



# UK Standards for Microbiology Investigations

## Investigation of Eye Swabs and Canalicular Pus



## 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 <http://www.hpa.org.uk/SMI/Partnerships>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <http://www.hpa.org.uk/SMI/WorkingGroups>).

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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UK Standards for Microbiology Investigations are produced in association with:



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NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

For full details on our accreditation visit: [www.nice.org.uk/accreditation](http://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 [standards@phe.gov.uk](mailto:standards@phe.gov.uk).

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

Amendment No/Date.	10/05.08.14
Issue no. discarded.	5.3
Insert Issue no.	5.4
<b>Section(s) involved</b>	<b>Amendment</b>
4.5.3 Culture media, conditions and organisms.	Type of specimen changed.

Amendment No/Date.	9/02.04.14
Issue no. discarded.	5.2
Insert Issue no.	5.3
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	<p>Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England.</p> <p>Front page has been redesigned.</p> <p>Status page has been renamed as Scope and Purpose and updated as appropriate.</p> <p>Professional body logos have been reviewed and updated.</p> <p>Standard safety and notification references have been reviewed and updated.</p> <p>Scientific content remains unchanged.</p>



## 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 <http://www.hpa.gov.uk/SMI/Partnerships>. 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> 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

[http://www.hpa.org.uk/webc/HPAwebFile/HPAweb\\_C/1317133470313](http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313).

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

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<http://www.hpa.org.uk/SMI/pdf>

## Scope of Document

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### Type of Specimen

Eye swabs, canalicular pus

## Scope

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This UK Standards for Microbiology Investigation (SMI) describe the processing and bacteriological investigation of specimens from the eyes; with the exception of those from keratitis, endophthalmitis, hypopyon and post-surgical infections, for these refer to [B 52 - Investigation of Intraocular Fluids and Corneal Scrapings](#). New 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

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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 involving a foreign body, following surgery, or simply by spread from adjacent sites<sup>1,2</sup>.

## Infections

Common mild eye infections include conjunctivitis (inflammation of the conjunctiva) and blepharitis (inflammation of the eyelid). Conjunctivitis may occur in association with infection of the eyelid (blepharoconjunctivitis) or of the cornea (keratoconjunctivitis). Less common, and more severe, infections include keratitis (inflammation of the cornea) and endophthalmitis (infection inside the eye itself). Haematogenous spread from a focus elsewhere in the body can also occur<sup>3</sup>. Other periorbital 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<sup>4</sup>. Invasive specimens may be required for optimal investigation of severe eye infections, and these are dealt with in [B 52 - Investigation of Intraocular Fluids and Corneal Scrapings](#). Separate swabs in appropriate transport media are needed for the diagnosis of viral and chlamydial infections.

Eye infections occurring in the first four weeks of life caused by *Chlamydia trachomatis* or *Neisseria gonorrhoeae* are notifiable as ophthalmia neonatorum.

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.

### Blepharitis

Blepharitis is associated with<sup>5,6</sup>:

- *Staphylococcus aureus*

- *Staphylococcus epidermidis*
- *Corynebacterium species*
- *Propionibacterium acnes*

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

## Conjunctivitis

Conjunctivitis may be acute or chronic. The conjunctiva is the most commonly infected ocular tissue, and infectious conjunctivitis is one of the most common causes of red or sticky eyes. Common bacterial causes include:

- *S. aureus*
- *Streptococcus pneumoniae*
- *Haemophilus influenzae*

Less common causes include Lancefield group A, C and G streptococci, *Neisseria cinerea*<sup>7,8</sup>.

*P. acnes*, *Moraxella* species, other Gram negative rods, and anaerobes such as *Eubacterium* species and *Peptostreptococcus* species<sup>9,10</sup>. *Moraxella catarrhalis* causes acute conjunctivitis and *Moraxella lacunata* causes a chronic infection<sup>10</sup>. However, many of these organisms may also be isolated from the surrounding areas (skin), and so the interpretation of the significance of their presence is difficult.

Conjunctivitis caused by *Neisseria* species is uncommon in developed countries. The most important ocular pathogen in this genus is *Neisseria gonorrhoeae*. In adults it is associated with concomitant genital infection. In neonates it is an important cause of ophthalmia neonatorum, which may cause blindness if left untreated. *Neisseria meningitidis* has also been implicated in hyperacute conjunctivitis. Treatment of this is important to reduce the risk of dissemination, and rifampicin prophylaxis may be indicated on household contacts and the patient to eliminate throat carriage.

