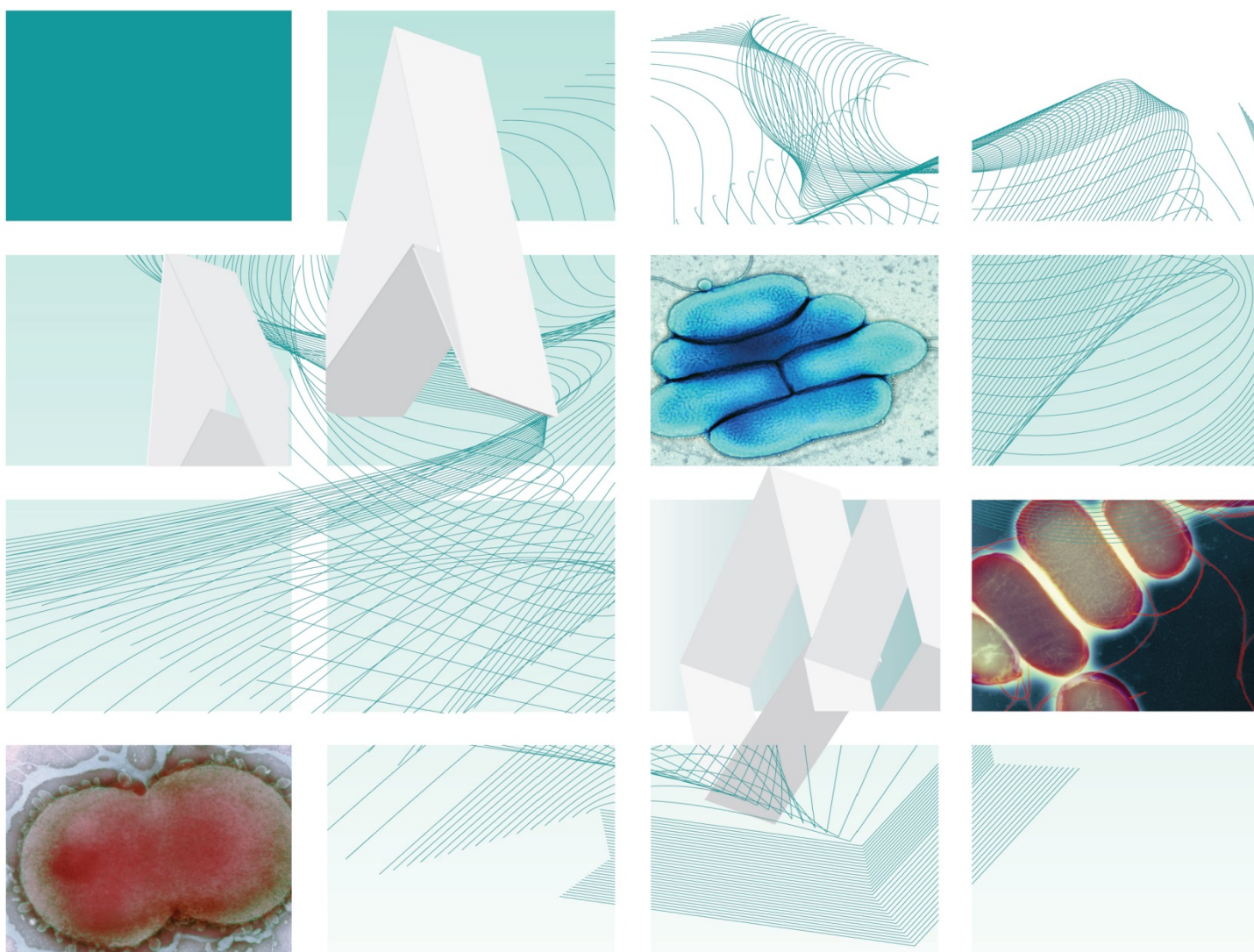




UK Standards for Microbiology Investigations

Investigation of bronchoalveolar lavage, sputum and associated specimens



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Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee>).

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Amendment table

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

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment no/date.	13/08.05.19
Issue no. discarded.	3.4
Insert issue no.	3.5
Section(s) involved	Amendment
Appendix 1.	Incubation time for Sabouraud plate changed from 40 hours to 5 days.

Amendment no/date.	12/01.03.18
Issue no. discarded.	3.3
Insert issue no.	3.4
Section(s) involved	Amendment
Appendix 2.	"Pneumonia or flu like symptoms" replaced by " <i>legionella</i> suspected" to be in agreement with sputum table.

Amendment no/date.	11/24.08.17
Issue no. discarded.	3.2
Insert issue no.	3.3
Section(s) involved	Amendment
Appendix 1.	Incubation time for <i>B. cepacia</i> has been clarified.

Amendment no/date.	10/28.12.16
Issue no. discarded.	3.1
Insert issue no.	3.2
Section(s) involved	Amendment

Introduction.	Subheading on <i>Nocardia</i> and <i>Actinomyces</i> infections has been updated to include bronchoalveolar lavage.
Safety Considerations.	Section has been strengthened with additional information on handling specimens suspected to be Containment level 3 organisms.
Specimen processing/procedure.	<p>Section 4.4.2 Supplementary</p> <ul style="list-style-type: none"> For Sputum, the appropriate staining technique for fungi has been updated. <p>Sections 4.5.3 and 4.5.4 have been updated with information on the incubation atmosphere and time for the <i>Legionella</i> species. Footnotes have been added for clarity.</p>
Appendix 1 and 2.	Flowcharts amended to reflect information on section 4.5.3.

Amendment no/date.	9/09.12.15
Issue no. discarded.	3
Insert issue no.	3.1
Section(s) involved	Amendment
Appendix 2.	Error in flowchart amended.

Amendment no/date.	8/02.10.15
Issue no. discarded.	2.5
Insert issue no.	3
Section(s) involved	Amendment
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Scope.	Cross links to other UK SMIs improved.
Introduction.	<p>Cystic fibrosis section reviewed and expanded. Information on <i>Legionella</i> included.</p> <p>The section on fungal infections reviewed and updated.</p> <p>Section on types of specimen reviewed and</p>

	<p>updated.</p> <p>Deliberate release organisms added to the introduction.</p> <p>Interpretation of gram stains section expanded.</p>
Safety considerations.	<p>Additional information on Hazard Group 3 Fungi inserted in to the document.</p>
Culture and investigation.	<p>Information for BAL expanded to clarify the semi-quantitative method.</p> <p>Information on Legionella culture inserted.</p> <p>Fungal section has been divided in to cystic fibrosis and non-cystic fibrosis patients.</p> <p>A section on molecular methods has been inserted.</p> <p>Culture for <i>M. abscessus</i> added to the cystic fibrosis section.</p>
References.	<p>References reviewed and updated.</p>

UK SMI[#]: scope and purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories><http://www.hpa-standardmethods.org.uk/>. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the SMI working groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI working groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs is subject to PHE equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>.

The SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Scope of document

Type of specimen

Bronchial aspirate, transthoracic aspirate, bronchoalveolar lavage, transtracheal aspirate, bronchial brushings, protected catheter specimens, bronchial washings, endotracheal tube specimens, sputum – expectorated

This SMI describes the isolation of organisms known to cause bacterial and fungal respiratory infection from sputum, bronchoalveolar lavage and associated specimens. Different tests are carried out on different sample types depending on the patient group. For information on *Bordetella pertussis* and *Bordetella parapertussis* see [B 6](#). For Investigation of specimens for *Mycobacterium* species see [B 40](#). For viruses see [S 2 – Pneumonia](#), and [G 8 - Respiratory viruses](#).

This SMI should be used in conjunction with other SMIs.

Introduction

Recovery and recognition of organisms responsible for pneumonia depends on:

- the adequacy of the lower respiratory tract specimen
- avoidance of contamination by upper respiratory tract flora
- the use of microscopic techniques and culture methods
- current and recent antimicrobial treatment

The expression lower respiratory tract infection (LRTI) includes pneumonia, where there is inflammation of the lung parenchyma, and infections such as bronchiolitis that affect 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.

Distinction between tracheobronchial colonisation and true pulmonary infection can prove difficult.

Pneumonia

Pneumonia can be classified according to whether it is community acquired or nosocomial (often defined as presenting more than 48 hours after hospitalisation). It may be primary, occurring in a person without previously identified risk factors, or secondary. Many conditions are associated with an increased risk of pneumonia. Common risk factors include chronic lung diseases such as chronic obstructive pulmonary disease (COPD), diabetes mellitus, cardiac or renal failure and immunosuppression (either congenital or acquired). Reduced level of consciousness and weakness of the gag and cough reflexes are risk factors for aspiration pneumonia. Recent infection with respiratory viruses, particularly influenza, is also a risk factor. There are clinical signs and laboratory indices that can be used to assess the severity of pneumonia in an individual patient, some of which are predictive of an increased risk of death if present¹.

The aetiology of pneumonia varies according to whether it has been acquired in the community or in hospital and the risk factors present. Many of the bacteria found as colonisers of the upper respiratory tract have been implicated in pneumonia. Antibiotic

treatment and hospitalisation affect the colonising flora, leading to an increase in numbers of aerobic Gram negative bacilli². These factors affect the sensitivity and specificity of sputum culture as a diagnostic test and results must always be interpreted in the light of the clinical information³. Sputum culture results are often unreliable and sensitivity of culture is poor for many pathogens, although culture and antibiotic sensitivities may be of value in sputum specimens from patients with severe exacerbation of COPD⁴.

Community acquired pneumonia⁵

The commonest cause of community acquired pneumonia is *Streptococcus pneumoniae*, which is responsible for up to 60% of cases in community based surveys and may be multi-drug resistant. It can affect individuals of any age, including those without known risk factors. Other bacterial pathogens tend to cause pneumonia in the presence of specific risk factors. Patients with COPD and patients infected with HIV are additionally at risk of pneumonia caused by *Haemophilus influenzae* and *Moraxella catarrhalis*. *Staphylococcus aureus* pneumonia occurs either in the context of recent influenza infection or, less commonly, as a result of blood borne spread from a distant focus, COPD or aspiration. Aerobic Gram negative rods are rare causes of community acquired pneumonia. Occasionally, *Klebsiella pneumoniae* causes severe necrotising pneumonia, typically in patients with a history of alcohol abuse and homelessness ("Friedländer's pneumonia").

A number of other pathogens cause atypical pneumonia within the community⁶. *Mycoplasma pneumoniae* causes up to 20% of community acquired pneumonia, second only to *S. pneumoniae*. Infection with *Mycoplasma pneumoniae* tends to occur in epidemics every 4-5 years and affects younger age groups. *Chlamydophila pneumoniae* is an exclusively human pathogen. Pneumonia in a minority of individuals is caused by *Chlamydophila psittaci* and *Coxiella burnetii* and occurs in individuals with a relevant exposure history (birds and farm animals). *Legionella pneumophila* is a rare cause of outbreaks of community acquired pneumonia usually where there is a recent history of travel. Respiratory viruses, such as respiratory syncytial virus (RSV), influenza and adenoviruses may commonly cause primary viral pneumonia (see [G 8 - Respiratory viruses](#))⁷.

Hospital acquired pneumonia⁸

Hospital acquired pneumonia is the second commonest type of nosocomial infection. Risk is increased by the presence of underlying disease and by various interventions and procedures⁹. Mechanical ventilation is a major risk factor. Patients with critical illnesses requiring prolonged mechanical ventilation are susceptible to multi-resistant *Pseudomonas aeruginosa* and *Acinetobacter* species (eg *Acinetobacter baumannii*). Aerobic Gram negative bacilli, including members of the Enterobacteriaceae (such as *Klebsiella* and *Enterobacter* species) and *P. aeruginosa* are implicated in up to 60% of cases¹⁰. Intravascular catheters and nasal carriage are risk factors for pneumonia caused by meticillin resistant *S. aureus* (MRSA). *Legionella* species are also an occasional cause of hospital-acquired pneumonia.

Aspiration pneumonia

Aspiration pneumonia occurs when oropharyngeal contents are introduced into the lower respiratory tract. Reduced level of consciousness, for instance following head injury or drug overdose is a risk factor, as are weak gag and cough reflexes which can follow a stroke or other neurological disease.

Lung abscess

Lung abscess may develop secondary to aspiration pneumonia, in which case the right middle zone is most frequently affected. Other organisms may give rise to multifocal abscess formation and the presence of multiple small abscesses (<2cm diameter) is sometimes referred to as necrotising pneumonia. Pneumonia caused by *S. aureus* and *K. pneumoniae* may show this picture. Nocardiosis, almost always occurring in a setting of immunosuppression, may present as pulmonary abscesses. Abscesses as a result of blood borne spread of infection from a distant focus may occur in conditions such as infective endocarditis.

Burkholderia pseudomallei may cause lung abscesses or necrotising pneumonia in those who have visited endemic areas (mainly south east Asia and northern Australia) especially in the presence of diabetes mellitus¹¹.

Lemierre's syndrome or necrobacillosis originates as an acute oropharyngeal infection. Infective thrombophlebitis of the internal jugular vein can lead to septic embolisation and metastatic infection. The lung is most frequently involved and multifocal abscesses may develop. *Fusobacterium necrophorum* is the most common pathogen isolated from blood cultures in patients with this syndrome¹².

Cystic fibrosis¹³

Cystic fibrosis (CF) is caused by a defect in the CF transmembrane conductance regulator gene that affects the transport of ions and water across the epithelium¹⁴. This leads to progressive pulmonary disease associated with pulmonary infections, which are the major cause of morbidity and mortality in CF patients. The major pathogens are *S. aureus*, *H. influenzae* (usually non-encapsulated in CF patients), *S. pneumoniae* and pseudomonads, particularly mucoid *P. aeruginosa* strains^{14,15}. Strains of *P. aeruginosa* with differing antibiotic susceptibilities may be isolated from a single sample. Anaerobes may also be present, together with *Aspergillus* species and mycobacteria other than *Mycobacterium tuberculosis* (MOTT)¹⁶.

