UK Health Security Agency

UK Standards for Microbiology Investigations

Investigation of samples for lower respiratory tract infections caused by fungal 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**

*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 used for the diagnosis of fungal infections of the lower respiratory tract including:

- Isolation and identification of fungi using culture and microscopy
- Detection of fungi in specimens using molecular testing including Nucleic Acid Amplification Tests (NAATs), Polymerase Chain Reaction (PCR) and immunological assays

The specific tests performed vary based on the patient group and sample type, and a combination of parallel tests are often required to support clinical diagnosis.

UK SMIs should be used in conjunction with other relevant UK SMIs, for example 'UK SMI B 57: Investigation of samples for lower respiratory tract infections caused by bacterial pathogens'.

Please note that following the recent update to fungal taxonomy, both the previous and updated names are included in this document as necessary (1-3).

4 Introduction

Respiratory fungal infections pose a significant burden on healthcare in immunocompromised patients or patients with underlying chronic medical conditions. Critically ill patients with severe respiratory viral infections are also at significant risk from secondary fungal infection. Such infections can present with a wide range of clinical symptoms, from sensitisation and asthma to bronchitis, pneumonia, and even invasive disease that may disseminate. Rapid and accurate laboratory diagnosis of lower respiratory tract infections (LRTIs) is essential to inform clinical decision-making and ensure appropriate patient management while preventing unnecessary antimicrobials use (4).

Note: Certain patient groups may be included in both the immunocompromised and immunocompetent sections below. This overlap is particularly relevant to fungal investigations, as immune status can vary depending on the underlying condition or clinical context which can alter the disease presentation and diagnostic requirements.

4.1 Respiratory fungal infections in immunocompromised patients

People with weakened immune systems (immunocompromised) have lower defences against infections, and their bodies may have a harder time building lasting protection from past immunisation or infection. People can be immunocompromised because of a medical condition or because they receive immunosuppressive medications or treatments (refer to CDC for more information).

Fungal pathogens, including Aspergillus (causing aspergillosis), Cryptococcus (leading to cryptococcosis), Pneumocystis jirovecii (causing Pneumocystis pneumonia (PCP)), and Mucoraceous moulds (causing mucormycosis) are the primary cause of life-threatening invasive respiratory diseases in immunocompromised patients, with the potential to disseminate to other organs. Endemic fungi (see section 4.2 below) can cause infection in both immunocompetent and immunocompromised individuals. However, the presentation is often more severe in immunocompromised, e.g. Histoplasmosis in HIV patients.

A. fumigatus is the most common cause of invasive aspergillosis, however other Aspergillus species (e.g., A. flavus, A. niger and A. terreus) can also cause the disease. In high-risk groups including patients with acute myeloid leukaemia (AML) and other haematological conditions, the prevalence is typically below 5% when mould-active prophylaxis is used, compared with 10–15% in its absence.

Cryptococcosis is a life-threatening fungal infection with a significant global burden. Infection is primarily acquired via inhalation but occurring mainly upon reactivation after a period of latency. Infection mostly affects the central nervous system, with lung infection typically mildly or asymptomatic. Dissemination via fungaemia to any organ can occur. It is primarily acquired via inhalation but occurring mainly upon reactivation after a period of latency. Cryptococcal meningitis is the most severe and fatal form of the disease. The Cryptococcus neoformans species complex, listed by the World Health Organization (WHO) as a top- priority fungal pathogen, predominantly causes disease in people living with HIV, while the Cryptococcus gattii species complex more frequently affects individuals who appear immunocompetent (5).

Pneumocystis jirovecii pneumonia (PJP) is still widely referred to in clinical practice as Pneumocystis carinii pneumonia (PCP). While P. jirovecii causes severe pneumonia in patients with advanced HIV infection, most cases now occur in HIV-negative patients receiving chronic immunosuppressive therapy. In HIV positive patients, disease presentation can be acute particularly, in newly diagnosed HIV cases or subacute illness. In contrast, HIV-negative immunocompromised patients often present with a more acute illness and may have lower organism burden. Established HIV treatment and PJP/PCP prophylaxis will reduce the pretest probability (baseline likelihood) of disease over time for a positive NAAT result.

Diagnosis is made by detection of the organism in either induced sputum or bronchoalveolar lavage fluid (BALF) and is supported with the detection in serum of 1-3-β-D-Glucan (BDG), a non-specific biomarker of fungal cell wall polysaccharides. Where diagnostic uncertainty persists despite non-invasive and lowerrespiratory testing, further sampling, including tissue sampling in selected cases, may be considered following specialist clinical discussion.

Infections involving the highly resistant species within the Mucorales order are increasing. These widespread environmental moulds cause an invasive, life-threatening infection in humans known as mucormycosis, the most common pathogens including species of Rhizopus, Mucor, Lichtheimia (formerly classified under Absidia and Mycocladus), Rhizomucor, Cunninghamella, Apophysomyces, and Saksenaea. Key risk factors for mucormycosis include diabetes mellitus, COVID-19, and increased immunosuppression (4). Clinical symptoms vary depending on the affected area, such as the sinuses, brain, lungs, skin, stomach, and intestines.

Candida species are commensals of the respiratory tract. The growth of Candida from respiratory secretions usually indicates colonisation and is not definitive of infection and therefore rarely requires treatment with antifungal therapy. Isolation of Candida species from respiratory samples in a severely immunosuppressed patient should prompt assessment for invasive candidiasis in the appropriate clinical context, rather than treatment of respiratory colonisation alone. For more information see guideline on the management of candidiasis (6).

Refer to Table 3 in the appendix for classification of patients at high risk of invasive pulmonary fungal infections based on their underlying conditions.

