunction with other UK SMIs.

4 Introduction

The genus *Mycobacterium* is a member of the family Mycobacteriaceae and consists of over 100 species and 10 subspecies of which a few have been reclassified to other species within the family⁴.

TB is caused by members of the *Mycobacterium tuberculosis* complex (MTBC); in humans this is predominantly *Mycobacterium tuberculosis*, though less often by other members of the complex such as *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium canettii* or *Mycobacterium caprae*. Strains of the live vaccine *Mycobacterium bovis* BCG, which are also used intra-vesically in the treatment of bladder cancer, can occasionally cause disease in patients who are immunocompromised. The two other species currently in the complex, *Mycobacterium microti* and *Mycobacterium pinnipedii* are almost exclusively associated with different mammalian hosts, but a few cases have been reported in patients who are immunocompromised⁵⁻⁷. Non-tuberculous mycobacteria (NTM) are also increasingly encountered as a cause of disease in humans. Not all persons infected with tubercle bacilli develop disease, and not all those that are infected become infectious to others.

Overt disease may develop months or years after the initial exposure. Patients with evidence of potential acquisition of MTBC, for example, on the basis of a positive tuberculin skin test and/or an interferon gamma blood test, but with no symptoms of active disease nor positive sample for MTBC may have latent TB infection (LTBI)¹. Disease due to tuberculosis may occur in virtually any organ of the body but is most common in the lungs and infection is characterised by caseating granuloma formation.

Initial infection in a person by MTBC organisms is termed primary tuberculosis. The lesion in the primary infection arises at the site of entry of the organism, which is usually the lung, although the tonsils, intestines or the skin may be involved. Lymph nodes will also be infected at this stage (primary complex). The tuberculin skin test (TST, Mantoux test) becomes positive at 3-8 weeks after infection, and marks the development of cellular immunity and tissue hypersensitivity. This test is useful in detecting latent disease. Interferon gamma release assays are also available. These tests have sensitivity at least equal to TSTs with superior specificity⁸. Foci developing in the endothelium of blood vessels may rupture leading to disseminated or miliary tuberculosis. Post-primary tuberculosis develops either as a result of reactivation of organisms in a 'healed' primary lesion or because of exogenous re-infection. Post-primary tuberculosis usually occurs five or more years after the primary infection and may affect children as well as adults. Infection with *M. tuberculosis* only progresses to clinical disease in a minority of cases. Patients who are infected with HIV are predisposed to reactivation of latent TB infection, and also to a rapid progression of recently acquired infection. Other predisposing factors may be vitamin D deficiency, which is frequently seen in immigrants and patients becoming immunocompromised due to other causes^{9,10}.

Mycobacterial cell walls contain mycolic acids, which are long chain fatty acids. These acids prevent ready access of commonly used dyes, and special procedures (for example, heat or detergent) are required to enable dye to penetrate mycobacterial cells. However, once the cells have taken up the stain, it is not subsequently removed by the usual acid-alcohol solvents – and hence mycobacteria are termed acid-alcohol fast bacilli (AAFBs), or now more commonly (AFBs)^{1,2,11,12}. This staining characteristic is very important in the microbiological and histological diagnosis of diseases caused by mycobacteria^{1,2,11,12}.

In view of the contribution of TB to human disease globally, international standards regarding the diagnosis and management of TB have been produced by a collaborative body, including the World Health Organization (WHO), which have then been adapted for application in the European Union; as well as there being authoritative American guidelines¹¹⁻¹⁴. UKHSA has produced a position statement on the use of molecular testing in the diagnosis of TB in England, Scotland and Wales¹⁵.

4.1 *Mycobacterium tuberculosis* complex

Pulmonary tuberculosis

Initial infection occurs by inhalation of droplet nuclei. Once *in situ*, the organisms may be ingested by host phagocytic cells where they may remain viable and may even continue to replicate. From this primary focus (Ghon focus), the organisms spread via the lymphatics to the lymph nodes and may reach the blood stream, infecting the lung and other organs. In the majority of individuals, the granulomatous foci may heal. However, they may continue to harbour viable organisms. Factors such as

immunosuppression, alcoholism, malnutrition and ageing may contribute to a failure to contain the infection.

Diagnosis of pulmonary TB is usually based on a combination of clinical and chest X-ray findings, with or without acid fast bacilli (AFB) being seen on microscopy of sputum (a positive smear), which may justify starting therapy whilst awaiting culture results^{1,11,16}. Every effort should be made to obtain adequate specimens for culture as *in vitro* susceptibility testing of *M. tuberculosis* isolates is becoming increasingly important in the context of emerging drug resistance and genotyping may provide evidence of epidemiological links^{1,12,17}. Three separate sputum samples for AFB microscopy and culture should normally be collected if available - although on the basis of the comparative increase in diagnostic yield, the WHO has now reduced its global recommendation from three to at least two serial samples for detection by direct smear so as to reduce time to diagnosis, accelerate initiation of treatment as well as reduce workload where laboratory resources are limited^{1,2,6,11,13,16,18,19}.

Extra-pulmonary tuberculosis

Lymphadenitis

Lymphadenitis is the most common form of extra-pulmonary mycobacterial infection, and when caused by tubercle bacilli it was previously referred to as scrofula. Cervical lymph nodes are most commonly infected, although any lymph node may be involved²⁰⁻²². Abscesses may develop with sinus formation. Cervicofacial lymphadenitis in young children may often be due to non-tuberculous mycobacteria, usually *Mycobacterium avium*^{23,24}.

Miliary tuberculosis

Miliary tuberculosis was the term first used to describe the resemblance of infected lesions (on chest X-ray) to millet seeds but is now generally used to describe all forms of progressive disseminated haematogenous tuberculosis²⁵. The number of cases seen is increasing due to the increase in patients who are immunocompromised as a result of HIV.

Neuro-tuberculosis - Tuberculous meningitis

Tuberculous meningitis (TBM) is usually caused by the rupture of a sub-ependymal tubercle to the sub-arachnoid space, rather than by direct haematogenous seeding of the meninges. The clinical findings usually begin with malaise, intermittent headache and low-grade fever; followed, within 2 to 3 weeks, by protracted headache, vomiting, confusion, meningism and focal neurological signs²⁵. Mortality is greatest in patients aged <5 years or >50 years, and in patients whose illness has been present for more than two months. Diagnosis of TBM relies heavily on clinical suspicion. Although the typical CSF picture in these cases is a raised white blood cell (WBC) count with a predominance of lymphocytes, white cells may be absent, or in some cases, polymorphonuclear cells may predominate. CSF protein is usually elevated. Diagnostic algorithms have been developed to assist in the early identification of patients at greatest risk of TBM²⁶. In such cases, the chances of obtaining a positive smear and culture result are increased when a large volume of CSF (> 6mL), or repeated CSF specimens are submitted for examination²⁶.

Gastrointestinal tuberculosis

Gastrointestinal tuberculosis was commonly found in the pre-antimicrobial era in patients with advanced pulmonary disease and resulted from swallowing infectious lung secretions²⁷. It can be caused by *M. tuberculosis*, or by *M. bovis* (which may follow ingestion of infected unpasteurised milk²⁸). Diagnosis of gastrointestinal TB is often made endoscopically. Biopsy tissue from the organ involved yields the highest numbers of organisms for AFB smear and culture¹.

Peritoneal TB

Peritoneal TB may occur in either the ascitic (exudative) or adhesive (dry) forms. The ascitic form is characterised by the presence of free fluid, the adhesive form resulting in fibrous adhesions and most frequently abdominal swelling²⁸.

Genitourinary tuberculosis

Genitourinary tuberculosis is rare before puberty, and is more common in males²⁹. The interval between infection and development of active renal disease is usually very long (years or even decades). As the infection progresses, kidney lesions may caseate, discharging viable AFBs to the renal pelvis and ureter, and infection may therefore spread to the bladder³⁰. The patient may complain of frequent micturition which may be accompanied by dysuria or haematuria, and urinalysis will often show proteinuria, haematuria (in up to 50% cases) and 'sterile' pyuria (in over 80%)^{12,31}.

Bone and joint, including spinal, tuberculosis

Bone and joint, including spinal, tuberculosis are usually a result of haematogenous spread to the bone from a primary pulmonary infection³². Predisposing factors include compromised immunity and intravenous drug use. Spinal TB is the commonest manifestation of bone and joint TB, followed by involvement of large weight bearing joints such as the knee or hip. Diagnosis of bone and joint TB is usually made clinically and radiographically, coupled with a suitable biopsy sample for histology and AFB smear and culture. Spinal TB (Pott's disease) occurs most commonly in the lower thoracic and upper lumbar areas³³. Occurring mainly in older children, young adults and the elderly it results in vertebral collapse with consequent spinal deformity. Tuberculous psoas abscess arises from disease of the thoracic or lumbar spine, and spreads within the sheath of the psoas muscle, sometimes as far as the thigh.

Bacteraemia

Low level bacteraemia often occurs with mycobacteria and is part of the natural history in an infected individual contributing to the occurrence of TB in other organs³⁴. However, detectable mycobacteraemia is comparatively rare except in patients who are otherwise markedly immunocompromised, such as those with HIV-AIDS³⁵⁻³⁸. In AIDs patients from developed countries, this was most often *Mycobacterium avium* complex; in those from sub-Saharan Africa it could be MTBC³⁷⁻³⁹. The incidence of these mycobacteraemic disseminated infections is now much less when effective anti-retroviral therapy is available⁴⁰. *Mycobacterium* species have also been isolated from the blood of other patients who are immunocompromised such as those with leukaemia, and occasionally from patients who are not overtly immunosuppressed³⁵.

Multi-drug-resistant tuberculosis (MDR-TB)

In recent years, cases of single drug and multi-drug resistance in TB have been increasingly reported throughout the world^{41,42}. *M. tuberculosis* becomes drug

resistant by spontaneous random mutation⁴³. Factors associated with drug resistance include incomplete and inadequate treatment, and failure to adhere to the prescribed treatment schedule. Primary resistance is defined as occurring in patients who are infected with a strain that is already resistant. Secondary resistance occurs when resistant mutants of an initially drug sensitive organism emerge during the course of an infection, usually due to inadequate chemotherapy. Multi-drug resistant (MDR) TB strains are resistant *in vitro* to isoniazid and rifampicin. In addition to conventional phenotypic sensitivity testing methods, a proportion of genetic mutations associated with resistance may be identified by various molecular methods. Such methods may be applied to an isolate or direct to a specimen; thus, providing earlier identification of the presence of drug resistance. Treatment regimens for MDR-TB tend to be less effective, and more prolonged, than therapy for drug sensitive TB. Strains of extensively drug resistant tuberculosis (XDR-TB) are also becoming more common particularly in certain geographical regions⁴⁴. These organisms, in addition to being MDR, are also resistant to any fluoroquinolone and at least one injectable 'second line' agent, namely amikacin, kanamycin or capreomycin⁴⁵. Cases of totally drug resistant tuberculosis have now also been reported⁴⁶.

