



# UK Standards for Microbiology Investigations

Investigation of Bacterial Eye Infections





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# Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website <a href="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</a>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <a href="https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee">https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee</a>).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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Bacteriology | B 2 | Issue no: 6.1 | Issue date: 04.05.17 | Page: 2 of 24 UK Standards for Microbiology Investigations | Issued by the Standards Unit, Public Health England

## Contents

ACKN	IOWLEDGMENTS	2		
AMENDMENT TABLE				
UK SMI: SCOPE AND PURPOSE				
SCOPE OF DOCUMENT7				
SCOPE				
INTRO	DDUCTION	7		
TECHNICAL INFORMATION/LIMITATIONS				
1	SAFETY CONSIDERATIONS	. 13		
2	SPECIMEN COLLECTION	. 13		
3	SPECIMEN TRANSPORT AND STORAGE	. 14		
4	SPECIMEN PROCESSING/PROCEDURE	. 14		
5	REPORTING PROCEDURE	. 19		
6	NOTIFICATION TO PHE OR EQUIVALENT IN THE DEVOLVED ADMINISTRATIONS	. 20		
APPENDIX: INVESTIGATION OF BACTERIAL EYE INFECTIONS				
REFERENCES				



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For full details on our accreditation visit: www.nice.org.uk/accreditation.

## **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 <u>standards@phe.gov.uk</u>.

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

Amendment No/Date.	12/04.05.17				
Issue no. discarded.	6				
Insert Issue no.	6.1				
Section(s) involved	Amendment				

Amendment No/Date.	11/29.12.14				
Issue no. discarded.	5.4				
Insert Issue no.	6				
Section(s) involved	Amendment				
Whole document.	Hyperlinks updated to gov.uk.				
Page 2.	Updated logos added.				
Whole document.	B 2 – Investigation of eye swabs and canalicular pus has been merged with B 52 – Investigation of intraocular fluids and corneal scrapings to form a new B 2 – Investigation of Bacterial Eye Infections.				
Types of specimen.	This section has been expanded to cover the new title.				
Introduction.	Expanded to include keratitis and endophthalmitis.				
4.5 Culture and investigation.	A note added to make it clear that <i>E. coli</i> seeded plates should not be sent to the patient's bedside.				
References.	References reviewed and updated.				

## UK SMI<sup>#</sup>: 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 <u>https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-qualityand-consistency-in-clinical-laboratories</u>. 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

<sup>&</sup>lt;sup>#</sup> 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.

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 <u>https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity</u>.

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.

SMIs are Crown copyright which should be acknowledged where appropriate.

#### **Suggested Citation for this Document**

Public Health England. (2017). Investigation of Bacterial Eye Infections. UK Standards for Microbiology Investigations. B 2 Issue 6.1. <u>https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories</u>

## **Scope of Document**

#### **Type of Specimen**

Eye swabs, canalicular pus, aqueous and vitreous humour, corneal scrapings, contact lens cases and cleaning fluid

## Scope

This SMI describes the processing and bacteriological investigation of specimens from the eyes.

Separate swabs in appropriate transport media are needed for the diagnosis of viral and chlamydial infections (see V 37 - Chlamydia trachomatis Infection - Testing by Nucleic Acid Amplification Tests (NAATs)). Invasive specimens may be required for optimal investigation of severe eye infections.

Molecular techniques are now available to diagnose chlamydia infections from eye swabs. These are not covered in this SMI.

This SMI should be used in conjunction with other SMIs.

## Introduction

Infections of the eye can be caused by a variety of microorganisms. Swabs from eyes may be contaminated with skin microflora, but any organism may be considered for further investigation if clinically indicated.

Exogenous organisms may be introduced to the eye via hands, fomites (eg contact lenses), traumatic injury, or following surgery. Haematogenous spread from a focus elsewhere in the body can also occur<sup>1</sup>.

The most common causes of viral conjunctivitis are adenoviruses, HSV and varicellazoster viruses. More information on this area can be found in  $\sqrt{21 - \text{Isolation of}}$ Viruses Associated with Infections of the Eye: Keratoconjunctivitis.

#### Infections

Common mild eye infections include conjunctivitis (inflammation of the conjunctiva). Conjunctivitis may occur in association with infection of the eyelid (blepharoconjunctivitis), inflammation of the eye lid (blepharitis) or of the cornea (keratoconjunctivitis).

