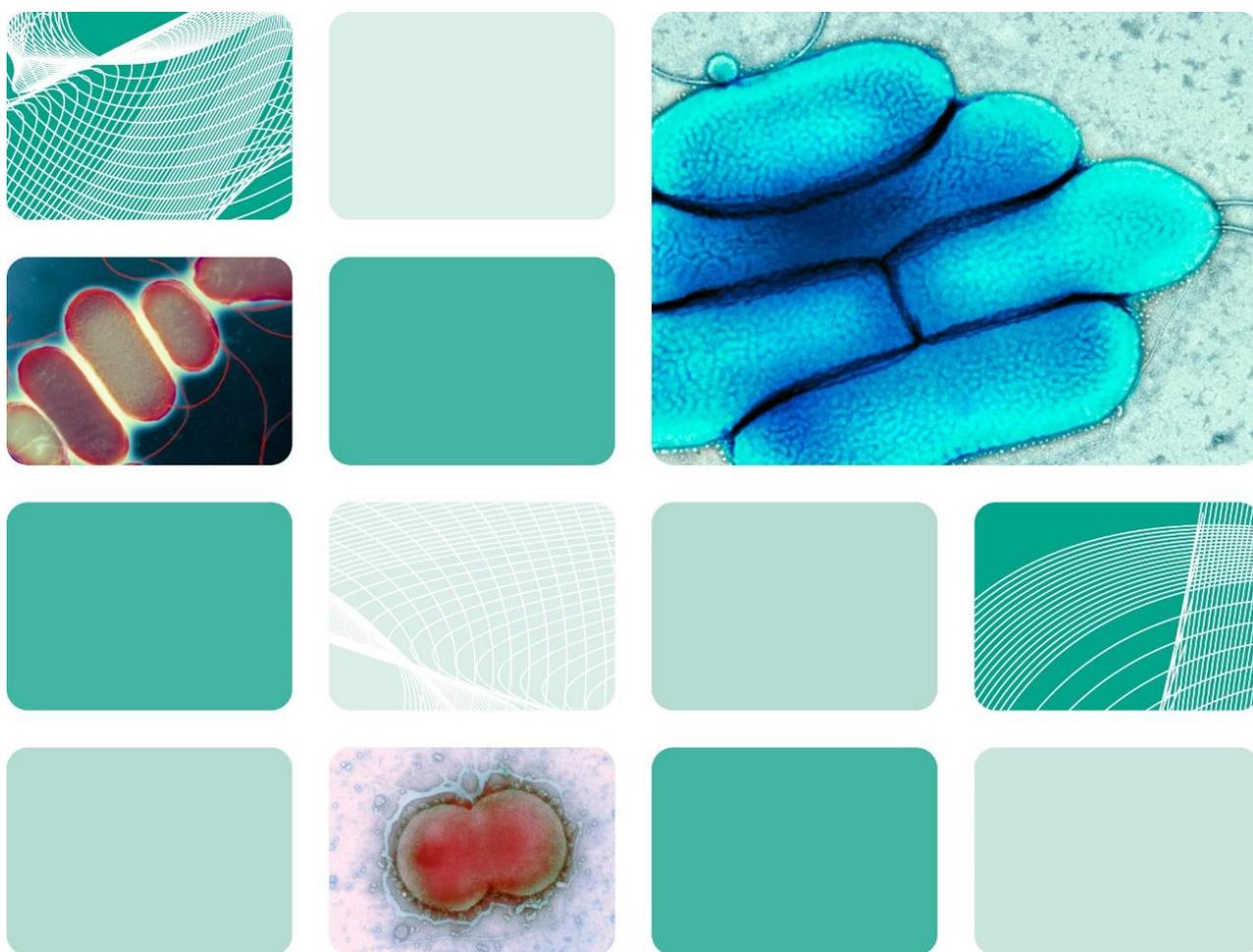




UK Health  
Security  
Agency

## UK Standards for Microbiology Investigations

### Investigation of samples from paranasal sinuses



## Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on [the UK SMI website](#). UK SMIs are developed, reviewed and revised by various working groups which are overseen by a [steering committee](#).

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Applied  
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British Infection Association

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Displayed logos correct as of December 2024.

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## Amendment table

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

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

Amendment number/date	13/06.02.26
Issue number discarded	8
Insert issue number	8.1
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	<p><b>This is an administrative point change.</b></p> <p><b>The content of this UK SMI document has not changed.</b></p> <p><b>The last scientific and clinical review was conducted on 16.07.2024</b></p> <p>Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms</p> <p>References to NICE accreditation removed.</p> <p>'Public health responsibilities of diagnostic Laboratories' section added.</p>

<b>Amendment number/date</b>	12/16.09.24
<b>Issue number discarded</b>	7.1
<b>Insert issue number</b>	8
<b>Anticipated next review date*</b>	16.09.27
<b>Section(s) involved</b>	<b>Amendment</b>
<b>Title</b>	The title has been changed from 'Investigation of nasal samples' to 'Investigation of samples from paranasal sinuses'.
<b>Whole document</b>	Hyperlinks updated to direct reader from UK SMIs webpages on GOV.UK to RCPATH website.

