UK Standards for Microbiology Investigations

Investigation of Throat Related Specimens

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Acknowledgments

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For further information please contact us at:

Standards Unit
Microbiology Services
Public Health England
61 Colindale Avenue
London NW9 5EQ
E-mail: standards@phe.gov.uk

Website: https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

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# Amendment Table

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

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

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| Whole document. | The title of the document has been broadened to include other clinical specimens apart from throat swabs. |
|                 | The protocol document P 3: Recommendations for screening specimens for Corynebacterium species for has been merged with B 9. |
|                 | The whole document has been updated to reflect these changes. |

| Scope of Document. | This has been updated with other throat related specimens that could be requested in upper respiratory infections. |

| Introduction. | Laryngitis has been added as one of the several types of inflammation of the upper respiratory tract. |
|               | The subheading “Rare Causes of Pharyngitis” has been updated with more information. |

| Safety considerations. | This section has been updated accordingly with information on Hazard Group 3 organisms. |

| Specimen Processing/Procedure. | Sections 2.1, 4.5, 4.6, 4.8 have been updated accordingly. |
|                               | All the links have been updated. |

| Reporting Procedure. | This section has been updated accordingly. |

| Appendix: Investigation of Throat Related Specimens. | The flowchart has been updated to reflect the information on the table in subheading 4.5. |
UK SMI#: Scope and Purpose

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

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

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

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

Quality Assurance
NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

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

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

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

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

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

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

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

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Suggested Citation for this Document
Scope of Document

Type of Specimen
Throat swab, posterior pharyngeal swab, nasopharyngeal swab, pharyngeal washings, pus aspirate, oropharyngeal swab, throat gargle

Scope
This SMI describes the examination of bacteria and fungi from throat related specimens known to cause upper respiratory tract infections.

For more information, refer to
ID 6 – Identification of Neisseria species
B 51 - Screening for Neisseria meningitidis
B 29 - Investigation of Specimens for Screening for MRSA, and
B 14 - Investigation of Abscesses and Deep-Seated Wound Infections.

For viruses that may be isolated from throat swabs, refer to G 8 – Respiratory Viruses.

This SMI should be used in conjunction with other SMIs.

Introduction
Throat related specimens are one of the most commonly performed procedures in patients with upper respiratory tract infections. This is usually carried out in primary care facilities and in emergency departments.

Upper respiratory tract infections are classified according to the type of inflammation they cause. As with many infections, the primary challenge in these conditions lies in identifying the causative pathogen and determining the extent of disease progression. There are several types of inflammation of the upper respiratory tract and they are as follows\(^1,2\):

- Pharyngitis (also known as sore throat)
- Tonsillitis
- Epiglottitis
- Laryngitis

Pharyngitis\(^1,2\)
Pharyngitis is inflammation of the pharynx. It is also known as “sore throat”. This infection may be acute or chronic. Most cases are caused by viruses but it can also be caused by bacteria. Clinically, it is difficult to differentiate between bacterial and viral cause of pharyngitis based on symptoms alone. The typical symptoms are a sore throat, fever and headache but may be associated with nausea and vomiting, abdominal pain, muscle pain, scarlet fever and rashes.
Organisms commonly isolated from pharyngitis are as follows;

**Streptococci**

The most common cause of bacterial pharyngitis is the Lancefield group A, *Streptococcus pyogenes*. Healthy carriers of group A streptococci are usually children in whom rates of up to 20% - 30% have been reported, but rates are much lower in adults (5% - 15%)\(^2\). In these individuals isolation of Lancefield group A streptococci does not necessarily imply a role in infection.

Extrapharyngeal manifestations of Lancefield group A streptococcus infection can be divided into those associated with acute infection and the nonsuppurative post streptococcal sequelae such as acute rheumatic fever and glomerulonephritis, which occur 2-3 weeks after pharyngeal infection\(^4\). In acute infection, bacteraemia and streptococcal toxic shock may occur. Post streptococcal sequelae appear to be limited to a circumscribed set of serotypes\(^5\).