4.2 Respiratory fungal infections in immunocompetent patients with or without co-morbidities

Invasive aspergillosis in immunocompetent individuals is rare. Prevalence amongst non-neutropenic, mechanically ventilated patients in intensive care is unclear, but may approach 12% amongst mechanically ventilated patients (7) with severe viral pneumonia/pneumonitis, such as patients with SARS-CoV-2 virus infection (8). Influenza-associated pulmonary aspergillosis (IAPA) is a more common (prevalence up to 20%) complication of severe influenza and is associated with increased mortality in critically ill patients. Therefore, early diagnosis and antifungal treatment are crucial for improving patient outcomes.

Chronic pulmonary aspergillosis (CPA) is the most common manifestation of aspergillosis in patients with pre-existing lung conditions such as tuberculosis or COPD. Aspergillus antibody measurement is the key diagnostic of CPA since symptoms and radiological appearances of CPA overlap with TB and other fungal infections. A chronic infection can become invasive when immunosuppressive therapies (e.g., prolonged high-dose corticosteroids) are used to manage the underlying respiratory condition.

Allergic bronchopulmonary aspergillosis (ABPA) is a notable complication of asthma and cystic fibrosis, affecting from 1 in 100 to 1 in 20 adults with asthma and approximately 18% of patients (adults and children) with cystic fibrosis. A key diagnostic criterion for ABPA is a positive *Aspergillus* specific IgE, representing sensitisation (or allergy) to *A fumigatus* (9).

It has been reported that approximately one-third of cryptococcosis cases occur in individuals with no readily identifiable immune defects (10,11). In particular, *Cryptococcus gattii* species complex more frequently affects individuals who appear immunocompetent (5). This highlights the importance of considering cryptococcosis in the differential diagnosis of mediastinal mass in an immunocompetent person (12).

Endemic mycoses are a group of diseases caused by dimorphic fungi that are endemic to specific regions of the world. Examples of endemic fungi that may have been imported into the UK include histoplasmosis (*Histoplasma capsulatum*), coccidioidomycosis (*Coccidioides* species), blastomycosis (*Blastomyces dermatitidis*), paracoccidioidomycosis (*Paracoccidioides brasiliensis*) and Talaromycosis (*Talaromyces marneffei*). These fungi are among the most common causes of infection in both immunocompetent and immunocompromised hosts within these regions and are the primary cause of life-threatening invasive respiratory diseases in immunocompromised patients, with the potential to disseminate to other organs. Although these infections have distinct characteristics, they can be difficult to differentiate clinically from other infectious and non-infectious respiratory illnesses, particularly in their early stages. Proper biosafety measures are crucial when handling these organisms. Laboratory diagnosis involves fungal microscopy, culture, serological testing, and NAAT. If a patient with a history of travel to an endemic area presents with symptoms consistent with an endemic mycoses and fails to respond to antibiotic therapy, fungal testing, including specific serological tests, should be considered (4) (13).

Refer to Table 4 in the appendix for the classification of patients at high risk of chronic pulmonary fungal infections based on their underlying conditions.

The subsequent sections will explore the types of samples collected and the laboratory investigations conducted for LRTIs.

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 (14-35).

5.1 Specimen collection, transport and storage

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 are essential.

5.2 Specimen handling, processing and examination

It is recommended that all respiratory samples with the potential to generate aerosols during processing be handled within a 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.

CL3 precautions are particularly important unless it can be definitively ruled out that the specimen contains a Hazard Group 3 pathogens 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 or mixing with lysis buffer for molecular testing, the specimen may be handled under Containment Level 2 conditions, providing the efficacy of inactivation has been demonstrated and absence of relevant travel/exposure history can be confirmed.

Note: Heat-fixing may not completely inactivate all *Mycobacterium* species, and slides should therefore be handled with caution.

Additionally, the type, selection and use of a microbiological safety cabinet as well as transport of biological agents should also be considered to further minimise the risk of transmission during handling of the organism.

Processing of low-risk community and cystic fibrosis samples without clinical suspicion of TB, can be conducted in Class I in CL2. However, any sputa where the clinical details indicate possible *Mycobacterium* species, endemic/dimorphic fungi or international travel or previous isolation of a hazard group 3 organism e.g. *Burkholderia pseudomallei* must only be processed at Containment level 3 until excluded.

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 lead to environmental contamination.

Avoid staining and microscopy if there is any clinical suspicion or travel history indicating possible endemic/dimorphic fungal infection.

All routine sputum with fungal infection suspected/requested should be incubated in CL3 laboratory.

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.

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). Also view WHO fungal priority pathogens list to guide research, development and public health action.

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.

6 Pre-laboratory processes

6.1 Specimen type

See table below for a list of specimen types included within the scope of this document along with recommended investigations relevant to the specimen type.

Specimen Type	Microscopy	Culture	Molecular (refer to Table 2 for tests)	Biomarkers (Ag)		Serology (Ab)
				Galactomannan antigen	1-3-β-D-Glucan (BDG)	
Lower Respiratory Tract						
Sputum: expectorated, induced	✓ [a]	✓	✓	See section 6.1.1		
Bronchoalveolar lavage (BALF) / washing (directed/non-directed)	✓	✓	✓	✓		
Endotracheal (ET) aspirate	✓	✓	✓	See section 6.1.1		
Bronchial brushing/aspirate		✓	✓	See section See 6.1.1		
Upper Respiratory Tract						
Nasopharyngeal aspirates			✓ [b]	See section 6.1.1		
Throat swabs			✓ [b]			
Other						
Plasma/serum			✓	✓ [c]	✓	✓
Urine				✓ [d]		

a) On clinical request for cases with high clinical suspicion. Where possible this should be performed from sputolysed, concentrated sputum.
b) Pneumocystis PCR only
c) Neutropenic patients only (36)
d) Primarily for diagnosis of Histoplasmosis

6.1.1 Testing non-BAL specimens by galactomannan antigen

Aspergillus Galactomannan assays are not validated by manufacturers for use on respiratory specimens other than BAL. However, in clinical scenarios where it is not possible to obtain BAL specimens, galactomannan testing of NBL, bronchial wash, ET aspirates, sputum or NPA specimens may be useful as part of an overall testing strategy in combination with other fungal diagnostic tests, provided that the result is not used as the sole diagnostic criterion and that the limitations of this approach and the clinical risks are understood:

- false positive results occur due to the complex nature of the specimen being tested.
- false negative results occur due to the limited sensitivity of the specimen being tested.

