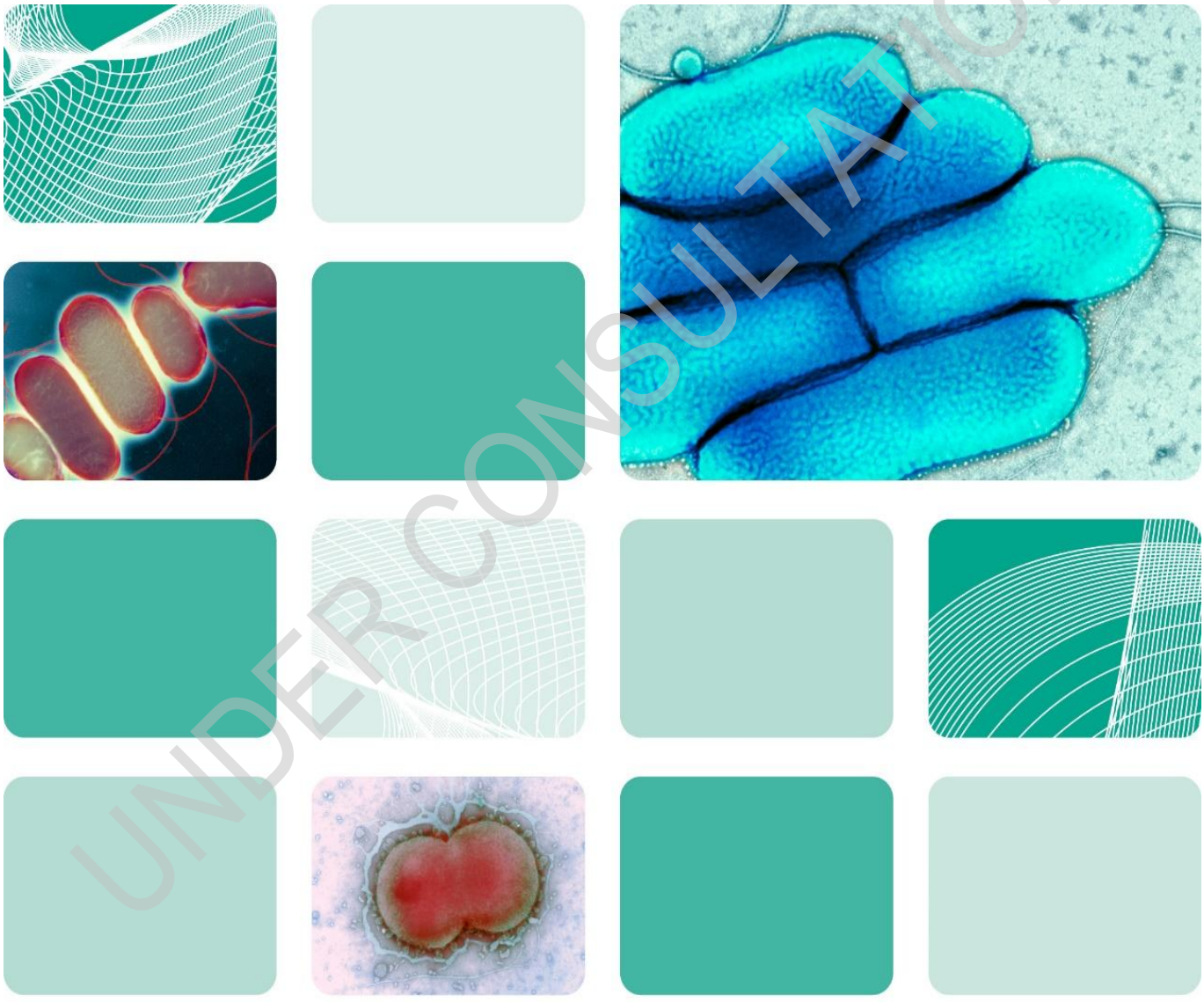
UK Health Security Agency

UK Standards for Microbiology Investigations

Investigation of samples for lower respiratory tract infections caused by bacterial pathogens



Acknowledgments

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Contents

Amendment table

The amendments since the previous version of this UK SMI document are listed in the amendments table below.

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

Amendment number/date	x/dd.mm.yy
Issue number discarded	
Insert issue number	
Anticipated next review date*	dd.mm.yy
Section(s) involved	**Amendment**
Title of the document	Title changed from “Investigation of bronchoalveolar lavage, sputum and associated specimens” to “Investigation of samples for lower respiratory tract infections caused by bacterial pathogens”
All document	All fungal-related information previously included in this document has been removed and consolidated into a new, dedicated mycology UK SMI document “B63: Investigation of samples for lower respiratory tract infections caused by fungal pathogens”.
Specimens type	Additional specimens have been included in the document

*Reviews can be extended up to 5 years where appropriate

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

This UK Standards for Microbiology Investigation (UK SMI) document outlines the microbiological methods for the diagnosis of bacterial causes of infection of the lower respiratory tract including culture, molecular tests and immunoassays.

The specific tests performed vary based on the patient group and sample type. While lower respiratory tract samples such as bronchoalveolar lavage fluid (BALF) are more sensitive and specific when testing for lower respiratory tract infections (LRTIs), upper respiratory tract samples, such as sputum and cough swabs are often taken to investigate LRTIs, particularly in patients where lower respiratory tract sample collection is not feasible. This is commonly the case for patients in the community or other clinic settings. By detailing the importance of specimen quality and collection methodology, this document aims to enhance diagnostic accuracy and improve the management of respiratory infections across diverse patient populations (e.g. paediatric patients, patients with cystic fibrosis, or immunocompromised patients).

Note: All fungal-related information previously included in this document has been removed and consolidated into a new mycology specific UK SMI document “B 63: Investigation of samples for lower respiratory tract infections caused by fungal pathogens”, which is currently in development.

UK SMIs should be used in conjunction with other relevant UK SMIs:

- for details on the investigation of *Bordetella pertussis* and *Bordetella parapertussis* refer to UK SMI B 6.
- for Investigation of specimens for *Mycobacterium* species refer to UK SMI B 40.
- for investigation of pus and exudates refer to UK SMI B 14.
- for information on tissues and biopsies from deep-seated sites and organs refer to UK SMI B 17.
- for the investigation of fluids from normally sterile sites refer to UK SMI B 26.
- for investigation of specimens other than blood for parasites refer to UK SMI B 31.

4 Introduction

Lower respiratory tract infection (LRTI) refers to infections affecting the lungs and airways, including the trachea, bronchi, and bronchioles.

LRTI includes conditions like bacterial pneumonia, characterised by inflammation of the lung parenchyma, and bronchiolitis, which affects the small airways. Lung abscess, where the lung parenchyma is replaced by pus filled cavities, and empyema, where pus occupies the pleural space, are less common manifestations of LRTI. Typically, LRTIs manifest as acute conditions, with symptoms emerging rapidly, within hours to days, following infection. Common respiratory symptoms include fever, cough, sore throat and difficulty breathing, with shortness of breath and wheezing.

Bacterial pneumonia is one of the most common examples of LRTIs. It is an infection of the lungs that causes air sacs to fill with microorganisms, fluid, and inflammatory cells. Symptoms include fever, cough, dyspnoea, and chest pain. Bacterial pneumonia can be primary, with no known risk factors, or secondary, associated with conditions like chronic lung disease, diabetes, and immunosuppression. It can be classified as community-acquired pneumonia (CAP), which occurs outside healthcare settings, or hospital-acquired pneumonia (HAP), which develops after 48 hours or more of hospitalisation or ventilator-associated pneumonia (VAP) that develops in a patient after 48 hours of receiving invasive mechanical ventilation. *Streptococcus pneumoniae* is common in CAP, while HAP often involves gram-negative organisms including *Pseudomonas aeruginosa*, *Acinetobacter species*, *Klebsiella pneumoniae* and the Gram-positive *Staphylococcus aureus*. For moderate to severe CAP, diagnosis can be conducted on sputum and blood/urine samples by molecular methods and immunoassays. In HAP, deeper respiratory samples such as endotracheal aspirates as well as blood cultures are used for microbiological testing (1,2).