Lancefield group C streptococci have been reported as a cause of pharyngitis\(^6\). The majority of the species, however, are zoonotic and rarely cause disease in humans; these include *Streptococcus equi* subspecies *zooepidemicus*, *Streptococcus equi* subspecies *equi* and *Streptococcus dysgalactiae* subspecies *dysgalactiae*. The beta-haemolytic group C streptococci infecting humans include the large colony form *Streptococcus dysgalactiae* subspecies *equisimilis* and the minute colony form or *Streptococcus anginosus* group (formerly the *S. milleri* group), which includes *Streptococcus constellatus* subspecies *pharyngis* and *Streptococcus anginosus*. These organisms are very rarely implicated in bacterial pharyngitis, and may express A, C, F or G Lancefield group antigens. The Lancefield group G streptococci are known to cause pharyngitis and are subdivided into the "large colony" form (which comprises the animal species *Streptococcus canis* and the human species *Streptococcus dysgalactiae* subspecies *equisimilis*, which is the only recognised causative agent of pharyngitis within the group) and the "minute colony" form (*S. anginosus*)\(^7\).

Most of the evidence for Lancefield groups C and G streptococci causing pharyngitis comes from reports of outbreaks\(^8\)-\(^11\).

**Corynebacterium diphtheriae**

Diphtheria is an acute infectious disease of the upper respiratory tract and occasionally the skin. It is caused by toxigenic strains of *Corynebacterium diphtheriae* (of which there are 4 biotypes - *gravis, mitis, intermedius* and *belfanti*) and some toxigenic strains of *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis*\(^12\). All can carry the phage-borne diphtheria toxin gene. In a fully developed case of diphtheria, this toxin damages the pharyngeal epithelium to produce a leathery membrane, giving the disease its name. This membrane may occlude the airway, sometimes causing death by respiratory obstruction. Systemic absorption by the host of the toxin from the primary site of replication may damage a wide range of cells, including those of the heart and nervous system. Myocarditis and neurological dysfunction may cause or contribute to disability or death.

The usual site of carriage or infection is the throat or nasopharynx, occasionally the nose.

Mild cases of the disease resemble streptococcal pharyngitis and the classic pseudomembrane of the pharynx may be lacking. It is thought that *C. diphtheriae* has additional virulence factors because invasive disease caused by non-toxigenic strains
has been reported\textsuperscript{13,14}. Non-toxigenic strains of \textit{C. diphtheriae} may be encountered in clinical specimens, especially those taken from persons previously immunised against diphtheria toxin. Non-toxigenic \textit{C. diphtheriae} has been suggested as a cause of sore throat, but does not cause a true diphtheritic membrane or symptoms attributable to systemic absorption of toxin\textsuperscript{12}. On re-introduction of the necessary gene, these organisms may, however, express toxin production. There is a suggestion that particular clones of non-toxigenic \textit{C. diphtheriae} may be especially virulent as described from Russia and other former Soviet states\textsuperscript{15,16}. Occasionally, humans will develop invasive infections with non-toxigenic strains of \textit{C. diphtheriae}\textsuperscript{13,14}. These conditions appear to be rare, and will be detected by blood culture rather than by culture of throat or nasopharyngeal swabs.

Although toxigenic \textit{C. ulcerans} generally causes mild pharyngitis without any associated sequelae, at least as many cases of clinical diphtheria are now caused by \textit{C. ulcerans} as by \textit{C. diphtheriae} in England and Wales since the 1990s\textsuperscript{17,18}. There is no direct evidence of person-to-person transmission of \textit{C. ulcerans}, but it is thought that this may occur. \textit{C. ulcerans} may infect the bovine udder and an association between human \textit{C. ulcerans} infection and drinking raw milk has been observed\textsuperscript{19}. However, molecular studies have indicated that domestic animals (livestock, pet cats and dogs) may be a more likely source of infection\textsuperscript{20-22}.

The pathogenic mechanism is unclear. However, as a consequence of the genome sequence being published, genes encoding adhesins, fimbriae and other products have now been identified and are thought to contribute towards pathogenicity\textsuperscript{23}. Non-toxigenic strains in pharyngeal flora have the potential to undergo lysogenic conversion to toxin production in vivo, which may lead to disease\textsuperscript{24}.

In the 1990s there was an increase in the incidence of diphtheria in Russia and other former Soviet states, although the situation is now improving\textsuperscript{25}. Diphtheria cases have continued to be reported from every WHO Region, especially the higher risk regions eg Africa, South East Asia and South America. Following enhancement or introduction of appropriate Public Health interventions, such as immunisation, case-finding and treatment of cases and carriers within these countries, there is now evidence that the situation has improved but there is still a strong need to maintain microbiological surveillance, laboratory expertise and an awareness of these organisms amongst public health specialists, microbiologists and clinicians\textsuperscript{25-27}.

