



NATIONAL STANDARD METHOD

INVESTIGATION OF THROAT SWABS

BSOP 9

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections



INVESTIGATION OF THROAT SWABS

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The reader is informed that all taxonomy in this document was correct at time of issue.

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AMENDMENT PROCEDURE

Controlled document reference	BSOP 9
Controlled document title	Investigation Of Throat Swabs

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
8/ 03.12.09	7	8	8	Introduction	Wording regarding <i>Fusobacterium necrophorum</i> amended
			8 All	Technical Information/Limitations 1.2 Specimen transport and storage	The term "CE marked leak proof container" replaces "sterile leak proof container"; endnote ^a added to clarify the change and referenced to IVD Directive 98/79/EC
			16	References	References reviewed and updated

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INVESTIGATION OF THROAT SWABS

Type of specimen: Throat swab

SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the isolation of bacterial and fungal organisms from throat swabs known to cause upper respiratory tract infections (also see [QSOP 53 - Recommendations for the screening of specimens for *Corynebacterium* species](#)). For viruses that may be isolated from throat swabs see [QSOP 60 – Respiratory viruses](#).

INTRODUCTION

Pharyngitis

The commonest 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% have been reported), but rates are much lower in adults. In these individuals isolation of the Lancefield group A streptococcus 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 infection². In acute infection, bacteraemia and streptococcal toxic shock may occur. Post streptococcal sequelae appear to be limited to a circumscribed set of serotypes³.

The isolation rate of Lancefield group A streptococci may be increased by incubating culture plates for 40-48 h⁴.

Lancefield group C streptococci have been reported as a cause of pharyngitis⁵. 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*)⁶.

Most of the evidence for Lancefield groups C and G streptococci causing pharyngitis comes from reports of outbreaks⁷⁻¹⁰.

Diphtheria

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 *pseudotuberculosis*¹¹. 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.

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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^{12,13}. Non-toxigenic strains of *C. diphtheriae* may be encountered in clinical specimens, especially those taken from persons previously immunised against diphtheria toxin. Although toxigenic *C. ulcerans* generally causes mild pharyngitis without any associated sequelae, at least as many cases of clinical diphtheria are now caused by *C. ulcerans* as by *C. diphtheriae* in England and Wales. There is no direct evidence of person-to-person transmission of *C. ulcerans* but it is thought that this may occur. However, molecular studies have indicated that domestic animals may be a more likely source of infection¹⁴⁻¹⁶.

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 pathogenicity¹⁷.

Non-toxigenic strains in pharyngeal flora have the potential to undergo lysogenic conversion to toxin production *in vivo*, which may lead to disease¹⁸.

In the 1990s there was an increase in the incidence of diphtheria in Russia and other former Soviet states, although the situation is now improving¹⁹. Diphtheria cases have continued to be reported from every WHO Region, especially the higher risk regions eg Africa, South East Asia and South America. 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 *C. diphtheriae* from the United Kingdom, but it might not have affected the carriage of non-toxigenic strains.

Criteria for screening throat swabs for *C. diphtheriae*

There are specific clinical associations and exposures which, if reported on request forms, should trigger examination of specimens for *C. diphtheriae* or *C. ulcerans*. These are based on recognised risk factors and information from enhanced diphtheria surveillance. Therefore this NSM recommends screening for *Corynebacterium* species in the following circumstances:

Throat or nose swabs from a patient with one or more of the following risk factors reported:

- Membranous or pseudomembranous pharyngitis/tonsillitis
- Travel overseas (especially Russia and Former Soviet States, Africa, South America and South-East Asia) within the last 10 days
- Recent contact with someone who has travelled overseas recently (especially Russia and Former Soviet States, Africa, South America and South-East Asia)*
- Recent consumption of raw milk products (*C. ulcerans*)
- Recent contact with farms/farm animals or domestic animals (*C. ulcerans*)
- The patient works in a clinical microbiology laboratory, or similar, where *Corynebacterium* species may be handled

* Travel or contact with travellers in the past 10 days is most likely to be relevant to the risk of diphtheria.