If galactomannan results are reported from non-validated respiratory specimens, they must also have an appropriate comment to state; **Note**: this assay is not validated for this specimen type and may result in false positive and false negative results. Results must be interpreted with caution and alongside other diagnostic biomarkers, radiological and clinical findings.

6.1.2 Additional sample types for consideration

- Protected catheter specimens: It is generally not recommended for routine testing but may be processed only upon specific clinician request.
- Cough swabs: These samples are not considered suitable for respiratory fungal investigation, as there is currently insufficient evidence for their reliability or diagnostic value or superiority over standard throat swab in detecting respiratory fungal pathogens. Cough swabs also pose an infection prevention and control risk due to the potential for aerosol generation and contamination during sample collection.
- Throat swabs have shown good specificity when performing P. jirovecii NAAT on patients specifically considered at risk of Pneumocystis pneumonia but have not yet been validated for the diagnosis of other respiratory fungal infections and its use with syndromic panels for the generic testing of respiratory infections is not fully validated. The presence of wooden applicators can also pose a source of fungal contamination (e.g., *Aspergillus* species).
- Transtracheal aspirate: It is a procedure that carries clinical risks and is therefore rarely performed in the UK.
- Transthoracic aspirate and tissues: 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.
- Sterile fluid including pleural fluid: refer to UK SMI B 26 - Investigation of fluids from normally sterile sites. For a sterile sample suspected of fungal infection, NAAT, galactomannan, and BDG tests can be used as adjuncts to fungal culture, especially in high-risk immunocompromised patients.

6.3 Specimen collection and handling

6.3.1 Respiratory specimens

Collect specimens as soon as possible after onset of symptoms.

Generally, the collection and transport requirements for respiratory specimens for fungal investigations are similar to those for bacteria. However, to improve fungal culture recovery, a high volume of respiratory sample is optimal (37,38). An ideal total volume for sputum / ETA / aspirate is 2 mL and for BALF 5 to 10 mL.

- Collect specimens before antifungal therapy where possible. If this is not possible, this must be clearly stated on the request form.
- Indication of travel throughout the patient's lifetime to any areas where dimorphic fungi are present should be stated on the request form.

Samples must be collected in a CE/UKCA marked leak-proof container, which has only been opened for the minimal amount of time. All containers must be properly labelled.

6.3.2 Other specimens

- Blood samples may be collected as described for the following:
    - Fungal biomarker detection should be taken in plasma or serum tubes. Avoid the use of gel clot tubes. A minimum sample volume of 4-6mL is optimal to facilitate combined biomarker testing.
    - Molecular testing including NAAT (see Table 2) especially in immunocompromised or neutropenic patients should be taken in plasma or serum tubes. A minimum sample volume of 2 mL is required for a single investigation, and ideally 2-5 mL if multiple tests are required.
- *P. jirovecii* NAAT is ideally performed on respiratory specimens. However, testing may be performed on blood samples following appropriate local validation and governance approval. Testing should only be requested in patients with a high clinical suspicion of PJP, supported by clinical and radiological findings and potentially by a strongly positive serum B-D-Glucan result. When included as part of a multiplex PCR assay, a positive result should not be released in the absence of clinical suspicion.
- A fresh <24 hours old urine is required for Histoplasma testing in a sterile, leak-proof container and must not contain boric acid. A minimum sample volume of 2mL is optimal.
- Throat swabs for *Pneumocystis* molecular testing should ideally be collected using synthetic swabs and transported as determined by local validated protocols, either in an empty swab container (dry) or in a variety of transport media. Charcoal swabs can be inhibitory to NAATs and should be avoided.

6.4 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.

To minimise the overgrowth of commensal, colonisation or contaminating fungi within the respiratory tract/samples, delays to the transport of specimens for fungal specimens should be avoided.

If a delay in transport is expected please store the sample in a refrigerator prior to transfer, except in cases where there is a specific risk of mucoraceous mould infection. In the respective instance ensure clear communication is given to the laboratory to process fungal cultures urgently to preserve the viability of the fungi within the specimen.

Urine for histoplasma antigen testing should be <24 hours old and sent to the laboratory urgently as freezing is required to maintain antigen stability and sample quality.

7 Laboratory processes

7.1 Sample preparation

- If a single specimen is received with multiple tests requested, ensure that the appropriate separation of the sample is conducted for parallel testing. A representative portion such as purulent material should be selected for each test to optimise culture sensitivity and avoid cross-contamination between procedures.
- Appropriate specimen collection and handling prior to examination are crucial for optimal results. Therefore, when a case is suspected, effective communication and close collaboration between clinicians and the microbiology laboratory are essential to ensure that every step of the diagnostic process is properly carried out.
- Salivary sputum specimens from immunocompetent patients may be rejected with a comment stating:

  ‘Sample not processed due to concerns over quality. If symptoms persist, please submit an expectorated sputum specimen’.

- Samples from immunocompromised patients should not be rejected on the basis of the specimen quality. 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’.

7.1.1 BALF/bronchial washings

- BALF specimens generally do not require pre-treatment with a mucolytic agent.
- Centrifuge samples >4mL at 1200 × g for 10 min.

  Note: in cases with suspected mucoraceous mould infection, separate a portion of the sample prior to centrifugation.

  Resuspend sediment in an appropriate residual volume of 1-2 mL of supernatant, according to starting volume and downstream testing requirements.