Nucleotide analysis of *recA* gene sequences suggests that *Burkholderia cepacia* complex consists of several closely related genomovars¹⁷. Transmission of *B. cepacia* complex between patients may occur and some patients succumb to "*B. cepacia* syndrome" which is a rapidly fulminating pneumonia sometimes accompanied by septicaemia¹⁸. Subsequent to early reports more species have been included within this title and the patients prognosis is poor^{13,19,20}.

Non-tuberculosis Mycobacteria are an increasing problem for this patient group; *M. abscessus* more so than the others²¹. Testing should be considered in patients who show deteriorating lung function where no clear pathogen has been identified²²⁻²⁵.

Resistance to antibiotics, particularly in *Burkholderia* spp., *Stenotrophomonas maltophilia* and *P. aeruginosa*, limits the options for treatment²⁶.

Organisms such as *Ralstonia*, *Achromobacter* and *Pandora* are emerging pathogens in chronic structural lung disease. Viruses have also been implicated^{27,28}.

For more information on this area refer to "Laboratory standards for processing Microbiological Sample from People with Cystic Fibrosis"²⁹.

Mycobacterial disease

Primary pulmonary infection with *Mycobacterium tuberculosis* may lead to the formation of the 'primary complex', particularly in childhood. The pulmonary focus may

be relatively small, but the draining hilar lymph nodes become greatly enlarged and may rupture, spreading infectious material into other areas of the lung. It is at this stage that miliary spread to other organs may occur via blood and lymphatics. Adolescents and adults may have asymptomatic primary infection, a typical primary complex or infection which progresses to typical chronic cavitating tuberculosis. Chronic cavitating disease is usually seen in reactivated primary infection and the lung apices are most commonly involved. The cough that accompanies this process produces aerosols of infectious particles, which is the route by which other persons may become infected. Mycobacteria other than tubercle bacilli have been recognised as causing human disease, particularly in those with immunosuppression or underlying disease. These include *Mycobacterium avium-intracellulare*, *Mycobacterium abscessus*, *Mycobacterium kansasii*, *Mycobacterium malmoense*, *Mycobacterium xenopi*, *Mycobacterium fortuitum* and *Mycobacterium haemophilum*. They are often resistant to standard antituberculous chemotherapy. Refer to [B 40 - Investigation of specimens for *Mycobacterium* species](#).

Legionella disease

Transmission is by inhalation of an aerosol of the organism, either from an environmental source or occasionally iatrogenically following a respiratory tract manipulation such as humidification or nebulisation of infected material.

Pneumonia is the most common manifestation of *Legionella* infections. Severity varies from mild to severe, life-threatening disease. Onset is usually abrupt with pyrexia, myalgia, headache and non-productive cough following, commonly, a 2-10 day incubation period. The incubation time has been found to be as long as 20 days in some cases involving whirlpool baths and spas³⁰. Watery diarrhoea may be present and neurological symptoms ranging from mild headache to encephalopathy may also occur³¹. Chest X-rays show pulmonary infiltrates progressing to consolidation often with pleural effusion³².

Pontiac fever/non-pneumonic disease is an acute febrile illness occurring 24 – 48 hours after exposure to any species, but particularly to *L. pneumophila*, *Legionella feeleyi*, *Legionella micdadei* and *Legionella anisa*³³⁻³⁶. Superficially, the disease resembles influenza and is usually self-limiting, without pneumonic involvement. It has been found that children have a shorter incubation period than adults and display symptoms such as ear ache and rashes, whereas common symptoms in adults included fever, dizziness, headaches, fatigue, arthralgia and abdominal pain³⁷.

Nocardia and Actinomyces infections^{38,39}

Nocardiosis and actinomycosis are rare conditions that may affect other systems apart from the lungs.

Nocardia species are most often seen in the lung where they cause acute, often necrotising pneumonia. This is commonly associated with cavitation. It may also produce a slowly enlarging pulmonary nodule and pneumonia that is often associated with empyema. Immune defects associated with alcoholism, organ transplantation and HIV infection are present in the majority (60% plus) of patients presenting with nocardiosis.

Actinomyces species cause a thoracic infection that may involve the lungs, pleura, mediastinum or chest wall. Cases often go unrecognised until empyema or a chest wall fistula develops. Aspiration of oral contents is a risk factor for the development of

thoracic actinomycosis, thus predisposing conditions include alcoholism, cerebral infarction, drug overdose, general anaesthesia, seizure, diabetic coma or shock.

The appropriate specimens for investigation of both these organisms are pus, tissue and biopsy (which include bronchoalveolar lavage) samples (see [B 14 - Investigation of abscesses and deep-seated wound infections](#) and [B 17 - Investigation of tissues and biopsies](#)).

Parasitic infections⁴⁰

Several helminth infections may give rise to the syndrome Tropical Pulmonary Eosinophilia, characterised by patchy pulmonary infiltrates and eosinophilia accompanied by symptoms of cough, fever and weight loss. These signs and symptoms are associated with passage of larval forms through the lungs and include *Ascaris lumbricoides*, hookworms and *Strongyloides stercoralis*.

The lung fluke, *Paragonimus westermanii* has a wide distribution and is particularly prevalent in the Far East, Indian subcontinent and West Africa. Human infection is acquired by consumption of uncooked freshwater crabs or crayfish that harbour encysted metacercariae. Although infection may be asymptomatic, heavy infestations are manifested by pulmonary infiltrates as above which may progress to chronic productive cough with pleuritic chest pain. Ova of *P. westermanii* are demonstrable in sputum (See [B 31 - Investigation of specimens other than blood for parasites](#)).

Fungal infections⁴¹

Candida species are extremely rare causes of LRTI. Occasionally infection occurs as a result of haematogenous seeding. Diagnosis is difficult given that the airways may become colonised in compromised patients treated with antibiotics.

Invasive aspergillosis still remains a life threatening infection in patients severely immunocompromised and contributes to the morbidity in cancer patients⁴². Underlying risk factors include patients receiving corticosteroids, individuals with haematological malignancies and those with previous pulmonary infections.

Aspergillus fumigatus species complex is one of the most prevalent species to cause fungal infections and a significant number of cases go undiagnosed, owing to the lack of sensitivity of tests available. Screening patients susceptible to fungal infections for the antigen galactomannan in serum and BAL in conjunction with molecular detection methods (eg 18SrRNA, ITS region) increase the diagnosis^{43,44}. However, detection of fungal DNA cannot determine colonisation from active infection⁴⁵.

