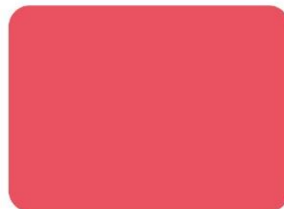
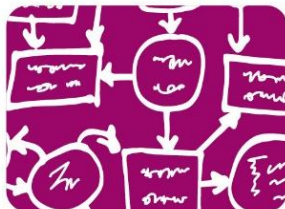
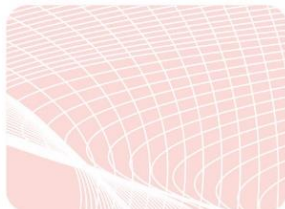
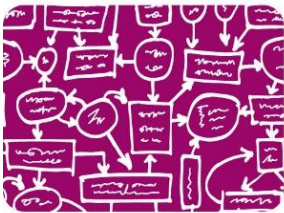
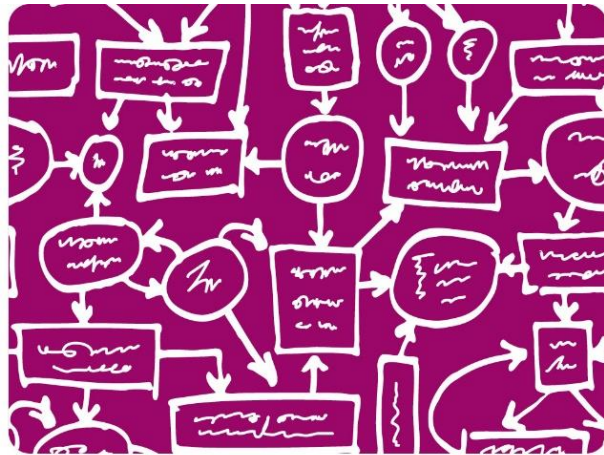
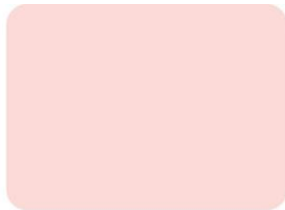




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UK Standards for Microbiology Investigations

Painful and/or discharging ear



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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Amendment table

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

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

Amendment number/date	2/24.04.25
Issue number discarded	1.1
Insert issue number	1.2
Section(s) involved	Amendment
Whole document.	<p>This is an administrative point change.</p> <p>The content of this UK SMI document has not changed.</p> <p>The last scientific and clinical review was conducted on 05.06.24.</p> <p>Hyperlinks throughout document updated to Royal College of Pathologists website.</p> <p>Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms</p> <p>Partner organisation logos updated.</p> <p>Broken links to devolved administrations replaced.</p> <p>References to NICE accreditation removed.</p> <p>Scope and Purpose replaced with General and Scientific information to align with current UK SMI template.</p>
Section 10: Public health responsibilities of diagnostic laboratories	This section has been added to UK SMI templates to highlight the public health responsibilities that diagnostic laboratories have as part of their duties.

Amendment number/date	1/16.09.24
Issue number discarded	1
Insert issue number	1.1
Section(s) involved	Amendment

Section 7.2 - Table 1: Culture media, conditions and organisms	Incubation time for anaerobes updated with a footnote "Review the plates at 48 hours. If there is no growth and there is a clinical suspicion of slow-growing facultative anaerobes, such as <i>Cutibacterium acnes</i> or <i>Actinomyces</i> species then re-incubate for 5 to 7 days".
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Amendment number/date	-/05.06.24
Issue number discarded	-
Insert issue number	1
Anticipated next review date*	04.06.27
Section(s) involved	Amendment
Whole document	This new syndromic document is based on <i>UK SMI B01: Investigation of ear infections and associated specimens</i> . The content and scope have expanded, and the document is presented in a new template with the relevant titles and headings.
Section 4	Background information updated
Section 5	Two new algorithms created
Table 1: Culture media, conditions and organisms	Table has been restructured and updated
Section 9	Antimicrobial susceptibility testing section updated

*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 document provides a comprehensive overview of infections related to painful and/or discharging ear, caused by bacteria, viruses, or fungi. It outlines relevant investigations, utilising molecular, culture, and serological techniques to identify common pathogens.

The scope of this document includes infections affecting the outer, middle, and, to a lesser extent, inner parts of the ear. Inner ear infections are briefly covered due to their significantly distinct clinical presentation.