7.1.2 Sputum/ETA

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

Notes:

- The use of mucolytic reagents may result in contamination with low levels of *Aspergillus* spp. This is important to consider when interpreting results, as *Aspergillus* spores may be present in reagents due to preparative measures such as lyophilisation.
- It is imperative that mucolytic reagents are batch tested prior to use in centres using molecular NAAT assays for the diagnosis of fungal infection to ensure no contamination fungal DNA/spores are present within the mucolytic agents. Negative process controls should also be inclusive of the specimen processing pathway prior to being aliquoted for molecular testing.
- If the sample is greater than or equal to 5mL centrifuge the entire sample at 1200 × g for 10 min. However, in cases with suspected mucoraceous mould infection, separate a portion of the sample prior to centrifugation.
- Sediment should be resuspended in 1-2 mL (dependant on how many aliquots are required for testing) of supernatant.

7.1.3 Tissue biopsy

Samples should be cut into multiple small pieces, do not grind or homogenise tissue to assure viability (See UK SMI B 17: Tissues and biopsies from deep-seated sites and organs

7.2 Microscopy

7.2.1 BALF/ bronchial washings

If fungal infection is suspected from the Gram stain, examine a representative portion of specimen for fungal hyphae at 10x and 40x magnification using fluorescent stain/ brighteners such as calcofluor white or blankophor white. Systematic examination of the whole microscopic sample is essential before reporting as negative, as the distribution of fungal elements can be uneven.

Microscopic examination using a fluorescent stain/brightener should be performed on all BALF samples with fungal investigation request, preferably from the original spun deposit. However, avoid staining and microscopy if there is any clinical suspicion or travel history indicating possible endemic/dimorphic fungal infection.

Refer to UK SMI TP 39 – Staining procedures for detailed protocols for bacterial and fungal staining.

Recommended diagnostic test for *Pneumocystis jirovecii* is NAAT (see section 7.5 'Molecular and immunological tests'). However, species specific immunofluorescence antibody tests can be done. For example, staining for *P. jirovecii* is more commonly done by specific immunofluorescence antibody methods, Periodic acid–Schiff staining or by silver staining.

7.2.2 Sputum

Sputum is generally not considered an appropriate specimen for fungal microscopy due to its limited sensitivity and the potential for contamination with upper respiratory tract flora including yeasts.

However, in cases with a high clinical index of disease where a BAL is unable to be collected and a liquified sputum or ET tube aspirate is available the test may be performed on clinical request. To concentrate the sample centrifuge the entire sample at 1200 × g for 10 min, carefully remove the supernatant and resuspend the pellet and process for Calcofluor as per BAL. Adding a drop of KOH to the slide with calcofluor can aid the mucous digestion.

If the sputum specimen is too mucoid for centrifugation a direct smear may be performed but will have reduced sensitivity.

7.3 Culture

Overall, the sensitivity of fungal culture can be enhanced by using media specifically designed to support fungal growth, rather than general bacterial media. For example, Sabouraud dextrose agar supplemented with chloramphenicol or gentamicin is commonly used to isolate fungi. For patients at higher risk of fungal infections, it is recommended to routinely inoculate specimens on fungal media. The selection of the appropriate medium for the primary isolation of fungi from clinical samples depends on the suspected species typically associated with a given site or clinical condition.

Incubation temperature influences recovery. Specimens with high loads of *Candida* species can obscure the growth of *Aspergillus* species. Incubating cultures at 42-45°C prevents the growth of most Candida and associated ascomycetous yeast, allowing *Aspergillus* species (particularly *A. fumigatus*) to grow. Refrigeration of specimens reduces the yield of mucoraceous moulds. To improve the recovery of dimorphic and other fastidious fungal pathogens, it is recommended to use additional enriched media, such as Brain Heart Infusion (BHI) agar.

Notes:

- Ensure fungal culture media used for respiratory specimens does not contain cycloheximide, as it may inhibit the growth of clinically significant fungi. Cycloheximide-containing media should only be used for the isolation of dermatophytes.
- *Pneumocystis* is not culturable, and therefore culture does not contribute to the diagnosis of *Pneumocystis* pneumonia.

7.3.1 Sputum/ETA

- To enhance fungal recovery, it is advised to use a pastette to inoculate a high volume of specimen onto Sabouraud agar plates or slopes, prepared in duplicate (37).
- In routine patient samples, each Sabouraud agar should be inoculated with a minimum of 100 to 500 µL of specimen (note: one drop from a pastette represents ~40µL so 3-12 drops per plate or slope is optimal).
- In high-risk patients, two plates or slopes should be set up and each should be inoculated with a minimum of 500 µL of specimen, to ensure at least 1mL of specimen is investigated.
- When using Sabouraud agar plates for culture:
    - Ensure the inoculum is spread evenly across the surface, either by tilting the plate, manual rotation, or using a sterile loop or sterile plate spreader.
    - Allow the sample to dry onto the agar surface where possible.
    - Where possible incubate plates inside individual bags to prevent drying out do not seal airtight to allow air to permeate.
- If a hazard group 3 fungi is suspected perform pure cultures on slopes rather than plate. incubate slopes for up to 8 weeks due to the protracted growth time of some endemic fungi
- For more information on culture media, conditions, and organisms for mycological investigation refer to Table 1.

7.3.2 BALF/ bronchial washings

- Use a pastette to inoculate a high volume of specimen onto each Sabouraud agar. This is recommended to increase the recovery of various moulds requiring a longer incubation period. For more information on culture media, conditions, and organisms for mycological investigation refer to Table 1.
- Process specimens as per sputum protocol.

Ensure that storage and retention of samples is in accordance with The Royal College of Pathologists guidance (39)

7.3.3 Tissue biopsy

Inoculate each agar plate with the cut pieces of tissue.

7.3.4 Brush specimens

Place the brush on the surface of a culture medium and gently rotate it on the surface of the medium using a sterile pair of forceps. If received in fluid refer to local protocols for testing both brush and/or fluid.