Less common and more severe infections include keratitis (inflammation of the cornea) and endophthalmitis (infection inside the eye itself)<sup>2</sup>. Other periocular infections include dacryoadenitis (inflammation of the lacrimal gland), dacryocystitis (inflammation of the lacrimal sac), canaliculitis (infection of the lacrimal puncta and canaliculi), and preseptal and orbital cellulitis. Hypopyon is a condition associated with a leukocytic exudate, seen in the anterior chamber of the eye, usually accompanied by redness of the conjunctiva and the underlying episclera.

Eye swabs may be received from patients with any of these conditions, but may need handling differently according to the type and severity of infection.

#### Conjunctivitis

Conjunctivitis may be acute or chronic. The conjunctiva is the most frequently infected ocular tissue, and infectious conjunctivitis is one of the causes of red or sticky eyes. Common bacterial causes in adults include<sup>1</sup>:

- S. aureus
- Streptococcus pneumoniae
- Haemophilus influenzae

Conjunctivitis in neonates is caused by the pathogens commonly found in adult cases<sup>3</sup>. Additional organisms include<sup>1</sup>:

- N. gonorrhoeae
- Haemophilus parainfluenzae
- Lancefield group B streptococci and enterococci
- Enterobacteriaceae eg Klebsiella pneumoniae and Proteus mirabilis
- Pseudomonas aeruginosa

#### Keratitis<sup>2,4,5</sup>

Keratitis is infection of the cornea. This is a serious condition requiring prompt and meticulous investigation, and may progress to perforation and blindness if treatment is unsuccessful. Initial infection with keratitis may progress to endophthalmitis if inappropriately treated<sup>6</sup>. Predisposing factors include contact lens wear followed by pre-existing ocular disease including herpes simplex keratitis, ocular trauma, ocular surgery, laser refractive surgery and use of topical steroids. The condition may be caused by a wide range of bacteria, fungi and parasites including:

- Staphylococci
- Streptococci
- Pseudomonads
- Enterobacteriaceae
- Corynebacterium species
- Moraxella species
- Serratia species
- Haemophilus
- N. gonorrhoea
- Acanthamoebae
- Aspergillus species
- Candida species
- Fusarium species
- Propionbacterium species

## Endophthalmitis<sup>7,8</sup>

**Infectious endophthalmitis** is a relatively uncommon but potentially sight-threatening infection of intraocular fluids and tissue. It may develop as a result of surgery, trauma, or by haematogenous spread of organisms. The most appropriate specimens for investigation of endophthalmitis are intraocular fluids (aqueous humour from the anterior chamber and vitreous humour from the vitreous cavity/body). Eye swabs may also be taken. The organisms most often involved include<sup>8</sup>:

- Coagulase negative staphylococci
- Staphylococcus aureus
- Streptococci
- Propionibacterium acnes
- Yeast and moulds

**Acute post-operative endophthalmitis** occurs within 1 to 7 days of intraocular surgery<sup>9</sup>. The source of infection is usually the patient's own ocular or eyelid surface flora making diagnosis by culture difficult. To address these issues quantitative PCR can now be used to diagnose infection and can help to clarify the causative organisms<sup>10</sup>. Although virtually any organism may be introduced and cause infection, the commoner causes include:

- Coagulase negative staphylococci
- S. aureus
- Streptococci
- Enterobacteriaceae
- P. aeruginosa
- P. acnes

**Glaucoma filtering-bleb-associated endophthalmitis** can be either localised to the bleb itself ('blebitis') or present as a fulminant intraocular infection<sup>9</sup>. It can occur within weeks or years after the original surgery including pars plana vitrectomy, penetrating keratoplasty and glaucoma drainage implant.

The range of organisms responsible is similar to that listed above.

**Chronic endophthalmitis** occurs months to years after intraocular surgery. Patients typically present with a persistent low-grade uveitis or they may present the same ways as acute-endophthalmitis. Causative organisms include<sup>8,9,11</sup>:

- P. acnes
- Coagulase negative staphylococci
- Corynebacterium species
- Yeasts and moulds
- P. aeruginosa
- S. aureus
- *Mycobacterium* species

Bacteriology | B 2 | Issue no: 6.1 | Issue date: 04.05.17 | Page: 9 of 24

**Post-traumatic endophthalmitis** occurs after penetrating or perforating ocular injuries. This condition is much worse if organic material is associated with the penetrating injury. Pathogens may include any organism, well recognised examples being<sup>8,9</sup>:

- Bacillus cereus
- Fungi
- Streptococci
- Clostridium species
- Microsporidium species