The document also covers infections associated with medical devices such as hearing aids, ventilation tubes, tympanostomy tubes and post-surgical infections.

This standard primarily targets laboratory professionals involved in diagnosing ear infections in secondary care settings, with some elements useful for primary care. The information presented here is also valuable for General Practitioners (GPs) when sample collection becomes necessary, such as in cases of otitis externa unresponsive to standard treatment.

Please note, following the recent update of fungal taxonomy, many species formerly part of the genus *Candida* now belong to a number of other genera. For the purposes of this document, both old and new names are mentioned as required and they are collectively referred to as '*Candida* and associated ascomycetous yeast' (1).

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

4 Background

The [NHS website](#) provides a table summarising the key differences between outer, middle, and inner ear infections. Otitis externa predominantly affects adults and is triggered by irritants like water, often affecting the ear canal. It can be caused by fungal, bacterial, and viral infections. Otitis media usually affects children and is caused by viruses, with secondary bacterial infection of the middle ear following an initial upper respiratory tract infection. It can also be caused by fungi, typically in more tropical climates. Inner ear infections impact both children and adults, arising from viral or bacterial pathogens, potentially leading to labyrinthitis with disturbances in the labyrinth and vestibular system. Further elaboration on each type of infection is provided in relevant sections below.

4.1 Outer ear infections – Otitis Externa

History and physical examination are essential in diagnosing the type of otitis externa and to initiate effective targeted treatment. In general, infection of the external auditory canal resembles infection of skin and soft tissue elsewhere. However, there are some notable differences. The canal is narrow making it susceptible to the entrapment of foreign materials, build-up of epithelial debris and fluids, leading to irritation and superficial tissue maceration. Otitis externa manifests in various forms, each with distinct characteristics; acute localised, acute diffuse, chronic, and necrotising otitis externa (also called skull base osteomyelitis and previously called 'malignant otitis externa') (2).

Possible complications of otitis externa include cellulitis, perichondritis, chondritis of the pinna and surrounding skin, abscess formation, and parotitis. In such cases early diagnosis and treatment is essential to avoid further complications.

Herpes zoster oticus is mentioned briefly under this section and is highlighted for consideration in individuals with a history of chickenpox. It is not covered in the flowchart as accurate diagnosis is made through Polymerase Chain Reaction (PCR) technique.

4.1.1 Acute localised otitis externa

Acute localised otitis externa, often resulting in a furuncle or pustule of a hair follicle, is primarily caused by *Staphylococcus aureus*. Erysipelas, associated with Group A Streptococcus, may be present in the concha and canal.

4.1.2 Acute diffuse otitis externa

Acute diffuse otitis externa, commonly known as "swimmer's ear," is prevalent among adults, particularly in hot and humid conditions. It is caused by variety of bacteria, the most common being *Pseudomonas aeruginosa* and *S. aureus* and occasionally anaerobes. Fungal pathogens, particularly *Aspergillus*, *Candida* and associated ascomycetous yeast and *Scedosporium* species, contribute to approximately 10% of cases. *Vibrio parahaemolyticus* should be taken into considered in cases where the patient has been swimming in coastal waters. Refer to [UK SMI ID 19 Identification of Vibrio and Aeromonas species](#). Individuals with dermatological conditions such as eczema are more susceptible to developing acute diffuse otitis externa. Conditions predisposing people to fungal otitis externa include trauma, diabetes, ENT surgery, high humidity or temperature, ear drum perforation and previous use of topical antibiotics and steroids (3,4).

4.1.3 Chronic otitis externa

Chronic otitis externa is inflammation lasting longer than 3 months. It is the result of recurrent otitis externa with bacterial or fungal infections and may be associated with underlying skin conditions. Fungal pathogens including *Aspergillus* species or *Candida albicans* are common causes. Skin disease such as atopic dermatitis, erysipelas, psoriasis and discoid lupus erythematosus involving the ear canal are predisposing factors (3). These conditions present similarly but can also become secondarily infected with bacteria and fungi.