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

Clinical details/ conditions	Specimen	Media	Incubation			Cultures read	Target organism(s)
			Temperature °C	Atmosphere	Time		
Fungal culture requested - no clinical details or patient determined as low risk for fungal infection	Routine sputum, aspirates, NBL, BALF	High volume [a] culture on Sabouraud dextrose agar supplemented with chloramphenicol or gentamicin	35-37	Air	7-10 days [b]	Daily	*Moulds*
Additional investigations to enhance fungal recovery in high-risk patients (see examples of high-risk patients in the appendix, should be determined locally)							
Clinical details/ conditions	**Specimen**	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temperature °C	**Atmosphere**	**Time**		
High risk patient	Sputum/ET aspirate/NBL	High volume [a] culture on Sabouraud dextrose agar supplemented with chloramphenicol or gentamicin	35-37 [c] 28-30 [d] (supplementary)	Air	14 days [b]	Every 24-48 hours	*Moulds* *Cryptococcus species*
	BALF				14 days		
Specific risk for Aspergillosis	All respiratory samples from high-risk patients (capacity permitting)	High volume [a] culture on Sabouraud dextrose agar supplemented with chloramphenicol or gentamicin	Process as high-risk patient with the addition of a culture at 40 - 45°C [e]	Air	14 days [b]	Every 24-48 hours	*Aspergillus* species

Clinical details/ conditions	Specimen	Supplementary media	Incubation		Cultures read	Target organism(s)	Clinical details/ conditions	Specimen
			Temperature °C		Atmosphere	Time		
Specific risk for Mucormycosis	BALF (unprocessed sample) [f]	High volume [a] culture on Sabouraud dextrose agar supplemented with chloramphenicol or gentamicin	28-30 and 35-37		Air	7 days [b]	Daily	*Mucorales*
Specific risk for Mucormycosis	Biopsies of affected tissue [g]	Sabouraud's Dextrose agar (unsupplemented)	28-30 and 35-37		Air	7 days [b]	Daily	*Mucorales*
Endemic mycoses (travel to or from endemic regions)	All respiratory samples	High volume [a] culture on Sabouraud dextrose agar supplemented with chloramphenicol or gentamicin [i]	28-30 and 35-37 [h] *(all procedures must be carried out in CL3, in accordance with HSE regulations)*		Air	6-8 weeks	Once weekly	*Histoplasma capsulatum, Coccidioides species, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Talaromyces marneffei*

Footnotes:

a High volume culture should be a minimum of 100 to 500 µL of specimen per plate for routine cultures, in high-risk patients a minimum of 500 µL is recommended with a desired volume of 1mL advised to enhance fungal recovery which can be divided across two agar plates/slopes.

b Carefully inspect the culture for any sign of visible growth before discarding. When there is any visible growth culture should be extended to allow identification.

c In high-risk patients the addition of a second culture at 35-37°C will increase the recovery of fungi and should be considered if an additional culture is not performed at 28-30 **°C.**

d The addition of a second plate at 28-30 °C may enhance the recovery of atypical fungal pathogens that do not grow well at higher temperatures but may still be implicated in infection.

e The addition of a culture at 40 - 45°C will supress yeast growth and therefore enhance the recovery of *Aspergillus fumigatus* complex isolates which are thermotolerant

f See section '7.1 sample preparation' for optimal BALF processing for the recovery of Mucorales species

g Sample is cut into multiple small pieces, do not grind or homogenise tissue to assure viability (See UK SMI B 17: Tissues and biopsies from deep-seated sites and organs)

h Dimorphic fungi may exhibit a mould form when incubated at 28-30°C and a yeast form at 35-37°C

i BHI or SABHI agar (supplementary for suspected infection with thermally dimorphic fungi, if available)

7.4 Identification

Refer to individual UK SMIs for organism identification.

7.4.1 Minimum level of identification in the laboratory

- **Moulds**: species level identification is required.
- **Yeast:** species level identification from respiratory samples is not required in the majority of cases.

a) Isolation of *Candida* species from respiratory secretions indicates colonisation rather than infection in the vast proportion of patients. If there remains clinical concern for *Candida* pneumonia (e.g., prolonged candidaemia or chronic disseminated candidiasis), confirmation should be conducted by histological examination of lung tissue (6).

b) When there is suspicion of cryptococcal pneumonia, and there are atypical slow growing yeast colonies species-level identification is necessary.

c) **Note**: After local validation, testing BALs for cryptococcal antigen (CrAg) may be useful for investigation of cryptococcal pneumonia.

d) If *Candidozyma auris* (formally known as *Candida auris*) screening requires a specific screening request and dedicated sample, and testing should follow the designated screening pathway as per UKHSA guidance 'Candidozyma auris: guidance for acute healthcare settings' and alongside local protocols.

7.4.2 Matrix-assisted laser desorption/ionisation – time of flight mass spectrometry (MALDI-TOF MS)

MALDI-TOF MS has demonstrated improved performance in mould identification particularly for *Aspergillus* and *Mucorales*. This progress is further supported by the use of ID-Fungi Plates (IDFP), which allow for direct and clean mycelium harvesting from the culture surface, leading to shorter incubation periods and a more streamlined laboratory workflow (40).

Note: if MALDI-ToF fails to identify a mould it may indicate a rarely encountered species and pan-fungal identification may be required.

7.5 Molecular and immunological tests

Molecular assays, including NAAT enables the rapid detection of pathogens in respiratory samples. When performing molecular testing, knowledge of the detection range, sensitivity and specificity of a specific 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 UK SMI Q 4: Good practice when performing molecular amplification assays and UK SMI Q 1 - Evaluations, validations and verifications of diagnostic tests for the validation and verification of diagnostic methods

A range of NAAT assays for DNA detection of fungal pathogens are available, for example *Pneumocystis jirovecii* (41)*, Aspergillus* species*, Mucorales* NAATs. For in-depth information refer to BSMM best practice guidance (9).