Chronic bacterial respiratory conditions include cystic fibrosis (CF) and bronchiectasis. Cystic fibrosis is caused by a defect in the CF transmembrane conductance regulator gene that affects the transport of ions and water across the epithelium. This leads to progressive pulmonary disease associated with pulmonary infections, which are the major cause of morbidity and mortality in CF patients. The common bacterial pathogens are *S. aureus*, *H. influenzae* (usually non-encapsulated in CF patients), *S. pneumoniae* and pseudomonads, particularly mucoid *P. aeruginosa* strains. Bronchiectasis is a long-term, progressive lung condition caused by inflammatory damage to the airways due to recurrent and/or severe lower respiratory tract infection. It can occur at any age, affecting both children and adults. The most commonly isolated pathogens are *H. influenzae*, followed by *P. aeruginosa*, *Moraxella catarrhalis*, *S. pneumoniae*, *S. aureus*, and members of the order Enterobacterales (3).

Although LRTIs caused by anaerobic bacteria are much less common than those caused by aerobic bacteria, anaerobes such as *Actinomyces* species, *Fusobacterium* species, *Prevotella* species and Gram-positive anaerobic cocci can also cause lower

respiratory tract infections. These usually follow aspiration of oral bacteria or contamination of the lung from trauma/surgery/abdominal infection where lung abscesses, necrotising pneumonia and empyema may ensue (4).

Rapid and accurate laboratory diagnosis of LRTIs is essential to inform clinical decision-making and ensure appropriate patient management while preventing the misuse of antimicrobials. The subsequent sections will explore the types of samples collected and the laboratory investigations conducted for LRTIs.

4.1 Types of specimens

For the most accurate diagnosis, samples should be collected directly from the site of infection in the lower respiratory tract. The procedures required to collect such samples including bronchoalveolar lavage and protected specimen brush, help minimise contamination with oropharyngeal contents, thereby enhancing test specificity. Where possible, if the disease presents with a specific focus (e.g. nodule or cavity) it is critical to obtain the sample from the respective lobe. However, in community settings, and in sampling from neonates, these procedures are often challenging or not feasible. As a result, less sensitive and specific sample types, such as sputum are collected, with results interpreted while considering the limitations of these samples. To improve sample quality, it is essential that the specimen contains freshly expectorated material with minimal contamination from saliva or other oropharyngeal contents.

4.1.1 Lower respiratory tract

- Bronchoalveolar lavage (BAL): Bronchoalveolar lavage is a widely used, and generally safe, diagnostic technique for diagnosing lung diseases. Unlike sputum analysis, a BAL allows precise sampling from the lower respiratory tract, minimising contamination from upper respiratory tract bacteria. The procedure involves insertion of a flexible bronchoscope, and a segment of lung is ‘washed’ with sterile saline which is then aspirated to obtain cellular and non-cellular components of the epithelial surface of the lower respiratory tract (5).

  BAL is often combined with other bronchoscopic techniques such as endobronchial or transbronchial biopsies, transbronchial needle aspiration, protected specimen brushings, and endobronchial ultrasound-guided needle aspiration to improve diagnostic accuracy (3). This method and the bronchial lavage fluid (BALF) is considered reliable for definitive diagnosis of pneumonia and other pulmonary infections (7,8).
- Non-directed bronchoalveolar lavage (NBL): Non-directed techniques have been found to give results comparable to bronchoscopic methods (6-8). A suction catheter, preferably a protected BAL catheter to minimise contamination, is passed down the endotracheal tube until resistance is met. An aliquot of sterile saline is injected and then aspirated. This method provides a lower respiratory tract sample

without the need for bronchoscopy and without the attendant risks of transtracheal aspiration. This technique is used mostly in intensive care settings, particularly for ventilated patients. Unlike BAL, it is not specifically directed to a particular lobe.

- Bronchial washings: Bronchial washings are collected in a similar fashion to BALFs, but the procedure involves the aspiration of small amounts of instilled saline, typically <20mL, from the large airways of the respiratory tract without wedging of the bronchoscope. These will sample the more proximal airways, with greater risk of contamination from colonising organisms and should be reported in a similar manner to endotracheal aspirates or sputum samples.
- Bronchial aspirate: Bronchial aspirates are collected by direct aspiration of material from the large airways of the respiratory tract by means of a flexible bronchoscope.
- Bronchial brushing: The technique of bronchial brushing uses a protected brush catheter in the bronchoscope (a brush within two catheters sealed at the end with a polyethylene glycol plug) to tease material from the airways (9).
- Protected catheter specimens: Material is collected from the lung via a bronchoscope in a similar way to bronchial brushing. An inner and outer catheter is used with a polyethylene glycol plug at the end to prevent contamination from the nasopharynx. When resistance is met the plug is expelled and the sample taken via the inner catheter.

4.1.2 Upper respiratory tract

- Expectorated sputum samples: Sputum samples are commonly used to identify the pathogens responsible for pneumonia, including community-acquired pneumonia (CAP). Several factors complicate their effectiveness, such as difficulties in obtaining quality samples from patients, prior antibiotic use, delays in transporting and processing samples, and contamination from upper respiratory tract bacteria (10). If tuberculosis (TB) is suspected, at least one early-morning sputum specimen is preferred, as these samples provide higher diagnostic value due to the overnight pooling of pathogenic bacteria, making them particularly useful for detecting *Mycobacterium* spp. in TB patients (11).
- Induced sputum: Induced sputum collection is a procedure typically performed to collect respiratory secretions for diagnostic testing. It involves the inhalation of a saline nebuliser, which induces coughing and helps to bring up secretions from the lungs. The process typically lasts for around 15 minutes, with assessments made at intervals by a physiotherapist to evaluate the patient's progress. During these assessments, the physiotherapist will guide the patient through various breathing exercises and provide appropriate physiotherapy to facilitate the expectoration of sputum. This technique is commonly used to diagnose respiratory infections and conditions such as CF and community-acquired pneumonia (12).

- Sputum trap: If a patient is unable to expectorate an adequate sputum sample, especially those who are intubated in intensive care units, oral suction can be performed using a catheter and sputum trap. The sputum trap is connected between the suction catheter and the suction tubing. The aspirated sputum sample can then be collected in the trap, and saline may be used to flush the sample into the trap for better collection.
- Tracheal aspirates: Tracheal aspirates are collected from the trachea, most commonly via endotracheal or tracheostomy tubes in patients with artificial airways. Such specimens including endotracheal aspirates (ETAs) are subject to the same limitations as sputum specimens, with the additional caveats regarding frequent colonisation of the trachea with Gram-negative bacteria in mechanically ventilated patients.
- Nasopharyngeal aspirates/swabs and oropharyngeal swabs: These are the optimal upper respiratory tract infection specimen collection methods. However, such specimens cannot be collected from infants, and many older patients may not allow a nasopharyngeal specimen to be collected.