4.1.4 Necrotising otitis externa

It is very important to identify necrotising otitis externa (also called skull base osteomyelitis and previously called 'malignant otitis externa'). This is a severe necrotising infection that spreads from the squamous epithelium of the ear canal into surrounding soft tissues, cartilage and bone. Primarily affecting elderly, diabetic, or immunocompromised individuals, and those who underwent radiotherapy. It is potentially a life-threatening condition with risk of neurological involvement including facial nerve paralysis. Early diagnosis and treatment is essential. *P. aeruginosa* is the most common causative agent, followed by *S. aureus*. If bacterial cultures cannot be obtained or have no growth, empiric treatment should cover *P. aeruginosa*. Fungal infections are rare with infrequent case reports of *Aspergillus*, *Candida* and associated ascomycetous yeast, Mucorales, and *Scedosporium* species as causative agents. In many cases the initiating infection in the ear canal may settle with topical treatment, but the skull base osteomyelitis may persist. In such cases ear swabs from the ear canal are inadequate in guiding the treatment. Biopsy of the granulation tissue is recommended for microbiological and histopathological examination to exclude other causes, such as malignancy or cholesteatoma (3,5,6).

Refer to the diagnostic and management algorithm provided by [ENT UK on necrotising otitis externa](#).

4.1.5 Herpes zoster oticus

Herpes zoster oticus, also known as Ramsay Hunt Syndrome, results from the reactivation of the dormant varicella-zoster virus (VZV) in individuals with a history of chickenpox. It presents with facial nerve paralysis, severe ear pain with vesicular rash in the ear and vertigo. The incidence and severity increase with age and in immunocompromised patients. Early diagnosis and management is recommended. Diagnosis is primarily based on clinical evaluation, with confirmation through Nucleic Acid Amplification Testing (NAAT) testing (7,8).

4.2 Middle ear infections – Otitis Media

Otitis media covers a spectrum of diseases, including acute otitis media, acute mastoiditis, chronic otitis media and otitis media with effusion. While less common in adults, the causative organisms and treatment parallel those in children. The more widespread uptake of the pneumococcal vaccination may impact the spectrum of the causative organisms for this condition (9).

An external ear swab is generally not useful in the investigation of otitis media unless eardrum perforation with purulent discharge into the ear canal occurs, and standard treatment is ineffective. Tympanocentesis is rarely necessary for sampling middle ear effusion.

It is essential to differentiate acute otitis media from otitis media with effusion ("glue ear") to prevent unnecessary antibiotic prescription. An alternative approach involves antimicrobial prophylaxis through myringotomy and tympanostomy tube placement (10).

A very common presentation in young children is turbid effusion in the ear, with chronic bacterial infection characterised by recurrent episodes of ear pain and ear or

nasal discharge. In these young children without fully developed paranasal sinuses the nasal discharge often reflects the bacterial flora in the middle ear.

For immunosuppressed patients with a history of otitis externa and evidence of necrosis or eardrum perforation, consider the presence of fungal infection.

4.2.1 Acute otitis media

Acute otitis media is defined by middle ear inflammation lasting less than 3 months and is characterised by the presence of purulent fluid in the middle ear usually with signs and symptoms of acute illness, such as fever and earache. It occurs when nasopharyngeal organisms ascend the eustachian tube, and are not eliminated by the defence mechanisms of the middle ear and may occur following a viral upper respiratory tract infection. The most common bacterial pathogens that cause this type of infection are *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. Viral pathogens include respiratory syncytial virus (RSV), rhinovirus, adenovirus, influenza virus, and parainfluenza virus (9,11). Diagnosis of acute otitis media can be based on the symptoms as the disease develops and through pneumatic otoscopic examination, with most cases of children resolving without treatment (12).

Consider otitis media as a common complication of measles in high-risk individuals such as infants, children aged less than 5 years and immunocompromised (13).

4.2.2 Acute mastoiditis

Acute mastoiditis is the most common complication of acute otitis media. It is an acute infection and inflammation in the mastoid, primarily affecting children. *S. pneumoniae* is the predominant pathogen, followed by *S. aureus*, *Streptococcus pyogenes*, and *H. influenzae*. *P. aeruginosa* is more common in patients with recent recurrent acute otitis media or recent antibiotic use. Surgical intervention is often necessary, providing an opportunity to collect samples for culture and antimicrobial susceptibility testing (14,15).

4.2.3 Chronic otitis media

Chronic otitis media also known as chronic suppurative otitis media (CSOM) is inflammation of the middle ear lasting over 3 months, exists in mucosal and squamous forms. Chronic mucosal otitis media results from ear drum perforation. Chronic squamous otitis media results from retraction of the ear drum into the middle ear with trapped squamous epithelium (often termed cholesteatoma). Chronic otitis media can be further subdivided into active and inactive according to the presence and absence of infection. Repeated infection can be destructive and, if longstanding, can be associated with complications such as hearing loss, facial palsy and intracranial infection. The most common bacterial pathogen is *P. aeruginosa* but may rarely result from meticillin resistant *staphylococcus aureus* (MRSA) with anaerobic bacteria found in 25% of patients (16).