- BAL fluids from patients at high risk of fungal disease can be used for Aspergillus galactomannan antigen (8,42) (normally referred to as only galactomannan) ELISA testing and fungal NAAT (e.g., *Aspergillus, Pneumocystis* and/or Mucorales NAAT).
- For the investigation for *P. jirovecii*, NAAT is recommended as the preferred diagnostic method, due to its high sensitivity and specificity and can be combined with 1-3-β-D-Glucan testing.
- Fungal cell wall biomarkers such as 1-3-β-D-Glucan (BDG) may provide an important approach in clinical settings such as ICU, haematology, and transplant. However, performing BDG testing on respiratory tract samples is not recommended due to the ubiquitous presence of commensal fungi (e.g., *Candida* species) within the respiratory tract. Serum BDG testing may be useful as a pre-emptive screen for invasive fungal disease and serum biomarkers to guide treatment of breakthrough infections.
- The use of fungal NAATs and biomarker testing (BDG/galactomannan) on plasma/serum of patients at high-risk of respiratory fungal infection when not on antifungal therapy can be a useful adjunctive test to complement the testing of respiratory tract samples.
- Pan-fungal NAAT is more appropriate for sterile-site specimens, tissue or histology-positive material, or culture-positive isolates where identification has failed, as its interpretation is challenging in non-sterile specimens such as BAL fluid and sputum and is therefore not recommended for use as a broad screening test in these samples.Commonly used galactomannan antigen assays are only validated for plasma/serum and BALF. Other specimen types, such as sputum, ET aspirates, and nasopharyngeal aspirates are known to be associated with false-positive results if standard interpretation cut-offs are used. However, with appropriate interpretation and use of combination testing as part of a broader biomarker strategy, they may still provide value when BALF samples are unavailable. All kits should undergo appropriate validation and verification for the relevant patient population and sample type.
- See table 2 below for more information on NAAT recommendations.

Table 2: Recommended Molecular Methods for Mycological Investigations

Investigation	Specimen	Targeting	Sample volume	Centrifugation (of concentrate)	Optimal fraction of sample to test	Mechanical lysis	Duplicate reactions	Elution volume	Paired biomarker	Internal control *b*
Aspergillus NAAT	BALF	Free and whole cell DNA	500uL - 1mL concentrate *a*	Recommended	Supernatant (free DNA) and pellet (whole cell)	Required for pellet	Recommended	≤100µL	Galactomannan	Yes
	Endotracheal aspirate	Whole cell DNA	500uL - 1mL digested	Dependant on extraction technique *a*	Pellet if centrifuged	Required	Recommended	≤100µL	Galactomannan (serum)	Yes
	Plasma/Serum	Free DNA	>0.5 mL	n/a	n/a	Not required	Recommended	≤100µL	Galactomannan	Yes
P. jirovecii NAAT	BALF	Whole cell DNA	500uL - 1mL concentrate *a*	Required	Pellet	Not required	Single reaction minimum	≤100µL	1-3-B-D-glucan (serum)	Yes
	Endotracheal aspirate / Sputum / NPA	Whole cell DNA	500uL - 1mL digested	Required	Pellet	Not required	Single reaction minimum	≤100µL	1-3-B-D-glucan (serum)	Yes
	Upper respiratory tract swab *c*	Whole cell DNA	n/a	n/a	Express swab into appropriate buffer	Not required	Single reaction minimum	≤100µL	1-3-B-D-glucan (serum)	Yes
	Plasma/Serum	Free DNA	>0.5 mL	n/a	n/a	Not required	Single reaction minimum	≤100µL	1-3-B-D-glucan (serum)	Yes
Mucorales NAAT	BALF	Free and whole cell DNA	500uL - 1mL concentrate *a*	No current recommendations – as Aspergillus	No current recommendations – as *Aspergillus*	Required for pellet	No current recommendations – as *Aspergillus*	≤100µL	None available	Yes
	Endotracheal aspirate / Sputum	Whole cell DNA	No current recommendations – as *Aspergillus*	No current recommendations – as *Aspergillus*	No current recommendations – as *Aspergillus*	Required	No current recommendations – as *Aspergillus*	≤100µL	None available	Yes
	Plasma/Serum	Free DNA	>0.5 mL	n/a	n/a	n/a	No current recommendations – as *Aspergillus*	≤100µL	None available	Yes

Considerations:

- Mucolytic agents should have control checks for fungal DNA contamination
- Fungal contamination control checks should be performed for every run and recorded as part of routine quality assurance. The use of aliquoting and single-use vials is recommended
- Conventional testing including microscopy and culture must be performed where appropriate on all samples processed for fungal NAAT
- Commercial assays are preferred over in-house assays for centres without an attached mycology reference unit or mycology specialist centre
- The choice of assay should consider; single target compared to multi copy target and the sensitivity of the NAAT and specificity of the target e.g. ITS or 18S
- Targeted NAAT is more sensitive than Pan-fungal NAAT. Pan-fungal NAAT typically lacks analytical sensitivity and negativity when performed directly on clinical specimens to exclude fungal disease. Furthermore, its ability to differentiate and identify fungal species is dependent on the specific gene targets and its utility is limited when testing non-sterile respiratory samples.
- The use of syndromic methods for the testing of respiratory samples that include fungal targets can generate false positive results and should only be used when there is clinical suspicion of respiratory fungal infection.

Footnotes:

a Ensure for qPCR sample volume tested is incorporated into copies/mL calculations

b As a minimum the IC should target a pre-defined quantity of non-fungal target to monitor for inhibition. Additionally, where possible a human endogenous gene control should also be included within the assay to monitor for the presence and quantity of human DNA as an indicator of specimen quality particularly for BALF specimens.

c Upper respiratory tract swabs used in combination with serum 1-3-B-D-glucan, has high specificity to diagnose PJP/PCP in the absence of BAL fluid. If swabs with transport medium are used instead, the extraction method must be adapted accordingly. However, data on the type of swab is limited (43,44).