4.2 Non-Tuberculous Mycobacteria (NTM)

These *Mycobacterium* species have been variously referred to as 'non-tuberculous mycobacteria,' 'environmental mycobacteria,' 'anonymous mycobacteria,' 'atypical mycobacteria,' and 'opportunistic mycobacteria.' NTM are ubiquitous in nature, have a varied spectrum of pathogenicity for humans, are not transmitted from person to person (except for *Mycobacterium abscessus* in which there is evidence to suggest that cross infection between patients is possible) and are often resistant to classical anti-tuberculous chemotherapy⁴⁷⁻⁵¹. However, correlation of *in vitro* resistance with *in vivo* efficacy remains much less defined for slow growing NTM than for MTBC⁵⁰. Over 100 species of mycobacteria have now been described with many recognised as obligate or opportunistic pathogens of man or animals^{50,52}. Unlike *M. tuberculosis*, isolation of an NTM species from specimens such as sputum does not equate to disease – the microbiology results need to be interpreted in conjunction with clinical and radiological findings⁵⁰. The taxonomy of this group is continually changing and expanding with the use of new techniques, such as comparative sequencing of the 16S rDNA⁵². The traditional distinction between 'rapid-growing' and 'slow-growing' species (on the basis of the ability of strains to demonstrate clearly visible colonies on subculture on a solid medium in 7 days or less of incubation) remains of clinical and taxonomic value^{50,52}.

Slow growing species

Mycobacterium avium – *intracellulare* group (MAI)

The term *M. avium* complex (MAC) is often used for convenience in clinical mycobacteriology to temporarily label strains phenotypically resembling *M. avium*⁵⁰. Separation of many species formerly assigned to this complex (including *M. intracellulare*) is now readily possible. There are currently three species within the MAC and they are *M. avium*, *M. intracellulare* and *M. chimaera*^{53,54}. Additionally, there are now three valid named subspecies of *M. avium*: *M. avium* subsp. *avium*; *M. avium* subsp. *paratuberculosis*; and *M. avium* subsp. *silvaticum*^{50,52,55}. In patients who are immunocompetent, MAI organisms, usually *M. intracellulare*, may invade the bronchial tree, pre-existing areas of bronchiectasis, or old cavities⁵⁰. In immunosuppressed patients *M. avium* and related organisms can cause disseminated infection^{40,50}.

Infections with *M. avium* may also cause cervical lymphadenitis in young children^{23,24,50}. These organisms are often present in water supplies and may contaminate specimens.

M. chimaera, a slow growing NTM found in the environment has been implicated recently in several cases of endocarditis or deep infection following cardiac surgery involving the use of cardiac bypass equipment^{56,57}.

Mycobacterium gordonae

This is a common aquatic species which has rarely, and disputably, caused disease in patients who are immunosuppressed⁵⁰. It is a common contaminant of clinical samples.

Mycobacterium kansasii

Pulmonary infection is the most common form of disease caused by *M. kansasii*, usually in patients with pre-existing chronic lung disease or pneumoconiosis, although infections can occur in other parts of the body⁵⁰. It is a photochromogen, that is, light is required for colonies to become pigmented.

Mycobacterium malmoense

M. malmoense usually causes pulmonary and lymph node diseases, but disseminated and other extra pulmonary disease has also been reported⁵⁰. Diagnosis of *M. malmoense* infection is as for other mycobacteria, although incubation times may need to be as long as 12 weeks before colonies become visible on solid media⁵⁸.

Mycobacterium marinum

M. marinum is the causative organism of 'fish tank' or 'swimming pool' granuloma, a localised skin lesion following contamination of an open wound or abrasion with water from fish tanks, swimming pools and natural areas of fresh or salt water. This species has an intermediate growth rate with an optimum growth temperature of 28 to 30°C⁵⁰.

Mycobacterium ulcerans

M. ulcerans is a cause of skin lesions in various global areas, including Australia ('Bairnsdale ulcer') and South-East Asia; Uganda and other parts of Africa ('Buruli ulcer'); and in Central/South America^{50,59}. Infection may lead to a chronic progressive painless ulcer, which can occasionally present in travellers from endemic areas^{50,59}. The organism can be difficult to isolate in the laboratory – it is more sensitive to standard decontamination methods than other mycobacteria, it is slow growing (6 to 12 weeks) and requires incubation at 30 to 33°C^{50,59}. Molecular methods can be of value in confirming the clinical diagnosis.

Mycobacterium xenopi

M. xenopi is another comparatively common cause of NTM pulmonary disease in certain geographic areas^{50,60}. It is thermophilic, with an optimum growth temperature of 45°C; and, similar to *M. malmoense*, grows comparatively slowly at 37°C. It can be isolated from various environmental sources, including hot water taps, and hence may also be a cause of specimen contamination⁵⁰.

Conspicuously fastidious species

Certain other mycobacterial species require specific additional supplements or conditions to be cultured successfully in the laboratory. These include *Mycobacterium*

genavense (for example, mycobactin J) and *Mycobacterium haemophilum* (haemin or other iron containing compounds), and both species have shown to be associated predominantly with patients who are immunocompromised, including those who are infected with HIV⁵⁰. However, *M. haemophilum*, which has an optimum growth temperature of 28 to 30°C, can also cause lymphadenitis in otherwise healthy paediatric patients^{24,50}. *Mycobacterium leprae*, the causative agent of leprosy, cannot currently be cultured *in vitro*.

Rapid growing species^{50,52,61}

Mycobacterium abscessus, *Mycobacterium chelonae*, *Mycobacterium fortuitum*

These, and related species, are well recognised as the cause of skin and soft tissue infections^{50,61,62}. These mycobacteria may infect long-term vascular catheters and other medical devices⁵⁰. Such organisms have been found in lavage fluids obtained by bronchoscopy and may be associated with false positive diagnoses. Although variation is found in some subspecies, the optimum growth temperature of these organisms lies between 30 to 33°C.

M. abscessus more so than the other non-tuberculous mycobacteria are an increasing problem for the cystic fibrosis patient group⁶³. Testing should be considered in cystic fibrosis patients who show deteriorating lung function but where no clear pathogen has been identified⁶⁴⁻⁶⁶. The Cystic Fibrosis Trust microbiology standards recommend routine screening of NTM at least once a year for all patients able to produce sputum and all *M. abscessus* positive isolates should be referred to the appropriate reference laboratory for strain typing⁴⁷.

Other slow growing and rapid growing *Mycobacterium* species isolated from clinical specimens have recently been identified using the molecular approach. For information and for update on the taxonomy of the *Mycobacterium* species, see link: <http://www.bacterio.net/mycobacterium.html>.

4.3 *Mycobacterium* and HIV/AIDS

Of all the people who are infected with HIV globally, a third are estimated to also be infected with *Mycobacterium tuberculosis*⁶⁷. Poverty, overcrowding and homelessness are socio-economic factors common to co-infection with both TB and HIV⁶⁸. Patients who are infected with HIV are predisposed to reactivation of latent TB infection, and also to a rapid progression of recently acquired infection⁶⁹. There is a need for a coordinated approach in the diagnosis and treatment of patients with co-infections⁷⁰.

A variety of non-tuberculous mycobacterial species have been isolated from systemic infections in patients who are HIV positive, the most common being MAI – however, the incidence of such disseminated infections is much reduced with the use of highly active anti-retroviral therapy^{40,50,71}.

4.4 New technologies for the diagnosis and typing of *M. tuberculosis*

Commercial blood-based assays for the diagnosis of latent tuberculosis

Detection of latent tuberculosis is essential for contact tracing and outbreak control. Traditionally the tuberculin skin test (TST, that is, the Mantoux or also previously the Heaf test) has been most commonly used for detection of latent *M. tuberculosis* infections. However, this procedure is fraught with problems, including; variations in interpretative criteria; false positive and false negative results; limited shelf life of the purified protein derivative (PPD); subjective reading of the results; and the unwillingness of some contacts to return for test interpretation⁷². Environmental mycobacteria and the *Mycobacterium bovis*-derived Bacille Calmette-Guérin (BCG) vaccine commonly cause false-positive results^{1,72}.

Consequently, there has been a need to develop a more reliable, sensitive and specific test for the diagnosis of latent tuberculosis. To address this need, whole blood-based assays have been developed which can detect *M. tuberculosis*-activated T cells or estimate γ -interferon production by these cells, termed interferon gamma release assays (IGRAs) or interferon gamma tests (IGTs)^{1,73}. Two commercially available tests are the QuantiFERON® - TB Gold In-Tube and the T-SPOT® TB assays. These are used to identify people who are at increased risk of developing TB and where treatment of the latent infection may be beneficial. Examples include healthcare workers, individuals who have had recent contact with a patient with active tuberculosis and those with underlying medical conditions such as HIV, leukaemia or lymphoma¹.

The assays detect the host response to infection using mycobacterial antigens which are present in *M. tuberculosis*, but not BCG; though they are found in some other mycobacteria, including *Mycobacterium szulgai*, *M. marinum* and *M. kansasii*. Thus they are unaffected by prior BCG vaccination or exposure to most non-tuberculous mycobacteria. However, these assays cannot distinguish latent TB infection from active or previously successfully treated disease. Both tests can provide a result within 24hr and can detect latent TB infection with a high level of sensitivity and specificity. Neither of these tests should be used in isolation to diagnose or define active TB disease^{73,74}. Revised recommendations regarding the roles of these tests in the diagnosis of LTBI has been published by NICE, and also by the Centers for Disease Control and Prevention in the United States in respect to their appropriate uses to detect *M. tuberculosis* infection^{1,73}.

Nucleic acid amplification tests

Real-time PCR has the potential to significantly change the current paradigm for mycobacteria identification by decreasing turnaround time for identification from weeks to hours while maintaining or improving upon diagnostic sensitivity and specificity.