4.2.4 Otitis media with effusion

Otitis media with effusion also known as 'glue ear' is characterised by the collection of non-infected fluid in the middle ear space without signs or symptoms of acute ear infection. In most instances, the fluid clears spontaneously and the hearing recovers. It may be asymptomatic, but it is the leading cause of childhood hearing impairment, often affecting children between 6 months and 4 years. Children with Down syndrome

or cranio-facial malformation (including cleft palate) are at increased risk of developing otitis media with effusion. Although organisms may be cultured, it is not considered an active infection requiring culture or antimicrobial treatment (17,18).

In adults with lymphadenopathy, consider chronic viral infections, including Human Immunodeficiency Virus (HIV), in addition to other differential diagnoses (19).

4.3 Inner ear infections – labyrinthitis and vestibular neuritis

4.3.1 Labyrinthitis

Labyrinthitis is inflammation of the membranous labyrinth, and can be caused by viruses, bacteria, or systemic diseases. It presents with vertigo, nausea, vomiting, tinnitus, and/or hearing loss. In most cases, labyrinthitis is caused by a viral infection such as varicella zoster, herpes simplex, measles, mumps. Hearing loss can be caused by congenital cytomegalovirus and rubella infection. Bacterial labyrinthitis is a complication of otitis media or bacterial meningitis. Diagnosis is supported by history, physical examination, and audiometry (20,21). Suppurative labyrinthitis is a severe form that requires management similar to meningitis.

4.3.2 Vestibular neuritis

Vestibular neuritis is inflammation of the vestibular nerve, often following a viral infection or secondary to ischaemia of the anterior vestibular artery. Viruses causing upper respiratory tract infections, such as influenza virus, adenovirus, herpes simplex virus, cytomegalovirus, Epstein-Barr virus, and parainfluenza virus are linked to vestibular neuritis. Herpes simplex virus type I is the most common cause of viral infection of the vestibular. Vestibular neuritis is characterised by acute spontaneous vertigo without hearing loss. Other symptoms include nausea, vomiting, and unsteadiness (22,23).

Hearing loss is a feature of labyrinthitis, but hearing is not affected in vestibular neuritis.

4.4 Infections associated with medical devices

4.4.1 Hearing aid

The use of a hearing aid can alter the ear canal flora, increasing the risk of fungal and bacterial otitis externa. Symptoms include debris/wax accumulation and irritation, itching and ear discharge. It is recommended to use appropriate hygiene routinely to clean and disinfect hearing aids and ear moulds regularly (24). Early sampling or swabbing is advised for patients with secondary ear canal complications related to hearing aid usage; this also applies to the use of earpods for prolonged periods of time.

4.4.2 Tympanostomy tube (grommet)

Tympanostomy tube insertion is a common procedure to improve hearing and reduce middle ear infections. The most common complication of tube insertion is otorrhea (25).

In addition, water precautions may be considered for children at risk of infection or complications.

4.4.3 Cochlear implant

The use of cochlear implants is common in patients with sensorineural hearing loss, particularly in children younger than 3 years. Surgical site infection and acute otitis media leading to bacterial meningitis are rare but severe complication of cochlear implant. It is recommended to have regular check-ups and look for possible signs and symptoms of meningitis and ear infection. In addition, ensure that the patient is up to date with their vaccination before having a cochlear implant (26). Early sampling may be useful in case of suspected infections.