	<p>The term 'Sinusitis' has been replaced with 'Rhinosinusitis' throughout the document.</p> <p>Subheadings have been revised and modified where needed.</p> <p>All sections have been updated with current and relevant information and supported with recent literature where available.</p> <p>Some sections have been restructured as appropriate to align with current laboratory practices.</p>
<b>Scope of document</b>	<p>Types of specimens have been moved to Section 6: Pre-laboratory processes (pre analytical stage).</p> <p>Other diagnostic methods are considered within the scope but not detailed in the document, emphasising that the diagnosis of rhinosinusitis involves multiple approaches.</p> <p>Topics that are outside the scope of this document have been mentioned and links to relevant UK SMIs were provided if available.</p> <p>The reader is also made aware of the updated taxonomy of <i>Candida</i> species.</p>
<b>Section 4: Introduction</b>	<p>Information updated and supported with recent literature where available.</p> <p>Section on nasal carriage has been removed and the link to UK SMI B 29: Investigation of specimens for screening for MRSA has been provided in the scope.</p>
<b>Technical Information/Limitations</b>	<p>This section has been removed.</p> <p>Information replaced with a link to refer reader to <a href="#">scientific information</a>.</p> <p>Information regarding limitations of diagnostic methods has been incorporated into the relevant sections specific to each method.</p>
<b>Table 1. Culture media, conditions, and organisms</b>	Table has been reviewed and updated.
<b>Table 2. Minimum level of identification in the laboratory</b>	Table has been reviewed and updated.
<b>Section: 7 Laboratory processes (analytical stage)</b>	Sections 7.5 molecular assays and 7.6 Antigen/antibody testing have been added.

<p><b>Section 9: Antimicrobial susceptibility testing</b></p>	<p>Information and hyperlinks reviewed and updated as appropriate.</p> <p>Hyperlink to British Society for Antimicrobial Chemotherapy (BSAC) removed.</p> <p>Hyperlink to Clinical and Laboratory Standards Institute (CLSI) added.</p>
<p><b>Section 10: Referral to reference or specialist testing laboratories</b></p>	<p>Hyperlinks were updated as appropriate.</p>
<p><b>Algorithm</b></p>	<p>The content of the algorithm has been reviewed and updated to align with the document.</p>
<p><b>References</b></p>	<p>References reviewed and updated.</p>

\*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 Investigations (UK SMIs) document describes the examination of sinus aspirates and associated samples for the detection and recovery of the organisms that cause the various forms of rhinosinusitis. This document does not cover investigations for conditions of viral, allergic or toxin origin.

Other diagnostic techniques such as Computed Tomography (CT) scans are commonly employed by healthcare professional for the diagnosis of rhinosinusitis and should be considered alongside the microbial methods mentioned in this document.

Please refer to [UK SMI B 29 - Investigation of Specimens for Screening for MRSA](#) for information on screening for MRSA nasal carriage.

Please note the following:

This document does not provide the details for the identification of organisms causing rhinosinusitis. The identification section in this document only provides information on the level to which organisms should be identified to as part of the reporting process with direct links to the relevant identification documents for reference.

This document addresses samples sent to clinical laboratories from both primary and secondary (hospital) healthcare. However, most cases of rhinosinusitis are diagnosed and managed in primary healthcare settings without the necessity of sending samples to clinical laboratories. Also, most cases are uncomplicated and tend to resolve on their own without further investigations or antimicrobial treatment. For more information, refer to the NICE guideline on [Sinusitis \(acute\): antimicrobial prescribing](#) and to their website for information on the management of [acute](#) and [chronic](#) rhinosinusitis and [prescribing information](#).

The advancement of molecular phylogenetic analysis has greatly influenced the taxonomy of microorganisms, especially fungi. This has resulted in the reclassification and renaming of many species. For the purpose of this document, both the previous and current nomenclature of reclassified species or species with updated nomenclature will be mentioned, as required. This also applies to the species formerly part of the genus *Candida* and will collectively be referred to as '*Candida* and associated ascomycetous yeast' (1).