Pneumocystis pneumonia is caused by *Pneumocystis jirovecii*. It is the commonest cause of severe pneumonia in patients with advanced HIV infection, and is considered an AIDS defining illness⁴⁶. Pneumocystis pneumonia also occurs in numerous other immunocompromised adults and children. It presents sub-acutely with cough, fever and hypoxia as the cardinal features, and is often subtle initially. The best diagnostic specimens are a BAL and transbronchial biopsies, but obtaining the latter carries some risk to the patient. BAL and induced sputum and mouthwash specimens are useful for molecular detection methods⁴⁷⁻⁴⁹.

Some rare fungal causative agents of LRTI are endemic to defined geographical areas. Although many infections are subclinical, clinically apparent infections are occasionally imported into the UK. These illnesses occur in immunocompetent individuals and are reported to be more severe in patients who are immunocompromised. The diagnosis should be considered in travellers returning from

endemic areas who present with respiratory illness or pneumonia, particularly if they fail to respond to standard therapy. These infections include: histoplasmosis, caused by *Histoplasma capsulatum* (south east USA, Central America, Africa, Australia and eastern Asia); Coccidioidomycosis, caused by *Coccidioides immitis* and *C. posadasii* (south west USA, Central and South America) and blastomycosis caused by *Blastomyces dermatitidis* (eastern USA, Central and South America and Africa). These infections do present with distinguishing characteristics, however it is often difficult to differentiate them clinically from other causes of respiratory infection, particularly in their early stages. Paracoccidioidomycosis caused by *Paracoccidioides brasiliensis* (Central and South America) usually causes asymptomatic primary pulmonary infection. *Talaromyces* (previously *Penicillium*) *marneffeii* (South east Asia, southern China) should also be considered when the travel history supports it. Fungal infections may reactivate if immune function declines.

Cryptococcosis is an unusual cause of pneumonia, usually in the immunocompromised host, and may be associated with meningitis, and is an AIDS defining illness. Pneumonia can be caused by *Cryptococcus neoformans*. This pathogen has worldwide distribution. Detection of the circulating cryptococcal antigen in the serum or BAL fluid is consistent with the diagnosis of cryptococcal pneumonia.

Types of specimen⁹

Expectorated sputum samples

Sputum samples are known to have issues with contamination. Early-morning sputum samples should be obtained because they contain pooled overnight secretions in which pathogenic bacteria are more likely to be concentrated. Ventilator associated pneumonia carries a high mortality but is difficult to diagnose clinically and microbiologically. The criteria for diagnosis remain controversial. The poor sensitivity and specificity of sputum culture in the diagnosis of pneumonia in hospital ventilated patients has led to the development of a variety of techniques for obtaining lower respiratory tract specimens some involving the use of fiberoptic bronchoscopy.

Bronchoalveolar lavage (BAL)

A segment of lung is 'washed' with sterile saline after insertion of a flexible bronchoscope, thereby allowing recovery of both cellular and non-cellular components of the epithelial surface of the lower respiratory tract⁵⁰. It is a reliable method for making a definitive aetiological diagnosis of pneumonia and other pulmonary infections^{51,52}.

Brush specimen results and bronchoalveolar lavage results are considered comparable by some authorities if a cut off of 10⁴cfu/mL is used for the bronchoalveolar lavage although this is not recommended in this SMI because it remains controversial⁵³.

Non-directed bronchoalveolar lavage (NBL)

Non-directed techniques have been found to give results comparable to bronchoscopic methods⁵³⁻⁵⁵. 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.

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. 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 pneumonia⁵².

Bronchial washings

Bronchial washings are collected in a similar fashion to bronchial aspirates, but the procedure involves the aspiration of small amounts of instilled saline from the large airways of the respiratory tract⁵⁰.

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.

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.

Transtracheal aspiration

Transtracheal aspiration is a procedure that carries clinical risks and is therefore rarely performed in the UK.

Tracheal aspirate

Tracheal aspirates are collected via the endotracheal tube. They are subject to the same limitations as sputum specimens.

Unusual organisms likely to be involved in a deliberate or accidental release of infection (bioterrorism or biological warfare)

In the absence of any other risk factor (eg foreign travel, clinical laboratory or veterinary work posing an infection hazard) cases or clusters of the organisms below could suggest the possibility of a deliberate or accidental release of micro-organisms. Such events require a rapid response; suspicion of deliberate or accidental release of micro-organisms must be notified urgently to the Public Health England 24hr Duty Doctor at Microbiology Services Colindale. Other arrangements exist in [Scotland](#)^{56,57}, [Wales](#)⁵⁸ and [Northern Ireland](#)⁵⁹.

If the following organisms are suspected, investigation should be carried out at containment level 3 unless otherwise stated. Suspect isolates should be sent to the appropriate reference laboratory for characterisation:

- *Bacillus anthracis* (Anthrax)
- *Brucella* species (Brucella)

- *Francisella tularensis* (Tularemia)
- *Burkholderia mallei* (Glanders)
- *Burkholderia pseudomallei* (Meliodosis)
- *Clostridium botulinum* (Botulism) may be investigated at Containment Level 2 in a Microbiological Safety Cabinet

Refer to [ID 8 - Identification of *Clostridium* species](#)

- *Coxiella burnetii* (Q fever)
- *Yersinia pestis* (Plague)

Note: *Brucella* species, *B. mallei*, *B. pseudomallei* and *Y. pestis* are listed in the databases of a number of commercially available kit-based identification systems; results should however be interpreted with caution.

Note: *B. anthracis*, *Brucella* species, *C. botulinum* and *Y. pestis* all cause disease which is reportable to the Local Authority Proper Officer 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:

<https://www.gov.uk/notifiable-diseases-and-causative-organisms-how-to-report>

Note: Brucellosis is reportable under the Zoonosis Order 1989.

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg 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^{60,61}

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 media

NAD-supplemented blood agar is inferior to combined use of blood and chocolate agars for isolation of *H. influenzae* and *S. pneumoniae*⁶². Slight improvement in isolation rates was demonstrated with prolonged incubation (48hr) of cultures.

Evaluations have shown that chocolate agar with bacitracin incorporated (or chocolate agar with a bacitracin disc) may be used in place of chocolate agar. Isolation rates of *H. influenzae* are not significantly different when this medium is used. Competing flora, however, are significantly reduced on bacitracin-incorporated agar and the quantity of growth of *H. influenzae* is greater, which eases follow-up picking of colonies⁶³.

If chocolate agar with bacitracin incorporated into the agar is used then blood agar incubated in 5-10% CO₂ must be included for the isolation of *M. catarrhalis* and *S. pneumoniae*⁶³. It may be difficult to differentiate between specific morphology of Streptococci on chocolate agar and in these instances a blood agar may be considered.

Burkholderia cepacia selective agar is recommended for use in the culture of specimens from patients with cystic fibrosis. It selectively supports the growth of *Burkholderia cepacia* and in this aspect is superior to CLED agar. *B. cepacia* selective agar may also grow *Burkholderia gladioli* and other pseudomonads.

BMPA α is recommended for clinical specimens, although there have been reports of cefamandole being inhibitory to some *Legionella* species⁶⁴⁻⁶⁶. Vancomycin sensitive strains have also been detected.