4.5 Treatment based on clinical judgment

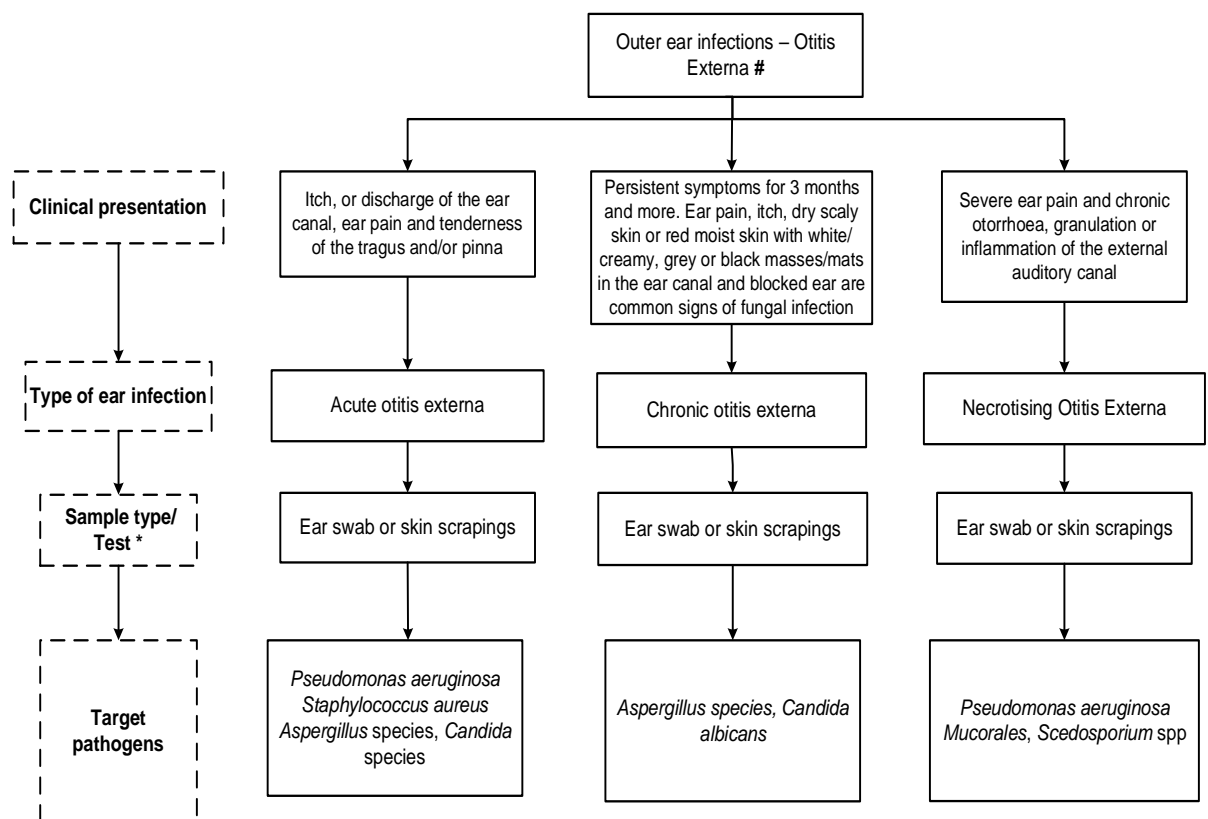
In a primary care setting, the management of otitis externa and otitis media is typically guided by clinical presentation, history and otoscopic examination. For additional details, consult the guidelines provided by NICE (12,27,28).

The most common complication of tympanostomy tube insertion is otorrhea due to bacterial ingress which is best managed with quinolone antibiotic eardrops (25).

Patients not responding to treatment or showing symptoms or signs of a more serious illness or condition may benefit from microbiological analysis of samples from the ear. Referral to secondary care should be considered in such cases.