Nucleic acid amplification tests (NAATs) for the detection of MTBC, when applied directly to a primary specimen, may be useful in certain situations, for example to confirm rapidly a diagnosis of tuberculous meningitis^{26,75}. Although, the accuracy of such NAATs has been found to be superior when applied to respiratory specimens in comparison with other sites, at this time, sensitivity (and to a lesser extent specificity) continues to limit the value of these tests. For TB disease, in an appropriate clinical setting, both in pulmonary and extra-pulmonary sites, a positive NAAT result could be used to rule in the diagnosis, but a negative one could not rule it out^{1,11,26}. The technology continues to develop, and a large multinational study demonstrated a sensitivity of 98% (for one platform) when testing sputum smear positive, and 73% for smear negative, MTBC culture confirmed patients with one NAAT per patient. In light of this and other studies, the WHO has made recommendations regarding the

application of this method – notably for it to be used as the initial diagnostic test for TB on at least one sputum for individuals who are suspected of having HIV associated TB or MDR-TB. The continuing need for conventional AFB microscopy and culture is acknowledged. In the US, nucleic acid amplification testing for TB is recommended as standard of care by CDC and the Association of Public Health Laboratories⁷⁶. The evidence was recently reviewed by NICE, and updated recommendations have been issued for the United Kingdom¹. Where pulmonary TB is clinically suspected, a NAAT, on a suitable primary specimen from someone 16 years or older, should be pursued in the setting of HIV; if rapid information about the presence (or absence) of MTBC DNA would alter the patient's care; or a large contact tracing exercise is being considered¹. A NAAT should be considered routinely in the setting of suspected pulmonary TB in children aged 15 years or less¹. In the setting of extra-pulmonary disease, a NAAT on certain primary specimen types; notably CSF, pericardial fluid and material from a lymph node; should be considered as an “additional” test - if the subsequent result would alter case management¹.

Genotypic methods are of critical value in enabling the preliminary rapid identification of a mycobacterial isolate to the complex or species level, as well as in elucidating the taxonomy of the genus^{2,52}. They can also be used to diagnose infections due to fastidious mycobacterial species which are difficult to culture in the laboratory^{50,59}.

NAATs are also of value for the rapid detection of *M. tuberculosis* genetic mutations associated with drug resistance (notably rifampicin, but also isoniazid; and potentially the fluoroquinolones or aminoglycosides if querying XDR)^{42,77-80}. In the United Kingdom it has been recommended that such a test to detect rifampicin resistance should be performed on a suitable sample from patients in whom not only TB is clinically suspected but also for whom one or more significant risk factors for MDR-TB is identified. The factors specified are if the patient has had prior treatment for TB, notably if known to have been poorly adherent; contact with a known case of MDR TB; or birth or residence in a country for which the WHO reports a high proportion (5% or more) of new TB cases are MDR¹.

A commercially available DNA line probe assay test, Genotype *Mycobacterium* common mycobacteria (CM), targeting the 23S rDNA region, has been developed. This assay has been reported to be sensitive and specific for the identification of most *Mycobacterium* species. An important advantage is the possibility to detect mixed infections. However, this assay has not been able to separate some members of the NTM group. It is also known to misidentify some strains of *M. abscessus* as *M. chelonae*^{81,82}.

Adenosine Deaminase (ADA) Assay

Adenosine deaminase (ADA) is a purine-degrading enzyme that catalyses the deamination of adenosine in an irreversible manner, which results in the production of inosine⁸³.



Adenosine deaminase (ADA) increases in TB because of the stimulation of T-cell lymphocytes by mycobacterial antigens. ADA can be detected in body fluids such as pleural, pericardial and peritoneal fluids. It has been developed and widely used for the rapid and early diagnosis of TB, especially in the case of negative AFB smears from body specimens^{84,85}.

Genomic typing analysis of *M. tuberculosis* isolates from TB outbreaks and clusters

Sporadic outbreaks of tuberculosis among human populations are a major threat to public health both in industrialised and developing countries. Early and accurate detection of the outbreak strains is paramount in the management and control of potential TB outbreaks. Various genomic based methods have been described⁸⁶. The chromosome of *M. tuberculosis* contains loci at which there are serially repeated genetic elements, with the number of repeats varying between different strains. These numbers can be determined for each isolate to generate a digital profile which can then be compared. These loci have been termed exact tandem repeats (ETR), variable number tandem repeats (VNTR) or mycobacterial interspersed repetitive units (MIRU), and the method has been called mycobacterial interspersed repetitive unit – variable number tandem repeat (MIRU-VNTR) typing^{87,88}. In the United Kingdom, 24 different loci are currently analysed for at least one *M. tuberculosis* isolate from each new patient. *M. tuberculosis* genotyping has been shown to be of value in various settings, including in the identification (or exclusion) of possible incidents of laboratory cross-contamination; the investigation of perceived outbreaks; as well as in the analysis of patterns of *M. tuberculosis* transmission within and between communities^{12,87-91}. Advice has been produced by UKHSA for the interpretation and investigation of clusters generated by the national strain typing programme in the United Kingdom, as well as there being guidance for the application of genotyping results by the CDC.

MALDI-TOF MS Identification of *Mycobacterium* species

Matrix-assisted laser desorption ionisation – time of flight (MALDI-TOF) mass spectroscopy analyses 16s ribosomal proteins. It compares the mass peaks achieved by the test strains against those of known reference strains to facilitate identification. It is possible for an organism to be identified within 20 min and is increasingly being used in microbiology laboratories to provide a robust organism identification system. Studies have been carried out on the benefit of this technique in the identification of mycobacterial species and it has been found to be a reliable means of distinguishing between closely related species within the group as well as between members of various mycobacterial complexes from culture of clinical specimens⁹²⁻⁹⁴. MALDI-TOF provides a rapid means of identification for this important group of pathogens, potentially allowing accurate treatment regimens to be started earlier, however further validation is required to enhance existing databases and standardise the method thereby improving interlaboratory reproducibility; and it is not currently used routinely⁹⁵.

Whole Genome Sequencing (WGS)

This is also known as “full genome sequencing, complete genome sequencing, or entire genome sequencing”. It is a laboratory process that determines the complete DNA sequence of an organism's genome at a single time. This sequencing method holds great promise for rapid, accurate, and comprehensive identification of bacterial transmission pathways in hospital and community settings, with concomitant reductions in infections, morbidity, and costs. This has been used successfully to detect microevolution within *M. tuberculosis* strains as well as to delineate outbreaks of tuberculosis with unprecedented resolution⁹⁶⁻¹⁰⁰. WGS also generates extended results regarding MTBC strain susceptibility to certain drugs – which currently could be

used as genotypic “predictions” whilst awaiting conventional phenotypic results¹⁰⁰. The use of WGS has also shown the frequent transmission of multidrug resistant NTM between patients with cystic fibrosis despite conventional cross-infection measures⁴⁹.

Note: There are specialist laboratories in the UK that offer the WGS service on request. It should also be noted that it is anticipated that this will form part of the routine analytical service on AFB isolates, provided by UKHSA’s national mycobacterium service. Other arrangements are in place in the other administrations.

5 Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (for example, sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers^{101,102}

UK SMIs use the term, “CE marked leak proof container,” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes.”

Culture of samples

Decontamination

Specimens submitted for mycobacterial culture fall into two categories: specimens normally contaminated with resident flora, and specimens from normally sterile sites. Contaminated specimens require a decontamination step before culture to reduce the likelihood of overgrowth by organisms other than mycobacteria. Excessive decontamination of specimens should be avoided as, although mycobacteria are more resilient than other bacteria to the decontaminating agents used, they are not entirely so, and hence this can produce false negative results¹². Five different methods are described for the decontamination of samples, but there is not much evidence that one method is optimum, and laboratories should select the method that they prefer – which may vary with patient and specimen types. Other processing techniques have also been described which perform well in comparison to the widely-used NALC-NaOH

procedure, described in 9.5.1.1; and may also be suitable if compliant with the liquid culture system in use, after local validation^{6,103,104}.

Sodium pyruvate

It should be noted that although sodium pyruvate enhances the growth of *M. bovis*, it may inhibit some non-tuberculous *Mycobacterium* species.

Concentration/centrifugation

When centrifuging specimens, appropriate time should be added to the total spinning time to allow the centrifuge to reach the appropriate speed and for subsequent braking time.

Concentration of sputum samples, for example by centrifugation, increases the sensitivity of initial microscopy¹⁰⁵.

It should be noted that when using a centrifuge (for all decontamination methods applied to clinical specimens), it is important to ascertain that the rotor reaches and maintains the required RCF of 3000g for 15 minutes in order to obtain good recovery of the mycobacteria. The rate at which mycobacteria sediment is critically dependent on time of centrifugation and relative centrifugal force applied to the specimen. A longer centrifugation time can offset a lower relative centrifugal force, but increased centrifugation time increases the temperature of the specimen, which leads to additional killing of mycobacteria (hence, a refrigerated centrifuge is highly recommended)¹⁰⁶.

Cross contamination

False positive cultures, due to cross contamination in laboratories, have been reported and the median false positive rate has been 3.1 %⁹⁰. Cross contamination should be avoided by the use of individual pipettes, single aliquots of decontaminants and other additives whenever possible. Any additives to the sample including water should be sterile.

It is recommended that reagents (such as mucolyse, NaOH and neutralising buffer) should not be dispensed directly from stock bottles but should be prepared and pre-dispensed into appropriate sterile containers aseptically, ready for use. This should be done in order to avoid problems with cross contamination due to inappropriate use of stock bottles of reagent. Laboratories can reduce potential contamination rates by ensuring that staff comply with the local guidelines set out on the preparation of reagents, handling of specimens, checking pH readings of solutions used in decontamination and sterilisation records of laboratory equipment. Contamination rates should be monitored on an ongoing basis.

Automated liquid culture systems

The automated liquid culture systems available in the UK have been tested for their ability to detect a wide range of both slow and rapidly growing mycobacteria; however, reliance should not be placed on these systems alone for the isolation of all mycobacterial species, particularly when investigating patients who are immunocompromised⁵⁰. Their limitations lie in a single incubation temperature and the difficulty of providing the growth additives necessary for certain very fastidious species. Advice may be sought from the Reference Laboratories or relevant system manufacturer. Rare isolates of *M. tuberculosis* are recovered only on egg-based media, such as a Löwenstein Jensen slope¹².

Incubation temperature

The temperature of heat mats should be monitored because plates that are too hot will result in loss of sensitivity in staining, and hence false negative smears^{107,108}.