Incubation in 2-5% CO₂ can enhance growth of some *Legionella* species such as *L. sainthelensi* and *L. oakridgensis*. This low level of CO₂ will not affect the growth of *L. pneumophila*, but CO₂ levels higher than 5% may inhibit growth⁶⁴.

All bacterial media are considerably inferior to fungal media, such as Sabouraud dextrose agar, for the detection of fungi. At risk patients should have specimens plated on fungal media routinely. Incubation temperature influences recovery: specimens with high loads of *Candida* species can obscure the growth of *Aspergillus* species, and culture at 42-45°C prevents *Candida* species growth, allowing *Aspergillus* species to grow. Refrigeration of specimens reduces the yield of mucoraceous moulds.

Interpretation of Gram stained smears

Gram stains on sputum specimens may be used for determining the quality of the specimen and for predicting likely pathogens by their characteristic appearance^{67,68}. Determining the quality of the specimen is based on the numbers of polymorphonuclear leucocytes and squamous epithelial cells (SECs) present: purulent specimens may be selected for culture and non-purulent specimens or specimens contaminated with squamous epithelial cells may be rejected.

Sputum specimens are often not evaluated before culture, and preparation of slides for Gram staining occurs in parallel with specimen processing. 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-viable⁶⁸. It may not be appropriate to identify organisms if gross contamination with oropharyngeal flora is evident for both BAL and sputum samples. The sensitivity of Gram stain can vary and is generally low and is often dependent on the individual reviewing the slide^{40,68,69}. Gram staining may identify yeasts or hyphae, but are inferior to potassium hydroxide (KOH) and fluorescent brighteners.

Various methods of interpreting Gram stained smears by white blood cell and organism counts have been proposed. In BAL specimens Gram staining may be useful to predict results of quantitative culture⁶⁹. In some cases, antimicrobial chemotherapy may be initiated on the results of the Gram stained smear before culture results are available.

Heat treatment of *Legionella* species

Some laboratories heat treat specimens when looking for *legionella* species. Although the method works well in certain contexts it has been shown to add very little to the clinical setting and is not included in this document^{66,70,71}.

1 Safety considerations^{60,61,72-86}

1.1 Specimen collection, transport and storage^{60,61,72-75}

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.

1.2 Specimen processing^{60,61,72-86}

Processing of diagnostic samples that are assessed to be at higher risk of containing hazard group 3 organisms must be undertaken under appropriate containment conditions as determined by risk assessment, as required by Biological agents: managing the risks in laboratories and healthcare premises. This will normally be under full CL3 conditions.

For other sample types as a minimum it is recommended that the processing of all samples, including respiratory samples, which may result in generation of aerosols should be processed in a microbiological safety cabinet in CL2 conditions with additional precautions to minimise risk of aerosols production in accordance with the relevant risk assessment, ACDP and HSE guidelines.

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.

Note: Heat-fixing may not kill all *Mycobacterium* species⁸⁷. Slides should be handled carefully.

Any mould isolated from patients with a travel history to areas where dimorphic or other Hazard Group 3 fungi are endemic should be processed at Category Level 3 until a hazard group 3 fungus is excluded.

Centrifugation must be carried out in sealed buckets which are subsequently opened in a microbiological safety cabinet.

Specimen containers must be placed in a suitable holder.

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

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

2 Specimen collection

2.1 Type of specimens

Bronchial aspirate, transthoracic aspirate, bronchoalveolar lavage, transtracheal aspirate, bronchial brushings, protected catheter specimens, bronchial washings, endotracheal tube specimens, sputum – expectorated

2.2 Optimal time and method of collection⁸⁸

For safety considerations refer to Section 1.1.

Where possible all specimens should be fresh and taken before antimicrobial treatment is started⁸⁸.

Early morning freshly expectorated sputum is recommended for *Mycobacterium* species ([B 40 - Investigation of specimens for *Mycobacterium* species](#)).

Culture for *Legionella* species may still be successful after antimicrobial therapy has been started ([ID 18 - Identification of *Legionella* species](#)).

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 be helpful. Saliva and pernasal secretions are not suitable.

Early morning specimens for examination of *Mycobacterium* species should ideally be collected on at least 3 consecutive days (see [B 40 - Investigation of specimens for *Mycobacterium* species](#)). BAL and associated specimens need specialist collection according to local protocols.

Unless otherwise stated, swabs for bacterial and fungal culture should then be placed in appropriate transport medium⁸⁹⁻⁹³.

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

2.3 Adequate quantity and appropriate number of specimens⁸⁸

Sputum - Ideally, a minimum volume of 1mL.

BAL - It is difficult to be specific on volume required; in principle, as large a volume as possible is preferred.

Numbers and frequency of specimens collected are dependent on clinical condition of patient.

Note: Consideration should be given to use of chain of evidence forms in view of the potential for legal action in the event of infection with *Legionella* species⁹⁴.

3 Specimen transport and storage^{60,61}

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Collect specimens before starting antimicrobial therapy where possible⁸⁸.

BAL 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 has to be 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^{88,95,96}.

4 Specimen processing/procedure^{60,61}

4.1 Test selection

Select a representative portion of specimen for appropriate procedures such as culture for *Mycobacterium* species ([B 40 - Investigation of specimens for *Mycobacterium* species](#)) and investigation of parasites ([B 31 - Investigation of specimens other than blood for parasites](#)) depending on clinical details.

Additional comments for sputum

Induced sputum may be sent for investigation for *P. jirovecii*.

Additional comments for BAL

Culture for *Mycobacterium* species should be performed on all BAL specimens unless special local arrangements do not require this.

Patients considered to be at risk of pulmonary aspergillosis, or in whom fungal infection is suspected, should have a portion of BAL fluid tested for *Aspergillus* galactomannan.

4.2 Appearance

Sputum

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.

BAL

N/A

4.3 Sample preparation

Sputum

Follow manufacturer's instructions for the addition of 0.1% solution of dithiothreitol or N-acetyl L-cysteine (NALC) to sputum.

Dilute 10µL of homogenised sputum in 5mL of sterile distilled water.

Note: For mucoid samples treat as sputum.

BAL^{40,97,98}

Centrifuge BAL at 1200 xg for 10 mins.

Tip off all but 0.5mL of supernatant and re-suspend centrifuged deposit in remaining fluid.

4.4 Microscopy

4.4.1 Standard

BAL

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.

Non-mucoid specimens

Using a sterile pipette place one drop of centrifuged specimen (see Section 4.3) on a clean microscope slide.

Spread this with a sterile loop to make a thin smear for Gram staining.

4.4.2 Supplementary

Sputum

Gram stain

Refer to [TP 39 - Staining procedures](#).

Using a sterile loop take a loopful of homogenised sputum (see Section 4.5.1) and make a thin smear on a clean microscope slide for Gram staining.

Salivary specimens may be rejected before homogenisation or on the basis of a ratio of <2:1 WBCs: SECs determined by a Gram stain at low power magnification (x100).

If a specimen is rejected on the basis of microscopy inform the ward, clinician or GP immediately.