It is recommended that those laboratories with a specific public health remit, such as Health Protection Agency laboratories, continue to screen all throat swabs for *Corynebacterium* species: this ensures that surveillance of the disease continues and appropriate public health action is taken (see [QSOP 53 - Recommendations for the screening of specimens for *Corynebacterium* species](#)).

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Epiglottitis

Most cases of epiglottitis in young children under the age of five used to be caused by *Haemophilus influenzae* type b²⁰. 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 organisms²¹.

H. influenzae type b should still be considered when treating epiglottitis, even in immunised children. Acute epiglottitis in young children is a rapidly progressive cellulitis 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.

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.

Other causes of pharyngitis

Non-toxigenic *C. diphtheriae*

Non-toxigenic *C. diphtheriae* can be a cause of sore throat, but does not cause a true diphtheritic membrane or symptoms attributable to systemic absorption of toxin. On re-introduction of the necessary gene, these organisms may, however, express toxin production. There is a suggestion that particular clones of non-toxigenic *C. diphtheriae* may be especially virulent as described from Russia and other former Soviet states^{22,23}. Occasionally, humans will develop invasive infections with non-toxigenic strains of *C. diphtheriae*. These conditions appear to be rare, and will be detected by blood culture rather than by culture of throat or nasopharyngeal swabs.

Arcanobacterium haemolyticum (previously *Corynebacterium haemolyticum*)

Although *Arcanobacterium haemolyticum* is recognised as a human pathogen, this NSM 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^{23,24}. It is suggested that in cases of treatment failure and recurrent tonsillitis, isolation of *A. haemolyticum* should be considered.

After 48 hours incubation on blood agar, *A. haemolyticum* colonies exhibit narrow zones of β -haemolysis and are approximately 0.5 mm in diameter. In cases where *A. haemolyticum* is suspected, incubation of culture plates may need to be extended up to 72 h. 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 revealed²⁵.

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 tissue²⁶. Recognition of oropharyngeal candidosis accompanied by dysphagia indicate the possibility of esophageal candidosis^{27,28} and this may be an AIDS-defining illness. Yeast and fungal isolates from patients who are immunocompromised usually require identification and susceptibility testing.

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Fusobacterium necrophorum

Acute pharyngitis and fever, sometimes accompanied by membranous tonsillitis, antibiotic is characteristic of the onset of infection caused by *Fusobacterium necrophorum*²⁹. 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 throat³⁰, 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 annually³¹. 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^{31,32}.

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 (see [BSOPID 6 – Identification of *Neisseria* species](#)). Pharyngeal colonisation may be found in patients with genital gonorrhoea, but the pharynx is rarely the only infected site³³.

Neisseria meningitidis

Neisseria 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 carriers³⁴.

Throat swabs may be an aid to diagnosis of meningococcal meningitis³⁵. *N. meningitidis* can be isolated from a throat swab in about half the cases of invasive meningococcal disease (see [BSOP 51 - Screening for meningococci](#)). The strain isolated from the throat is likely to be of the same group and type as that isolated from cerebrospinal fluid and blood³⁴. 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 cases³⁶⁻³⁸.

Staphylococcus aureus

Throat (and nose) swabs may be used to investigate carriage of *Staphylococcus aureus*, for example in pre-operative cardiac patients.

Throat swabs are also used to screen for carriage of Meticillin Resistant *Staphylococcus aureus* (MRSA)³⁹ (see [BSOP 29 - Investigation of specimens for screening for MRSA](#)). *S. aureus* has sporadically been reported as a cause of peritonsillar abscess (quinsy). Pus may be aspirated from the abscess and sent for culture (see [BSOP 14 - Investigation of abscesses and post-operative wound and deep-seated wound infections](#)).

Screening of neonates

Surveillance screening of neonates may include a throat swab ([BSOP 23 - Investigation of gastric aspirates and infection screen swabs from neonates](#)).