Staining of slides

Laboratories that perform TB smear tests should ensure that the smeared slides are fixed at the correct temperature (65 to 75°C) for a suitable period (until dry, approximately 10 minutes in most circumstances, and not exceeding one hour) as recommended in the [UK SMI TP 39 – Staining Procedures](#). This is because prolonged heat fixation or use of higher temperatures than recommended may decrease the sensitivity of detecting alcohol and acid fast bacilli (AAFBs), with associated risk of false negative smears¹⁰⁷.

Volume of sample

A minimum volume of 5mL is required for reliable NAAT testing of clinical specimens^{2,19}. However, submitting more volume of clinical specimens will increase sensitivity.

Accreditation

Laboratories should be registered with UKAS. They must include mycobacteriology investigations within their declared scope².

Quality control

The quality of commercially available egg-based solid media and liquid media should be guaranteed by the manufacturer. However, storage conditions especially during transit to the laboratories may not be optimal and so it is advisable to revalidate new batches of media received into the laboratory before routine use.

Egg-based media are robust and retain their sensitivity unless exposed to direct sunlight or prolonged high temperatures. Liquid media are more prone to contamination by mycobacteria other than *Mycobacterium tuberculosis* or by other bacteria.

It should be noted that in-house preparation of liquid media and reagents are very susceptible to contamination and so aseptic techniques and utmost care must be taken when preparing these.

Misidentification using GenoType *Mycobacterium* (CM/AS) assays

This assay has been reported to be sensitive and specific for the identification of most *Mycobacterium* species. However, this assay has not been able to separate the members of the NTM such as some strains of *M. abscessus* as *M. chelonae*^{81,82}.

6 Safety considerations^{101,102,109-123}

6.1 Specimen collection, transport and storage^{101,102,109-112}

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

6.2 Specimen processing^{101,102,109-123}

Hazard Group 3 organisms.

All *Mycobacterium tuberculosis* complex organisms are notifiable to UKHSA under the Health Protection (Notification) Regulations 2010.

Mycobacterium tuberculosis can cause laboratory-acquired infections (LAIs). The most important route for transmitting LAIs is through aerosols. As a minimum it is recommended that the processing of all samples, including respiratory samples that may result in generation of aerosols should be processed in a microbiological safety cabinet in Containment Level 2 conditions with additional precautions to minimise risk of aerosols production in accordance with the relevant risk assessment, ACDP and HSE guidelines¹¹⁵.

However, if processing diagnostic samples that are assessed to be at higher risk of containing hazard group 3 organisms such as *M. ulcerans*, *M. microti* and *M. tuberculosis*, this must be undertaken under appropriate containment conditions as determined by local risk assessment. The nature of the work that may dictate that full Containment Level 3 conditions should be used are; the preparation of sputum smear slides for microscopy, treatment of specimens (such as sputum) before culture, processing of positive blood cultures (MGIT) and DNA extraction from clinical specimens (for molecular techniques such as DNA identification, detection of gene mutations related to drug resistance or possibly genotyping) in order to comply with COSHH 2004 Schedule 3 (4e). If performing the MALDI-TOF test procedure as an example, ensure that the manufacturer's instructions are adhered to on how to use the different types of matrices for MALDI-TOF MS extraction as some are associated with significant occupational hazards such as eye, skin and respiratory toxicity. For more information, see [UK SMI TP 40: Matrix-assisted laser desorption/ionisation - time of flight mass spectrometry \(MALDI-TOF MS\) test procedure](#).

Use disinfectants according to manufacturer's instructions to disinfect surfaces of microbiological safety cabinets, and for wiping the exterior of items of equipment. Phenolic disinfectants are not supported by the Biocidal Products Directive 2001, and therefore no longer available in the UK¹²⁴. Alternatives are available which have varied efficacy of inactivation against *Mycobacterium* species¹²⁵. Public Health England has made recommendations for possible alternatives to phenolic disinfectants including hypochlorites and chlorine dioxide¹²⁶. There is also evidence to suggest that 0.35% peracetic acid (Nu-cidex A and B) is rapidly mycobactericidal¹²⁷. Contact times and concentration for various disinfectants can vary and should be considered. Disinfectants with rapid contact times are desirable.

The NALC-NaOH reagent contains strong alkali and causes severe burns. Gloves and eye/face protection must be worn. NaOH is irritating to the eyes and skin.

The use of hot plates in microbiological safety cabinet in a Containment Level 3 room may alter the airflow through the cabinet. It is important to verify that the air flow is not affected by the hot plate operation and to ensure that operator protection is not compromised.

In settings where equipment/ kit designed for point of care testing (POCT) is used in the laboratory, a risk assessment should be undertaken as to where it can be placed.

Use sealed buckets for centrifugation. After centrifugation, open the buckets in a microbiological safety cabinet.

Transport the discarded material directly to the autoclave when ready for disposal and autoclave immediately.

Use plastic consumables in preference to glass wherever possible.

Note: Heat fixation of smears does not kill *Mycobacterium* species. Handle the slides with care¹²⁸ (see section 9.4).

Place and transport specimen containers in holders designed to minimise breakage and spillage.

Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

Staff must wear personal protective equipment at all times when processing clinical samples in the Containment Level 3 laboratory as well as maintaining safe working conditions.

The above guidance should be supplemented with local COSHH and risk assessments.

7 Specimen collection

7.1 Type of specimens

Sputum, sterile site body fluids (CSF, pleural fluids etc), urine, skin or tissue biopsies, bronchoalveolar washings, post-mortem specimens, gastric washing, bone marrow, blood, bone

7.2 Optimal time and method of collection¹²⁹

For safety considerations refer to Section 6.

Collect specimens before antimicrobial therapy where possible¹²⁹.

For the initial diagnosis of mycobacterial infection all specimens should be fresh and taken, whenever possible, before anti-tubercular treatment is started. 'Other' antimicrobials may also have significant anti-mycobacterial activity, notably the fluoroquinolones such as ciprofloxacin, levofloxacin or moxifloxacin, and the macrolides such as clarithromycin or azithromycin.

Use appropriate hazard labelling according to local policy.

Refer to the relevant HSE/COSHH guidelines on the collection and safe handling of specimens likely to contain Hazard Group 3 organisms.

Aerosol generating procedures, such as bronchoscopy or sputum induction, should be performed in an appropriately engineered and ventilated area¹.

Specimens other than blood

Specimens other than blood or bone marrow should be refrigerated if transport to the laboratory or specimen processing is delayed for >1hr.

Gastric washings

Gastric washings should be neutralised by adding approximately 100mg of sodium carbonate to approximately 50mL of the specimen if processing is delayed for >4hr¹³⁰.

Blood and bone marrow cultures

Blood and bone marrow aspirate cultures should be transported and loaded into the automated culture system as soon as possible.

Note: These samples should not be collected in EDTA tubes as this inhibits the growth of mycobacteria. Lithium Heparin tubes are recommended.

7.2.1 Correct specimen type and method of collection

Sputum specimens

Sputum specimens should be relatively fresh (less than 1 day old) to minimise contamination. Purulent specimens are best. Two to three samples of ≥5mL should be collected approximately 8 to 24 hours apart with at least one from early morning^{1,2,11,13,19}.

Samples taken early morning (that is, shortly after patient waking) have the greatest yield. When the cough is dry, physiotherapy, postural drainage or inhalation of nebulised saline ('sputum induction') before expectoration may be helpful.

Note: Decontaminated and neutralised samples are not recommended as they may lose viability during transit to the laboratory.

Bronchoalveolar lavage/bronchial washings

These may be sent if spontaneous or induced sputum is unavailable or if such specimens are AFB smear negative.

Note: Contamination of the bronchoscope with tap water, which may contain environmental *Mycobacterium* species, should be avoided. Minimum sample size is preferably 5mL.

Gastric washings

Gastric washings are usually used for children where there are problems obtaining sputum. Young children will often swallow their respiratory secretions rather than cough them up. Induced sputum is considered preferable to gastric washings, if possible¹. Collect samples early in the morning (before breakfast) on 3 consecutive days¹³¹. Preferably, a minimum volume of 5mL should be collected. Aspirates should be promptly delivered and processed to avoid acidic deterioration of organisms (see under neutralisation, section 9.5). Results of direct microscopy on gastric washings can be misleading because other acid-fast bacilli are normally present in the stomach.

Sterile site body fluids

Sterile site body fluids (CSF, pleural fluid, etc) will normally not require decontamination, and can be inoculated directly to neutral media. However, these samples can be assessed for contamination by setting up purity plates. If contaminated, they can be treated with acid and if pure they can be directly inoculated. Collect aseptically as much (for example >6mL in adults) CSF sample as possible into a CE Marked leak proof container in a sealed plastic bag. If only a small volume is available after initial lumbar puncture, and the findings of cell counts and protein suggest TB meningitis, a second procedure should be considered to obtain a larger volume to improve chances of achieving positive cultures²⁶.

It should be noted that pleural or pericardial fluids are not very sensitive samples for the detection of *M. tuberculosis*, and that a concurrent pleural or pericardial biopsy taken with the fluid is more useful¹². A negative result on these fluids does not rule out the diagnosis.

Urine specimens

Urine specimens should be collected in the early morning on three consecutive days in a CE marked leak proof container (that does not contain boric acid) and placed in a sealed plastic bag. If there are no appropriate containers for a whole Early Morning Urine (EMU) sample, a midstream EMU sample is an acceptable, but not ideal alternative.

Skin, bone, and tissue including post mortem specimens¹³²

Specimens of such type should be homogenised, with the exception of bone. It may be necessary to select and cut out a suitable piece of tissue if a large piece is received. Similarly, some pieces of tissue may need to be 'minced' using sterile scissors and forceps before they can be successfully homogenised. Specimens should be collected aseptically and placed in a CE Marked leak proof container without preservatives in a sealed plastic bag, and sterile distilled water added to prevent desiccation. A caseous portion should be selected if possible: the majority of organisms will be found in the periphery of a caseous lesion.

Tissue biopsy specimens received in formalin are unacceptable and should not be processed¹³³.

Faecal samples

Mycobacterium tuberculosis and *Mycobacterium avium-intracellulare* group have been isolated from faeces, notably in patients who are immunocompromised such as those with HIV-AIDS. However, NTMs can often be isolated from healthy individuals, representing colonisation only¹³⁴. If *M. tuberculosis* is isolated, this may well be due to the ingestion of infected respiratory secretions rather than intestinal disease¹³⁴. The isolation procedure is unreliable and has a low success rate due to the heavy contamination with other bacteria; hence culturing faecal samples for mycobacteria is not recommended in this UK SMI. *M. tuberculosis* and NTMs, including MAI, may be isolated from blood cultures in disseminated infection.