4.1.3 Additional specimen types

- Urine: Detection of *Streptococcus pneumoniae* and *Legionella pneumophila* serogroup 1 antigens
- Blood:
    - Blood culture (see UK SMI S 12 - Sepsis, and systemic or disseminated infections).
    - Serum or plasma: Immunological testing
- Tissue: See UK SMI B 17 - Tissues and biopsies from deep-seated sites and organs
- Cough Swab: Cough swabs should only be used if a patient is unable to expectorate sputum or sputum induction is not feasible at that time of sampling. This may occur in young children or patients with CF who may have difficulty producing sputum. However, sputum induction is generally well tolerated by children with CF. As sputum has a higher sensitivity in detecting bacterial pathogens, cough swabs are not a recommended sample. In children who cannot cough on request, the swab may be gently rubbed at the back of the throat immediately after a spontaneous cough.
- Transthoracic aspirate: Samples of transthoracic aspirates are obtained through the chest wall via a needle passed between the ribs. This procedure may be undertaken to sample, for instance, an aspergilloma, abscess or any focal lung lesion that is accessible. For guidance on handling tissues and biopsies from deep-seated sites, refer to UK SMI B 17- Tissues and biopsies from deep-seated sites and organs. If the sample is collected as fluid then, it should be processed as a BALF, as mentioned above.

- Transtracheal aspiration: Transtracheal aspiration is a procedure that carries clinical risks and is therefore rarely performed in the UK.
- Pleural fluids: refer to UK SMI B 26 - Investigation of fluids from normally sterile sites.
- Lymph node: refer to UK SMI B 17 - Tissues and biopsies from deep-seated sites and organs.

5 Safety considerations

The section covers specific safety considerations related to this UK SMI, and should be read in conjunction with the general safety considerations (13-34).

- it is recommended that all respiratory samples with the potential to generate aerosols during processing be handled within a class I microbiological safety cabinet under Containment Level 3 (CL3) conditions. This should be done in accordance with a local risk assessment and the relevant guidance from the Advisory Committee on Dangerous Pathogens (ACDP) and the Health and Safety Executive (HSE), with additional precautions to minimise aerosol production.
- given the severity of the disease and the risks associated with generating aerosols of the organism, any manipulation of suspected isolates of *Neisseria meningitidis* should always be undertaken in a microbiological safety cabinet until *N. meningitidis* has been ruled out (as must any laboratory procedure giving rise to infectious aerosols), for more information refer to UK SMI B 51 - Screening for Neisseria meningitidis.
- CL3 precautions are particularly important unless it can be definitively ruled out that the specimen contains a Hazard Group 3 pathogen such as *Mycobacterium tuberculosis*, *Brucella* species, *Francisella* species, *Yersinia pestis*, *Burkholderia mallei*, *Burkholderia pseudomallei*, or certain endemic fungi (e.g. *Histoplasma* and *Blastomyces* species).
- however, if the processing step involves pathogen inactivation, such as heat-fixing, the specimen may be subsequently handled under Containment Level 2 conditions.

  Note: Heat-fixing may not kill all *Mycobacterium* species (35). Slides should be handled as infectious up to the point of staining which will kill any bacteria present.
- additionally, the type, selection and use of a microbiological safety cabinet (MSC), as well as transport of biological agents, should also be considered to further minimise the risk of transmission during handling of the specimen/organism.
- prior to staining for Mycobacteria, the smeared material should be fixed by placing the slide on an electric hotplate (65 to 75°C), inside the safety cabinet, until dry, and then placed in a rack or other suitable holder.

- the list of agents is regularly reviewed and updated by the Advisory Committee on Dangerous Pathogens (ACDP) and the resulting publication The Approved List of biological agents is issued by the Health and Safety Executive (HSE)
- centrifugation must be carried out in sealed buckets which are subsequently opened in a class I MSC.
- specimen containers must be placed in a suitable holder.
- refer to current guidance on the safe handling of all organisms documented in this UK SMI.
- the above guidance should be supplemented with local COSHH and risk assessments and waste procedure.
- following vortexing or any similar mixing process, containers should be left to stand for an appropriate period before opening to allow any aerosols generated to settle. Opening containers prematurely may increase the risk of operator exposure to aerosolised biological or chemical agents and could lead to environmental contamination.
- use plastic consumables in preference to glass wherever possible.

6 Pre-laboratory processes

6.1 Specimen type

- Bronchoalveolar lavage fluid
- Non-directed bronchoalveolar lavage fluid
- Bronchial washing
- Brushings aspirate
- Bronchial brushing
- Protected catheter specimens
- Endotracheal tube specimens
- Tracheal aspirate
- Sputum: expectorated, induced and endotracheal (trap)
- Nasopharyngeal aspirates/swabs and oropharyngeal swabs

6.2 Specimen collection and handling

For safety considerations refer to Section 5.

- use aseptic technique.
- collect specimens before antimicrobial or antifungal therapy where possible. If this is not possible, this must be stated on the request form.

- culture for *Legionella* species may still be successful after antimicrobial therapy has been started (UK SMI ID 18 - Identification of Legionella species).
- the best specimens are those produced early in the morning from a deep cough, before the patient eats, drinks or cleans their teeth.
- to collect sputum, use appropriate cleansing procedures to reduce oral contamination.
- for sputum specimens the material required is from the lower respiratory tract, expectorated by deep coughing. When the cough is dry, physiotherapy, postural drainage, or inhalation of an aerosol before expectoration may aid in inducing sputum. Saliva from the mouth, without deep coughing, and pernasal secretions are not suitable.
- early morning freshly expectorated sputum is recommended for *Mycobacterium* species. Ideally, specimens should be collected on at least 3 consecutive days (see UK SMI B 40 - Investigation of specimens for Mycobacterium species). BALF and associated specimens need specialist collection according to local protocols.
- unless otherwise stated, swabs for bacterial and fungal culture should be placed in appropriate transport medium.
  - collect specimens, other than swabs, into appropriate UKCA/CE marked leak proof containers and transport in sealed plastic bags.
  - some respiratory pathogens (e.g. *Streptococcus pneumoniae*) easily become non-viable if exposed to low temperatures or other adverse environmental conditions, and so recovery of these agents will be less likely if samples are stored for prolonged periods before processing.

6.2.1 Adequate quantity and appropriate number of specimens

- Sputum**:** Ideally, a minimum volume of 2 mL should be collected for bacterial investigation and 2 mL for fungal investigation. The need for higher volumes or repeat testing depends on tests requested and clinical progression (36).
- BALF, NBLF and bronchial wash: It is difficult to be specific on the volume required. In principle, as large a volume as possible should be provided. For fungal pathogens, a minimum of 5 to 10 mL of sample should be collected to allow a range of investigations. For more information, refer to ‘UK SMI B 63: Investigation of samples for lower respiratory tract infections caused by fungal pathogens’. The need for higher volumes or repeat testing depends on tests requested and clinical progression (36).
- Urine: concentrated urine has better yield, collect at least 1 mL of specimen.

6.3 Specimen transport and storage

This section covers specimen transport and storage consideration related to this UK SMI, and should be read in conjunction with the scientific information on our webpages.

For safety considerations refer to Section 5.

- specimens should be transported and processed as soon as possible.
- compliance with postal, transport and storage regulations is essential.
- BALF and sputum should be processed promptly to give the best opportunity to culture pathogenic organisms and reduce the risk of overgrowth with contaminants. If processing is delayed up to 24 hours, refrigeration is preferable to storage at ambient temperature. If specimens are not processed on the same day that they are collected, this should be noted on the report and interpretation of results should be made with care.

7 Laboratory processes

7.1 Sample preparation

Note: If a single specimen is received with multiple tests requested, ensure that the sample is appropriately separated before centrifugation for parallel fungal testing. A representative portion such as purulent material should be selected for each test to optimise culture sensitivity and avoid cross-contamination between procedures. If a small volume is received with multiple tests requested, please discuss with the requestor/managing clinical team to allow prompt prioritisation of testing.

It is generally advised not to reject bronchial wash specimens, BALF specimens, or lung aspirates, as these samples are collected following an invasive procedure and are not easily repeatable.