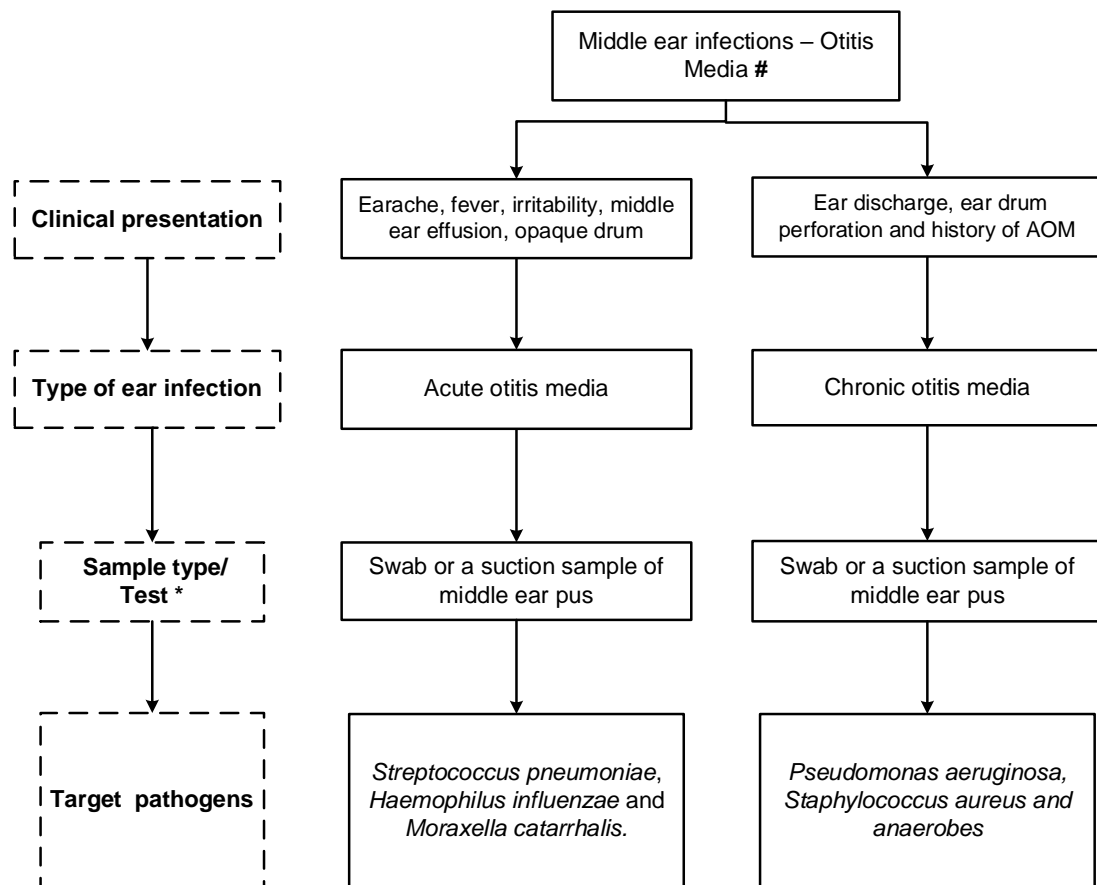
5 Clinical presentations of painful and/or discharging ear

5.1 Outer ear infections – Otitis Externa



1. * Microbiological testing should be pursued if clinically indicated
2. # Some infections can also be caused by upper respiratory viral infections, refer to section 4 for more information

5.2 Middle ear infections – Otitis Media



1. * Microbiological testing should be pursued if clinically indicated
2. # Some infections can also be caused by upper respiratory viral infections, refer to section 4 for more information

Note: Microbiological testing is not required for otitis media with effusion

6 Pre-laboratory processes (pre-analytical stage)

6.1 Specimen type

The main indications for microbiological diagnosis are: severe or unusual presentation; post-surgical and device associated infections; poor response to standard treatment; and infection in immunocompromised patients.

The type of specimens include (if clinically indicated):

- Otitis Externa:
 - Ear swab
 - Skin scrapings from pinna for fungal diagnostics
 - Herpes zoster oticus: swabs from skin lesions are preferred for detecting VZV from the external ear (29).

Notes:

Swabs taken from the nasopharynx for diagnosis of ear infections are inappropriate and should be discarded according to local protocols.

Send a swab sample from the affected ear for culture and antimicrobial susceptibility testing.

If seborrheic dermatitis of auricle is suspected skin scrapings may be required. Please refer to [UK SMI B 39 – investigation of dermatological specimens for superficial mycoses](#).

In the case of skull base osteomyelitis, early sampling particularly in vulnerable patients, is recommended.

- Otitis Media:
 - Swab or a suction sample of middle ear pus.
 - Under specialist guidance by the ENT: a nasal swab may be useful for a young child suffering from recurrent episodes of painful ear with nasal discharge.
 - For the investigation of complex fungal infections, scrapings of material from the ear canal are preferred as they allow direct microscopy. However, swabs can also be used if direct microscopy is not required.
- Device associated infections:
 - Swab

Note:

Under specialist guidance: for hearing aid and cochlear implant users early sampling or swabbing of the affected area may be useful.