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

## 4 Introduction

### 4.1 Rhinosinusitis

Rhinosinusitis refers to inflammation in the nasal mucosa and one or more of the paranasal sinuses: maxillary, ethmoid, frontal and sphenoid, with the maxillary being the commonest sinus to get infected (2). It can result from viral, bacterial, or fungal infections (2,3). Clinical presentations of rhinosinusitis are diverse and can overlap with other conditions such as hay fever. Symptoms include nasal congestion and discharge, facial pain, pressure or fullness, headaches and fatigue. Factors that predispose an individual to rhinosinusitis include impaired mucociliary function, obstruction of the sinus entrance (e.g., by nasotracheal intubation or by mucosal oedema as a result of viral infection) and defects in the immune system (4-6). The sinus cavities are usually sterile or may contain small numbers of bacteria that are continuously removed by the mucociliary system.

Rhinosinusitis aspiration is generally the preferred method for specimen collection as it allows more targeted sampling and reduces risk of contamination by upper respiratory tract flora (2,7). Specimens should be collected by Ear, Nose and Throat (ENT) surgeons and obtained through rigid endoscopy and careful aspiration of the sinus cavity avoiding contamination by upper respiratory tract flora.

In the case of suspected invasive fungal rhinosinusitis, a biopsy sample may also be obtained for direct microscopic examination prior to culture to identify the presence of fungal elements (8,9).

#### 4.1.1 Acute rhinosinusitis

Acute rhinosinusitis is often suspected if the symptoms meet clinical criteria and deteriorate after 5 days or persist beyond 10 days (less than 12 weeks) (6). The aetiology of community acquired infections can be viral, bacterial, mixed (viral and bacterial) or occasionally fungal (particularly in immunosuppressed patients) with viral upper respiratory tract infections being the most common cause of acute rhinosinusitis.

For acute rhinosinusitis, routine culture and antibiotic prescription is not recommended as acute rhinosinusitis often resolves on its own without the need for antibiotics (7,10).

Culture is selectively obtained in individuals at high risk of complications or antibiotic resistance. Antibiotic prescription is often reserved for patients with persistent or worsening symptoms or complications of acute rhinosinusitis (10). Serious complications of acute rhinosinusitis resulting in the formation of abscess such as subperiosteal abscess or brain abscess may also require surgical intervention and drainage.

#### Community rhinosinusitis

The most common bacteria isolated from cases of acute community acquired rhinosinusitis are *Streptococcus pneumoniae*, non-typeable *Haemophilus influenzae* (NTHi) and *Moraxella catarrhalis* which is more prevalent in children than adults (2,11,12). Other less common organisms isolated are streptococci of the “anginosus” group (*Streptococcus anginosus*, *Streptococcus constellatus* and *Streptococcus*

*intermedius*), group A streptococcus, other  $\alpha$ -haemolytic streptococci, *Staphylococcus aureus* and anaerobic bacteria (which are infrequent in children) (2,13).

### Nosocomial rhinosinusitis

Nosocomial rhinosinusitis is often a complication in endotracheal intubation and mechanical ventilation and often shows no clinical signs of infection (14,15). The causative organisms can vary and infections are often polymicrobial. *Pseudomonas aeruginosa* and other Gram-negative rods commonly cause nosocomial rhinosinusitis in intensive care and immunocompromised patients (2,16). Similar pathogens and polymicrobial infections have been identified in children with more anaerobes being isolated (17,18). Acute fungal rhinosinusitis caused by *Aspergillus* or Mucorales species can occur in immunosuppressed patients, such as haematology patients undergoing chemotherapy, allogeneic or solid-organ transplantation recipients and patients with poorly controlled diabetes.

Nosocomial infections are often underestimated and difficult to diagnose in intensive care patients, therefore close collaboration among physicians, ENT surgeons, microbiologists and histopathologists is necessary to reach a diagnosis.

#### 4.1.2 Chronic rhinosinusitis

Chronic rhinosinusitis is a long-term inflammation of the sinuses that last for longer than 12 weeks without complete resolution (6). Chronic rhinosinusitis is multifactorial in nature and can include allergies, anatomical abnormalities, environmental irritants, and infections including odontogenic infections and those associated with biomaterials such as dental implants. It may also be a feature of some congenital immunodeficiency syndromes and disorders of mucociliary function, although most patients do not have these conditions. In addition, chronic rhinosinusitis can persist in some patients even after surgical intervention.

Chronic rhinosinusitis is clinically sub-categorised by the presence or absence of nasal polyps (CRSwNP or CRSsNP respectively) (5,6). Further research and understanding of chronic rhinosinusitis have led to the proposal of additional categorisation models to enhance the accuracy of diagnosis and management of different subgroups of patients (5,6,19,20).

Mucosal inflammation in chronic rhinosinusitis can persist based on the immune responses. Type 2 immune response can lead to persistent mucosal inflammation and is a characteristic of CRSwNP, especially in western countries. Type 1 inflammation is more commonly associated with CRSsNP. Biofilm formation by microorganisms in the sinonasal cavity can exacerbate Type 1 inflammation, contributing to persistent infections (5). Biofilms are difficult to treat and often necessitate surgical intervention.