TECHNICAL INFORMATION/LIMITATIONS

In National Standard Methods, the term “CE Marked leak proof container” is used to describe containers bearing the CE marking and which are used for the collection and transport of clinical specimens. The requirements of the EU *in vitro* Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1)⁴⁰ state that such devices must “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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1 SAFETY CONSIDERATIONS⁴¹⁻⁴⁷

1.1 SPECIMEN COLLECTION

N/A

1.2 SPECIMEN STORAGE AND COLLECTION

CE Marked leak proof container^a in a sealed plastic bag

1.3 SPECIMEN PROCESSING

C. diphtheriae/C. ulcerans is in Hazard Group 2, although in some cases the nature of the work may dictate full Containment Level 3 conditions.

Suspected isolates of *C. diphtheriae/C. ulcerans* should always be handled in a microbiological safety cabinet. For the urease test a urea slope is considered safer than a liquid medium.

C. diphtheriae/C. ulcerans causes severe and sometimes fatal disease. Laboratory acquired infections have been reported¹¹. The organism infects primarily by the respiratory route. Vaccination against diphtheria is available; guidance is given in the Health Protection Agency immunisation policy.

N. meningitidis is in Hazard Group 2 although in some cases the nature of the work may dictate full Containment Level 3 conditions.

N. meningitidis causes severe and sometimes fatal disease. Laboratory acquired infections have been reported. The organism infects primarily by the respiratory route. An effective vaccine is available for some meningococcal groups.

Suspected isolates of *N. meningitidis* should always be handled in a microbiological safety cabinet.

Refer to current guidance on the safe handling of all Hazard Group 2 organisms documented in this NSM.

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

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

Compliance with postal and transport regulations is essential.

2 SPECIMEN COLLECTION

2.1 OPTIMAL TIME OF SPECIMEN COLLECTION

At onset of symptoms.

Before antimicrobial therapy where possible.

2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION

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

2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

N/A.

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3 SPECIMEN TRANSPORT AND STORAGE

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens should be transported and processed as soon as possible.

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 possible⁴⁸.

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

Swabs should be transported in Amies transport medium with charcoal⁴⁹.

If processing is delayed, refrigeration is preferable to storage at ambient temperature⁵⁰.

Delays of over 48 h are undesirable.

4 SPECIMEN PROCESSING

4.1 TEST SELECTION

N/A

4.2 APPEARANCE

N/A

4.3 MICROSCOPY

Stain for Vincent's organisms if clinically indicated. (See [BSOPTH 39 - Staining procedures](#))

4.4 CULTURE AND INVESTIGATION

4.4.1. PRE-TREATMENT

N/A

4.4.2. SPECIMEN PROCESSING

Inoculate each agar plate with swab (see [BSOP 54 – Inoculation of culture media](#)).

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4.4.3 CULTURE MEDIA, CONDITIONS AND ORGANISMS FOR ALL SPECIMENS^{11,51,52}