In a susceptible population the introduction of a toxigenic strain can result in direct spread by droplet infection. Mass immunisation has resulted in the virtual disappearance of toxigenic \textit{C. diphtheriae} from the United Kingdom, but it might not have affected the carriage of non-toxigenic strains. Most cases of toxigenic \textit{C. diphtheriae} reported in the UK are imported from South East Asia and the Indian sub-continent and these diphtheria cases continue to be reported in South-East Asia, South America, Africa and India. A large number of UK citizens travel to and from these regions, maintaining the possibility of the reintroduction of \textit{C. diphtheriae} into the UK\textsuperscript{26}. However, according to the UK schedule, all travellers to epidemic or endemic areas should ensure that they are fully immunised. It also highlights the need to maintain UK routine vaccination coverage at the 95% level in the UK as recommended by the World Health Organization\textsuperscript{17,28}.

\textbf{Note:} For more information on the new diphtheria guidelines, see the Public Health Control and Management of Diphtheria in England and Wales publication. The

**Criteria for screening throat swabs for C. diphtheriae**

There are specific clinical associations and exposures which, if reported on requests, should trigger examination of specimens for *C. diphtheriae* or *C. ulcerans*. These are based on recognised risk factors and information from enhanced diphtheria surveillance. For more on relevant information that requests may have, see ID 2 – Identification of Corynebacterium species.

However, this SMI recommends testing for *Corynebacterium* species on samples from symptomatic patients for which the following information is provided:

- Membranous or pseudomembranous pharyngitis/tonsillitis
- Contact with a confirmed case within the last 10 days
- Travel abroad to high risk area within the last 10 days
- Contact with someone who has been to a high risk area within the last 10 days
- Contact with any animals (including household pets, visiting a farm or petting zoo) within the last 10 days
- Recent consumption of any type of unpasteurised milk or dairy products
- The patient works in a clinical microbiology laboratory, or similar occupation, where *Corynebacterium* species may be handled

**Other causes of pharyngitis**

**Vincent’s angina**

*Borrelia vincentii* and *Fusobacterium* species are associated with the infection known as Vincent’s angina. It is characterised by ulceration of the pharynx or gums and occurs in adults with poor mouth hygiene or serious systemic disease ²⁹.

While there is some evidence that *Fusobacterium* species can be detected frequently from throat swabs, its prevalence in cases of acute tonsillitis has not yet been established ³⁰,³¹.

**Arcanobacterium haemolyticum** (previously *Corynebacterium haemolyticum*)

Although *Arcanobacterium haemolyticum* is recognised as a human pathogen, this SMI does not recommend routine investigation for the organism. It has been associated with tonsillitis, pharyngitis and may cause a rash in young adults and occasionally in children ¹⁶,³². It is suggested that in cases of treatment failure and recurrent tonsillitis, isolation of *A. haemolyticum* should be considered.

After 48hr incubation on blood agar, *A. haemolyticum* colonies exhibit narrow zones of ß-haemolysis and are approximately 0.5mm in diameter. In cases where *A. haemolyticum* is suspected, incubation of culture plates may need to be extended up to 72hr. The organism’s presence may be indicated by the pitting of the agar underneath the colony; when the colony is pushed aside a minute dark pit is revealed ³³.

**Fungal throat and pharyngeal infections**

These infections are common in patients who are immunocompromised, particularly during episodes of severe neutropenia. Patients receiving antibiotics are also prone to
fungal infections. *Candida* species may rarely cause severe invasive oesophagitis which can result in desquamation and expulsion of tissue. Recognition of oropharyngeal candidosis accompanied by dysphagia indicate the possibility of oesophageal candidosis and this may be an AIDS-defining illness. Yeast and fungal isolates from patients who are immunocompromised usually require identification and susceptibility testing.

**Fusobacterium necrophorum**

*Fusobacterium necrophorum* infection may be characterised by acute pharyngitis and fever, sometimes accompanied by membranous tonsillitis. In the absence of therapy, a small number of these patients may develop the bacteraemia and metastatic infection characteristic of Lemierre’s disease, which can be life threatening.