The microbiology of chronic rhinosinusitis differs significantly from that of acute rhinosinusitis and is commonly caused by anaerobes, Gram-negative bacteria, *Staphylococcus aureus*, and fungi (usually *Aspergillus* species and occasionally other moulds). The persistence of infection or extension of unresolved acute rhinosinusitis can lead to a shift in the types of bacteria present from aerobic to anaerobic bacteria. This includes *Pseudomonas aeruginosa*, particularly from patients with cystic fibrosis, *Enterobacterales*, *Peptostreptococcus* species, *Cutibacterium* (Formerly

*Propionibacterium*) species, *Fusobacterium* species, *Prevotella* species and other anaerobic Gram-negative bacteria (21-24).

Chronic rhinosinusitis can lead to serious and life-threatening complications with the most common being orbital infections (25-27). Intracranial infections are less common but may cause significant morbidity and mortality. Another rare complication is osteomyelitis (see [UK SMI B 42 - Investigation of Bone](#)), involving the frontal bone (Pott's puffy tumour). *Staphylococcus aureus* and anaerobes are the predominant isolates from such cases and are also recovered from children with severe rhinosinusitis symptoms requiring surgical intervention or with protracted rhinosinusitis (lasting over one year) (12).

### 4.1.3 Fungal rhinosinusitis

Fungal rhinosinusitis can be acute or chronic and is categorised into non-invasive and invasive fungal rhinosinusitis depending on the extent of fungal involvement in the sinuses and the severity of the infection. Non-invasive fungal rhinosinusitis may take the form of a fungus ball in the sinus, saprophytic fungal rhinosinusitis or allergic fungal rhinosinusitis (28-30). Invasive fungal rhinosinusitis can be either acute or chronic and may form granulomas within the sinus cavities. Granulomatous invasive rhinosinusitis may be confused with Granulomatosis with polyangiitis (formerly known as Wegener's granulomatosis) or squamous cell carcinoma (28). Therefore, examination of tissue rather than pus is important in fungal rhinosinusitis and close co-operation among the surgeon, microbiologist and histopathologist is also necessary (8,28). Acute invasive rhinosinusitis can spread rapidly from the involved sinuses and is to be regarded as a medical emergency. Aggressive surgical debridement is often required in addition to systematic antifungal therapy and treatment of the underlying cause.

Fungal rhinosinusitis is predominantly caused by moulds, with *Aspergillus* species being the most common pathogens. Other moulds that are less commonly encountered include *Mucor* and *Rhizopus* which are associated with the fatal invasive fungal infection, mucormycosis (31,32). Also, less common are thermally dimorphic fungi such as *Sporothrix schenckii*, one of the causative agents of endemic mycoses. These exist as tissue invasive yeast at human body temperature and as moulds in the environment (33). They are limited by geographical distribution and rare in the UK.

Community-acquired chronic fungal rhinosinusitis is a relatively common problem in some tropical and subtropical countries which can in some instances lead to invasive disease, e.g., in Africa and India, and imported cases may be encountered (32). The commonest cause overall is *Aspergillus flavus*, but other fungi should be considered.

It is important to take into consideration the country of origin and travel history of patients in suspected cases of fungal rhinosinusitis to help determine the causative agent of infection. Rhino-orbital cerebral mucormycosis, which became more prevalent in India during COVID-19 pandemic, highlights the importance of such considerations in diagnosis and treatment planning (34).

## 5 Safety considerations

The section covers specific safety considerations (35-55) related to this UK SMI, and should be read in conjunction with the general [safety considerations](#).

### 5.1 Specimen collection, transport and storage:

Use aseptic technique.

Collect all specimens before antimicrobial or antifungal therapy where possible.

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

Compliance with postal, transport and storage regulations is essential.

### 5.2 Specimen handling, processing and examination:

The majority of diagnostic work can be conducted at Containment Level 2. However, specific procedures involving mycology work and certain hazard group 2 bacterial isolates should be conducted within a microbiology safety cabinet.

When Hazard Group 3 organisms are suspected, or clinical details regarding travel history are not available for a risk assessment, all specimens must be processed in a microbiology safety cabinet under full containment level 3 conditions. General containment level 3 procedures are also recommended for the examination of cultures that may contain dimorphic fungi and other pathogenic fungi. Sealed containers such as screw-capped bottles should be used for culture. Culture plates are not suitable.

Any laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet.

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

Specimen containers must also be placed in a suitable holder.

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

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

## 6 Pre-laboratory processes (pre analytical stage)

### 6.1 Specimen type:

Antral washout, sinus aspirate, sinus washout, and tissue.