Pus or pus swabs

Pus, or pus swabs, should be collected aseptically, and the largest practical sample submitted in CE marked leak-proof container in a sealed plastic bag. Pus is the sample type of choice. Swabs are less preferable as mycobacteria, if present, may adhere to the swab rather than be transferred successfully to the culture media¹³⁵.

Bone marrow

As large a sample of bone marrow as possible should be aspirated and added directly to the culture medium in accordance with the manufacturer's instructions.

Blood

For more information on blood cultures, refer to [UK SMIs](#) but microscopy, sub-culturing and further testing should be handled in accordance with the methods outlined in this UK SMI.

Note: EDTA, even in trace amounts, inhibits the growth of some *Mycobacterium* species and so is not acceptable.

Cultures

Culture specimens could be sent as egg medium slopes or aliquots of liquid medium in appropriate transport containers. At least 5mL is required if grown in liquid medium. For details of how to prepare a culture specimen for referral to the appropriate Reference laboratory, see the link in section 9.9 of this UK SMI.

7.3 Adequate quantity and appropriate number of specimens¹²⁹

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

8 Specimen transport and storage^{101,102}

8.1 Optimal transport and storage conditions

For safety considerations refer to Section 6.

Specimens should be transported and processed as soon as possible¹²⁹.

Specimens should be transported and received in the laboratory within one working day of collection and processed as soon as possible². Requirements of individual testing laboratories should be referred to.

If processing is delayed, refrigeration is preferable to storage at ambient temperature¹²⁹.

8.1.1 Transportation

The terms Category A (for positive cultures) and Category B (for specimens) are limited to classifying samples / microbial cultures being transported to another laboratory (see Table below).

Sample description	Packaging requirement
Category A samples are known or suspected to contain a microbial agent with the following definition "an infectious substance which is transported in a form that if exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease to humans or animals". The majority are Hazard Group 3 or 4	Assign to UN2814 (Humans) Packaging Instructions P620 Supporting documentation as per ADR Transport as category A ADR licensed courier

For practical reasons to allow referral / reference services to continue a limited number of Category A agents have exempted from being transported as Category A. These are Vero-cytotoxin producing <i>Escherichia coli</i> (VTEC), <i>Mycobacterium tuberculosis</i> and <i>Shigella dysenteriae</i> 1	Assign UN3373 Packaging instruction P650 Send by courier Royal Mail will NOT accept
Category B samples are those that do not meet the definitions of Category A	Assign UN3373 Packaging instruction P650 Post or courier Royal Mail WILL accept

9 Specimen processing/procedure^{101,102}

9.1 Test selection

If there is sufficient volume, specimens being processed for AFBs should normally have AFB microscopy (a 'smear') prior to, and in addition to, culture; with the exception of samples received already pre-inoculated into a mycobacterial culture bottle. Direct microscopy of urine is also of questionable value; and moreover, depending on the clinical setting, can be misleading due to the presence of non-tuberculous mycobacteria^{12,31}.

If sample volume is insufficient for both, culture is usually preferred to microscopy due to greater sensitivity.

It may be appropriate to process a sample for AFBs, depending on specimen type and available clinical details, even if these tests were not specifically requested when submitted¹. Such specimen types in particular would include pleural biopsies or material from lymph nodes¹.

In addition to conventional AFB investigations, nucleic acid amplification test(s) (NAAT), such as by PCR, may also have been requested - either to detect presence of MTBC DNA and/or genetic mutation associated with drug resistance (see 4.5.4 and the introduction for more details when appropriate to consider¹). **Note:** NAAT on a primary specimen is an "additional" test to consider - if limited specimen volume, conventional tests should normally take priority.

For certain body fluids, such as CSF or pleural, pericardial or ascetic fluids, where TB is being considered, adenosine deaminase (ADA) assay may also be requested¹.

9.2 Appearance

Sputum specimens

The sputum samples should appear thick and mucoid or clear but with purulent grains. The colour varies from opaque white to green.

Bloody sputum specimens will appear reddish or brown.

Note: clear saliva or nasal discharge is not accepted as a TB specimen.

Sterile site body fluids

Note the presence of any clot and, if present, include it in the processing.

Tissue biopsies

Process the entire tissue if the sample is small.

For larger samples, select caseous portions (if present) as well as the tissue immediately surrounding the caseating areas for smears and culture.

9.3 Sample preparation

For safety considerations refer to Section 6.

Please see relevant sections for sample preparation for smear and culture investigation below.

9.4 Microscopy

9.4.1 Standard

Microscopy should be performed, and the result issued within one working day of receipt of the specimen².

Microscopy should be performed after homogenisation and before decontamination of samples, or directly from samples.

1. Centrifuge homogenised samples in sealed buckets at 3000 x g^* for 15 min.
2. Carefully discard the supernatant to a discard pot containing an appropriate disinfectant.
3. Prepare a thin smear of the deposit on a single microscope slide and heat-fix on the hotplate (65 to 75°C), for a minimum period of 10min and a maximum of 1hr then place in a rack or other suitable holder¹⁰⁷.
4. Stain using the auramine-phenol method.

*It is important that centrifugation is carried out at an appropriate Relative Centrifugal Force (RCF). This will vary with the centrifuge and is a factor of rotor arm length and rotation speed. This should not be confused with Revolutions per Minute (RPM).

Note: At least a six day service during the normal working days should be provided for smear examination of appropriate samples².

9.4.2 Preparation of smears

Sputum smears

Using a plastic loop, spread the treated centrifuged deposit over the slide keeping away from the slide edges^{11,12,108}. Avoid making the smear too thick.

Sterile site body fluids

Prepare smears as for sputum above from the spun deposits. In the case of CSF, it may be appropriate to build up several layers of material, if sufficient volume.

Tissue

Smears from tissue may be more sensitive when processed by histology, that is, serial sections that are stained by modified Ziehl-Neelsen stain. Direct smears from tissue are possible, but they are usually insensitive. However, where the amount of diagnostic material is limited, culture of fresh tissue is the most sensitive means of making the diagnosis and provides most information for patient management.

Swabs

Swabs that are received singly should not be examined by direct microscopy as this may lead to contamination. If a pair of swabs is received, or if the tissue is accompanied by a pus swab, then microscopy can be carried out. Swabs are not the specimen type of choice. If a single swab is received, then suspend the contained material in 1mL of sterile distilled water and treat as sputum.

Blood / bone marrow aspirate

Perform microscopy on any broth bottle from an automated culture system which 'flags' positive or which is visually positive. With an appropriate safety needle or device, remove a few drops of blood/broth mixture and place on a clean microscope slide. Spread this with a sterile loop to make a thin smear for acid-fast staining.

Urine

Do not pool urine specimens together when prepared for inoculation.

9.4.3 Staining smears

Auramine-phenol staining is more sensitive than that by the Ziehl-Neelsen method and is therefore more suitable for assessment of smears from clinical specimens^{2,11,12,50,105,108,136}. Ziehl-Neelsen staining provides morphological details and is useful for the examination of AFB in positive cultures and may be used to review results from clinical specimens that are positive with auramine-phenol.

Stain previously fixed positive and negative control slides, with each batch of auramine-phenol and Ziehl-Neelsen stains, before the stains are routinely used.

Note: When staining slides, to reduce the risk of cross-contamination, it is advisable to ensure that they do not touch each other. Both a negative and a positive control slide should be present and tested correctly. Also ensure they are not splashed with tap water prior to staining – this may produce false positive results due to NTMs present in the plumbing. When using stains, the methodology should be supplemented with local COSHH and risk assessments.

For more information on the staining of heat fixed films by auramine-phenol in clinical specimens and staining of heat fixed films by Ziehl-Neelsen stain in positive cultures, refer to [UK SMI TP 39 – Staining procedures](#).

9.5 Culture and investigation

9.5.1 Treatment of specimens

All methods go through one or more of the stages outlined below:

Pre-treatment (for example centrifugation):

This is not suitable for all specimen types.

Homogenisation:

Improves the sensitivity of culture but is not required for all sample types.

Decontamination and neutralisation:

Removes contamination and balances pH. The timing of the various stages should be reviewed in light of individual laboratory contamination rates. Laboratories using automated culture systems should refer to manufacturer's recommendations for

compatible decontamination methods. Five decontamination/digestion methods which are currently used to process specimens are:

1. Decontamination of specimens using 1N Sodium Hydroxide (NaOH 4% w/v) / modified Petroff method
2. Decontamination of specimens using *N*-acetyl-*L*-cysteine sodium hydroxide (NALC-NaOH)
3. Decontamination of specimens using trisodium phosphate and benzalkonium (TSPB)
4. Decontamination of specimens using H₂SO₄ (0.5N)
5. Decontamination of specimens using oxalic acid (5%)

There is not enough evidence or evaluation data available to recommend an optimum method. The choice of the most suitable method and length of decontamination will vary with the level of contaminants in the specimens. Laboratories should treat samples according to the level of contamination expected in the sample.

There are commercially available specimen digestion/decontamination kits. Follow manufacturer's instructions.

Note: It should be noted that the success of homogenisation and decontamination depends on the appropriate concentration of homogenisation or decontamination solution, the length of exposure time to the organisms, the centrifugation speed and time used to sediment the tubercle bacilli as well as the temperature build-up in the specimen during centrifugation.

Concentration (for example centrifugation):

This is not appropriate for all sample types.

9.5.1.1 Specimens from non-sterile sites

The specimens for non-sterile sites include:

- Sputum
- Gastric washing
- Bronchoalveolar washing/bronchial washings
- Laryngeal swabs (material should be eluted from the swabs using NaOH and the product treated as for sputum. If microscopy is required, two swabs should be sent)

Note: Specimens from otherwise sterile sites such as (CSF, bone marrow, tissue biopsies, pleural, pericardial and peritoneal fluids, surgically resected specimens (excluding autopsy material) pus from cold abscesses, spinal fluid, synovial or other internal body fluids) do not need decontamination prior to culture.

Pre-treatment:

Centrifuge fluid samples, if sufficiently liquid, for example gastric lavage, bronchoalveolar washings. Centrifuge at 3000 x *g* for 15min and discard supernatant into disinfectant, leaving 1mL to re-suspend the pellet.

Homogenisation

Homogenisation can be achieved by one of the following methods:

1. Repeatedly vortexing during the decontamination process until the specimen is fully homogenised
2. Treatment with dithiothreitol (DTT): Liquefy samples with an excess volume of dithiothreitol, shaking or vortexing intermittently until the specimen is homogenised. Centrifuge at 3000 x g for 15min and discard supernatant into disinfectant, leaving 1mL to re-suspend the pellet

Note: The use of DTT allows dilution and facilitates retrieval of micro-organisms present in the sample¹³⁷.