7.1.1 Assessing acceptability of sputum samples for culture

- specimens should not be rejected solely on macroscopic appearance. They may be described using the following terms: salivary, mucosalivary, mucoid, mucopurulent, purulent and/or bloodstained.
- salivary sputum specimens without mucous plugs from immunocompetent patients may be rejected with a comment stating:

  ‘Salivary specimen received unlikely to represent the site of infection: sample not processed. If symptoms persist, please submit an expectorated sputum specimen’.
- the performance of sputum as a diagnostic sample type depends on both macroscopic and microscopic examination.

- sputum Gram staining may be useful as a preliminary screening method to assess the quality of the sample to determine if it is acceptable for culture. See section 7.3.2.
- as above, specimens from the following patients should not be rejected on the basis of the specimen quality:
    - immunocompromised
    - neutropenic
    - intubated
    - cystic fibrosis
    - *Legionella* culture requested
    - *Mycobacterium* species investigation requested
- specimens should be processed with a comment indicating that: 'Salivary specimen received. This is unlikely to represent the site of infection. If symptoms persist, please submit an expectorated sputum specimen'.

7.1.2 Processing BALF for culture

- a minimum total volume of 4mL is required: 2mL bacterial and 2mL fungal culture and molecular testing.
- if additional tests on uncentrifuged BALF (e.g. fungal culture) are required, take a sufficient aliquot prior to centrifugation.

  Note: This is essential in cases with suspected mucoraceous mould infection.
- BALF specimens generally do not require pre-treatment with a mucolytic agent.
- for BALF/NBLF, if the sample is thick or mucopurulent do not centrifuge and select the most purulent part of the sample for testing.
- centrifuge all suitable samples at 1200 ×g for 10 min. Where investigation for *Mycobacterium* species is also requested, the centrifugation speed and time are typically increased, refer to UK SMI B 40 - Investigation of specimens for Mycobacterium species.
- tip off all but 1-2mL of supernatant and re-suspend centrifuged deposit in remaining fluid.

7.1.3 Processing sputum/ endotracheal aspirates for culture

- if sputum sample is too thick, 0.1% solution of dithiothreitol or N-acetyl L-cysteine (NALC) also known as sputasol or mucolyse should be added to homogenise the sample.

Laboratories may culture sputum using diluted samples, which suppress normal flora and highlight organisms present at high levels (e.g. ≥10^6 CFU/mL), homogenised 'neat' sputum samples, which preserve mixed flora for contextual interpretation, or a combination of both.

The approach used is defined by local laboratory policy and may vary depending on the culture media in use and the patient group (e.g. routine vs specialist populations).

- diluted samples: transfer 10µL of homogenised sputum in 5mL of sterile distilled water and mix gently before inoculating onto routine media.

7.2 Molecular and immunological tests

When performing molecular testing, knowledge of the detection range, sensitivity and specificity of the assay is required, along with an understanding of the limitations and risks of genomic amplification. Molecular assays for the detection of pathogens are widely available. Some multiplex molecular testing may give results for organisms not requested. Under these circumstances laboratories should follow local procedures. Please refer to UK SMI Q 4 - Good practice when performing molecular amplification assays.

Molecular assays, including nucleic acid amplification tests (NAAT), i.e. Polymerase Chain Reaction (PCR), enable the rapid detection of pathogens in respiratory samples (37). Real-time PCR, which allows for diagnoses in just a few hours, or less, is more sensitive and faster than traditional methods, offering a significant impact on treatment decisions. Molecular techniques address challenges of culture-based methods, such as false negatives and difficulties in identifying rare bacterial and fungal species. Please see section 8.1 for guidance on NAAT result interpretation in this setting.

Rapid multiplex NAAT tests (also called syndromic panels) are designed to detect specific pathogens associated with a particular clinical syndrome, with respiratory infections being one of the most common applications. As an example, multiplex molecular diagnostic platforms for rapid detection of atypical respiratory pathogens, including *Mycoplasma pneumoniae, Chlamydophila pneumoniae* and *Legionella pneumophila* are used in many clinical microbiology laboratories.

Respiratory syndromic panels are rapid, accurate methods for detecting multiple bacterial and viral respiratory pathogens simultaneously with a single sample. This is particularly beneficial when collecting difficult samples, such as nasopharyngeal aspirates. These rapid multiplex panels are useful in the timely and accurate diagnosis of respiratory infections, enhancing patient management and outcomes as well as potentially reducing unnecessary antibiotic consumption by guiding targeted therapy. Although molecular methods are generally fast, they can have lower throughput, may require batch testing and are more expensive per sample (for consumables) than conventional tests (38,39).

- **Urinary antigen test:**
    - *Legionella pneumophila* serogroup 1: Follow manufacturer's instructions. Positive specimens should be sent to the reference laboratory for confirmation. For legionella urine testing please refer to UK SMI B 41 - Investigation of urine. This assay should not be used in isolation as infections can be caused by other serogroups of *L. pneumophila* and other species of *Legionella*.

- *Streptococcus pneumoniae*: Follow manufacturer's instructions.

- **Serum serology test:**

Serological testing can be performed to detect antibody, but it is not recommended due to poor performance. Molecular testing is preferred for the diagnosis of acute infection.

- **Molecular typing:**

Typing is essential to identify transmission events, detect outbreaks, and monitor the spread of high-risk or antimicrobial-resistant *Pseudomonas aeruginosa* clones, particularly in vulnerable patient groups such as those with cystic fibrosis and in intensive care settings. Historically, diagnostic laboratories have used variable number tandem repeat (VNTR) analysis to generate comparable strain profiles. More recently, whole genome sequencing (WGS) has been increasingly adopted, providing higher-resolution analysis at the single nucleotide polymorphism (SNP) level. While WGS is becoming the preferred approach in specialist centres, many laboratories continue to use VNTR, either alone or alongside WGS, depending on local resources and workflows (40).

7.3 Microscopy

7.3.1 BALF

Refer to UK SMI TP 39 – Staining procedures.

It is important that microscopical examination is performed on all BALF samples as Gram staining can be useful to predict results of quantitative culture (41).

- mucoid specimens: Using a sterile loop select the most purulent or blood-stained portion of specimen and make a thin smear on a clean microscope slide for Gram staining and fungal microscopy (if required).
- non-mucoid specimens: Using a sterile pipette place one drop of centrifuged deposit or neat specimen on to a clean microscope slide. Spread this with a sterile loop to make a thin smear for Gram staining and fungal microscopy (if required).

7.3.2 Sputum/ETA

Although Gram stain on sputum/ETA specimens is not routinely performed in many laboratories as the sensitivity can vary, it may still be useful in some patient groups (see section 7.1.1) and to determine the quality of the specimen. Specimen quality is determined by the relative numbers of polymorphonuclear leucocytes and squamous epithelial cells (SECs) present: purulent specimens may be selected for culture whereas non-purulent specimens or those heavily contaminated with SECs unlikely represent the site of infection and may be rejected.

In addition, Gram staining of good-quality sputum samples may help predict likely pathogens based on their characteristic morphology (42-44). Studies have shown that,

in good-quality specimens, Gram stain findings can predict the detection of Streptococcus *pneumoniae* and *Haemophilus influenzae* in community acquired pneumonia (CAP). However, evidence supporting its diagnostic accuracy for other organisms and other clinical scenarios remains limited.

In some cases, antimicrobial therapy may be initiated based on Gram stain findings before culture results become available. However, in many laboratory workflows, sputum specimens are processed without prior evaluation, with Gram stain preparation occurring in parallel with culture. As a result, the opportunity to use Gram stain findings to guide specimen rejection or early clinical decision-making may be limited.

Refer to UK SMI TP 39 - Staining procedures.