**Endogenous endophthalmitis** is rare and occurs in patients with bacteraemia or fungaemia, often associated with treatment with immunosuppressive therapy, intravenous drug abuse or invasive surgical procedures. Blood cultures are indicated in these conditions. Causative organisms include<sup>9</sup>:

- Yeasts
- Moulds
- S. aureus
- Streptococci
- Enterobacteriaceae
- Bacillus species

#### **Orbital cellulitis**

Orbital cellulitis is the infection of orbital tissue. It can result from trauma, surgery, or an extension of paranasal sinus infections. It is a serious infection and may cause blindness, septic thrombosis of the cavernous sinus or intracranial infections. The most common pathogens in adults are *S. aureus*, streptococci and anaerobes. In children *H. influenzae* still remains prevalent, but the capsulated (type b) strain is rarely seen following the introduction of the vaccine programme<sup>12</sup>. Streptococci, staphylococci, peptostreptococci and *P. aeruginosa* may cause necrosis. Eye swabs are of limited value in the investigation of orbital and preseptal cellulitis. Ideally aspirates from the affected tissues should be obtained and treated according to the procedures outlined in <u>B 26 - Investigation of Fluids from Normally Sterile Sites</u>. Blood cultures are also useful in diagnosis (refer to <u>B 37 - Investigation of Blood Cultures (for Organisms other than *Mycobacterium* species)).</u>

#### Canaliculitis

Canaliculitis is a rare condition. Infections are usually chronic and caused by anaerobic actinomycetes such as *Actinomyces israelii* or by *Propionibacterium propionicum*<sup>13,14</sup>. Swabs of samples of the canalicular pus are preferable to eye swabs for diagnosis.

#### **Blepharitis**

Blepharitis is an acute or chronic inflammatory process involving the eyelids that can be associated with conjunctivitis and other eye infections<sup>15</sup>. Patients are not routinely

swabbed for this condition unless it is associated with other ocular infections. Bacteria associated with this infection include<sup>16</sup>:

- Staphylococcus aureus
- Staphylococcus epidermidis
- Streptococcus species
- Moraxella species
- Corynebacterium species
- P. acnes

However, these organisms may be isolated from the eyelids of normal healthy individuals, necessitating careful interpretation of such cultures.

#### **Bacteria**

#### Chlamydia<sup>1</sup>

Inclusion conjunctivitis and trachoma are caused by various serotypes of *Chlamydia trachomatis*. Trachoma is associated with serotypes A-C. These serotypes are associated with sexual transmission and can also cause infection in neonates.

#### Protozoa

#### Acanthamoeba species<sup>17</sup>

*Acanthamoeba* species can cause severe keratitis, usually in contact lens wearers or after ocular trauma. If not treated promptly, it may lead to corneal ulceration, and eventually to blindness. These protozoa may be isolated from corneal scrapings, as well as from contact lenses and storage cases. However, *Acanthamoeba* species may be isolated from contact lens fluid from individuals with no signs or symptoms of disease<sup>18</sup>. PCR is becoming the tool of choice for diagnosis.

## **Technical Information/Limitations**

Superficial swabs, although not ideal, may be all that is available. Deep-seated samples if available should be sought.

#### 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.

Bacteriology | B 2 | Issue no: 6.1 | Issue date: 04.05.17 | Page: 11 of 24 UK Standards for Microbiology Investigations | Issued by the Standards Unit, Public Health England

## Specimen Containers<sup>19,20</sup>

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".

# **1** Safety Considerations<sup>19-35</sup>

## **1.1** Specimen Collection, Transport and Storage<sup>19-24</sup>

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<sup>19-35</sup>

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet<sup>27</sup>.

If infection with a Hazard Group 3 organism, for example *Mycobacterium tuberculosis*, *Brucella*, or an agent of exotic imported mycosis, is suspected, all work must be undertaken in a microbiological safety cabinet under full Containment Level 3 conditions<sup>27</sup>.

Although *N. meningitidis, Cryptococcus neoformans* and *Acanthamoeba* species are in Hazard group 2, suspected and known isolates of *N. meningitidis, Cryptococcus neoformans* and *Acanthamoeba* species should always be handled in a microbiological safety cabinet<sup>25</sup>. Sometimes the nature of the work may dictate that full containment level 3 conditions should be used eg for the propagation of *N. meningitidis* in order to comply with COSHH 2004 Schedule 3 (4e).