Retain specimens at 4°C for at least 48hr.

Note: Specimens from patients who are immunocompromised, neutropenic or intubated or for culture of *Mycobacterium* species should not be rejected on the basis of the quality of specimen.

Microscopy for *Mycobacterium* species ([B 40 - Investigation of specimens for *Mycobacterium* species](#)) and parasites ([B 31 - Investigation of specimens other than blood for parasites](#)).

KOH - Calcofluor white preparation for fungi ([TP 39 – Staining procedures](#)).

BAL

Indirect immunofluorescent antibody test for *P. jirovecii* using a commercial kit.

Legionella

Fluorescent staining technique.

Homogenised specimens.

Using a sterile pipette place one drop of homogenised specimen (see Section 4.3) on to a clean PTFE microscope slide.

Spread the drop with a sterile loop to make a thin smear for fluorescent staining.

Follow kit manufacturers' instructions.

4.5 Culture and investigation

4.5.1 Standard

Sputum

Inoculate 1µL loopful of the final dilution prepared in 4.3 to each type of media plate (see Section 4.5.2).

For CF and patients who are immunocompromised also inoculate 1µL of the more concentrated sputasol/sputum dilution on the same plates. The dilutions may be plated on to half plates to allow easier comparison of 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 plate⁹⁹.

BAL

Using a sterile loop inoculate each agar plate with the deposit of the centrifuge sample (see [Q 5 – Inoculation of culture media for bacteriology](#)).

Semi-Quantitative method

Centrifuged BAL is re-suspended in the fluid and three serial dilutions are made (1/10, 1/1000 and 1/100,000. Of these dilutions 0.1mL of each is plated out⁹⁷.

	Volume plated to blood and chocolate	Final dilution
Vortexed BAL sample	0.1mL	1:10
Dilute 0.1mL in to 9.9mL saline	0.1mL	1:1000
Dilute 0.1mL in to 9.9mL saline	0.1mL	1:100,000

Quantitate each morphotype present and express as a colony forming unit.

Alternatively a calibrated loop is used. For BAL fluids samples, quantitative calibrated loops designed for the delivery of 0.010 and 0.001 mL are used. After incubation, the colonies are counted on the plates and the number of CFU per millilitre is determined by multiplying the number of colonies by the dilution factor. When using calibrated loops it is important to verify the calibration of the loop. Calibrations should be performed with BAL fluid as the test solution and borderline quantitative culture results should be interpreted with knowledge of the inaccuracy values of the loop¹⁰⁰.

Note: Do not delay between diluting the specimen and inoculating agar plates.

Diagnostic thresholds are 10⁵-10⁶cfu/mL for bronchoscopic aspirates, 10³cfu/mL for protected brush specimens and 10⁴cfu/mL for BAL⁴⁰. 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 may also give false-negative results.

4.5.2 Supplementary

Legionella

Sputum

Inoculate plates directly with 0.1mL of digested sputum (see section 4.3).

Bronchoalveolar lavages

Centrifuge at a minimum of 2000 x g for 15 mins. Use the deposit as the inoculum.

For other respiratory tract specimens select any milky or blood stained portion, if present, for use as the inoculum.

Heavily contaminated specimens should be heat-treated and diluted to decrease the numbers of yeasts, pseudomonads and *Proteus* species and then re-cultured.

Dilution

Dilute the original specimen 1:100 in distilled water and re-culture.

Note: when diagnosing legionellae the use of urinary antigen test can prove useful^{66,101}.

Note: heat treatment does not improve diagnostic yield and is therefore not included in the document⁷¹.

Fungi

Non-CF patients ie immunocompromised and others:

After treating with mucolytic agent if required, spin entire sample. Examine part of residue with KOH and calcofluor white staining and culture the remainder.

CF Patients

After treating with a mucolytic agent plate culture one aliquot of 10uL and one aliquot of 100uL and spread well over the plate. Spin the remaining sample and examine part of the residue with KOH and calcofluor white staining and culture the remainder.

Other

Mycobacterium species ([B 40 - Investigation of specimens for *Mycobacterium* species](#)) and parasites ([B 31 - Investigation of specimens other than blood for parasites](#)).

Molecular detection methods

Numerous pathogens can be detected in respiratory samples by nucleic acid amplification or polymerase chain reaction (PCR) methods¹⁰². The advent of real-time PCR has allowed diagnoses to be made in a few hours. Many tests are available as commercial kits. PCR methods are always quicker than conventional methods and are usually more sensitive as well, potentially having a significant impact on treatment decisions.

4.5.3 Culture media, conditions and organisms for BAL samples

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Bronchitis	BAL	Chocolate agar*	35-37	5-10%	40-48hr	Daily	<i>H. influenzae</i>

Investigation of bronchoalveolar lavage, sputum and associated specimens

Chest infection Chronic obstructive airways disease Community-acquired pneumonia Hospital-acquired pneumonia		+ Bacitracin disc or incorporated in the medium		CO ₂			<i>M. catarrhalis</i> <i>S. aureus</i> <i>S. pneumoniae</i> Other organisms in pure growth may be significant
		Sabouraud agar (Screw-capped Universals should be used If dimorphic fungi suspected)	35-37 42-44	air	5d‡ 5d‡	≥40hr	Fungi
		CLED or MacConkey agar	35-37	air	40-48hr	Daily	Enterobacteriaceae Pseudomonads

For these situations, add the following:

Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Bronchiectasis Cystic fibrosis	BAL	Mannitol Salt/Chromogenic Agar	35-37	air	40-48hr	Daily	<i>S. aureus</i>
Cystic fibrosis ²⁹	BAL	<i>B. cepacia</i> selective agar Broth or solid medium as per B40	35-37	air	5d	Daily for five days	<i>B. cepacia</i> complex <i>M. abscessus</i> **
Pneumonia or flu like symptoms	BAL	<i>Legionella</i> selective agar***	35-37	Moist Atmos	Up to 10d	at 3d, 7d and 10d	<i>Legionella</i> species

Other organisms for consideration –

Mycobacterium species ([B 40 - Investigation of specimens for *Mycobacterium* species](#)) and parasites ([B 31 - Investigation of specimens other than blood for parasites](#)).

* If chocolate agar with bacitracin incorporated into the agar is used then blood agar incubated in 5-10% CO₂ must be included for the isolation of *M. catarrhalis* and *S. pneumoniae*⁶³. It may be difficult to differentiate between specific morphology of Streptococci on chocolate agar and in these instances a blood agar may be considered.

** Testing for *M. abscessus* should be carried out on request or at a patients Annual Review²¹⁻²⁴.

*** Buffered cefamandole, polymyxin, anisomycin, α-ketoglutarate medium (BMPAα) or Buffered charcoal yeast extract, anisomycin agar (BCYE) ⁶⁴⁻⁶⁶. It should also be noted that incubation in 2-5% CO₂ can enhance growth of some *Legionella* species such as *L. sainthelensi* and *L. oakridgensis*. This low level of CO₂ will not affect the growth of *L. pneumophila*, but CO₂ levels higher than 5% may inhibit growth ⁶⁴. The incubation of plates in 2-5% CO₂ is not compulsory; this is only mentioned for laboratories that may want 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.