*Fusobacterium necrophorum* has been isolated in cases of recurrent or persistent sore throat, and is a common cause of peritonsillar abscess or quinsy. It is believed that up to half a million patients may present with pharyngitis due to this organism annually. The literature, however, also suggests that the organism may form a minor part of the normal microflora of the upper airways in some individuals, although it has proven to be difficult to obtain primary evidence for this.

**Neisseria gonorrhoeae**

Pharyngeal specimens contain a variety of microorganisms including saprophytic *Neisseria* species. Identification of *Neisseria gonorrhoeae* from extragenital sites such as the oropharynx must be carefully performed and checked as a positive result can have important clinical and medico-legal implications (refer to ID 6 – Identification of *Neisseria* species). Pharyngeal colonisation may be found in patients with genital gonorrhoea, but the pharynx is rarely the only infected site.

**Rare causes of Pharyngitis**

**Francisella tularensis**

Oropharyngeal tularemia (Type B tularemia) is contracted by ingestion of contaminated food or water and it presents as stomatitis and pharyngitis. The primary ulcer is localised in the mouth, and lymph nodes of the neck region are enlarged. Physical examination shows redness and pustular changes in the mouth and pharyngeal mucous membranes, together with enlargement of regional neck lymph nodes. If tularemia is not suspected for epidemiological reasons, the diagnosis will most likely be missed and appropriate therapy not prescribed. Identification of *Francisella tularensis* from oropharyngeal specimens should be carefully done in a microbiological safety cabinet level 2 whereas work on colonies and manipulations that might involve aerosol formation requires biological safety cabinet level 3 conditions.

For diagnosis, culture is more often performed for type B tularemia in regions where this is endemic. It can be grown from pharyngeal washings, sputum specimens, and even fasting gastric aspirates in a high proportion of patients with inhalational tularemia. It is only occasionally isolated from blood. When growth of *F. tularensis* is suspected, a reference laboratory should be consulted for safe handling and further identification.

Tularaemia occurs endemically in most countries of the Northern hemisphere, within a range of 30 to 71° latitude. The countries where the disease has been reported are Canada, the USA and Japan. Tularaemia is widely distributed over the Eurasian
continent. A high prevalence is found in the former Soviet Union and the Nordic countries, whereas the British Islands seem to be free from the disease.

**Yersinia enterocolitica**

*Y. enterocolitica* commonly causes enteric infections but may also infect other body sites such as lungs, bone joints, etc. Although rare, this organism has been responsible for some sporadic cases of pharyngitis. This has been isolated from throats of patients with enteritis from an outbreak due to contaminated pasteurised milk. The signs and symptoms are characterised by sore throat and fever without enteritis.

Throat swabs may be used to investigate carriage of *Yersinia enterocolitica* in patients.

**Other uncommon organisms**

Pathogens such as *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* are also uncommon causes of acute pharyngitis.

**Screening for carriage in contacts**

*Neisseria meningitidis*

*N. meningitidis* can be spread from carrier to carrier, probably via the oral-respiratory route. A susceptible person is at risk when close contacts such as family members are identified as carriers.

*N. meningitidis* is carried on the posterior pharyngeal wall and can be detected from oropharyngeal or nasopharyngeal swabs. However, posterior pharyngeal swabs seem to be better than nasopharyngeal swabs for detecting *N. meningitidis* carriage in large epidemiological studies because they identify a significantly larger number of pathogen carriers and recover a significantly larger amount of bacterial DNA. Throat swabs may be an aid to diagnosis of meningococcal meningitis. *N. meningitidis* can be isolated from a throat swab in about half the cases of invasive meningococcal disease (refer to B 51 - Screening for *Neisseria meningitidis*). The strain isolated from the throat is likely to be of the same group and type as that isolated from cerebrospinal fluid and blood. However, other reports have described throat swabs from contacts as having no value as an aid to diagnosis because the strains from contacts are often different from those isolated from index cases.

*Staphylococcus aureus*

Throat swabs may be used to investigate carriage of *Staphylococcus aureus*, for example in pre-operative cardiac patients as well as to screen for carriage of Meticillin Resistant *Staphylococcus aureus* (MRSA) refer to B 29 - Investigation of Specimens for Screening for MRSA.

*S. aureus* has sporadically been reported as a cause of peritonsillar abscess. Pus may be aspirated from the abscess and sent for culture (refer to B 14 - Investigation of Abscesses and Post-Operative Wound and Deep-Seated Wound Infections).