#### Notes:

Nose swabs are not a suitable sample type for the diagnosis of rhinosinusitis although they can be used for screening for nasal carriage of e.g., MRSA or *Candida auris*. However, superficial nasal suction samples and swabs can be used to investigate nasal discharge in patients with nasogastric tube or post ENT surgery.

Endoscopic aspiration is recommended for areas that are challenging to access directly and for reducing the risk of iatrogenic trauma and blood contamination of the samples that may be caused by blind aspiration (56).

The choice of specimen is influenced by the patients' clinical details and the investigation aim. For example, sinus aspiration may be sufficient for the diagnosis of bacterial rhinosinusitis while additional biopsy may be required for the identification of fungal structure and more detailed analysis of tissue changes.

### 6.2 Specimen collection and handling:

For safety considerations refer to Section 5.

Collect specimens before antimicrobial therapy where possible.

Collect specimens other than swabs into appropriate containers and place in sealed plastic bags.

Ideally, a minimum volume of 1mL for washouts is required to ensure adequate sensitivity.

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

### 6.3 Specimen transport and storage:

Specimens should be transported and processed as soon as possible.

The volume of specimen influences the transport time that is acceptable. Large volumes of purulent material maintain the viability of anaerobes for longer.

The recovery of fastidious organisms and anaerobes in particular is compromised if the transport time is delayed.

Samples should be kept at room temperature if they are to be processed immediately. Otherwise, refrigeration is preferable to storage at ambient temperature.

## 7 Laboratory processes (analytical stage)

### 7.1 Specimen processing:

For safety considerations refer to Section 5.

#### Standard

Non-mucoid sinus or antral washouts are processed as follows:

- Centrifuge specimen (for antral washouts), unless very mucoid, at 1200 x *g* for 10 minutes
- Discard most of the supernatant, leaving approximately 0.5mL
- Resuspend the centrifuged deposit in the remaining fluid
- Carry out microscopy and culture

Mucoid specimens are processed by digestion as follows:

- Carry out microscopy
- Add equal volume of a 0.1% solution of N-acetyl cysteine to specimen
- Agitate gently for approximately 10 seconds
- Incubation at 35-37°C for 15 minutes followed by gentle agitation for approximately 15 seconds will assist homogenisation

**Note:** do not exceed 20 minutes of incubation to avoid overprocessing the sample.

- Inoculate plates

### 7.2 Microscopy

Refer to [UK SMI TP 39 – Staining procedures](#)

For information on technical limitations refer to [scientific information](#).

Direct microscopic examination of fresh or stained specimens can provide pre-diagnostic information on the presence of fungal structures as culture results may not be available for a few days. This is particularly important for the prompt administration of anti-fungal therapy in immunocompromised patients. However, this method is insensitive and negative results do not rule out a fungal infection as may be the case in the early stages of the infection.

#### Standard

For mucoid specimens:

Using a sterile loop select the most purulent or blood-stained portion of the specimen and make a thin smear on a clean microscope slide for Gram staining.

For non-mucoid specimens:

Using a sterile pipette place one drop of centrifuged deposit (see section 6.1.2) or neat specimen on to a clean microscope slide. Spread this with a sterile loop to make a thin smear for Gram staining.

**Note:** If fungal infection is suspected or seen in the Gram stain carry out the supplementary examination.

### Supplementary

Using a sterile pipette, place one drop of centrifuged deposit (see section 6.1.2) or neat specimen on a clean microscope slide.

Examine at 10x and 40x magnification using potassium hydroxide (KOH) or enhanced with calcofluor white or blankophor white staining for fungal hyphae.

## 7.3 Culture

Using a sterile loop inoculate each agar plate with centrifuged deposit (see [UK SMI Q 5 - Inoculation of Culture Media for Bacteriology](#)).

For fungal culture on Sabouraud slopes use a pastette to place 2 - 3 drops of deposit onto the agar.

Table 1: Culture media, conditions, and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temperature °C	Atmosphere	Time		
Rhinosinusitis	Antral washout/ Sinus aspirate/ Sinus washout	Blood agar and / or Staph / Strep selective agar	35 to 37	5 to 10 % CO <sub>2</sub>	40 to 48hrs	daily	<b>Common pathogens:</b> <i>M. catarrhalis</i> <i>S. pneumoniae</i> <i>S. aureus</i> Lancefield Groups A, C, G and F Streptococcus anginosus group Other organisms in pure growth may be significant.
			35 to 37	Air	40 to 48hrs	daily	