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s) [‡]
		Temp °C	Atmos	Time		
Sore throat Pharyngitis Tonsillitis	Blood agar*	35-37	anaerobic	40-48 h	≥48 h	Lancefield group A,C and G streptococci
For these situations, add the following:						
Clinical details/ Conditions	Supplementary media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
Membrane formation or membranous pharyngitis/tonsillitis Foreign travel	Hoyle's tellurite agar	35-37	air	24-48 h	daily	Toxigenic <i>C. diphtheriae</i> and <i>C. ulcerans</i>
<i>S. aureus</i> carriage	Blood agar*	35-37	5-10% CO ₂	16-24 h	≥16 h	<i>S. aureus</i>
GUM clinic, gonorrhoea, <i>N. meningitidis</i> case or contact	GC selective agar	35-37	5-10% CO ₂	40-48 h	≥40 h	<i>N. gonorrhoeae</i> <i>N. meningitidis</i>
Tonsillitis, treatment failure, pharyngitis, and rash	Blood agar	35-37	5-10% CO ₂	40-48 h**	≥48 h	<i>A. haemolyticum</i>
Epiglottitis	Chocolate agar	35-37	5-10% CO ₂	40-48 h	daily	<i>H. influenzae</i>
Diabetes Immunosuppressed oral candidosis	Sabouraud agar	35-37	air	40-48 h	≥40 h	Yeasts
Optional Media		Incubation			Cultures read	Target organism(s)
Clinical Conditions		Temp C	Atmos	Time		
Persistent sore throat or Quinsy	Fastidious Anaerobic Agar (FAA) containing nalidixic acid and vancomycin	35-37	anaerobic	5-7 d	≥48 h	<i>F. necrophorum</i> ³⁰
Other organisms for consideration - MRSA (BSOP 29 - Investigation of specimens for screening for MRSA) and non-toxigenic <i>C. diphtheriae</i>						
<p>* - Staphylococcus/streptococcus selective agars may be used for Lancefield group streptococci ** - May be extended to 72 hours ‡ - For appearance of relevant target organism see relevant BSOP ID</p>						

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4.5 IDENTIFICATION

4.5.1. MINIMUM LEVEL IN THE LABORATORY

C. diphtheriae	species level; urgent toxigenicity test/refer to Ref Lab
C. ulcerans	species level; urgent toxigenicity test/refer to Ref Lab
H. influenzae	species level; type b or not
β haemolytic streptococci	Lancefield group level
A. haemolyticum	species level
N. gonorrhoeae	species level
N. meningitidis	species level
S. aureus	species level
Yeasts	"yeasts" level
Anaerobes	species level

Organisms may be further identified if clinically or epidemiologically indicated.

A medical microbiologist must be informed of all suspected isolates of *C. diphtheriae* as soon as possible (toxigenicity testing is available from the reference laboratory).

4.5.2. REFERRAL TO REFERENCE LABORATORIES

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

Isolates associated with outbreaks, where epidemiologically indicated, 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.

4.6 ANTIMICROBIAL SUSCEPTIBILITY TESTING

See [BSOP 45 - Susceptibility testing](#)

5 REPORTING PROCEDURE

5.1 MICROSCOPY

Stain for Vincent's organisms: report on Vincent's organisms detected.

5.1.1. MICROSCOPY REPORTING TIME

16 – 24 h for Vincent's organisms.

5.2 CULTURE

Negatives

"β-haemolytic streptococci of Lancefield groups A, C and G not isolated".

"*Corynebacterium diphtheriae* not isolated".

Also, report results of supplementary investigations.

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Positives

Report clinically significant organisms isolated.

5.2.1. CULTURE REPORTING TIME

Clinically urgent culture results to be telephoned or sent electronically.

Written report, 16 – 72 h stating, if appropriate, that a further report will be issued.

Supplementary investigations, toxigenicity testing of *C. diphtheriae*.

5.3 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Report susceptibilities as clinically indicated.

6 REPORTING TO THE HPA (LOCAL AND REGIONAL SERVICES AND CENTRE FOR INFECTIONS)

For public health management of cases, as diphtheria is a notifiable disease in the UK contacts and outbreaks, all suspected cases should be notified to the local Health Protection Unit immediately. Refer to the following:

<http://www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/GreenBook/fs/en>).

Individual NSMs on organism identification

Health Protection Agency publications:

"Reporting to the CDR: A guide for diagnostic laboratories"

"Hospital infection control: Guidance on the control of infection in hospitals"

Local guidelines

Isolation of possible *C. diphtheriae* should be reported urgently to Cfl Immunisation Department and the Reference Laboratory immediately, along with submission of the suspect isolate.

In cases of suspected meningococcal disease (and contacts) the isolation of *N. meningitidis* should be reported to the Cfl Immunisation Department urgently.