3. Treatment with N-acetyl-L-cysteine (NALC): Include NALC at decontamination stage (see decontamination of specimens using NALC-NaOH)

Decontamination of specimens using 4% NaOH (modified Petroff method)¹³⁸⁻¹⁴⁰

NaOH is a commonly used decontaminant and serves as a mucolytic agent but strict adherence to the reduced indicated timing for the modified Petroff method is required as it is slightly more harmful to the tubercle bacilli than to contaminating organisms. However, if using the original Petroff method, timing is extended to 30 minutes and the specimen is neutralised by HCL using a drop of phenol red as indicator before inoculation onto appropriate media.

1. Add the specimen (3-5mL) into the centrifuge tube and an equal volume of 4% NaOH with 0.04g/l phenol red indicator already in it
2. Allow the NaOH to act for 15min at room temperature (20 to 25°C), vortexing at regular intervals (for example every five minutes)
3. Neutralise the specimen with sterile 0.067 M phosphate buffer (pH 6.8) or 20mL sterile distilled water
4. Centrifuge at 3000 x g for 15min

Note: if using 2% NaOH as a decontaminating agent, this concentration is toxic to both contaminants and to some mycobacteria.

Decontamination of specimens using NALC-NaOH¹³⁸:

This method is a “preferred method” for the digestion step because it is the least toxic to mycobacteria and therefore provides the highest yield of positives. It is the most commonly used method in clinical laboratories.

1. Add an equal volume of working NALC-NaOH solution to the specimen in the centrifuge tube. Tighten the screw-cap

Note: Mix equal volumes of 0.5 N NaOH* solution and Na₃C₆H₅O₇.2H₂O** solution (trisodium citrate. 2H₂O). These can be premade before the day of use. Add 2% NALC on the day of use to the mixed solution when it will be used, as its activity wanes if pre-made and stored.

*Add 4g of NaOH to 100ml of distilled water to make the NaOH working solution.

** Add 2.9g Na₃C₆H₅O₇.2H₂O (or 2.6g if using anhydrous sodium citrate) to 100ml of distilled water to make the Na₃C₆H₅O₇.2H₂O working solution.

2. Agitate the tube on a vortex mixer for not more than 20sec. Invert the tube so that the NALC-NaOH comes in contact with the entire inner surface of the tube. Avoid excessive agitation

3. Allow the tube to stand for 15min at room temperature (20 to 25°C) to decontaminate the specimen
4. Dilute the mixture with sterile 0.067 M phosphate buffer (pH 6.8) containing phenol red indicator and invert several times to mix the contents
5. Centrifuge at 3000 x g for 15min

Note: If processing sputum, three washes should be done instead of one.

Decontamination of specimens using trisodium phosphate and benzalkonium (TSPB)^{139,141}

This method has been found to be a useful procedure as these chemicals are fairly non-toxic to mycobacteria and provide reasonably better mucolysis.

The timing of this process is not critical for viability of tubercle bacilli. Its limitations are its laboriousness, and the unavailability of the materials needed as benzalkonium is not easily available.

- Add the specimen into the centrifuge tube and add an equal volume of the TSPB solution
- Tighten the screw-cap and vortex. Allow to stand at room temperature for 30min
- Neutralize the mixture with sterile 0.067 M phosphate buffer (pH 6.8) and vortex briefly to mix the contents

Concentration

Centrifuge at 3000 x g for 15min using sealed buckets within the centrifuge. Discard supernatant into disinfectant, leaving 1mL to re-suspend the pellet or re-suspend in 1-2mL sterile 0.067 M phosphate buffer (pH 6.8). (The latter has the added effect of increasing the neutralisation activity).

9.5.1.2 Specimens that are heavily contaminated with Gram negative bacteria

The specimens that are heavily contaminated with Gram negative bacteria include:

- Urine
- Skin or tissue biopsies from non-sterile sites
- Post mortem specimens
- Pus, aspirates and fluids

Pre-treatment

- Centrifuge fluid specimens, for example urine or pus if suitably liquid and sufficient volume in sealed buckets at 3000 x g for 15min. Do not pool urine samples
- Open centrifuge buckets and carefully decant supernatant into a discard pot containing a suitable disinfectant
- Cut tissue into small pieces with a sterile scalpel and homogenise down all tissue specimens in a sterile porcelain mortar or tissue grinder. Transfer to a sterile universal. Place a portion of the sample in a sterile bijoux and store at ≤ -20°C to allow culture to be repeated if contaminated. Plate out directly

Decontamination of specimens using (0.5N) H₂SO₄^{139,142}

This method is used for specimens that consistently produce contaminated cultures when processed with one of the alkaline digestants. A major limitation of this method is that it may inhibit most of the tubercle bacilli in the specimen.

1. Add an equal volume of H₂SO₄ (0.5N) to all fluid/tissue specimens and allow to act for 20 to 30min. Timing of the various stages should be reviewed in light of individual laboratory contamination rates for different specimen types
2. Top up the container with sterile 0.067 M phosphate buffer (pH 6.8) after treatment and centrifuge at 3000 x g for 15min
3. Discard the supernatant into discard pot leaving the deposit in approximately 1mL of liquid or re-suspend in 1 to 2mL sterile 0.067 M phosphate buffer pH 6.8 (The latter has the added effect of increasing the neutralisation activity)
4. Neutralise the sediment with 4% NaOH containing a phenol red indicator (the phenol indicator is added so that neutralisation can be verified visually. However, the use of lower working concentrations of NaOH (2%) together with a dilution step should obviate the need for this step)
5. Inoculate the sediment directly onto culture medium (in urine specimens)

Note: It should be noted that the use of phenol red indicator is not compulsory. Users omit the use of phenol red indicator where they have validated their methods. However, some users use this to ensure that neutralisation has taken place especially when training is given to staff.

9.5.1.3 Specimens contaminated with *Pseudomonas* species

The specimens include sputum or other respiratory samples from patients with cystic fibrosis or bronchiectasis that are likely to be consistently colonised with *Pseudomonas* species.

Homogenisation

See Section 4.5.1.1 Specimens from non-sterile sites.

Decontamination of specimens using 5% oxalic acid¹³⁹

This method is used for specimens consistently contaminated with *Pseudomonas* species.

1. Add sufficient amount of 5% oxalic acid to the specimen in a plastic universal container to almost fill the container and vortex
2. Allow the acid to act for 30min (or longer if necessary) shaking intermittently to aid homogenisation and decontamination
3. Centrifuge the specimen at 3000 x g for 15min
4. Decant the supernatant into a discard can containing appropriate concentration of disinfectant
5. Neutralise the specimen with NaOH (0.5N) or re-suspend in 1-2mL sterile 0.067 M phosphate buffer (pH 6.8).

Or

Note: If recovering *M. tuberculosis* from swab samples,

1. Cover the swab with 5% oxalic acid for 15min and then transfer into sterile saline for a few minutes
2. This can be later removed and allowed to drain.
3. Neutralise the specimen (swab) with 2 to 3mL of 5% sterile sodium citrate for 5min before inoculation in media

9.5.1.4 Specimens from sterile sites

Cerebrospinal fluid (CSF), blood and other samples from normally sterile sites, or specimens already shown to be free of viable bacteria by culture, do not require decontamination (see below if heavily blood-stained fluid sample, such as from pleura). In addition to culture for mycobacteria, these samples should also be cultured for other pathogens (see [UK SMI B 27 - Investigation of cerebrospinal fluid](#), or other UK SMIs as appropriate) to eliminate other causes of infection. This also indicates the absence of other bacteria or reveals the need for decontamination procedures. At this stage consider the need for molecular tests prior to processing.

Centrifuge CSF and other suitable fluid specimens from otherwise sterile sites prior to culturing and smear preparation (3000 x *g* for 15min, as described above).

'Decontamination' (as in 9.5.1.2) of heavily blood-stained non-CSF fluids may also be appropriate prior to incubation in an automated liquid culture system, to reduce false positive signalling of the fluorescence detection system. Use the remainder of both the deposit and the supernatant (if available) for culture. For CSF specimens, after inoculation of appropriate culture media add a liquid culture medium (for example Kirchner's) if available to the original container and incubate with the other inoculated media. Alternatively, tuberculosis growth media from an automated liquid culture system may be used to flush the container.

Homogenise tissues and biopsies and decontaminate if required. Using aseptic procedures, inoculate tissue biopsies and bone that have been cut into small pieces directly to the surface of solid media and to enrichment media.

9.5.2 Automated monitoring systems

Automated culture systems are recommended for faster and easier detection of growth of mycobacteria². Automated culture systems indicate mycobacterial growth by detecting oxygen consumption or CO₂ production. These systems reduce the mean time for detection of growth of mycobacteria, notably in comparison with solid slopes¹⁴³⁻¹⁴⁵. Solid media should be used in addition^{2,12,50,146}. Rare isolates of *M. tuberculosis* are recovered only on egg-based media, such as a Löwenstein Jensen (LJ) slope¹². A single pyruvate incorporated LJ slope (or other suitable egg-based medium) is recommended to optimise growth of *M. bovis*¹². Some non-tuberculous mycobacteria may not signal in commercial liquid media, and work is needed to establish an adequate evidence base for when to use liquid culture alone. Solid culture is also needed for some specimen types when a range of incubation temperatures are indicated, for example if from superficial lesions.

Automated culture systems can also be used for sensitivity testing, reducing the time to availability of results to 4 to 12 days after inoculation.

Note: If there is insufficient volume of sample for all investigations, tests should be prioritised following medical microbiological advice (see [UK SMI B 27 - Investigation of cerebrospinal fluid](#)).

To reduce the risk of missed positive cultures, and following the manufacturer's instructions for use, liquid cultures that are ultimately negative on an automated system should be visually inspected for evidence of growth before being discarded.