Investigation of samples from paranasal sinuses

Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temperature °C	Atmosphere	Time		
Rhinosinusitis	Antral washout/ Sinus aspirate/ Sinus washout	Chocolate agar with or without bacitracin <sup>a</sup>	35 to 37	5 to 10% CO <sub>2</sub>	40 to 48hrs	daily	<b>Common Pathogens:</b> β-haemolytic streptococci Enterobacterales <sup>b</sup> <i>H. influenzae</i> <i>M. catarrhalis</i> Pseudomonads <i>S. aureus</i> <i>S. anginosus</i> group <i>S. pneumoniae</i>
		Neomycin fastidious anaerobe agar with 5 µg metronidazole disc	35 to 37	Anaerobic	48hrs or 5 to 7 days <sup>c</sup>	48hrs and at 5 to 7 days <sup>c</sup>	<b>Common Pathogens:</b> <i>Fusobacterium</i> species <i>Peptostreptococcus</i> species <i>Cutibacterium</i> species <i>Prevotella</i> species

Investigation of samples from paranasal sinuses

Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temperature °C	Atmosphere	Time		
Chronic rhinosinusitis or Rhinosinusitis in immunocompromised patients <sup>d</sup>	Antral washout/ Sinus aspirate/ Sinus washout/ Tissue <sup>e</sup>	Sabouraud dextrose agar supplemented with chloramphenicol or gentamicin <sup>f</sup>	30 to 37 <sup>g</sup>	Air	5 to 7 days <sup>h</sup>	Greater than 40 hrs and at 5 to 7 days <sup>h</sup>	Moulds <sup>i</sup>

**a** May include either a bacitracin 10-unit disc or bacitracin incorporated in the agar. When bacitracin is incorporated into the plate, a separate blood agar plate incubated in 5 to 10% CO<sub>2</sub> will need to be put up to detect *M. catarrhalis* and *S. pneumoniae*.

**b** Gram-negative selective plate (CLED or MacConkey) could be added for the isolation and differentiation of Gram-negative organisms, including those in the Enterobacterales order.

**c** Review the plates at 48 hours. If there is no growth and there is a clinical suspicion of slow-growing facultative anaerobes, such as *Cutibacterium acnes* or *Actinomyces* species then re-incubate for 5 to 7 days.

**d** Immunocompromised patients groups include haematology, oncology, diabetes (uncontrolled), neutropenic (other).

**e** When testing tissue biopsies, specimens should not be overly processed (e.g., crushed) as this may damage hyphal elements and minimise the chances for isolating the fungus.

**f** Supplemented with chloramphenicol or gentamicin; chromogenic media may be useful for identifying mixed fungal infections.

**g** In case of suspected mucormycosis or observation of hyphae of Mucorales in direct microscopy, it is recommended to incubate the cultures at two separate temperatures, 30 °C and 37 °C to increase the yield.

**h** Moulds are the main fungal pathogens for rhinosinusitis whilst yeast growth normally represents colonisation. Therefore, extended incubation is advocated for a minimum of 5 to 7 days, for all surgical samples and for the investigation of fungal rhinosinusitis in high-risk patients. Plates should be read after 40 hours and at 5 to 7 days. Some fungal pathogens may require extended incubation up to 14 days.

If based on travel history endemic mycoses such as histoplasmosis is suspected, samples should be processed in containment level 3 and all yeasts identified to species level.

i Differentiation between yeast and filamentous forms may require microscopic examination. Wet mounts or stain with lactophenol cotton blue (LPCB) should be used.

For fungal culture, one SAB with chloramphenicol or gentamicin plate should be used per sample and streaked as per routine and standard bacteriology practice. However, increasing the number of plates will increase the sensitivity. It is highly recommended that SAB with chloramphenicol or gentamicin plates be sealed with gas-permeable tape or alternatively placed inside a sealable plastic bag during incubation to avoid cross contamination. Incubation of SAB with chloramphenicol or gentamicin plates in 'automated incubation and imaging' modules may lead to fungal contamination of modules and other cultures. No fungal isolate should be dismissed as a 'contaminant' without full identification.

If microscopy is suggestive of organisms not listed in the target list additional and/or other media may be required.

Laboratory should follow local protocols or manufacturer's instructions for optimal incubation conditions.

## 7.4 Identification

Refer to individual UK SMIs for organism identification.

**Table 2. Minimum level of identification in the laboratory**

Target organism	Identification level
<a href="#"><i>Peptostreptococcus</i> species</a>	“anaerobes” level
<a href="#"><i>Cutibacterium</i> species</a>	“anaerobes” level
<a href="#"><i>Fusobacterium</i> species</a>	“anaerobes” level
<a href="#"><i>Prevotella</i> species</a>	“anaerobes” level
<a href="#">β-haemolytic streptococci</a>	Lancefield group level
<a href="#">Enterobacteriaceae</a>	species level
Fungi*	species level (if clinically significant)
<a href="#"><i>H. influenzae</i></a>	species level
<a href="#"><i>M. catarrhalis</i></a>	species level
<a href="#"><i>Pseudomonas</i> species</a>	species level
<a href="#"><i>S. aureus</i></a>	species level
<a href="#"><i>S. anginosus</i></a>	<i>S. anginosus</i> group level
<a href="#"><i>S. pneumoniae</i></a>	species level

\* Moulds are more commonly associated with rhinosinusitis.