‡Fungal culture may need to be prolonged (up to 6 weeks) if dimorphic fungal pathogens are suspected; in such cases the screw-capped bijoux bottles should be read at 40hr and then left in the incubator/cabinet.

4.5.4 Culture media, conditions and organisms for sputum specimens

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Bronchitis Chest infection Chronic obstructive airways disease Pneumonia	Sputum	Chocolate agar* + Bacitracin disc or incorporated in the medium	35-37	5-10% CO ₂	40-48hr	Daily	<i>H. influenzae</i> <i>M. catarrhalis</i> <i>S. aureus</i> <i>S. pneumoniae</i> Other organisms in pure growth may be significant
For these situations, add the following:							
Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Bronchiectasis Cystic fibrosis Immunocompromised/ ITU	Sputum	CLED agar or MacConkey agar	35-37	air	40-48hr	Daily	Enterobacteriaceae Pseudomonads
		Mannitol Salt / Chromogenic Agar	35-37	air	40-48hr	Daily	<i>S. aureus</i>
		Sabouraud agar	35-37	air	40-48hr†	≥40hr	Fungi
Cystic fibrosis ²⁹	Sputum	<i>B. cepacia</i> selective agar Broth or solid medium as per B40	35-37	air	5d	Daily	<i>B. cepacia</i> complex <i>M. abscessus</i> **
Mycological investigations	Sputum	Sabouraud agar (Screw-capped Universals should be used if dimorphic fungi suspected)	35-37	air	40-48hr†	≥40hr	Fungi
Legionella suspected	Sputum	<i>Legionella</i> selective agar***	35-37	Moist Atmos	Up to 10d	at 3d, 7d and 10d	<i>Legionella</i> species
Other organisms for consideration - <i>Mycobacterium</i> species (B 40 - Investigation of specimens for <i>Mycobacterium</i> species) and parasites (B 31 - Investigation of specimens other than blood for parasites).							
<p>*If chocolate agar with bacitracin incorporated in the agar is used then blood agar incubated in 5-10% CO₂ must be included for the isolation of <i>M. catarrhalis</i> and <i>S. pneumoniae</i>⁶³. It may be difficult to differentiate between specific morphology of Streptococci on chocolate agar and in these instances a blood agar may be considered.</p> <p>** Testing for <i>M. abscessus</i> should be carried out on request or at a patients Annual Review²¹⁻²⁴.</p>							

*** Refer to the information on Table 4.5.3.

†Fungal culture may need to be prolonged (up to 6 weeks for *P. brasiliensis*) if clinically indicated; in such cases the screw-capped bijoux bottles should be read at ≥40hr and then left in the incubator/cabinet.

4.6 Identification

Refer to individual SMLs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Burkholderia and related species	species level (see ID 17 – Identification of Pseudomonas species and other non-glucose fermenters)
S. maltophilia	species level
Enterobacteriaceae	From community samples to coliform level From inpatients to species level
Klebsiella pneumoniae	species level
Moulds	genus level
H. influenzae	species level
M. catarrhalis	species level
N. meningitidis	species level
Pasteurella	species level
Pseudomonads	"pseudomonads" level
P. aeruginosa	mucoid or non-mucoid species level
S. aureus	species level
S. pneumoniae	species level
Yeasts	"yeasts" level
Legionella	species level
<i>Mycobacterium</i>	see B 40 - Investigation of specimens for Mycobacterium species
Parasites	see B 31 - Investigation of specimens other than blood for parasites

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

4.7 Antimicrobial susceptibility testing

Refer to [British Society for Antimicrobial Chemotherapy \(BSAC\)](#) and/or [EUCAST](#) guidelines.

4.8 Referral for outbreak Investigations

N/A

4.9 Referral to reference laboratories

Legionella species obtained from clinical material must be referred for identification and serogrouping.

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, or associated with 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 and Wales

<https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services>

Scotland

<http://www.hps.scot.nhs.uk/reflab/index.aspx>

Northern Ireland

<http://www.belfasttrust.hscni.net/Laboratory-MortuaryServices.htm>

5 Reporting procedure

5.1 Microscopy

If the patient is immuno-competent, report poor quality or salivary specimens as:

"Poor quality specimen/salivary specimen received. Please repeat if clinically indicated".

Gram stain (if performed).

Report on epithelial cells, WBCs and organisms detected.

Report on fungal hyphae detected.

Legionella pneumophila detected by immunofluorescence or

Legionella pneumophila not detected by immunofluorescence.

P. jirovecii immunofluorescence

P. jirovecii cysts detected by immunofluorescence or

P. jirovecii cysts NOT detected by immunofluorescence.

Microscopy for *Legionella*, *Mycobacterium* species ([B 40 - Investigation of specimens for *Mycobacterium* species](#)) and parasites ([B 31 - Investigation of specimens other than blood for parasites](#)).

5.1.1 Microscopy reporting time

All results should be issued to the requesting clinician as soon as they become available, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

5.2 Culture

Report clinically significant organisms isolated and their amount if BAL and semi-quantitative method employed or

Report other growth, eg: Mixed upper respiratory tract flora or

Report absence of growth or

Report absence of growth of specifically targeted organism at a 10^{-6} dilution of the specimen (for CF and bronchiectasis patients)

Report results of supplementary investigations.

5.2.1 Culture reporting time

Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

Supplementary investigations, *Mycobacterium* species ([B 40 - Investigation of specimens for *Mycobacterium* species](#)) and parasites ([B 31 - Investigation of specimens other than blood for parasites](#)).

5.3 Antimicrobial susceptibility testing

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

6 Notification to PHE^{103,104}, or equivalent in the devolved administrations⁵⁶⁻⁵⁹

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) 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 PHE 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.