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

Eye swabs, canalicular pus, aqueous and vitreous humour, corneal scrapings, contact lens cases and cleaning fluid

#### 2.2 Optimal Time and Method of Collection

For safety considerations refer to Section 1.1.

Unless otherwise stated, swabs for bacterial and fungal culture should be placed in appropriate transport medium<sup>36-40</sup>.

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

Collect specimens before antimicrobial therapy where possible<sup>41</sup>.

Corneal scrapings and intraocular fluids will be collected by an ophthalmic surgeon. Because of the small amounts of material involved, inoculation of plates and preparation of slides may need to be done at the patients' side. Laboratories should agree a protocol for the collection of specimens, inoculation of media, and transport to the laboratory with their local ophthalmologists, and supply kits for this purpose when required.

Under appropriate agreed protocols consider issuing corneal scrape kits to the ophthalmologists. They would scrape the cornea and send the blade in 1mL BHI broth in a bijou (inside an appropriate CE marked leakproof containers and placed in sealed plastic bags) and this is cultured.

Any available pus should be sampled as well as the lesion of interest.

It may also be useful to sample the contact lens itself and the contact lens case if still available and cleaning solutions.

Separate samples must be collected into appropriate transport media for detection of viruses or chlamydiae (see <u>V 37 – Chlamydia trachomatis Infection – Testing by</u> <u>Nucleic Acid Amplification Tests (NAATs)</u>). Alcohol or acetone fixed smears for immunofluorescence is also used for chlamydial investigations.

#### 2.3 Adequate Quantity and Appropriate Number of Specimens<sup>41</sup>

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

Culture should be the priority with corneal scrapings if there is insufficient sample for both an impression smear and inoculation of plates.

# **3** Specimen Transport and Storage<sup>19,20</sup>

#### 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<sup>41</sup>.

If processing is delayed, refrigeration is preferable to storage at ambient temperature<sup>41</sup>.

If specimens for investigation for amoebae cannot be processed within 8hr, it is preferable to store them at ambient temperature.

Do not freeze specimens.

# 4 Specimen Processing/Procedure<sup>19,20</sup>

#### 4.1 Test Selection

Separate samples should be collected for detection of viruses. Corneal scrapings should be inoculated to media for culture of *Acanthamoeba* species if indicated by clinical details (eg the use of contact lenses or corneal ulceration).

Smears and media for detection of *Mycobacterium* species should be processed according to <u>B 40 - Investigation of Specimens for *Mycobacterium* species</u>.

#### 4.2 Appearance

N/A

#### 4.3 Sample Preparation

For safety considerations refer to Section 1.2.

#### 4.4 Microscopy

#### 4.4.1 Standard

Refer to TP 39 – Staining Procedures.

#### Gram stain

Eye swabs (from neonates with sticky eyes and others as appropriate) and canalicular pus.

Prepare a thin smear from the swab or pus on a clean microscope slide for Gram staining.

#### 4.5 Culture and Investigation

#### 4.5.1 Standard

Inoculate each agar plate with swab or pus (<u>Q 5 - Inoculation of Culture Media for</u> <u>Bacteriology</u>).

For inoculation methods performed at the patient's side, refer to local protocols.

#### Aqueous and vitreous humour

If fluids are received, one or two drops of fluid should be inoculated to each of the agar plates and streaked out with a sterile loop for the isolation of individual colonies. Enrichment media should also be inoculated if sufficient specimen is available (see Q <u>5 - Inoculation of Culture Media for Bacteriology</u>).

Agar plates maybe inoculated directly at the patient's side and should be streaked out with a sterile loop for the isolation of individual colonies, and immediately incubated on receipt in the laboratory (see Q 5 - Inoculation of Culture Media for Bacteriology).

#### **Corneal scrapings**

Agar plates for bacterial culture, inoculated directly at the patient's side, should be streaked out with a sterile loop for the isolation of individual colonies, and incubated immediately on receipt in the laboratory (see  $Q \ 5$  - Inoculation of Culture Media for Bacteriology).

Note: *E. coli* seeded plates should not be sent to the patient's bedside.

#### 4.5.2 Supplementary

#### Culture for free-living amoebae<sup>42</sup>

Cultures for Acanthamoeba species must be processed in a safety cabinet.

A non-pathogenic control strain of *Acanthamoeba* species, *A. castellani* (Neff strain), can be obtained from the Culture Collection of Algae and Protozoa (CCAP) on request (CCAP Ref. 1501/1A).