**Note:** Any organism considered to be a contaminant may not require identification to species level. Organisms may be identified further if clinically or epidemiologically indicated.

## 7.5 Molecular assays

Molecular methods such as Nucleic Acid Amplification Test (NAAT) and fluorescence *in situ* hybridization have been developed for the rapid detection of fungal species directly from patient specimen. These assays allow for the timely diagnosis of invasive fungal rhinosinusitis from tissue samples and subsequently the prompt initiation of antifungal therapy (57). This is crucial due to the high risk of complications and mortality associated with these types of infections in immunocompromised and diabetic patients. In addition, molecular methods can help overcome some of the challenges encountered with culture-based methods such as false negative results and the identification of rare fungal species (57,58).

There are numerous commercially available assays that have been developed for the detection of fungal organisms ranging from pan fungal to pathogen specific (e.g., *Aspergillus*, Mucorales) assays, with pathogen specific assays having higher sensitivity and faster turnaround time (59,60). The quality of these assays is largely dependent on the analytical range of detection provided by the specific assay, type and volume of specimen, the DNA fungal extraction method and automation of the process to increase sensitivity (60,61). Currently, the validation of NAAT for the diagnosis of fungal rhinosinusitis is relatively limited and it is essential that these assays are validated for the specimen type before their use in laboratories.

Despite the advancement in molecular methods, they are not routinely used for the detection of fungal rhinosinusitis and have not yet replaced culture-based methods in clinical laboratories. These methods would often be used in conjunction with culture-based methods as appropriate or utilised largely by reference laboratories (57,60). Where fungal elements are visualized in specimens but are culture negative, molecular testing may be a useful adjunct test to provide identification (61).

## 7.6 Antigen/antibody testing

Antigen/antibody tests have many limitations and are not regularly used as diagnostic tools for rhinosinusitis, including invasive fungal rhinosinusitis. Factors that can influence the performance of these assays include variation in sensitivity and specificity depending on the clinical setting e.g., underlying conditions and immunosuppression as well as false positive results due to cross-reactivity e.g., individuals with yeast colonisation (62-64).

Furthermore, antibody tests have limited applicability in the diagnosis of invasive fungal rhinosinusitis, because of the reduced or delayed antibody production in immunocompromised individuals.

# 8 Post-laboratory processes (post analytical stage)

## 8.1 Microscopy

### 8.1.1 Reporting microscopy

Report microscopy results as:

#### Gram's stain

- Report presence of WBCs.
- Report if organisms detected.

**Note:** The presence of yeast cells e.g., *Candida* is very rare and usually a reflection of oropharyngeal colonisation. In such cases further histopathological evidence is required.

## Fungal stain

- Report presence or absence of fungal elements.
- Differentiate between yeasts and filamentous fungi (moulds).
- Where possible provide a description of the filamentous fungi/element observed.

### Notes:

- The presence of fungal structures in biopsy specimens from deep tissues should be reported as clear/proven evidence of fungal infection.
- The presence of broad, aseptate or pauci-septate hyphae with wide-angle branching is consistent with Mucorales. The presence of regularly septate hyphae with 45° branching is consistent with *Aspergillus* species. but could represent other hyaline fungi such as *Scedosporium* species.
- Reports simply stating fungal elements seen with no differentiation, are of limited clinical utility and should be avoided.

## 8.1.2 Microscopy reporting time

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

In immunocompromised patients or when fungal investigation is specifically requested, microscopy positive fungal results indicating 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

Report culture results as:

#### Bacterial Culture

- Clinically significant organisms with antimicrobial susceptibility results
- No growth of clinically significant organisms\*
- No growth

\* Identification should not be reported for organisms of no clinical significance.

## Fungal culture

- Yeasts should be reported along with an indication of growth quantity of scanty/light, moderate or heavy to allow for interpretation of significance.
- Isolation of any filamentous fungi should be reported, irrespective of burden.
- No fungal growth.

### Notes

- Fungal growth may be reported as negative after 48 hours incubation although cultures will continue to be incubated up to 5 - 7 days and extended culture (up to 14 days may be required where fungal rhinosinusitis is specifically suspected). 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 noted in the result and not reported as 'fungi not isolated'.
- All clinically significant fungal isolates should be identified to species level (for yeast species, level identification is essential in recurrent or recalcitrant infections).

## 8.2.2 Culture reporting time

Interim or preliminary results should be issued on detection of 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.

See appropriate UK SMIs for supplementary investigations.

## 8.3 Reporting other tests including molecular testing

As newer and more novel methods are becoming available, their validation and reporting should follow local protocols or manufacturer's instructions.