- using a sterile loop take a loopful of homogenised sputum (see section 7.1) and make a thin smear on a clean microscope slide for Gram staining. Care must be taken in interpreting a Gram stained sputum smear as the use of antimicrobials may render organisms, which are visible in the smear, non-viable (45).
- record the number of polymorphonuclear leucocytes and squamous epithelial cells (SECs) present (46).
- retain specimens at 4°C for at least 48hr.

Refer to UK SMI B 40 - Investigation of specimens for Mycobacterium species for microscopy of *Mycobacterium* species, and refer to UK SMI B 31 - Investigation of specimens other than blood for parasites for parasitic investigations.

7.4 Culture

The choice and combination of culture media used for investigating respiratory specimens should support the recovery of the main bacterial pathogens associated with lower respiratory tract infection (see table 1) and should be based on local validation, patient population, and laboratory requirements.

Selective and differential media may be used to aid the differentiation and isolation of specific organisms and to reduce interference from competing flora. This includes the following (46,47):

- Chocolate agar with bacitracin or chocolate agar supplemented with a bacitracin disc may be used to enhance the recovery of *H. influenzae*. Bacitracin inhibits many competing organisms commonly found in respiratory flora, including streptococci, staphylococci, *Neisseria* species, and *Micrococcus* species, thereby reducing overgrowth by commensal flora and facilitating the detection and isolation of clinically significant *Haemophilus* species (48).
- MacConkey agar and CLED agar may be used to aid the isolation and differentiation of Enterobacterales and non-fermenting Gram-negative bacilli, particularly in mixed cultures.

- Staphylococcal differential media, such as mannitol salt agar, may be used to aid the detection and presumptive identification of *Staphylococcus aureus* in mixed cultures.
- *Burkholderia cepacia* selective agar is recommended for the culture of specimens from patients with cystic fibrosis. It selectively supports the growth of *Burkholderia cepacia* and is superior to CLED agar. *B. cepacia* selective agar may also grow *Burkholderia gladioli* and other pseudomonads.
- BMPAα is recommended for clinical specimens when testing for *Legionella*, although there have been reports of cefamandole being inhibitory to some *Legionella* species (49-51). Vancomycin sensitive strains have also been detected.
- Incubation in 2-5% CO_2 can enhance the growth of some *Legionella* species such as *L. sainthelensi* and *L. oakridgensis*. This low level of CO_2 will not affect the growth of *L. pneumophila*, but CO_2 levels higher than 5% may inhibit growth (49). The incubation of plates in 2-5% CO_2 is not compulsory; it is described for laboratories that choose to use it to enhance the growth of *Legionella* species. If laboratories choose to use *Legionella* selective agar plates as supplementary media, its inclusion should be subject to the results of local validation.

Chromogenic media may be used as supplementary culture media, particularly for specimens from patients with cystic fibrosis, to aid the detection and differentiation of pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and selected Gram-negative organisms. Their use should be subject to local validation and interpretation within the clinical context.

For *Mycobacterium* species, culture should be performed as outlined in UK SMI B 40 - Investigation of specimens for Mycobacterium species, and should be performed on all BAL specimens unless alternative local arrangements are in place.

Semi-quantification using quadrant streaking is used to estimate bacterial load based on the extent of growth across sequential dilution streaks on an agar plate. Growth extending into later quadrants indicates a higher organism burden and supports the assessment of clinical significance, particularly in mixed cultures. See section 7. Calibrated loops or dilution methods are used in quantitative cultures to provide precise colony-forming unit (CFU) counts that support diagnostic thresholds for infection.

Standard:

7.4.1 BALF

Semi-quantitative approach to BALF culture

In most cases, semi-quantitative culture is performed for BALF specimens.

If centrifugation is performed the deposit should be re-suspended in 1-2 mL of supernatant (this is dependent on how many aliquots are required for testing).

A known volume of sample, usually 10µL of deposit, is inoculated directly onto culture media (see UK SMI Q 5 – Inoculation of culture media for bacteriology).

Quantitative approach to BALF culture

This method is rarely utilised in routine diagnostic laboratories but may be useful when there are validated clinical thresholds for interpretation of culture in CFU/mL concentrations to either differentiation between infection, contamination or colonisation or used to monitor treatment efficacy.

a) A known volume of BALF i.e. 10mL should be centrifuged and the deposit resuspended in a known volume of supernatant i.e. 1mL giving a calculated (10-fold) concentration.
b) A known volume is plated out onto agar i.e. 1uL (1000th of the 1mL concentrate)
c) The colonies on culture are counted and used to calculate the initial CFU/mL in the sample, working example below.

$$\text{CFU/mL} = \frac{\text{Number of colonies counted on plate}}{\text{Volume plated (µL)}} \times \text{(volume of deposit / neat volume)}$$

$$\text{CFU/mL} = \frac{10}{1} \times (1000 / 10) = 1000$$

Serial dilutions can also be prepared in accordance with local policy, for specimens with high concentrations of microorganisms by preparing serial dilutions of the 'neat' specimen in sterile saline and culturing (52).

Notes:

- do not delay between diluting the specimen and inoculating agar plates.
- the measurement uncertainty of the quantitative method should be applied in reporting when applying thresholds for interpretation.

The method of routine culture of BALFs is defined by local policy and reporting requirements and should be subject to robust validation and risk assessment.

7.4.2 Sputum/ETA

- inoculate 1µL loopful of the diluted sample prepared in section 7.1 or neat (homogenised) sample to each type of media. Laboratories may choose to use larger aliquot such as 10 µL, however, dilution calculations and interpretation of results must be adjusted accordingly.
- for certain patient populations such as CF and immunocompromised patients, it is recommended that a 1 µL aliquot of the more concentrated neat (homogenised) sputum is also cultured. Both specimens' preparations may be plated onto split (half) plates to facilitate direct comparison of growth.

Using both methods provides the best balance, allowing detection of significant pathogens while retaining insight into the original microbial context and reducing the risk of misinterpreting apparently pure growth.

- for patients with cystic fibrosis who have no previous *B. cepacia* colonisation, inoculate 100µL of the liquefied sputum onto a *B. cepacia* plate and spread inoculum over the entire surface of the agar plate (53).
- if cough swabs are used, they are only suitable for direct culture on solid media. For processing of swabs, refer to UK SMI Q 5 - Inoculation of culture media for bacteriology.

Supplementary:

7.4.3 *Legionella* culture

The use of molecular testing platforms is increasingly replacing culture due to high sensitivity in detecting all species and serogroups of Legionella.

Culture does continue to play an important role in the following scenarios:

- confirming the presence of viable organisms.
- identifying the source of infection.
- comparing the genetics of clinical and environmental isolates.
- typing strains for possible outbreak investigation and epidemiological study.

BALF is the preferred specimen as is more representative of the site of disease, but sputum is acceptable

Routine centrifugation is not essential for Legionella culture. Some laboratories may choose to concentrate BALF to maximise Legionella

- recovery, but this is a local validation decision. Sputum specimens are typically processed without centrifugation.
- if centrifugation is conducted, ensure that the sample is not too thick or mucopurulent and spun at a minimum of 1200 x *g* for 10 mins. Carefully discard the supernatant and resuspend the deposit in 1 mL of sterile water.
- inoculate 10µL of direct specimen or deposit onto the BCYE and/or another agar selective for Legionella.
- a further serial dilution can be conducted using the original dilution if required.
- culturing of heat-treated samples may be considered routine alongside a culture of the untreated sample or restricted to only heavily contaminated samples. Heat treatment should be conducted at 50 ºC for 30 min.
- dilution (1:100) may help to decrease the number of yeasts, pseudomonads, and *Proteus* species.