8 Post-laboratory processes

A range of diagnostic tests is available for fungal diagnosis, and all results should be interpreted collectively as part of a comprehensive diagnostic approach, including the clinical presentation and overall patient context.

8.1 Microscopy

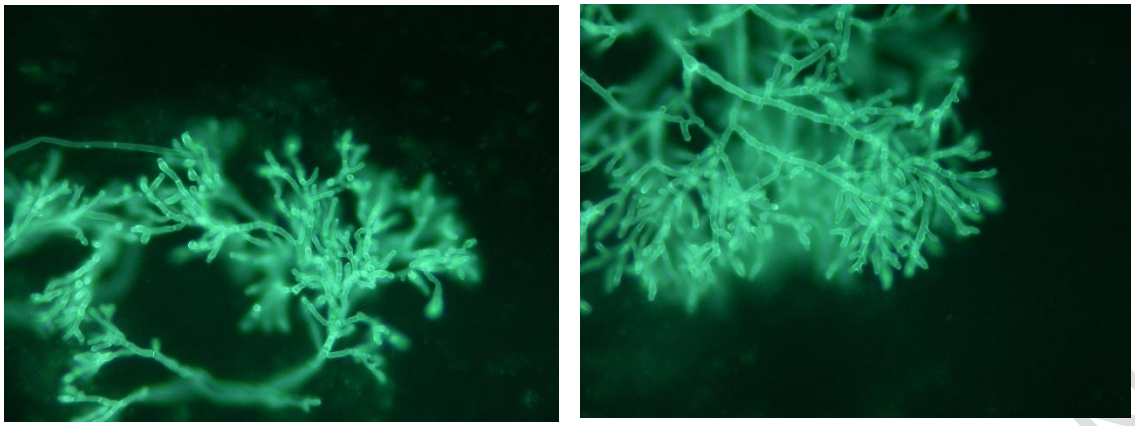
8.1.1 Reporting microscopy

Fungal stain

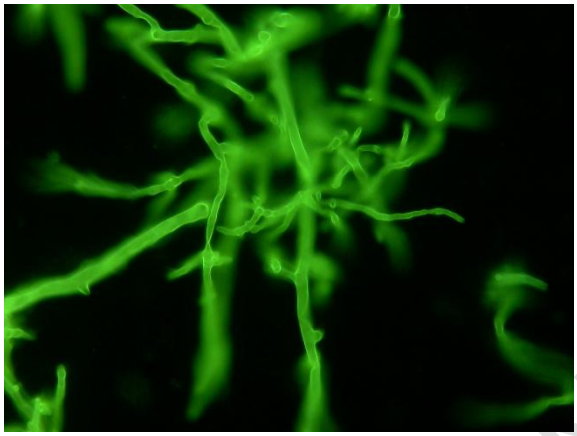
- Only report fungal elements consistent with moulds, examples in figure 1a.
- The reporting of "fungal elements seen", in the absence of descriptive details provides little diagnostic benefit. Please see table below for best practice reporting for fungal microscopy

Reporting strategy for direct fungal microscopy from respiratory specimens			
Observation	**Image**	**Result**	**Comment**
Negative	n/a	Filamentous fungal elements NOT seen	n/a
Positive	Figure 1 a	Filamentous fungal elements SEEN	n/a
Positive with branching at 45°	Figure 1 b	Filamentous fungal elements SEEN	Microscopy consistent with *Aspergillus* species, but is not definitive, and may be other fungal species
Positive – broad aseptate	Figure 1 c	Filamentous fungal elements SEEN	Microscopy consistent with *Mucorales* fungal species

- All positive microscopy results are to be communicated to the clinical team without delay.
- The presence of regularly septate hyphae with 45° branching is consistent with *Aspergillus* species, figure 1b. but could represent other hyaline fungi such as *Scedosporium* species.
- The presence of broad, aseptate or pauci-septate hyphae with wide-angle branching is consistent with Mucorales, figure 1c.



a b



c

Figure 1. Calcofluor white fluorescence microscopy demonstrating filamentous fungal elements: (a) septate hyphae; (b) septate hyphae with acute-angle branching (c. 45°); (c) broad aseptate or pauci-septate hyphae.

Images courtesy of H. van der Lee, Department of Medical Microbiology, Radboud University Medical Centre, Nijmegen, The Netherlands.

Note: Unless encapsulated, the presence of yeast cells should not be reported in the overwhelming majority of cases as *Candida* species and associated ascomycetous yeasts in actual infection is very rare and is usually a reflection of oropharyngeal colonisation, figure 2. In such cases further histopathological evidence may be required.

Images awaiting permissions

Figure 2 Example of *Candida albicans* calcofluor staining with yeast cells present

8.1.2 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.

In immunocompromised patients or when fungal investigation is specifically requested, microscopy results positive for the presence of filamentous hyphae indicative of mucoraceous mould (members of Mucorales) or *Aspergillus* species should be immediately communicated to the consultant looking after the patient or an infection consultant liaising with the clinical teams.

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.

8.2 Culture

8.2.1 Reporting culture

- Isolation of any filamentous fungi should be reported, irrespective of burden.
- Closely related cryptic species cannot always be distinguished phenotypically; reporting to species-complex level may therefore be appropriate for some fungal pathogens (e.g., Mucorales complex or *Aspergillus fumigatus* complex).
- To prevent possible clinical confusion, species which have transitioned to new names, should be reported with the new name alongside the previous name in brackets.
- Yeasts should not routinely be reported/speciated from respiratory specimens unless identified as:
    a) *Cryptococcus neoformans* or *Cryptococcus gattii*, or if there is suspicion of cryptococcal pneumonia
    b) Dimorphic fungus which fungi may be isolated in yeast or mould form at varying temperatures, and this must be considered when reporting.
    c) Clinically significant Yeast like moulds *e.g. Exophiala*
    d) Do not routinely report/speciate common *Candida* from respiratory specimens unless clinically indicated.
- Report a culture negative for fungal growth as ‘No fungal growth’.