9.5.3 Culture

All specimens are processed as follows:

1. Prepare bottles according to manufacturer's instructions
2. Inoculate the surface of a pH neutral pyruvate-based Löwenstein Jensen (LJ) slope (or other suitable egg-based medium) with 0.2mL of treated specimen and a liquid culture medium (with an appropriate volume as defined by the manufacturer)
3. Inoculate specimens taken from surface sites, for example skin, to two sets of media, one of which is incubated at 28 to 30°C. Two incubation temperatures should also be used for smear positive bronchial washings, as the mycobacteria which most commonly contaminate bronchoscopes and endoscopes prefer the lower temperature for growth. Bone and joint fluid samples may also need to be incubated at two temperatures to optimise recovery of all NTM species⁵⁰. Consider for example, if the sample is from a limb extremity and/or direct smear positive and tuberculosis is not expected
4. Briefly angle slopes to allow the specimen to inoculate the entire surface. Ensure that the caps are tightly fitted
5. Incubate slopes at 35 to 37°C for 8 weeks, extending to 12 weeks if necessary, reading every week to check for possible acid-fast growth⁵⁸
6. Log automated liquid culture bottles to the incubation system and incubate as instructed by the manufacturer. For small volumes, consider extending the incubation period. Isolation rates for CSF can also be increased by adding a liquid medium, such as Kirchner's or Middlebrook 7H9, to the original container and incubating at 35 to 37°C; as mycobacteria are known to adhere to the walls of plastic containers. Alternatively, one can flush the original container with the broth from an automated liquid culture tube
7. Store the unused treated deposit in case the samples need to be decontaminated. If not needed for other tests, the entire CSF specimen should be cultured to maximise the recovery rate
8. Confirm the presence of Acid Fast Bacilli (AFB) in positive cultures with the Ziehl-Neelsen or auramine-phenol stain. The former may be preferred due to the greater morphological detail obtained. When following up liquid culture positive bottles, it is recommended that a Gram stain is carried out to enable the detection of possible contamination and/or a blood agar plate set up for the same reason
9. Send aliquots of the confirmed positive cultures (in CE Marked leak proof containers in a sealed plastic bag) to the relevant Reference Laboratory in accordance with the postal and transport regulations¹¹⁰
10. Check the bacterial overgrowth at the post-decontamination stage by using purity plates

Note: Automated liquid culture plus conventional solid culture on at least one sample of each suitable specimen type should be set up within one working day of receipt. A

six day service should be provided by laboratories to meet the diagnostic standard recommended in the Department of Health Guidelines².

9.5.4 Nucleic acid amplification tests

Nucleic acid amplification tests (NAATs) are appropriate as a primary diagnostic method in certain circumstances¹. For a more detailed discussion, see introduction and the NICE recommendations.

1. Molecular detection for diagnosis of tuberculosis is increasingly becoming standard of care, whenever a clinical diagnosis is being seriously entertained
2. As a minimum, rapid diagnostic nucleic acid amplification tests for the *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. africanum*) should be requested on a suitable primary specimen if there is clinical suspicion of TB disease, and:
 - patient has HIV or
 - rapid information about mycobacterial species would alter the patient's care or
 - the need for a large contact-tracing initiative is being explored
3. In children and young people aged 15 years or younger with suspected pulmonary TB, offer rapid diagnostic nucleic acid amplification tests for the *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. africanum*). Usually only one nucleic acid amplification test will be necessary per specimen type (for example, spontaneous sputum, induced sputum or gastric lavage).
4. In young people aged 16–18 years use the same criteria as in adults to decide whether to request rapid diagnostic nucleic acid amplification tests
5. Clinicians should think about a diagnosis of extrapulmonary TB even if rapid diagnostic tests in, for example, cerebrospinal fluid, pleural fluid or ascitic fluid are negative
6. Offer treatment for TB meningitis if clinical signs and other laboratory findings are consistent with the diagnosis, even if a rapid diagnostic test is negative
7. Ideally, rapid diagnostic nucleic acid amplification tests for rifampicin (and possibly isoniazid resistance) should be undertaken routinely on all AAFB smear positive cases or if *M. tuberculosis* is detected on a NAAT test directly on sputum.
8. As a minimum, for people with clinically suspected TB, rapid diagnostic nucleic acid amplification tests for rifampicin resistance on primary specimens should be undertaken if a risk assessment for multidrug resistance identifies any of the following risk factors:
 - history of previous TB drug treatment, particularly if there was known to be poor adherence to
 - contact with a known case of multidrug-resistant TB
 - birth or residence in a country in which the World Health Organization reports that a high proportion (5% or more) of new TB cases are multidrug-resistant

All NAATs should be carried out in accordance with manufacturer's instructions.

Suitable clinical samples should ideally be sent for conventional AFB microscopy and culture investigations as outlined above.

Note: It should be noted that if there is not enough specimen volume for PCR and culture, then only culture should be done. All samples, even if PCR positive, should be submitted for culture.

9.5.5 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s) ‡	
			Temp °C	Atmos	Time			
All conditions	Sterile samples:	Automated liquid systems	If automated monitoring systems are used, after decontamination of clinical samples, refer to local protocols and manufacturer's recommendations. These should be checked continuously for bottles that flag positive. A blood agar plate should be set up for all bottles that are flagged positive to check for contamination.				<i>Mycobacterium</i> species	
	Blood							
	All bone marrow aspirates							
	Wound swab	LJ + pyruvate	35 – 37	Air	8 weeks extending to 12 weeks if required ⁵⁸	Weekly		
	Pus or Pus swabs*							
	CSF							
	Ascites							
	Joint fluid							
	Pleural fluid							
	Non-sterile samples:	Automated liquid systems	If automated monitoring systems are used, after decontamination of clinical samples, refer to local protocols and manufacturer's recommendations. These should be checked continuously for bottles that flag positive. A blood agar plate should be set up for all bottles that are flagged positive to check for contamination.				<i>Mycobacterium tuberculosis</i>	
	Sputum							
	Brochoalveolar lavage/bronchial washings							
	Gastric washings	LJ + pyruvate**	35 – 37	Air	8 weeks extending to 12 weeks if required ⁵⁸	Weekly		
Genitourinary tuberculosis	Urine	Automated liquid systems	If automated monitoring systems are used, after decontamination of clinical samples, refer to local protocols and manufacturer's recommendations. These should be checked continuously for bottles that flag positive. A blood agar plate should be set up for all bottles that are flagged positive to check for contamination.				<i>Mycobacterium</i> species	
		LJ + pyruvate	35 – 37	Air	8 weeks extending to 12 weeks if required ⁵⁸	Weekly		

Investigation of specimens for *Mycobacterium* species

Skin infections - ‘fish tank granuloma’ - cutaneous ulcer (or “Buruli” or “Bairnsdale ulcer”)	Skin lesions Tissue Biopsy	LJ + pyruvate	28 - 30	Air	8 weeks extending to 12 weeks if required ⁵⁸	Weekly	<i>M. marinum</i> <i>M. ulcerans</i> <i>M. chelonae</i>
Cardiac surgery	Post-surgery sites eg sternotomy	Automated liquid systems	If automated monitoring systems are used, after decontamination of clinical samples, refer to local protocols and manufacturer’s recommendations. These should be checked continuously for bottles that flag positive. A blood agar plate should be set up for all bottles that are flagged positive to check for contamination.				<i>M.chimaera</i> / <i>M. avium</i> – intracellular group
		LJ + pyruvate	35 - 37	Air	8 weeks extending to 12 weeks if required ⁵⁸	Weekly	
For these situations, add the following:							
Clinical details/ Conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Tuberculous meningitis	CSF and other non-sputum specimens	Kirchner’s liquid media OR Middlebrook 7H9	35 – 37	Air	8 weeks extending to 12 weeks if required ⁵⁸	Weekly	<i>Mycobacterium</i> species
Clinical details/ Conditions	Specimen	Optional media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Osteomyelitis	Consider for bone and joint fluids eg from extremities and/or direct smear positive	Automated liquid systems*	If automated monitoring systems are used, after decontamination of clinical samples, refer to local protocols and manufacturer’s recommendations. These should be checked continuously for bottles that flag positive. A blood agar plate should be set up for all bottles that are flagged positive to check for contamination.				Non-tuberculous mycobacteria
		LJ + pyruvate	28 - 30	Air	8 weeks extending to 12 weeks if required ⁵⁸	Weekly	
Pulmonary tuberculosis	Smear +ve BAL/washing	LJ + pyruvate	28 – 30	Air	4-6 weeks	Weekly	Rapidly growing mycobacteria

Investigation of specimens for *Mycobacterium* species

Pulmonary tuberculosis	Sputum Brochoalveolar lavage/bronchial washings Gastric washings	LJ + glycerol Note: <i>M.xenopi</i> do not grow well on medium with pyruvate preferring glycerol.	42	Air	8 weeks extending to 12 weeks if required ⁵⁸	Weekly	<i>M. xenopi</i>
* Pus swabs are not the specimen type of choice for mycobacteria. This is because if present, may adhere to the swab rather than be transferred successfully to the culture media¹³⁵. ** BCG and <i>M.xenopi</i> do not grow well on medium with pyruvate preferring glycerol. Additional supplements may be required for isolating conspicuously fastidious mycobacterial species. Note: In certain circumstances NAAT testing is appropriate see section 4.5.4.							

9.6 Identification

Most laboratories refer their isolates for identification and susceptibility testing to the reference laboratories.

Organisms may be further identified if this is clinically or epidemiologically indicated.

Note: For some NTM isolates, especially rapidly growing mycobacterial (RGM) isolates (*M. fortuitum*, *M. abscessus*, and *M. chelonae*), other identification techniques may be necessary including extended antibiotic in vitro susceptibility testing, DNA sequencing or polymerase chain reaction (PCR) restriction endonuclease assay (PRA). Due to differences in antimicrobial susceptibility that determine treatment options, species-level identification of the NTM is becoming increasingly clinically important. Several factors increase the likelihood of clinical significance of NTM isolates, including the recovery from multiple specimens or sites, recovery of the organism in large quantities (AFB smear–positive specimens), or recovery of an NTM isolate from a normally sterile site such as blood. For initial clinical mycobacterial isolates, however, it is sometimes difficult to determine the clinical significance of the isolate without species identification. Therefore, identification of most mycobacterial isolates to the species level and not merely as groups, such as *M.chelonae/abscessus* group is strongly recommended⁵⁰.

In the event that a specific laboratory does not have the necessary technology for species identification of an NTM isolate, the isolate could be sent to a Mycobacterium reference laboratory (see 4.9) for further analysis.

9.6.1 Minimum level of identification in the laboratory

Mycobacterium genus level (based on Ziehl-Neelsen or auramine-phenol stain from culture).

At least one AFB isolate from each new patient should be identified to complex/species level, and suitable susceptibility tests performed if identified as MTBC². Such tests are usually performed at a mycobacterial reference laboratory (listed below).

Repeat AFB isolates from the same patient should usually be identified again and susceptibility tests performed for MTBC, if cultured from a sample collected three months or more after a previously referred MTBC isolate².