Urinary antigen tests detecting *Legionella pneumophila* serogroup 1 can help support culture results (51,54)

For parasitic investigations, refer to UK SMI B 31 - Investigation of specimens other than blood for parasites. An example is pulmonary cryptosporidiosis which may be suspected in patients with profound T-cell immune deficiency and unexplained respiratory symptoms, depending on the clinical details. The method used, such as microscopy, enzyme immunoassay, or NAAT will depend on the specimen type and the capabilities of the local laboratory. Alternatively, specimens can be referred to a Reference Laboratory. Different culture techniques are applied depending on whether semi-quantitative or quantitative reporting is required in respiratory samples.

Table 1: Culture media, conditions, and organisms for bacterial investigations

Clinical details/ conditions	Specimen	Media	Incubation [a]			Cultures read	Target organism(s)
			Temperature °C	Atmosphere	Time		
All respiratory conditions	All samples	Blood agar [b]	35-37	5-10% CO_2	40-48hr	Daily	*Moraxella catarrhalis* *Streptococcus pneumoniae* Other organisms in pure growth may be significant in certain clinical contexts, i.e., *Staphylococcus aureus* in long term ventilation, necrotising pneumonia and Group B streptococci (GBS) for neonates
		Chocolate agar with or without bacitracin [c]					All organisms above and *Haemophilus influenzae*
All respiratory conditions	Bronchoalveolar lavage (BAL) non-directed bronchoalveolar lavage (NBL) bronchial washing brushings aspirate bronchial brushing	Selective/ differential agar, CLED or MacConkey agar	35-37	Air	>16 - 24hr	>16 hr	Enterobacterales Pseudomonads

Clinical details/ conditions	Specimen	Media	Incubation [a]			Cultures read	Target organism(s)
			Temperature °C	Atmosph-ere	Time		
For these situations, add the following:							
Bronchiectasis Cystic fibrosis Immunocompro-mised/ ITU	Sputum [d]	Selective/differential agar, CLED or MacConkey agar	35-37	Air	>16-24hr [e]	> 16hr	Enterobacterales Pseudomonads *Pseudomonas aeruginosa*
Cystic fibrosis Bronchiectasis [f]	Sputum/BAL	*Burkholderia cepacian* selective agar Broth or solid medium	35-37	Air	5 days	Daily	*Burkholderia cepacia complex*
Cystic fibrosis Immunocompro-mised	Sputum/BAL	*Staphylococcus aureus* differential media	35-37	Air	40-48hr	Daily	*Staphylococcus aureus*
Pneumonia/ flu-like symptoms If Legionella suspected	Sputum/BAL	*Non-selective and selective agars for Legionella* [g]	35-37	Moist atmosphere Air or CO_2 [h]	Up to 10 days	3, 7 and 10 days	*Legionella* species
Empyema, lung abscess, abdominal infection, trauma/surgery	All samples	Neomycin fastidious anaerobe agar (FAA) with metronidazole 5µg disc [i,j]	35-37	Anaerobic	40-48hrs	40-48hrs	Anaerobes

Footnotes:

a Follow the manufacturer's instructions or validated local procedures.

b An optochin disc may be added.

c Depending on the local laboratory workflow, use either chocolate agar containing incorporated bacitracin or chocolate agar supplemented with a 10 µg bacitracin disc.

d Laboratories may process cough swabs using the same media as those used for sputum samples for Cystic Fibrosis.

e For special patient groups, incubation times may need to be extended to 40 to 48hrs, refer to the medium manufacturer's instructions.

f Burkholderia cepacia selective agar may be used for the investigation of specimens from patients with bronchiectasis when there is clinical suspicion of Burkholderia infection or upon request from the specialist clinical team.

g Buffered cefamandole, polymyxin, anisomycin, α-ketoglutarate medium (BMPAα), Glycine–Vancomycin–Polymyxin–Cycloheximide agar (GVPC) or buffered charcoal yeast extract, anisomycin agar (BCYE).

h It should also be noted that incubation in 2 to 5% CO_2 can enhance the growth of some *Legionella* species such as *L. sainthelensi* and *L. oakridgensis* (for more information, see section 7.4).

i Anaerobic plates should be incubated as soon as possible to avoid oxygen sensitive strains losing viability. Maintaining and monitoring the anaerobiosis (using a real-time anaerobic indicator) are essential for the isolation of strict anaerobes.

j Due to intrinsic, and increasing acquired metronidazole resistance in anaerobes, colonies within a metronidazole zone should not automatically be disregarded as non-anaerobic bacteria. To help prevent possible treatment failure and poor clinical outcomes, identification of possible metronidazole resistant isolates from within the indicator zone is encouraged, where feasible.

Notes:

- consider additional rare pathogens and refer to the relevant UK SMI documents, for example *Nocardia* spp. and *Actinomyces* spp.. The appropriate specimens for investigation of both these organisms are pus, tissue and biopsy (which include bronchoalveolar lavage) samples (see UK SMI B 14 - Investigation of abscesses and deep-seated wound infections and UK SMI B 17 - Tissues and biopsies from deep-seated sites and organs).
- other organisms for consideration, such as *Mycobacterium* species and non-tuberculous mycobacteria (NTM) (UK SMI B 40 - Investigation of specimens for Mycobacterium species), parasites (UK SMI B 31 - Investigation of specimens other than blood for parasites), *Bordetella pertussis*/*Bordetella parapertussis* (UK SMI B 6 - Investigation of whooping cough) and *Corynebacterium diphtheriae* (UK SMI B 9 - Investigation of throat related specimens).
- for neonatal sepsis, neonatal respiratory infections, pneumonia or meningitis in neonates, consider *Mycoplasma hominis*, *Ureaplasma* species and Group B streptococci as the most common pathogens.
- for blood culture, refer to UK SMI S 12 - Sepsis, and systemic or disseminated infections.
- for pleural fluids, refer to UK SMI B 26 - Investigation of fluids from normally sterile sites.
- for lymph node, refer to UK SMI B 17 - Tissues and biopsies from deep-seated sites and organs.

7.5 Identification

Refer to individual UK SMIs for organism identification.

Semi-quantitative culture can guide which organisms should be prioritised for identification and susceptibility testing, especially in mixed cultures. Organisms present in moderate or heavy growth, particularly when predominant, are more likely to represent clinically significant pathogens, whereas light growth may reflect colonisation or contamination depending on specimen type and clinical context.

7.5.1. BALF and other LRT samples

Mixed cultures in BALF and other LRT samples are less common and more clinically meaningful. If multiple organisms appear but one is clearly predominant, interpretation focuses on that organism.

Mixed flora in BALFs combined with high WBCs may indicate polymicrobial infection (e.g., in aspiration, severe bronchiectasis).

If quantitative culture is conducted the following thresholds may indicate possible infection (2,55,56):

- BALF- ≥10^4 CFU/mL)
- bronchoscopic aspirates - 10^5-10^6 CFU/mL (10^8–10^9 CFU/L)
- protected brush specimens- 10^3 CFU/mL (10^6 CFU/L)

  A pure bacterial count of greater than 10^3 CFU/mL in a brush specimen obtained bronchoscopically has been found to correlate with a histological diagnosis of pneumonia.

Note: The diagnostic threshold may not be met if the infection has just started, if infectious bronchiolitis is present or if patients have received antibiotics.

Even if a pathogen is below 10^4 CFU/mL, dominant Gram-stain morphology plus clinical correlation warrants investigation.

7.5.2 Sputum/ETA

Diluted specimen culture:

Any growth of isolates in diluted specimen culture is assumed to be present at clinically significant levels i.e. ≥10^6 CFU/mL, so selection for identification should be based on the following:

- prioritisation of recognised target respiratory organisms such as *S. pneumoniae.*
- high level growth (higher numbers of colonies) and predominance.
- clinical context i.e. clinical details and location of the patient (inpatient status, immunosuppression, chronic lung disease).