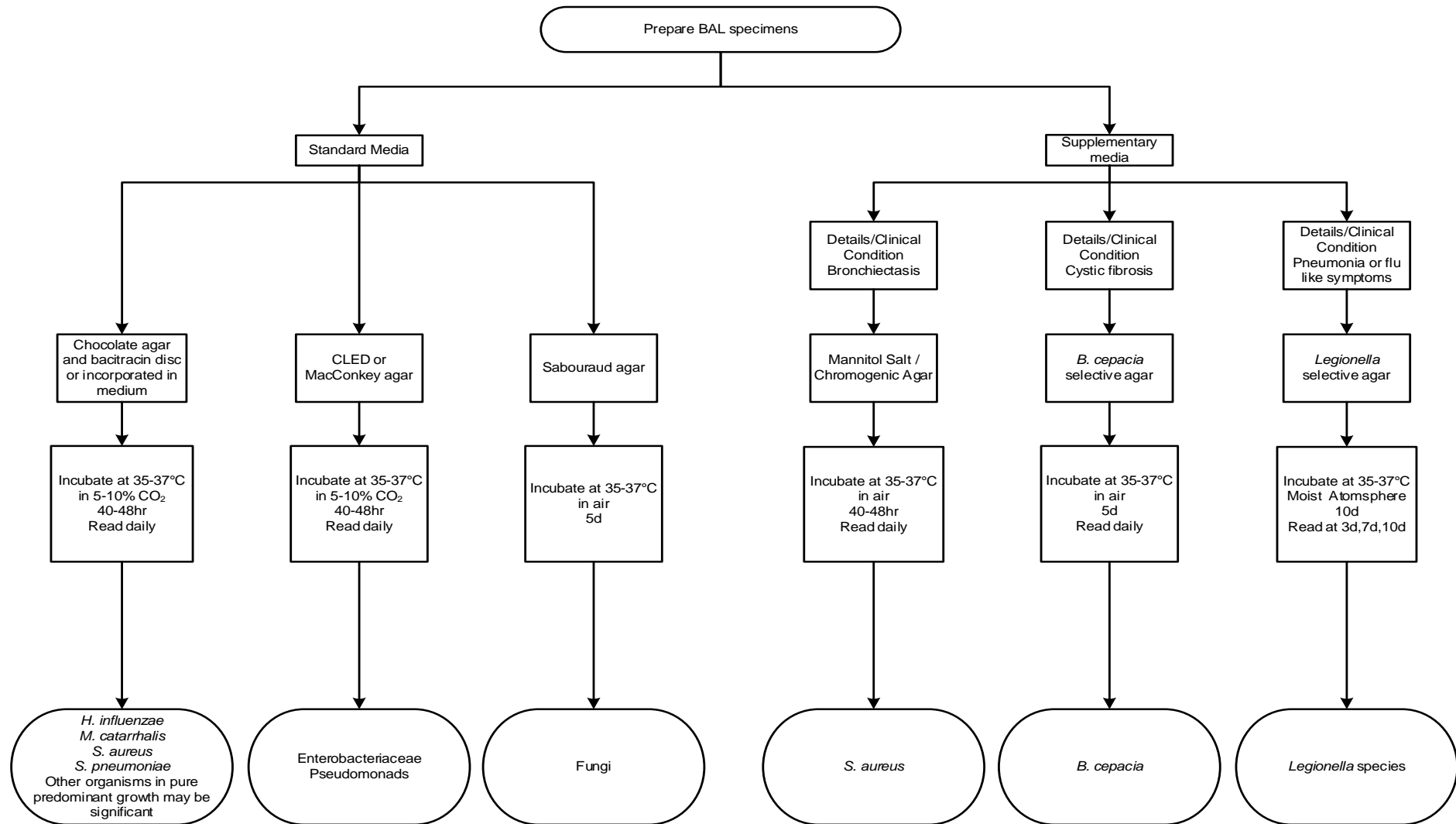
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local 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 (HCAs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

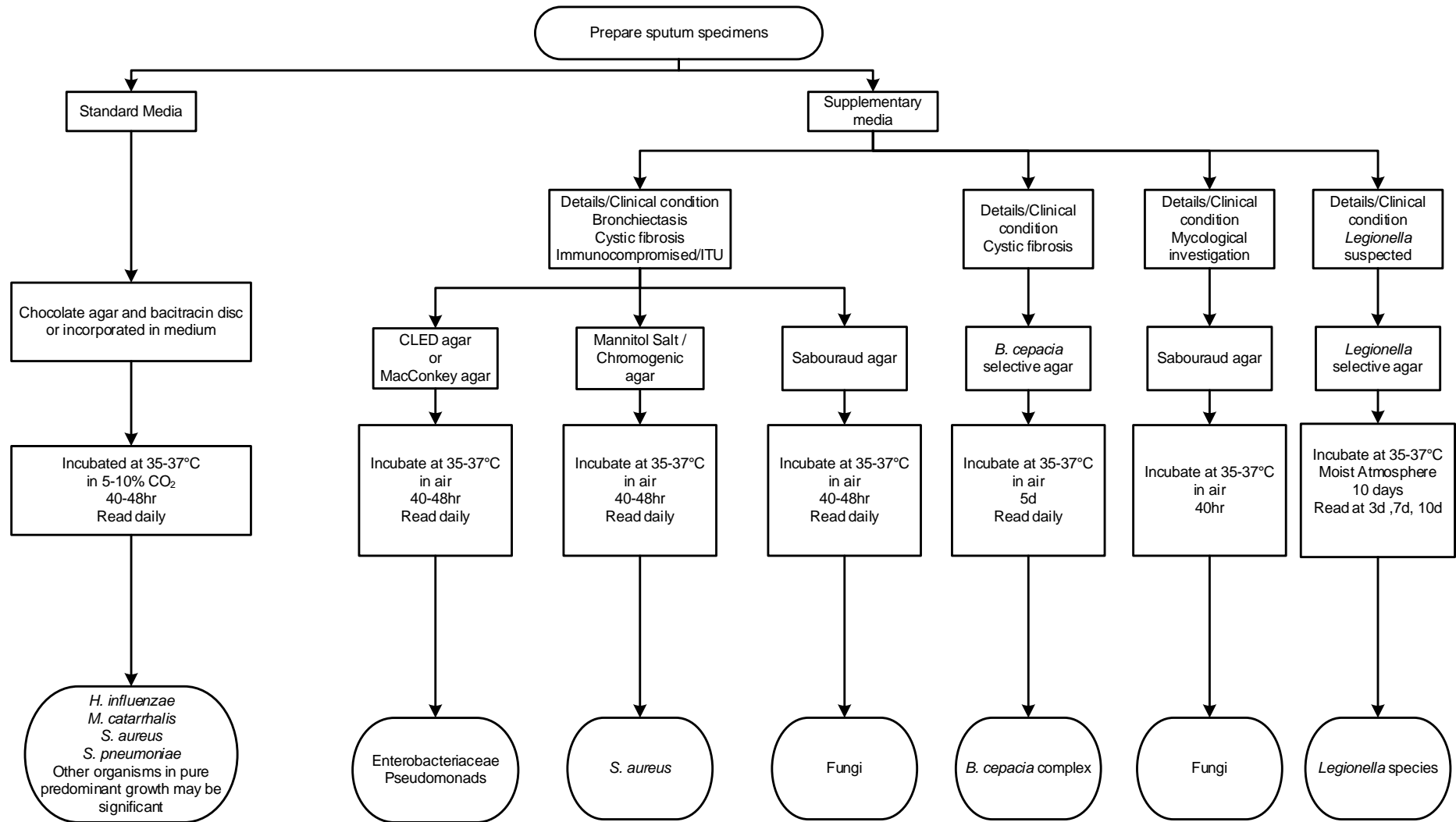
<https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010>

Other arrangements exist in [Scotland](#)^{56,57}, [Wales](#)⁵⁸ and [Northern Ireland](#)⁵⁹.

Appendix 1: BAL specimens for culture



Appendix 2: Sputum specimens for culture



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