9.7 Antimicrobial susceptibility testing

Refer to the National Mycobacterium Reference Service (NMRS) as required.

For other devolved national reference regions, refer to section 4.9 for more information.

At least one AFB isolate from each new patient should be identified to complex/species level and suitable susceptibility tests performed if identified as MTBC².

Repeat AFB isolates from the same patient should be identified again and susceptibility tests performed for MTBC if cultured from a sample collected three months or more after a previously referred MTBC isolate².

9.8 Referral for outbreak investigations

As tuberculosis is a notifiable disease in the UK, for public health management of cases, contacts and outbreaks, all suspected cases should be notified immediately to the local Public Health England Centres.

All clinically significant isolates should be notified by the diagnostic laboratories to ensure urgent initiation of proper procedures and all such isolates should be referred to the national reference laboratory for further testing.

9.9 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory [click here for user manuals and request forms](#).

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should, be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

[England](#)

[Wales](#)

[Scotland](#)

[Northern Ireland](#)

Note: Only send AFB positive isolates. If sending anything else, discuss with the reference laboratory first. Retain an aliquot or culture pending final report.

Isolates for identification and susceptibility testing should be sent to the appropriate RCM within one working day of the culture becoming positive². If the Mycobacteria Growth Indicator Tube (MGIT[®]) culture system is used, the culture should be incubated for a further 48 hours before despatch to achieve suitable biomass. Isolates should reach the RCM within one working day of despatch².

Isolates associated with outbreaks, where epidemiologically indicated, organisms with unusual or unexpected resistance and whenever there is a laboratory or clinical problem or anomaly that requires elucidation should be sent to the appropriate reference laboratory – if not been sent already.

Investigation of specimens for *Mycobacterium* species

Advice on rapid culture techniques or other diagnostic services may also be sought from the following:

National Mycobacterium Reference Service South

UK Health Security Agency (UKHSA),
61 Colindale Avenue,
London
NW9 5HT
Tel: 0208 3276957

National Mycobacterium Reference Service North and Central

UK Health Security Agency (UKHSA), West Midlands, Birmingham Laboratory
Birmingham Heartlands Hospital
Bordesley Green East
Birmingham
B9 5SS
Tel: 0121 424 3247

Wales Centre for Mycobacteria (WCM)

Public Health Wales, Microbiology Cardiff
Llandough Hospital
Penlan Road
Penarth
CF64 2XX
Tel: 029 2071 6408

<http://www.wales.nhs.uk/sites3/page.cfm?orgId=457&pid=25286>

Northern Ireland Mycobacterium Reference Laboratory

Department of Microbiology
Kelvin Building
Royal Victoria Hospital
Grosvenor Road
Belfast
BT12 6BA
Tel: 028 9063 4125

Scottish Mycobacteria Reference Laboratory

Clinical Microbiology
Royal Infirmary of Edinburgh
51 Little France
Old Dalkeith Road
Edinburgh
EH16 4SA
Tel: 0131 242 6016

Refer to local Regional Reference Laboratory procedures for strain typing.

10 Reporting procedure

10.1 Microscopy

Report the presence or absence of AFBs.

10.1.1 Microscopy reporting time

Microscopy result should be reported within one working day of receipt of the specimen^{2,11}.

New positive results should be transmitted to a member of the clinical team responsible for the patient's care².

10.2 Culture

Positives

Mycobacterium species isolated (together with comment on potential non-tuberculous mycobacterial identification, if appropriate, according to local protocols).

Negatives

Mycobacterium species not isolated.

10.2.1 Culture reporting time

New culture positives and clinically urgent results: communicate when available.

To meet internationally accepted criteria, mycobacterial samples should be cultured, and acid fast bacilli isolated and identified within 21 days of the source laboratory receiving a specimen for at least 90% of such samples².

Issue negative reports from automated liquid system according to manufacturer's instructions.

If solid culture media is used, issue negative report at 8 to 12 weeks.

10.3 Molecular

NAATs reporting

Positives

MTBC complex /non -tuberculous mycobacteria (NTM) /MDR-TB detected.

Negatives

MTBC complex // non -tuberculous mycobacteria (NTM) / MDR-TB not detected.

NAATs reporting time

PCR results are reported as they are done.

Strain typing reporting

The 24 MIRU-VNTR loci are reported by the Mycobacterial Reference Laboratories via Mycobnet (Mycobacterial Surveillance Network) as a 24-digit string in the same order, namely:

- 5 ETRs: A,B,C,D,E ; followed by ten MIRUs: 2,10,16,20,23,24,26,27,39,40; followed by
- 9 VNTRs: 424,1955,2163b,2347,2401,3171,3690,4052,4156

These helps minimise false clustering and enable international standardisation.

If a locus contains over 9 repeats, capital letters are used to indicate copy number (A for 10 and so on).

If a partial deletion of a repeat in ETR-D is present, then all the repeats are counted including the partial repeat, and presence of the partial repeat in the count is indicated by a´.

It has been recommended that an MIRU-VNTR genotype for each new MTBC isolate should be available, and entered on the national database, within 21 days of mycobacterial reference laboratory receipt for ≥95% isolates².

Note: UKHSA initially developed an on-line tool to provide a shorthand “cluster name” for identification of a molecular cluster rather than the full 24-digit code but it has been recommended that this is discontinued in order to reduce costs, improve efficiency and increase effectiveness. However, there are currently advancing technologies that will help inform the future of strain typing¹⁴⁷.

10.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated.

For MTBC, the results of susceptibility testing of primary therapeutic agents (that is, isoniazid, rifampicin, pyrazinamide, ethambutol) should be available within 30 days of receipt of the relevant sample in the source laboratory for ≥95% of such samples².

These results should be available within 14 days of MTBC isolate receipt by the relevant sensitivity testing laboratory².

11 Notification to UKHSA^{148,149}, or equivalent in the devolved administrations¹⁵⁰⁻¹⁵³

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify UK Health Security Agency (UKHSA) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local UKHSA Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to UKHSA. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to UKHSA

and many UKHSA Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

<https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010>

Other arrangements exist in [Scotland](#)^{150,151}, [Wales](#)¹⁵² and [Northern Ireland](#)¹⁵³.

12 Public health responsibilities of diagnostic laboratories

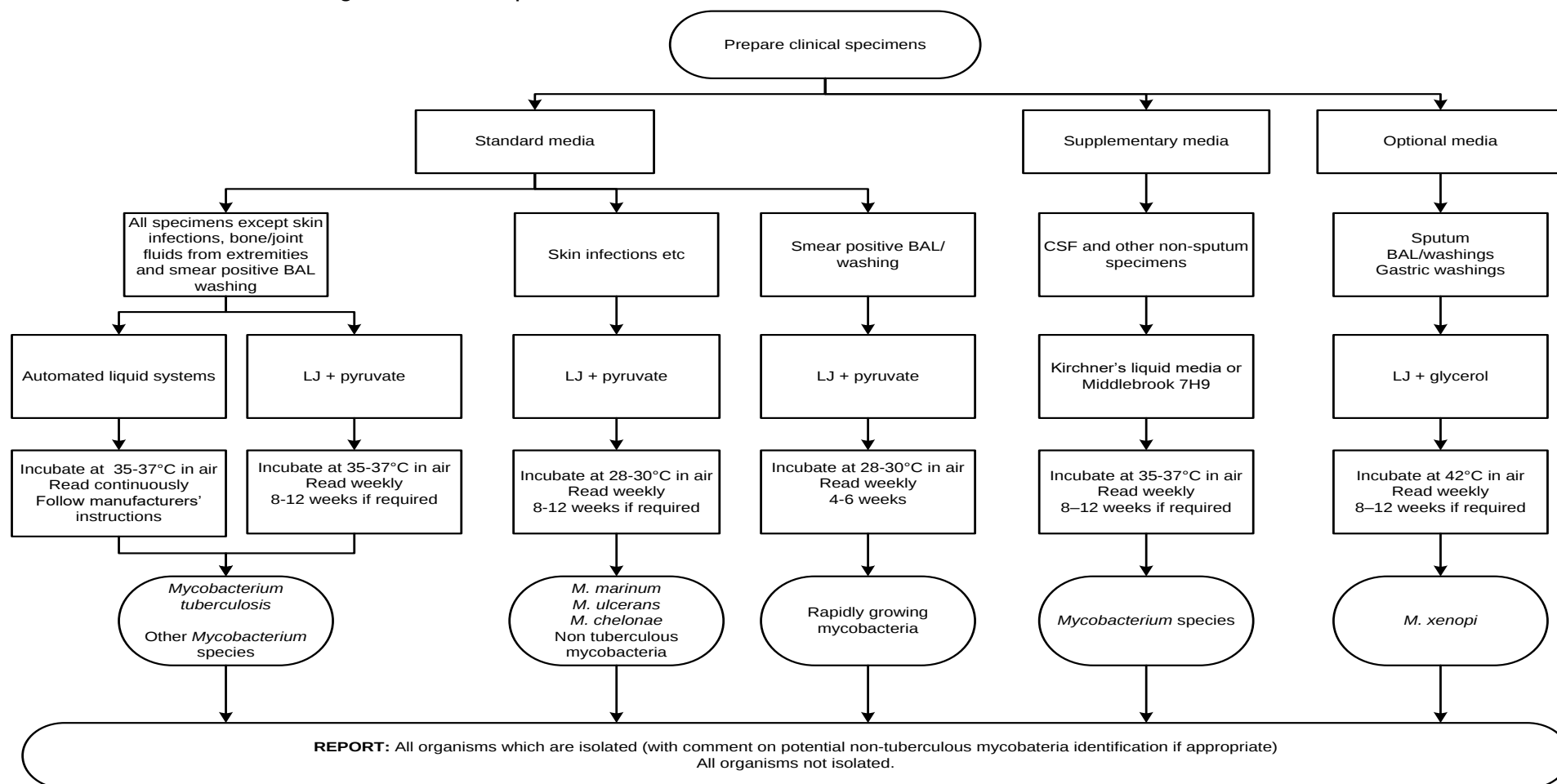
Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral to further characterise the organism as required, primarily for public health purposes e.g. routine cryptosporidium detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance forming an essential component of healthcare. It is recognised that additional testing and referral of samples may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

Algorithm: Investigation of specimens for *Mycobacterium* species

The information within this algorithm is also presented in table 9.5.5



In certain circumstances NAAT testing is appropriate see section 9.5.4.

References

An explanation of the reference assessment used is available in the [scientific information section on the UK SMI website](#).

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