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7 ACKNOWLEDGEMENTS AND CONTACTS

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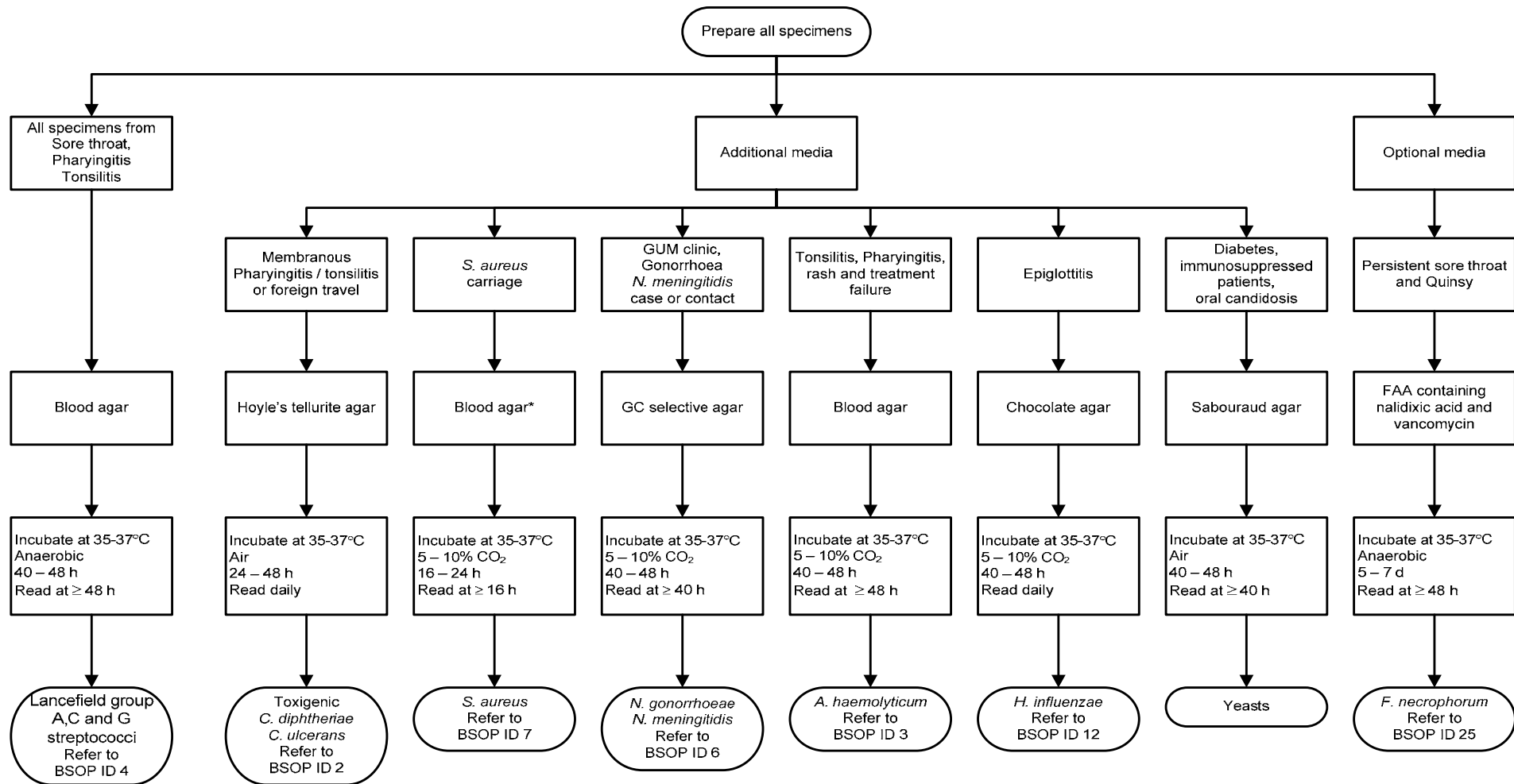
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APPENDIX



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

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^a *The requirements of the EU in vitro Diagnostic Medical Devices Directive⁴⁰ (98/79/EC Annex 1 B 2.1) state that such devices must “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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