## 9 Antimicrobial susceptibility testing

For interpretation of susceptibility testing results, laboratories should test and interpret according to the 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 \(clsi.org\)](#).

Alternatively, isolates can be sent to an appropriate specialist or reference laboratory. Antifungal susceptibility testing should be performed for fungal rhinosinusitis, when a culture is available.

### 9.1 Reporting of antimicrobial susceptibility testing

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

## 10 Referral to reference or specialist testing laboratories

In case of 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](#)

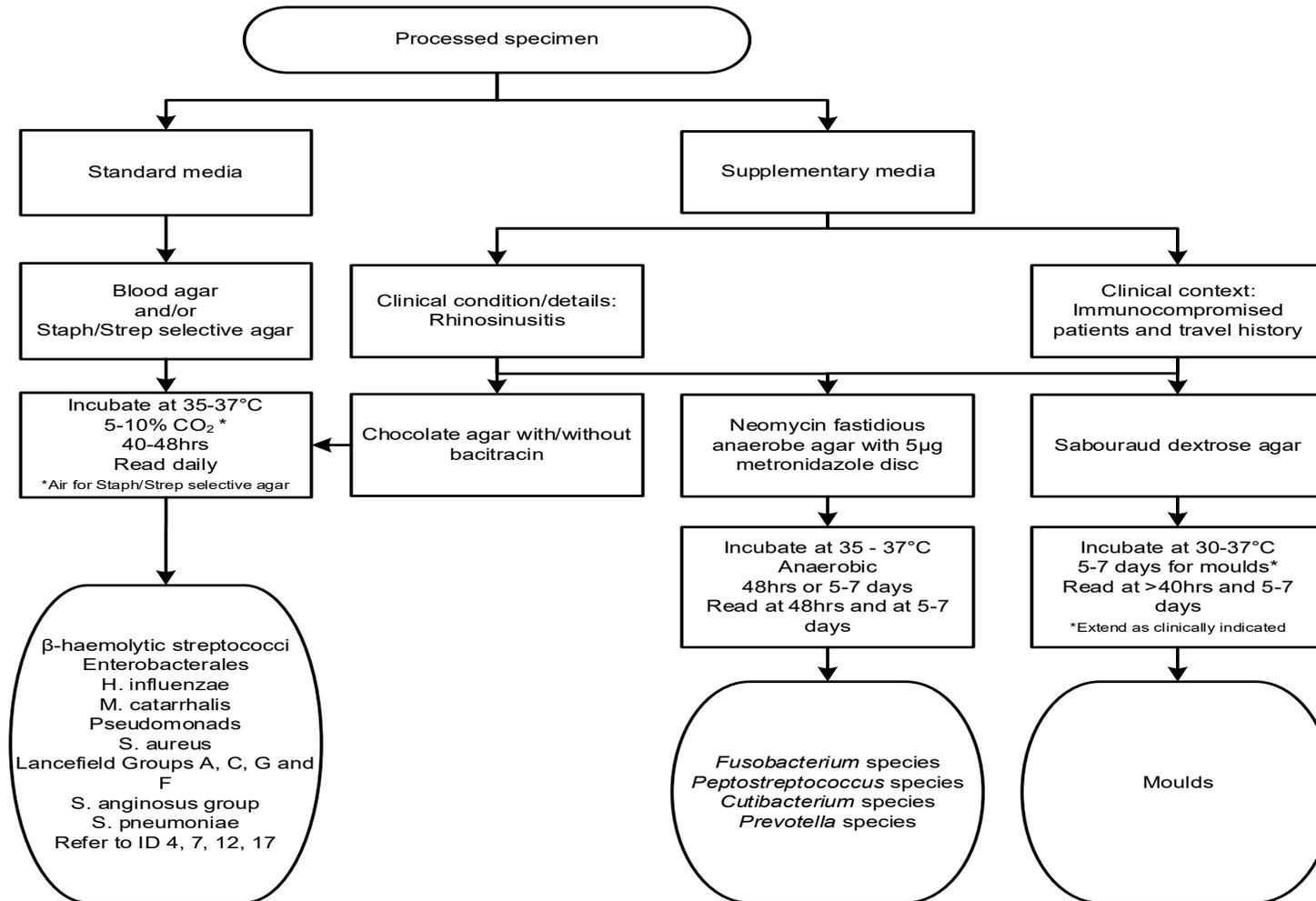
## 11 Public health responsibilities of diagnostic laboratories

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.

## Algorithm: Investigation of samples from paranasal sinuses



Please see Table 1 for algorithm details. If microscopy is suggestive of organisms not listed in target list, additional and/or other media may be required. In addition, if fungal elements are seen but the culture is negative, molecular identification may be useful.

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

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