Conjunctivitis in neonates is caused by the pathogens commonly found in adult cases<sup>9,11</sup>. Additional organisms include<sup>10</sup>:

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

## Chlamydial and viral conjunctivitis

Chlamydial and viral conjunctivitis also occur. Inclusion conjunctivitis and trachoma are caused by various serotypes of *Chlamydia trachomatis*. Trachoma is associated with serotypes A-C. This occurs in rural under-developed areas, whereas inclusion conjunctivitis is associated with types D-K, and is a feature of developed urban communities<sup>12</sup>. These serotypes are associated with sexual transmission. The most common causes of viral conjunctivitis are adenoviruses.



### ***Acanthamoeba* species**

*Acanthamoeba* species can cause severe keratitis, usually in contact lens wearers or after ocular trauma. These protozoa may be isolated from corneal scrapings, as well as from contact lenses and storage cases ([B 52 - Investigation of Intraocular Fluids and Corneal Scrapings](#) and [B 31 - Investigation of Specimens other than Blood for Parasites](#)).

### **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. Streptococci, staphylococci, peptostreptococci and *P. aeruginosa* may cause necrosis<sup>13</sup>. 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 [B 26 - Investigation of Fluids from Normally Sterile Sites](#). Blood cultures are also useful in diagnosis (refer to [B 37 - Investigation of Blood Cultures \(for Organisms other than Mycobacterium Species\)](#)).

### **Canaliculitis**

Canaliculitis is a rare condition. Infections are usually chronic and caused by anaerobic actinomycetes, such as *Actinomyces israelii* or by *Propionibacterium propionicus*<sup>14,15</sup>. Swabs of samples of the canalicular pus are preferable to eye swabs for diagnosis.

For further information about serious eye infections, including examination for *Acanthamoeba* species, refer to [B 51 - Investigation of Intraocular Fluids and Corneal Scrapings](#).

## **Technical Information/Limitations**

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### **Limitations of UK SMIs**

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) when 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.

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

### **Specimen Containers<sup>16,17</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.”

UNDER REVIEW

# 1 Safety Considerations<sup>16-32</sup>

## 1.1 Specimen Collection, Transport and Storage<sup>16-21</sup>

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport specimens 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>16-32</sup>

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet<sup>24</sup>.

If infection with a Hazard Group 3 organism, for example *M. 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>24</sup>.

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

## 2.2 Optimal Time and Method of Collection<sup>34</sup>

For safety considerations refer to Section 1.1.

Collect before antimicrobial therapy, where possible, and preferably before application of local anaesthetic.

Unless otherwise stated, swabs for bacterial and fungal culture should be placed in appropriate transport medium<sup>33,35-38</sup>.

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

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

Separate samples must be collected into appropriate transport media for detection of viruses or chlamydiae. Alcohol or acetone fixed smears for immunofluorescence is also used for chlamydial investigations.

For *Acanthamoeba* investigations refer to [B 52 - Investigation of Intraocular Fluids and Corneal Scrapings](#).

### 2.3 Adequate Quantity and Appropriate Number of Specimens<sup>34</sup>

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

## 3 Specimen Transport and Storage<sup>16,17</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>34</sup>.

If processing is delayed, refrigeration is preferable to storage at ambient temperature<sup>34</sup>.

## 4 Specimen Processing<sup>16,17</sup>

### 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

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.

#### 4.4.2 Supplementary

N/A

### 4.5 Culture and Investigation

#### Swabs

Inoculate each agar plate with swab (refer to [Q 5 – Inoculation of Culture Media for Bacteriology](#)).

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

### **Middle ear effusion**

Using a sterile pipette inoculate each agar plate with specimen (refer to [Q 5 – Inoculation of Culture Media for Bacteriology](#)).

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

#### **4.5.1 Pre-treatment**

N/A

#### **4.5.2 Specimen processing**

Inoculate each agar plate with swab or pus ([Q 5 - Inoculation of Culture Media for Bacteriology](#)).