**Epiglottitis**

Epiglottitis is an inflammation of the epiglottis. It commonly affects children and is associated with fever, hoarseness of voice, stridor and difficulty in swallowing. Most cases of epiglottitis in young children under the age of five used to be caused by *Haemophilus influenzae* type b but since the introduction of *H. influenzae* type b (Hib)
vaccine in October 1992, a decline in the number of cases of acute epiglottitis in children has occurred, although a minor resurgence of cases was seen in the early part of the 21st century. Epiglottitis in adults is unusual and the numbers have been largely unaffected by the vaccination programme, in keeping with the more diverse range of causative organisms.

Capsulated *H. influenzae* type b, as well as other types should still be considered when treating epiglottitis, even in immunised children. Acute epiglottitis in young children is a rapidly progressive inflammation of the epiglottis and surrounding tissues and may result in complete airways obstruction. Because trauma from the swab may precipitate obstruction, throat swabs are contraindicated in cases of suspected acute epiglottitis. Blood cultures should be taken in all cases of suspected epiglottitis.

Treatment of *H. influenzae* type b invasive disease may not eliminate pharyngeal carriage of the organism. Failure to eradicate upper airway colonisation may impose a risk to the patient and to susceptible family contacts.

Throat swabs to determine upper airway colonisation with *H. influenzae* type b are usually only taken for epidemiological studies.

Other bacterial causes of epiglottitis include Group A beta-haemolytic streptococci, *Pseudomonas* species and *Mycobacterium tuberculosis*. *Candida* species and *Aspergillus* species are seen in immunocompromised patients.

### Tonsillitis

Tonsillitis is inflammation of the tonsils, usually due to a viral infection or, less commonly, a bacterial infection. It is a common type of infection in children, although it can sometimes affect adults. Symptoms of tonsillitis include sore throat that can feel worse when swallowing, fever, coughing and headache. These symptoms will usually pass within 3-4 days.

Quinsy (peritonsillar abscess) is an acute infection located between the capsule of the palatine tonsil and the superior constrictor muscle of the pharynx. Peritonsillar abscess is rare and forms, usually on one side of the throat only, with the swelling behind the tonsil near the back of the roof of the mouth. Symptoms are similar to that of tonsillitis, including dribbling, generally feeling unwell and neck swelling because of the abscess. This disease can occur in all age groups, but teenagers and young adults are most frequently affected. It is usually caused by *Streptococcus* species as a complication of tonsillitis. The *Streptococcus anginosus* group (also known as *Streptococcus milleri* group) and Group A Streptococci have been established as key organisms in peritonsillar abscesses.

*Fusobacterium necrophorum* and *Fusobacterium nucleatum* are also comparatively common causes of quinsy. Anaerobic organisms predominantly isolated in peritonsillar abscesses include *Prevotella*, *Porphyromonas* and *Peptostreptococcus* species.

*S. aureus* has sporadically been reported as a cause of quinsy. Pus may be aspirated from the abscess and sent for culture (refer to **B 14 - Investigation of Abscesses and Deep-Seated Wound Infections**).

Arcanobacterium haemolyticum has been associated with tonsillitis, pharyngitis and may cause a rash in young adults and occasionally in children. It is suggested that in cases of treatment failure and recurrent tonsillitis, isolation of *A. haemolyticum* should be considered.
Laryngitis

Laryngitis is inflammation of the larynx (voice box). In most cases, laryngitis is caused by a viral infection (such as a cold), or voice strain or by bacteria such as Corynebacterium diphtheriae, although this is rare\(^3\). There is also a recent case report that suggests that MRSA has been implicated in laryngitis\(^60\). This eases without treatment within a week. This is known as acute laryngitis. Symptoms of laryngitis include hoarseness, loss of voice and sore throat.

Laryngitis can occasionally have other causes, such as smoking, alcohol misuse, voice overuse, reflux of acid from the stomach (also called gastroesophageal reflux disease (GERD)), rare infections or allergies, or inhalation or irritants or chemicals\(^61\). The symptoms do last much longer. This is known as chronic laryngitis.

Other less common causes of chronic laryngitis are bacterial (Group A streptococci, Streptococcus pneumoniae, Haemophilus influenzae and Mycobacterium tuberculosis) or fungal (Candida species, Blastomyces species) infections and parasite infections.