#### Medium for inoculation of specimens

- 1. Autoclave a 1.5% concentration (15g/L) of purified agar in quarter-strength Ringer's solution (or preferably Page's saline if available). Allow to cool and pour into small Petri dishes. Dry plates before use
- 2. Flood the blood agar plate with *Escherichia coli*\* (NCTC 10418) and incubate at 37°C overnight
- 3. Recover all the growth with a sterile cotton-tipped swab and suspend in 2mL of the preferred saline (as used in 1) or sterile distilled water
- 4. Add two drops of the suspension to the surface of the purified agar plates and spread evenly over the surface with a swab. The plates are now ready for inoculation with the specimen
- 5. On grounds of patient safety it is preferred to spread the coliform suspension after receipt of plates by the laboratory after sampling has been performed in theatre. In which case the plates should be flooded on their return to the laboratory and excess fluid removed.

Note: An even distribution of the organisms is required on the plate.

\*Klebsiella species and Enterobacter aerogenes are acceptable alternatives to E. coli.

#### Inoculation of specimen<sup>42</sup>

- 1. Inoculate the specimen to a clean microscope slide (examine with a low-power objective) and to the surface of a bacteria-coated purified agar plate. Ring the inoculated area on the base of the plate with a permanent felt-tipped pen for easy reference. Include a plate with a non-pathogenic *Acanthamoeba* control
- 2. Place the plate in a sealable bag or moist box
- 3. Incubate plates at 30°C. Incubation at 37°C is not recommended as some strains grow poorly at this temperature
- 4. Examine the surface of the plate after 24hr and then daily for up to 7 days with an inverted microscope with a low-power objective. The plate need not be opened
- 5. Trophozoite stage amoebae may be seen to have made tracks in the bacterial layer. Characteristic polygonal cysts may also be seen

#### Contact lens solutions<sup>42</sup>

- Transfer fluid from contact lens storage case to a sterile universal container. Rub the inside of the storage case with a sterile cotton-tipped swab moistened with sterile distilled water. Express the liquid from the swab into the fluid in the sterile universal container
- 2. Centrifuge at 800 x g for 5 mins
- 3. Using a sterile pipette discard the supernatant into disinfectant, leaving approximately 0.5mL of centrifuged deposit
- 4. Resuspend the centrifuged deposit in the remaining fluid and place 2 drops in the centre of a bacteria-coated purified agar plate

After the fluid has been absorbed incubate and examine the plate as described for corneal scrapings (above).

Bacteriology | B 2 | Issue no: 6.1 | Issue date: 04.05.17 | Page: 16 of 24 UK Standards for Microbiology Investigations | Issued by the Standards Unit, Public Health England For *Mycobacterium* species – see <u>B 40 - Investigation of Specimens for</u> <u>Mycobacterium</u> species.

Clinical details/	Specimen	Standard	Incubation			Cultures	Target organism(s)	
conditions		ons r	media	Temp ℃	Atmos	Time	read	
Conjunctivitis	All	Chocolate	35-37	5-10%	40-48hr	daily	H. influenzae	
Sticky eye	samples	agar Blood agar	35-37	CO <sub>2</sub> 5-10% CO <sub>2</sub>	40-48hr	daily	Lancefield group A,B,C and G streptococci	
If no clinical details available.							Moraxella species	
treat as a 'sticky							N. gonorrhoeae	
eye							N. meningitidis	
Dianharitia							P. aeruginosa	
(associated with							S. aureus	
other infections)							S. pneumoniae	
							Other organisms (see section 4.6.1)	
For these situations	s, add the follo	wing:						
Clinical details/	Specimen	Supplement	Incubation		Cultures	Target organism(s)		
conditions		ary media	Temp ℃	Atmos	Time	- read		
GUM clinic sticky eye	Swabs	GC selective agar	35-37	5-10% CO2	40-48hr	≥40hr	N. gonorrhoeae	
Neonates								
Immunocomprom ised	Swabs	Sabouraud agar	28-30	air	40-48hr*	≥40hr	Fungi	
Chronic blepharitis								
Canaliculitis †	canalicular	Fastidious	35-37	anaero	40-48hr*	≥40hr	Anaerobes	
Orbital cellulitis	pus, aqueous	anaerobe agar		DIC				
Dacryocystitis †	and vitreous							
Dacryoadenitis †	humour,							
Keratitis‡	corneal scrapings							
Endophthalmitis	1 3							
Hypopyon					10d	≥40hr, at	Actinomycetes	
Post surgery						10d		
Post trauma		Sabouraud agar	28-30	air	40-48hr*	≥40hr	Fungi	
If Gram negative rods seen in Gram film	All samples	CLED agar	35-37	air	16-24hr	≥16hr	Enterobacteriaceae	
Other organisms for consideration - Chlamydia species and viruses and Mycobacterium species.								