6.2 Specimen collection and handling

- Collect specimens as soon as possible after onset of symptoms.
- Collect all specimens before antimicrobial therapy where possible.
- Swabs should be slim enough to comfortably fit in the ear canal.
- When collecting samples from the ear to aid in diagnosis, the tip of the microbiology swab should only touch the site of infected debris to minimise risk of contamination with normal commensal bacteria. When using a swab to collect middle ear pus the outer ear canal should be first cleaned for the same reason. It is not necessary to wear sterile gloves or prepare the surrounding skin.
- For the investigation of fungal infections, the same swabbing technique should be applied as for bacterial infections.
- For intact ear drum, clean ear canal to remove any scabbing and superficial debris and collect fluid via syringe aspiration technique
- For ruptured ear drum, collect fluid on flexible shaft swab via auditory speculum
- For the outer ear, use a moistened swab to remove any debris or crust from the ear canal. Obtain a sample by firmly rotating a swab in the outer canal (30).

Note: Wooden swabs are not recommended as they can be contaminated with fungi, in particular *Aspergillus* and other mould spores.

Refer to current guidance on the safe handling of all organisms in the [safety considerations section](#).

6.3 Specimen transport and storage

This section covers specimen transport and storage consideration related to this UK SMI, and should be read in conjunction with the [scientific information on the UK SMI website](#)

- Unless otherwise stated, swabs for bacterial and fungal culture should be placed in appropriate transport medium.
- Pus samples should be collected in CE marked leak-proof containers and placed in sealed plastic bags.

All specimens should be transported and processed as soon as possible. If processing is delayed, refrigeration is preferable to storage at ambient temperature.

For safety considerations refer to Section 6.5.

6.4 Relevant clinical details needed on patient request forms when referring samples to the laboratory

Full clinical details and information on patient history should be provided with clinical requests.

Clinical details should include:

- specimen date and time of collection
- where the sample has been taken from, such as the outer ear and middle ear
- type of infection suspected

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- type of swab or sample sent to the laboratory
- immune status
- other relevant information (travel history, occupation, trauma, ENT surgery, presence of grommets, hearing aid wearer, water exposure)

6.5 Safety considerations

The section covers specific safety considerations (31-52) related to this UK SMI, and should be read in conjunction with the general [safety considerations on the UK SMI website](#).

All Hazard group 2 organisms must be confirmed at Containment Level 2.

Containment level 3 organisms are extremely rare causes of painful and or discharging ear.

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

Collect swabs into appropriate transport medium.

Compliance with postal, transport and storage regulations is essential.

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

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.

7 Laboratory processes (analytical stage)

7.1 Microscopy

7.1.1 Specimen processing:

- Swab: microscopy is not recommended for external ear swabs
- Pus: middle ear pus should be assessed by microscopy
- Skin Scrapings: should be assessed by microscopy

If skin scrapings from the ear canal are sent specifically for fungal investigation a fungal stain (potassium hydroxide – calcofluor white (KOH-CFW) preparation) should be performed. Refer to [UK SMI TP 39 – Staining procedures](#) for detailed protocols for bacterial and fungal staining.

For safety considerations refer to Section 6.5.

7.2 Culture

7.2.1 Specimen processing:

- Swab: Inoculate each agar plate directly by rolling the swab on a part of the plate (refer to [UK SMI Q 5 - Inoculation of Culture Media for Bacteriology](#)). Swabs taken from the nasopharynx for diagnosis of ear infections are inappropriate and should be discarded according to local protocols.
- Pus: Using a sterile pipette inoculate each agar plate with the specimen (refer to [UK SMI B 14 – Investigation of pus and exudates](#)).
- Skin Scrapings: If scrapings of material from the ear canal are sent for investigation use a sterile loop to inoculate the material onto agar.

For the isolation of individual colonies, spread inoculum with a sterile loop.

For safety considerations refer to Section 6.5.

Table 1: Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target Organisms
			Temperature °C	Atmosphere	Time		
Outer ear infections – Otitis Externa: Acute localised otitis externa Acute diffuse otitis externa Chronic otitis externa Necrotising otitis externa	All swabs / Pus / Tissue	Chocolate agar with or without bacitracin ^a	35 to 37	5 to 10 % CO ₂	40 to 48hrs	Daily	Common Pathogens: <i>H. influenzae</i> <i>M. catarrhalis</i> <i>P. aeruginosa</i> <i>S. pneumoniae</i> <i>S. aureus</i> <i>Lancefield group A streptococcus</i> Other organisms in pure growth may be significant. Consider <i>Neisseria meningitidis</i> when bacterial meningitis suspected
	Swabs Tissue / Pus	Neomycin fastidious anaerobe agar with metronidazole 5µg disc	35 to 37	Anaerobic	48hrs or 5 to 7 days ^b	48hrs and at 5 to 7 days ^b	Anaerobes Polymicrobial infections possible, extended incubation recommended for osteomyelitis