Mixed flora is expected in these specimens, especially in patients recently exposed to antibiotics or with chronic lung disease.

Below are some examples for guidance only of selected groups where growth at higher levels (higher numbers of colonies) indicate significance:

- ***Streptococcus pneumoniae-*** significant in all patients.
- ***Haemophilus influenzae-*** particularly in COPD/bronchiectasis.
- ***Moraxella catarrhalis-*** more likely to be significant in COPD/elderly patients.
- ***Streptococcus pyogenes-*** significant in all patients. Particularly relevant with compatible clinical syndrome or in neonates.
- ***Streptococcus agalactiae-*** significant in neonates or immunocompromised patients.
- ***Staphylococcus aureus-*** significant in post-influenza, ventilated, or immunocompromised patients.
- ***Pseudomonas aeruginosa-*** significant in CF, bronchiectasis, HAP and high-risk groups.
- ***Enterobacterales (e.g. Klebsiella, E. coli) and Acinetobacter-*** significant in hospitalised patients.

While mixed upper respiratory flora does not need treatment, it may be clinically significant in selected groups (e.g. bronchiectasis, immunosuppression, or suspected HAP/VAP), especially when one organism is predominant or present in high quantity.

Neat (homogenised) specimen culture:

In neat specimen culture, organisms should be selected for identification based on clinical relevance, predominance and quantity using semi-quantitative assessment within mixed flora, rather than just presence.

Below are some examples for guidance only:

- ***Streptococcus pneumoniae***
  - significant in heavy or predominant growth.
  - high numbers of neutrophils if microscopy is conducted indicates likely infection and should be fully investigated.
  - light/mixed growth in low quality samples may represent carriage.
  - please refer to UK SMI ID 4 - Identification of Streptococcus species, Enterococcus species and morphologically similar organisms.
- ***Haemophilus influenzae***
  - significant in moderate/heavy growth, especially in chronic lung diseases, such as COPD or bronchiectasis.
  - light/mixed growth can represent colonisation.

  - please refer to UK SMI ID 12 - Identification of *Haemophilus* species and the HACEK group of organisms.

- ***Moraxella catarrhalis***
  - predominant or heavy growth may be significant in older adults and COPD patients.
  - please refer to UK SMI ID 11 - Identification of *Moraxella* species and morphologically similar organisms.

- ***Streptococcus pyogenes***
  - not part of normal lower respiratory tract flora; therefore, its isolation in respiratory specimens should be interpreted with caution and in clinical context; except in neonates, where any growth is clinically significant and warrants prompt investigation.
  - significant in heavy or predominant growth (moderate to heavy growth) with high numbers of neutrophils indicates likely infection and should be fully investigated.
  - a compatible illness may include rapidly progressive pneumonia, particularly following viral infection, or features of invasive disease such as concurrent pharyngitis or scarlet fever.
  - please refer to UK SMI ID 4 - Identification of *Streptococcus* species, *Enterococcus* species and morphologically similar organisms.
- ***Streptococcus agalactiae***
  - light growth in mixed cultures is likely to represent colonisation.
  - light pure growth in neonates should always be investigated.
  - heavy or predominant growth in culture from immunocompromised patients or those with severe pneumonia.
  - a compatible illness may include severe or post viral pneumonia and/or immunocompromised patient.
  - please refer to UK SMI ID 4 - Identification of *Streptococcus* species, *Enterococcus* species and morphologically similar organisms.

- ***Staphylococcus aureus***
  - a common coloniser and significance is usually case dependent.
  - significant in predominant or heavy growth in mainly post-influenza, in aspiration or long termed ventilated and/or immunocompromised patients.
  - please refer to UK SMI ID 7 - Identification of *Staphylococcus* species, *Micrococcus* species and Rothia species.

- ***Pseudomonas aeruginosa***
  - more clinically significant in bronchiectasis, suspected HAP/VAP and other high-risk groups i.e., immunocompromised, ECMO and lung transplant patients.
  - heavy growth increases the likelihood of significance
  - in CF patients note whether the isolate is mucoid Please refer to UK SMI ID 17 - Identification of *Pseudomonas* species and other non glucose fermenters.
  - surveillance of different strains of *P. aeruginosa* within CF clinics is useful to establish the prevalence of transmissible strains such as the Liverpool (LES), Manchester (MAN) and Midlands 1 (Mdl1) strains, and to monitor the prevalence of other shared strains for cross-infection purposes (40).
  - VNTR typing is recommended for all newly identified *P. aeruginosa* isolates, with repeat testing performed annually. Where multiple strains are present within a culture, individual isolates should be submitted separately to the reference laboratory on nutrient agar slopes.

- ***Klebsiella pneumoniae, E. coli, Acinetobacter***
  - Significant in predominant or heavy growth in hospitalised patients when isolated from higher quality sputum samples. Please refer to UK SMI ID 17 - Identification of *Pseudomonas* species and other non glucose fermenters and UK SMI ID 16 - Identification of Enterobacteriaceae.

8 Post-laboratory processes

8.1 Interpretation and reporting other tests, including molecular testing

As newer and more novel methods are becoming available, their validation and reporting should follow local protocols or manufacturer's instructions, with the caveat that some tests will lack the subsequent guidance on reporting/interpreting of a result for pathogens in certain circumstances.

- indicating the strength of positivity is encouraged; higher burdens are more likely to represent disease.
- results should be interpreted according to sample type and within the clinical context of the individual patient.
- attention should be paid to the reporting of very low positive results that may indicate environmental contamination e.g. *Legionella* NAAT results.
- respiratory sample quality should be assessed, especially for BAL specimens.

a) poor sample quality should be indicated alongside negative results to allow users to interpret that result in the context of the sample's poor quality.

b) the impact of poor sample quality on weak positive results should be indicated where they may have influenced signal strength.

c) an indication of poor sample quality, as identified by molecular testing, should be applied to all other tests performed on that specimen, providing sample processing is broadly comparable.

8.1.1 Reporting multiplex molecular results

NAAT results should be reported as soon as they are performed and analysed.

When multiplex diagnostic platforms are used for pathogen detection, the approach to reporting for each pathogen in the assay should be agreed with a specialist with domain-specific expertise. True competency (defined as both formal training and practical experience) should guide the interpretation of results.

Note: not all respiratory sample types are suitable for detection of infection by some pathogens included in multiplex panels. This limitation may impact the diagnostic sensitivity and/or specificity and is important to consider when results are reported.

8.1.2 Reporting *Legionella* Urinary antigen testing:

- Positive*:* provisional positive for *Legionella pneumophila* serogroup 1 antigen in urine. Please send a respiratory sample for *Legionella* culture. Specimen has been referred to the reference laboratory for confirmatory testing.
- Negative: *Legionella* pneumophila serogroup 1 urine antigen not detected.

Note: a negative urinary antigen does not exclude the diagnosis of *Legionella* infection, because it primarily only identifies serogroup 1. If there is still a high suspicion of *Legionella* infection, then other confirmatory tests may be performed, such as culture.

8.2 Reporting microscopy results

8.2.1 Gram stain on BALF

- report presence of WBCs. Numerous neutrophils can indicate true infection.
- few/ no squamous epithelial cells can indicate a high sample quality indicative of lower respiratory origin. This may not be reported externally but should be recorded to aid investigative decisions.
- report if organisms detected. High numbers of organisms strongly suggest infection

Refer to relevant UK SMIs for reporting of microscopy results for *Legionella, Mycobacterium* species (UK SMI B 40 - Investigation of specimens for Mycobacterium

species) and parasites (UK SMI B 31 - Investigation of specimens other than blood for parasites).