## 4.5.3 Culture media, conditions and organisms

#### Bacteriology | B 2 | Issue no: 6.1 | Issue date: 04.05.17 | Page: 17 of 24

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

\*incubation may be extended to 5 days; in such cases plates should be read at  $\geq$ 40hr and then left in the incubator/cabinet until day 5.

†extend incubation time to 10 days if clinically suspected or Gram positive branching rods present in Gram stain.

#### 4.6 Identification

Refer to individual SMIs for organism identification.

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

Actinomycetes	"actinomycetes" level				
Anaerobes	"anaerobes" level				
Bacillus species	genus level				
Coagulase negative staphylococci	"coagulase negative" level				
Diphtheroids	"diphtheroid" level				
Enterobacteriaceae	"coliforms" level				
Enterococci	species level				
Yeast and Moulds	genus level				
<u>Haemophilus influenzae</u>	species level				
Lancefield groups A, B, C and G streptococci	Lancefield group level				
Moraxella species	species level				
Neisseria meningitidis	species level				
<u>P. aeruginosa</u>	species level				
Pseudomonads	"pseudomonads" level				
<u>S. aureus</u>	species level				
<u>S. pneumoniae</u>	species level				
<u>α-haemolytic streptococci</u>	"α-haemolytic" level				
Yeasts	"yeasts" level				
Mycobacterium species	B 40 - Investigation of Specimens for Mycobacterium species				
Parasites	B 31 - Investigation of Specimens other than Blood for Parasites				

**Note 1**: Organisms may be further identified if clinically or epidemiologically indicated. Any organism isolated from a normally sterile site should be identified to species level.

**Note 2:** Any organism considered to be a contaminant (or part of normal flora) may not require identification to species level.

**Note 3**: All work on suspected *N. meningitidis* and *C. neoformans* isolates which is likely to generate aerosols must be performed in a microbiological safety cabinet<sup>43</sup>.

#### 4.7 Antimicrobial Susceptibility Testing

Refer to <u>British Society for Antimicrobial Chemotherapy (BSAC)</u> and/or <u>EUCAST</u> guidelines.

#### 4.8 Referral for Outbreak Investigations

N/A

#### 4.9 Referral to Reference Laboratories

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory <u>click here for user manuals and request</u> <u>forms</u>.

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, turn around times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-andservices

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

## 5 Reporting Procedure

#### 5.1 Microscopy

Report on WBCs and organisms detected.

Microscopy for *Mycobacterium* species (<u>B 40 - Investigation of Specimens for</u> <u>*Mycobacterium* species</u>) and parasites (<u>B 31 - Investigation of Specimens other than</u> <u>Blood for Parasites</u>).

#### 5.1.1 Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically.

Written report, 16–72hr.

#### 5.2 Culture

Report:

Clinically significant organisms isolated or

other growth, eg "No significant growth" or

absence of growth

Report presence or absence of Acanthamoeba species if applicable.

Bacteriology | B 2 | Issue no: 6.1 | Issue date: 04.05.17 | Page: 19 of 24

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

#### 5.2.1 Culture reporting time

Clinically urgent requests: telephone when available.

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

For *Acanthamoeba* species written report on day 4 stating if appropriate, that a further report will be issued.

Supplementary investigations: *Mycobacterium* species (<u>B 40 - Investigation of</u> <u>Specimens for *Mycobacterium* species</u>).

#### 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<sup>44,45</sup> or Equivalent in the Devolved Administrations<sup>46-49</sup>

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'.

Note: Isolation of *N. meningitidis* should be reported to the CCDC.

https://www.gov.uk/government/organisations/public-health-england/about/ourgovernance#health-protection-regulations-2010

Other arrangements exist in <u>Scotland</u><sup>46,47</sup>, <u>Wales</u><sup>48</sup> and <u>Northern Ireland</u><sup>49</sup>.

## **Appendix: Investigation of Bacterial Eye Infections**



Bacteriology | B 2 | Issue no: 6.1 | Issue date: 04.05.17 | Page: 21 of 24

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

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#### Bacteriology | B 2 | Issue no: 6.1 | Issue date: 04.05.17 | Page: 22 of 24

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