For viruses that may be isolated from throat swabs, refer to G 8 – Respiratory Viruses.

Screening of neonates

Surveillance screening of neonates may include a throat swab.

Technical Information/Limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective Media in Screening Procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen Containers\(^62,63\)

SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

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UK Standards for Microbiology Investigations | Issued by the Standards Unit, Public Health England
Incubation

The duration of incubation can affect the throat culture result. Once plated, a culture should be incubated at 35°C–37°C for 18–24 hours before reading. Additional incubation overnight at room temperature may identify a number of additional positive throat culture results. However, although initial therapeutic decisions may be made on the basis of overnight culture, it is advisable to re-examine plates at 48 hours that yield negative results at 24 hours\textsuperscript{64}.
1 Safety Considerations

1.1 Specimen Collection, Transport and Storage

Use aseptic technique.
Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.
Collect swabs into appropriate transport medium and transport in sealed plastic bags.
Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing

Hazard Group 2 Organisms

C. diphtheriae and C. ulcerans are in Hazard group 2; suspected and known isolates of C. diphtheriae/C. ulcerans should always be handled in a microbiological safety cabinet. Sometimes the nature of the work may dictate that full containment Level 3 conditions should be used eg for the propagation of C. diphtheriae/C. ulcerans in order to comply with COSHH 2004 Schedule 3 (4e). For the urease test a urea slope is considered safer than a liquid medium.

N. gonorrhoeae and N. meningitidis are also Hazard group 2 organisms. Although for N. meningitidis, the processing of diagnostic samples can be carried out in a microbiological safety cabinet at Containment Level 2 but due to the severity of the disease and the risks associated with generating aerosols, any manipulation of suspected isolates of N. meningitidis should always be undertaken in a microbiological safety cabinet in a containment level 3 facility until N. meningitidis has been ruled out (as must any laboratory procedure giving rise to infectious aerosols)71.

Haemophilus influenzae is a Hazard Group 2 organism, and, and in some cases the nature of the work may dictate full Containment Level 3 conditions80.

Hazard Group 3 Organisms

F. tularensis is a Hazard group 3 organism, one of the most potent pathogens known in human medicine and evokes great concern as a bioterrorism agent81.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet71.

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

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

2 Specimen Collection

2.1 Type of Specimens

Throat swab, posterior pharyngeal swab, nasopharyngeal swab, pharyngeal washings, pus aspirate, oropharyngeal swab, throat gargle

2.2 Optimal Time and Method of Collection

For safety considerations refer to Section 1.1.
Collect specimens before antimicrobial therapy where possible. Unless otherwise stated, swabs for bacterial and fungal culture should be placed in appropriate transport medium.

Throat swabs should be taken from the tonsillar area and/or posterior pharynx, avoiding the tongue and uvula.

Throat culture should not be taken if the epiglottis is inflamed as sampling may cause serious respiratory obstruction.

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

2.3 Adequate Quantity and Appropriate Number of Specimens

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

3 Specimen Transport and Storage

3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible.

If processing is delayed, refrigeration is preferable to storage at ambient temperature.

Ideally, inoculation of specimens for N. gonorrhoeae should be made directly on to culture media at the time of collection and these should be incubated without delay. Transport time should be as short as possible.

4 Specimen Processing/Procedure

4.1 Test Selection

N/A

4.2 Appearance

N/A

4.3 Sample Preparation

For safety considerations refer to Section 1.2.

4.4 Microscopy

4.4.1 Standard

Stain for Vincent's organism if clinically indicated (refer to TP 39 – Staining Procedures).

4.4.2 Supplementary / Preparation of smears

N/A
### 4.5 Culture and Investigation

Inoculate each agar plate with swab (refer to Q 5 – Inoculation of Culture Media in Bacteriology).