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Middle Ear infections – Otitis Media: Acute otitis media Acute mastoiditis Chronic otitis media	All swabs/ Pus / Tissue/ tissue fluid	Blood agar ^a And / Or Staph/strep selective agar	35 to 37 35 to 37	5 to 10 % CO ₂ Air	40 to 48hrs 40 to 48hrs	Daily Daily	<i>M. catarrhalis</i> <i>S. pneumoniae</i> <i>S. aureus</i> <i>H. influenzae</i> Lancefield Groups A, C, G and F Streptococcus anginosus group Other organisms in pure growth may be significant
	All swabs	CLED or MacConkey agar	35 to 37	Air	16 to 24hrs	greater than 16hrs	Common Pathogen: <i>P. aeruginosa</i>
	Swabs Tissue / Pus	CLED or MacConkey agar	35 to 37	Air	16 to 24hrs	greater than 16hrs	Common Pathogens: <i>P. aeruginosa</i> Clinical circumstances determine the significance of the following isolates. consider reporting for pus /tissue samples or when necrotising infection present Pseudomonads Enterobacterales Other nonfermenting Gram negative bacilli

Painful and/or discharging ear

Otitis Externa and Otitis Media	Tissue / Pus / Swabs / Skin scrapings	Sabouraud dextrose agar supplemented with chloramphenicol or gentamicin ^c	30 to 37	Air	5 to 7 days for moulds 40 to 48hrs for yeasts	Daily	Moulds and yeasts
<p>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% CO₂ will need to be put up to detect <i>M. catarrhalis</i> and <i>S. pneumoniae</i>.</p> <p>b Review the plates at 48 hours. If there is no growth and there is a clinical suspicion of slow-growing facultative anaerobes, such as <i>Cutibacterium acnes</i> or <i>Actinomyces</i> species then re-incubate for 5 to 7 days.</p> <p>c Supplemented with chloramphenicol or gentamicin, chromogenic media may be useful for identifying mixed fungal infections.</p> <p>d In cases where extended incubation is clinically indicated then incubation should be extended to 5 to 7 days for isolation of moulds. In such cases plates should be read at greater than 40 hours for early sign of growth and then left in the incubator/cabinet until day 5 to 7. Certain opportunistic pathogens will require extended incubation.</p> <p>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.</p> <p>In patients coming from endemic regions, tuberculous granuloma of the middle ear should be considered and appropriate cultures set up, see UK SMI B 40 – Investigation of Specimens for Mycobacterium species.</p>							

7.3 Identification

Refer to individual UK SMLs for organism identification.

All clinically significant isolates should be identified to species level.

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.

7.4 Molecular testing

Investigation of varicella-zoster virus (VZV):

- Nucleic Acid Amplification Testing (NAAT) is the most sensitive method for confirming a diagnosis of varicella to detect VZV in skin lesions (29).
- Deep sterile site samples may benefit 16S rRNA and or panfungal PCR.

8 Post-laboratory processes (post analytical stage)

8.1 Microscopy

8.1.1 Reporting microscopy

Report microscopy results as:

Gram stain

1. Report presence of WBCs
2. Report if organisms detected.

Fungal stain

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

Notes:

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

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

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.
- Any isolation of filamentous fungi should be reported
- No fungal growth
- Fungal growth may be reported as negative after 48 hours incubation although cultures will continue to be incubated 5-7 days. In the event of fungal growth, a further report will be issued

Note:

The presence of fungi should be documented even when a fungal culture is overgrown by chloramphenicol-resistant Gram-negative bacterial (e.g., *Pseudomonas* species). This should be noted in the result and not reported as 'fungi not isolated'.

All clinically significant isolates should be identified to species level (in cases of recurrent or persistent infections, precise identification at the species level is crucial for yeasts).

8.2.2 Culture reporting time

Interim or preliminary results should be issued promptly upon 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 conveyed through telephone or transmitted electronically in accordance with local policies.

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

8.3 Reporting other tests including molecular testing

As newer and more novel methods are becoming available, their validation and reporting would follow local laboratory testing protocols.

9 Antimicrobial susceptibility testing

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

Laboratories should test and interpret antimicrobial susceptibility using the criteria in The European Committee on Antimicrobial Susceptibility Testing (EUCAST), 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.

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 Public health responsibilities of diagnostic laboratories

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

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

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

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

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