8.2.2 Gram stain on sputum

If conducted as discussed in section 7.3.2, report the following in accordance with the requirements of the clinical service and local protocols:

- numbers of polymorphonuclear leucocytes present.
- numbers of squamous epithelial cells (SECs) present.

8.3 Reporting BALF, and other LRT culture results

8.3.1 Negative culture

- if no growth is observed, report as: “No growth”.

8.3.2 Non-significant growth

- although BALF and other LRT samples are considered low contamination samples this may occasionally occur, and if growth of normal upper respiratory tract flora is present. This should be reported as “No pathogenic organisms isolated”.
- when significant amount of upper respiratory tract flora is present, this may be reported as “Mixed upper respiratory tract flora isolated – likely contamination. No predominant pathogen identified. Please correlate clinically.”

8.3.3 Significant growth

- significant isolates should be explicitly reported with semi-quantification or as CFU/mL
- significant isolates should be reported alongside antimicrobial susceptibility test results.
- organisms which are rarely associated with respiratory tract infections and are typically considered normal commensal organisms should be reported with caution. Susceptibility test results may be suppressed due to their low clinical significance.
- if quantitative culture is conducted the following thresholds indicate possible infection (55,56):
    - BAL- ≥10^4 CFU/mL
    - bronchoscopic aspirates - 10^5-10^6 CFU/mL
    - protected brush specimens- 10^3 CFU/mL A pure bacterial count of greater than 10^3cfu/mL in a brush specimen obtained bronchoscopically has been found to correlate with a histological diagnosis of pneumonia.

- **Note:** The diagnostic threshold may not be met if the infection has just started or if infectious bronchiolitis is present. Specimens from patients who have received antibiotics.

8.4 Reporting of sputum/ETA culture results

8.4.1 Negative culture results

Diluted cultures:

- the term 'no growth' should not be used for diluted cultures due to the possible suppression or elimination of normal respiratory flora during dilution.
- absence of growth indicates no organisms detected at high concentrations (e.g. ≥10^6 CFU/mL) rather than true absence of bacteria, therefore an example report includes:
  - "No organisms present at clinically significant levels (e.g. ≥10^6 CFU/mL)"
  - "No growth of specifically targeted organism at a 10^{-6} dilution of the specimen" (Bronchiectasis and CF patients)

Neat (homogenised) cultures:

- a 'no growth' culture result must be interpreted more cautiously and in the context of sample quality and clinical factors. It is more accurate to use for example:
  - "No significant respiratory pathogens isolated "
  - "No growth of clinically significant organisms"

8.4.2 Non-significant growth

- the same terminology can be applied to both neat and diluted samples, such as:
  - "No significant bacterial growth,"
  - "No respiratory pathogens isolated,"
- however, results for diluted samples often reflect successful reduction of commensal flora, so more contextual statements may be more helpful, i.e.,
  - "No significant growth following dilution"
- if commensal or organisms of no clinical significance are present, these should not be reported independently, but using comments based on the following examples:
  - "Upper respiratory tract flora/microbiota"
  - "Mixed upper respiratory tract flora/microbiota only"
  - "Mixed commensal (normal) flora/ microbiota"
  - "Mixed normal flora/microbiota including coliforms" or "Mixed normal flora/microbiota including Pseudomonas" (low risk patients)

- neat samples may require additional context, particularly where oropharyngeal contamination is likely, using comments such as “consistent with normal upper respiratory flora.”

8.4.3 Significant growth

Interpretation of culture must consider the processing method, as this directly influences both the apparent quantity and context of organisms recovered.

- growth may be of varying clinical significance and represent colonisation rather than disease in certain combinations of specimen quality, patient type and clinical condition. The exact mechanism of reporting is dependent on local clinical policy and risk assessment.
- significant isolates should be reported in context of the full culture results wherever possible. If significant organisms are isolated alongside commensal respiratory flora, consider using a reporting comment such as, “*Haemophilus influenzae* and *Streptococcus pneumoniae* isolated. Commensal upper respiratory flora also present.”

Diluted cultures:

When diluted sputum is used growth generally reflects organisms present at higher concentrations (e.g. ≥10^6 CFU/mL) and therefore results should not be interpreted as representing pure growth.

Neat (homogenised) cultures:

In contrast, culture of neat specimen preserves the original microbial composition, allowing assessment of mixed flora and relative abundance using semi-quantitative methods as described in Section 7.

- reporting significant isolates with semi quantification provides further context, especially if there are multiple significant organisms isolated.
- significant isolates should be reported with AST results when pure or heavy in culture and where clinically appropriate
- if organisms are reported but AST results are suppressed, comments should be used to clarify the report, i.e.,

  “Mixed upper respiratory tract flora including *Pseudomonas aeruginosa* and Enterobacterales. No significant respiratory pathogen isolated. Significance uncertain; please correlate clinically.”

Note: Cough swabs may be reported as above; however, it is recommended to include a comment indicating that this is not the optimal sample type and results may be of reduced quality.

8.5 Reporting time

If interim or preliminary results are required as per local policy, these should be issued as soon as actionable findings are available (significant microscopical results or initial culture growth), unless alternative arrangements have been agreed with the requestor.

Final reports should be issued as soon as all investigations are complete and authorised by competent staff.

Any notifiable disease should also be notified to the relevant body in accordance with statutory requirements.

See appropriate UK SMIs for supplementary investigations, for example for Mycobacterium species refer to UK SMI B 40 - Investigation of specimens for Mycobacterium species and for parasites refer to UK SMI B 31 - Investigation of specimens other than blood for parasites.

9 Antimicrobial susceptibility testing

All clinically significant isolates (bacterial and fungal) should be tested for antimicrobial susceptibility, particularly in cases of poor treatment response.

For interpretation of susceptibility testing results, laboratories should test and interpret according to the relevant EUCAST breakpoint, refer to EUCAST guidelines for breakpoint information.

Alternatively, the Clinical and Laboratory Standards Institute (CLSI) method along with the corresponding CLSI breakpoints can be used: Susceptibility Testing Subcommittees (clsi.org).

Note: Laboratories must ensure that the appropriate methodology and corresponding breakpoints are used consistently. EUCAST and CLSI methods are not interchangeable, and combining elements from both may lead to inaccurate interpretation. Laboratories should validate or verify all methods used, as appropriate

Alternatively, isolates can be sent to an appropriate specialist or reference laboratory.

9.1 Reporting of antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

Only clinically significant isolates should have susceptibility results reported. Avoid reporting from likely contaminants or colonising organisms unless deemed clinically necessary (e.g. in immunocompromised patients or sterile sites).

If molecular methods are used to detect mutations associated with resistance this should be stated in the report.

10 Referral to reference or specialist testing laboratories

In the absence of any other risk factor (for example foreign travel, clinical laboratory or veterinary work posing an infection hazard) cases or clusters of certain organisms could suggest the possibility of a deliberate or accidental release. Such events require a rapid response. Suspicion of deliberate or accidental release of micro-organisms must be notified urgently. The organisms are reportable to UKHSA under The Health Protection (Notification) Regulations 2010. A comprehensive list of diseases notifiable to the Local Authority Proper Office under the Health Protection (Notification) Regulations 2010 is available at: Notifiable diseases and how to report them.

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

When sending away isolates to reference or specialist testing laboratories for processing, ensure that the specimen is placed in the appropriate package and transported accordingly. Follow local regulations and instructions provided by the reference or specialist testing laboratories for sending isolates.

Contact the appropriate reference laboratory (refer to the links provided below) for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission.

England

Wales

Scotland

Northern Ireland

11 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.

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An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

For suggested citation of UK SMIs, refer to UK SMI Development.

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