Notes

- A culture negative for fungal growth may be reported after 48 hours incubation, although cultures will continue to be incubated up to 5 - 7 days. In the event of fungal growth, a further report will be issued.
- The presence of fungi should be documented even when a fungal culture is overgrown by chloramphenicol-resistant Gram-negative bacterial (e.g., *Pseudomonas* spp.). This should be reported as filamentous mould isolated, unable to obtain identification due to bacterial overgrowth.

8.2.2 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.

If a mould has been isolated but is awaiting identification due to subculture the clinical teams must be informed of 'fungal growth under investigation'.

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.

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

8.3 Reporting other tests including molecular testing

Diagnostic performance of quantitative PCR (qPCR) on bronchoalveolar lavage fluid provides the highest sensitivity and specificity particularly for detecting PJP/PCP in immunocompromised patients.

As more novel methods are becoming increasingly accessible, it is essential to ensure that reporting and authorisation of results follow local protocols or manufacturer's instructions. Some tests may require a caveat as they lack the subsequent guidance on reporting/interpreting a result for pathogens in certain circumstances.

1. Indicating the strength of positivity is encouraged; higher burdens are more likely to represent disease.
2. Results should be interpreted according to sample type and within the clinical context of the individual patient including additional mycological evidence (see below).
3. Attention should be paid to the reporting of very low positive results that may indicate environmental contamination e.g. *Aspergillus* or *Pneumocystis* NAAT results.

4. Correlation with other mycological evidence is required to help determine the significance of any biomarker/molecular result.
    a) Samples that are positive by multiple mycological investigations are typically more indicative of disease
    b) Respiratory samples where biomarker and molecular results are discordant require further diagnostic and clinical work-up and can reflect test false positivity.
    c) Respiratory samples that are negative by multiple mycological investigations are typically useful for excluding a respiratory fungal infection caused by a specific pathogen (e.g., Aspergillosis when a BAL fluid is galactomannan, *Aspergillus* NAAT and culture/microscopy negative) but in the presence of radiological evidence typical of respiratory fungal infection additional diagnostic testing (e.g., Mucorales NAAT) is recommended.
5. Respiratory sample quality should be assessed, especially for BALF 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 and minimise the reporting of false negative results.
    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.

9 Antimicrobial susceptibility testing

Clinically significant fungal isolates which may cause severe deep-seated or life-threatening invasive disease should be tested for antifungal susceptibility. Avoid reporting from likely contaminants or colonising organisms unless deemed clinically necessary (e.g. in immunocompromised patients or sterile sites).

This is particularly important in cases where antifungal therapy is being considered or already underway, in cases of poor treatment response and in breakthrough infections (9).

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.

Organism	Examples of agents to be included within primary test panel	Examples of agents to be considered for supplementary testing	Notes
Aspergillus species	Voriconazole Posaconazole Itraconazole Amphotericin B Echinocandin	Isavuconazole	Fluconazole is not a mould active agent Therapeutic Drug Monitoring (TDM) is mandatory for itraconazole and voriconazole. Posaconazole and isavuconazole may require TDM in certain clinical circumstances. Refer to the appropriate reference or specialist laboratory England, Wales, Scotland or Northern Ireland.
Mucorales species	Posaconazole Amphotericin B	Isavuconazole	
Cryptococcal species	Fluconazole Amphotericin B + Flucytosine	N/A	Refer to the appropriate reference or specialist laboratory England, Wales, Scotland or Northern Ireland.

9.1 Reporting of antimicrobial susceptibility testing

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

If molecular methods are used to detect mutations associated with resistance this should be stated in the report, together with interpretative comments related to the limitations of such an approach.

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.

Appendix

Table 3 - Patients at high risk of invasive pulmonary fungal infection according to underlying conditions

Patients group *(please consider accumulation of risk factors)*	**Fungal disease/pathogens**				
	Aspergillosis -*Aspergillus fumigatus* and other species	Pneumocystis pneumonia - *Pneumocystis jirovecii*	Mucormycosis - Mucorales	Cryptococcosis- Cryptococcus species	Endemic mycoses
Haematology and neutropenic patients (in/outpatients) Qualitative or quantitative neutrophil abnormality (inherited neutrophil deficiency, absolute neutrophil count of ≤500 cells/mm3)	high	moderate	low	low	low (do not investigate unless the patient has travelled to or from an endemic region)
Haematology with lymphopenia	moderate	high	low	low	
Treatment with recognised immunosuppressants (eg, high dose steroids, calcineurin or mammalian target of rapamycin [mTOR] inhibitors, blockers of tumour necrosis factor [TNF] and similar antifungal immunity pathways, alemtuzumab, ibrutinib, nucleoside analogues) during the past 90 days	moderate	moderate	low	low	
Solid organ transplant (highest risk within 6 months of transplant)	high in lung transplant. Moderate in others	moderate	low (predominantly lung, heart and liver transplant)	low	
HIV with CD4 count <200 cells/mm³	low	high	low	moderate	
Severe influenza/COVID-19 critically ill patients	high	low	low (considered moderate when combined with uncontrolled diabetes)	low	

Ventilated critically ill patients lacking other conditions	low	low	N/A	N/A	
Liver disease in decompensated cirrhosis	moderate	indeterminate	N/A	N/A	

Table 4: Patients at high risk of chronic pulmonary fungal disease

Patients group *(please consider accumulation of risk factors)*	**Fungal disease/pathogens**				
	Aspergillosis- *Aspergillus fumigatus* and other species	Pneumocystis pneumonia- *Pneumocystis jirovecii*	Mucormycosis- Mucorales	Cryptococcosis- Cryptococcus species	Endemic mycoses
Cystic Fibrosis	moderate	low	low	low	low (do not investigate unless the patient has travelled to or from an endemic region)
Chronic respiratory airway abnormality (e.g. bronchiectasis or COPD)	moderate	low	low	low	
Fungal asthma	moderate	low	low	low	

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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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