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

UNDER REVIEW



## 4.5.3 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Blepharitis Conjunctivitis Sticky eye  If no clinical details available, treat as a 'sticky eye'	Eye swabs, canalicular pus	Chocolate agar	35-37	5-10% CO <sub>2</sub>	40-48hr	daily	<i>H. influenzae</i>  Lancefield group A,B,C and G streptococci  <i>Moraxella</i> <i>speciosa</i>  <i>N.</i> <i>gonorrhoeae</i>  <i>Meningitidis</i>  <i>P. aeruginosa</i>  <i>S. aureus</i>  <i>S. pneumoniae</i>  Other organisms (see section 4.6.1)
		Blood agar	35-37	5-10% CO <sub>2</sub>	40-48hr	daily	
<i>For these situations, add the following:</i>							
Clinical details/ conditions	Specimen	Suppleme ntary media	Incubatio			Cultures read	Target organism(s)
			Tem °C	Atmos	Time		
GUM clinic sticky eye Neonates	Eye swabs, canalicular pus	GC selective agar	35-37	5-10% CO <sub>2</sub>	40-48hr	≥40hr	<i>N.</i> <i>gonorrhoeae</i>
Immunocompromis ed Chronic blepharitis	Eye swabs, canalicula pus	Sabourau agar	28-30	air	40-48hr*	≥40hr	Fungi
Canaliculitis † Orbital cellulitis Dacryocystitis † Dacryoadenitis Keratitis ‡ Endophthalmitis ‡ Hypopyon † Post-surgery ‡ Post trauma	Eye swabs, canalicular pus	Fastidious anaerobe agar	35-37	anaerobic	40-48hr*	≥40hr	Anaerobes
		Sabouraud agar	28-30	air	40-48hr*	≥40hr	Fungi
If Gram negative rods seen in Gram film	Eye swabs, canalicular pus	CLED agar	35-37	air	16-24hr	≥16hr	Enterobacteria ceae
<b>Other organisms for consideration - <i>Chlamydia</i> species and viruses</b>							

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

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

‡ Refer to [B 52 - Investigation of Intraocular Fluids and Corneal Scrapings](#).

## 4.6 Identification

Refer to individual SMIs for organism identification.

### 4.6.1 Minimum level of identification in the laboratory

Actinomycetes	"actinomycetes" level
<a href="#">Anaerobes</a>	"anaerobes" level <a href="#">ID 14 - Identification of Anaerobic Cocci</a> <a href="#">ID 8 - Identification of <i>Clostridium</i> species</a> <a href="#">ID 25 - Identification of Anaerobic Gram Negative Rods</a>
<a href="#">Coagulase negative staphylococci</a>	"coagulase-negative" level
<a href="#">Diphtheroids</a>	"diphtheroid" level
<a href="#">Enterobacteriaceae</a>	"coliforms" level
<a href="#">Enterococci</a>	species level
Fungi	genus level
<a href="#">Haemophilus influenzae</a>	species level
<a href="#">Lancefield groups A, B, C and G streptococci</a>	Lancefield group level
<a href="#">Moraxella species</a>	species level
<a href="#">Neisseria meningitidis</a>	species level
<a href="#">P. aeruginosa</a>	species level
<a href="#">Pseudomonads</a>	"pseudomonads" level
<a href="#">S. aureus</a>	species level
<a href="#">S. pneumoniae</a>	species level
<a href="#"><math>\alpha</math>-haemolytic streptococci</a>	" $\alpha$ -haemolytic" level
Yeasts	"yeasts" level

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

## 4.7 Antimicrobial Susceptibility Testing

Refer to [British Society for Antimicrobial Chemotherapy \(BSAC\)](#) and/or [EUCAST](#) guidelines.

## 4.8 Referral for Outbreak Investigations

N/A

## 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

<http://www.hpa.org.uk/web/HPAweb&Page&HPAwebAutoListName/Page/1158313434370?p=1158313434370>

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.

### 5.2 Culture

Report:

Clinically significant organisms isolated **or** other growth, eg, “no significant growth,” **or** absence of growth

#### 5.2.1 Culture reporting time

Clinically urgent results: to be telephoned or sent electronically.

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

### 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>39,40</sup> or Equivalent in the Devolved Administrations<sup>41-44</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 (HCAs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

<http://www.hpa.org.uk/Topics/InfectiousDiseases/infectionsAZ/HealthProtectionRegulations/>

Other arrangements exist in [Scotland](#)<sup>41,42</sup>, [Wales](#)<sup>43</sup> and [Northern Ireland](#)<sup>44</sup>.

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