#### 4.5.1 Culture media, conditions and organisms

<table>
<thead>
<tr>
<th>Clinical details/conditions</th>
<th>Specimen</th>
<th>Standard media</th>
<th>Incubation</th>
<th>Cultures read</th>
<th>Target organism(s)‡</th>
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<tbody>
<tr>
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<td>Temp °C</td>
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<tr>
<td>Pharyngitis (Sore throat)</td>
<td>Throat swab</td>
<td>Blood agar*</td>
<td>35–37</td>
<td>Anaerobic</td>
<td>≥18hr</td>
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<tr>
<td>Epiglottitis</td>
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<tr>
<td>Tonsillitis</td>
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<td>35–37</td>
<td>Aerobic</td>
<td>≥24hr</td>
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<tr>
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<tr>
<td>Epiglottitis</td>
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<td></td>
<td>OR</td>
<td></td>
<td>18-48hr</td>
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<td></td>
<td>Nasopharyngeal swab</td>
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<tr>
<td>Pharyngitis</td>
<td>Throat swab</td>
<td>Blood agar**</td>
<td>35–37</td>
<td>5-10% CO₂</td>
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</tr>
<tr>
<td></td>
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</tr>
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<td>Air</td>
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<td>Nasopharyngeal agar</td>
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</tr>
<tr>
<td>S. aureus (MSSA) carriage</td>
<td>Throat swab</td>
<td>Blood agar**</td>
<td>35–37</td>
<td>5-10% CO₂</td>
<td>≥18hr</td>
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<td></td>
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<td>35–37</td>
<td>5-10% CO₂</td>
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<td>OR</td>
<td></td>
<td>40-48hr</td>
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<tr>
<td>contact</td>
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For these situations, add the following:

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<th>Supplementary media</th>
<th>Incubation</th>
<th>Cultures read</th>
<th>Target organism(s)</th>
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<tr>
<td>Membrane formation or</td>
<td>Throat swab</td>
<td>Hoyle’s tellurite agar</td>
<td>35–37</td>
<td>Air</td>
<td>daily</td>
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<tr>
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<td>Nasopharyngeal</td>
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<td>contact</td>
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<tr>
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</tbody>
</table>

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UK Standards for Microbiology Investigations | Issued by the Standards Unit, Public Health England
### 4.6 Identification

Refer to individual SMIs for organism identification.

#### 4.6.1 Minimum level of identification in the laboratory

<table>
<thead>
<tr>
<th>Organism</th>
<th>Level</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. diphtheriae</td>
<td>species level; urgent toxigenicity test / refer to Ref Lab</td>
<td></td>
</tr>
<tr>
<td>C. ulcerans</td>
<td>species level; urgent toxigenicity test / refer to Ref Lab</td>
<td></td>
</tr>
<tr>
<td>H. influenzae</td>
<td>species level; type b or not if epiglottitis, refer to Ref Lab</td>
<td></td>
</tr>
<tr>
<td>β haemolytic streptococci</td>
<td>Lancefield group level</td>
<td></td>
</tr>
<tr>
<td>A. haemolyticum</td>
<td>species level</td>
<td></td>
</tr>
<tr>
<td>N. gonorrhoeae</td>
<td>species level</td>
<td></td>
</tr>
<tr>
<td>N. meningitidis</td>
<td>species level</td>
<td></td>
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<tr>
<td>S. aureus</td>
<td>species level</td>
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</tr>
<tr>
<td>Yeasts</td>
<td><em>yeasts</em> level *</td>
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</tr>
<tr>
<td>Fusobacterium species</td>
<td>species level</td>
<td></td>
</tr>
</tbody>
</table>

* Yeast and fungal isolates from patients who are immunocompromised usually require identification and susceptibility testing.

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

**Note:** All work on suspected isolates of *C. diphtheriae* which is likely to generate aerosols must be performed in a safety cabinet.
A medical microbiologist must be informed of all suspected isolates of *C. diphtheriae* as soon as possible, so that a risk assessment can be undertaken for rapid referral for toxin testing. Toxigenicity testing is available and undertaken only by the Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU) PHE Colindale.

4.7 Antimicrobial Susceptibility Testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

4.8 Referral for Outbreak Investigations

**Diphtheria**

As diphtheria is a notifiable disease in the UK, for public health management of cases, contacts and outbreaks, all suspected clinical cases should be notified immediately to the local Public Health England Centres.

Isolates of *C. diphtheriae* for which information available is suggestive of clinical diagnosis of diphtheria should be notified by the diagnostic laboratories to ensure urgent initiation of proper procedures and all such isolates should be referred to the national reference laboratory for toxigenicity testing.

**Group A Streptococci (GAS) infection**

Clinicians, microbiologists and health protection teams (HPTs) should be mindful of potential increases in invasive disease and maintain a high index of suspicion in relevant patients as early recognition and prompt initiation of specific and supportive therapy can be lifesaving. Invasive disease isolates and those from suspected clusters or outbreaks should be submitted immediately to the Respiratory and Vaccine Preventable Bacteria Reference Unit at Public Health England, 61 Colindale Avenue, London NW9 5EQ. For more information, refer to [https://www.gov.uk/streptococcal-infections](https://www.gov.uk/streptococcal-infections).

4.9 Referral to Reference Laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory [click here for user manuals and request forms](#).

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

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

**England and Wales**


**Scotland**


**Northern Ireland**

5   Reporting Procedure

5.1 Microscopy

5.1.1 Microscopy reporting time
Report results for Vincent's organisms as soon as available within 24hr of receipt.

5.2 Culture

Negatives
"β-haemolytic streptococci of Lancefield group A, C and G not isolated".
"Corynebacterium diphtheriae not isolated".
Also, report results of supplementary investigations.

Positives
Report clinically significant organisms isolated.

5.2.1 Culture reporting time
Clinically urgent culture results to be telephoned or sent electronically stating, if appropriate, that a further report will be issued.

Written report, 16–72hr.

Supplementary investigations eg toxigenicity testing of C. diphtheriae to be issued when available.

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

6   Notification to PHE or Equivalent in the Devolved Administrations

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many
PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

**Note:** The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.


Other arrangements exist in Scotland[^97][^98], Wales[^99] and Northern Ireland[^100].
Appendix: Investigation of Throat Related Specimens

Prepare all specimens

**Pharyngitis (sore throat)**
- Tonsillitis
- Epiglottitis
- Laryngitis

**Supplementary media**

- Membranous Pharyngitis / tonsillitis or foreign travel
- **S. aureus** (MSSA) carriage
- **GUM clinic, Gonorrhoea N. meningitidis case or contact**
- Treatment failure and recurrent tonsillitis
- Epiglottitis
- Diabetes, immunosuppressed patients, oral candidosis
- Persistent sore throat and Quinsy

**Optional media**

- Blood agar or Staph/strep selective agar*
- **Blood agar**
  - **Blood agar**
  - Hoyle’s tellurite agar
  - **Blood agar**
  - Or Staph/strep selective agar*
  - Chromogenic agar
- **GC selective agar**
- **Blood agar**
  - Incubate at 35-37°C
  - Anaerobic
  - 18 – 24hr
  - Read at ≥ 16hr
- **Blood agar**
  - Incubate at 35-37°C
  - Air
  - 18 – 48hr
  - Read daily
- **GC selective agar**
  - Incubate at 35-37°C
  - Air
  - 40 – 48hr
  - Read at ≥ 40hr
- **Blood agar**
  - Incubate at 35-37°C
  - 5 – 10% CO₂
  - 18 – 24hr
  - Read at ≥ 16hr
- **Blood agar**
  - Incubate at 35-37°C
  - 5 – 10% CO₂
  - 40 – 48hr
  - Read at ≥ 48hr
- **Blood agar**
  - Incubate at 35-37°C
  - 5 – 10% CO₂
  - 40 – 48hr
  - Read at ≥ 48hr
- **Blood agar**
  - Incubate at 35-37°C
  - 5 – 10% CO₂
  - 24 – 48hr
  - Read daily
- **Blood agar**
  - Incubate at 35-37°C
  - Air
  - 40 – 48hr
  - Read at ≥ 40hr
- **Blood agar**
  - Incubate at 35-37°C
  - 5 – 10% CO₂
  - 24 – 48hr
  - Read at ≥ 48hr
- **Blood agar**
  - Incubate at 35-37°C
  - Anaerobic
  - 5 – 7 d
  - Read at ≥ 48hr

**Lancefield group**
- A, C and G streptococci
  - Refer to ID 4

**Toxicogenic**
- C. diphtheriae
  - C. ulcerans
  - Refer to ID 2

**S. aureus**
  - Refer to ID 7

**N. gonorrhoeae**
  - Refer to ID 6

**N. meningitidis**
  - Refer to ID 5

**A. haemolyticum**
  - Refer to ID 3

**H. influenzae**
  - Refer to ID 12

**Yeast**
- Refer to ID 25

*Staphylococcus/Streptococcus selective agars may be used for Lancefield group streptococci
References


31. Wren M. Throat swabs: what are we missing? The Biomedical Scientist 2008;221-3.


62. European Parliament. UK Standards for Microbiology Investigations (SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".


