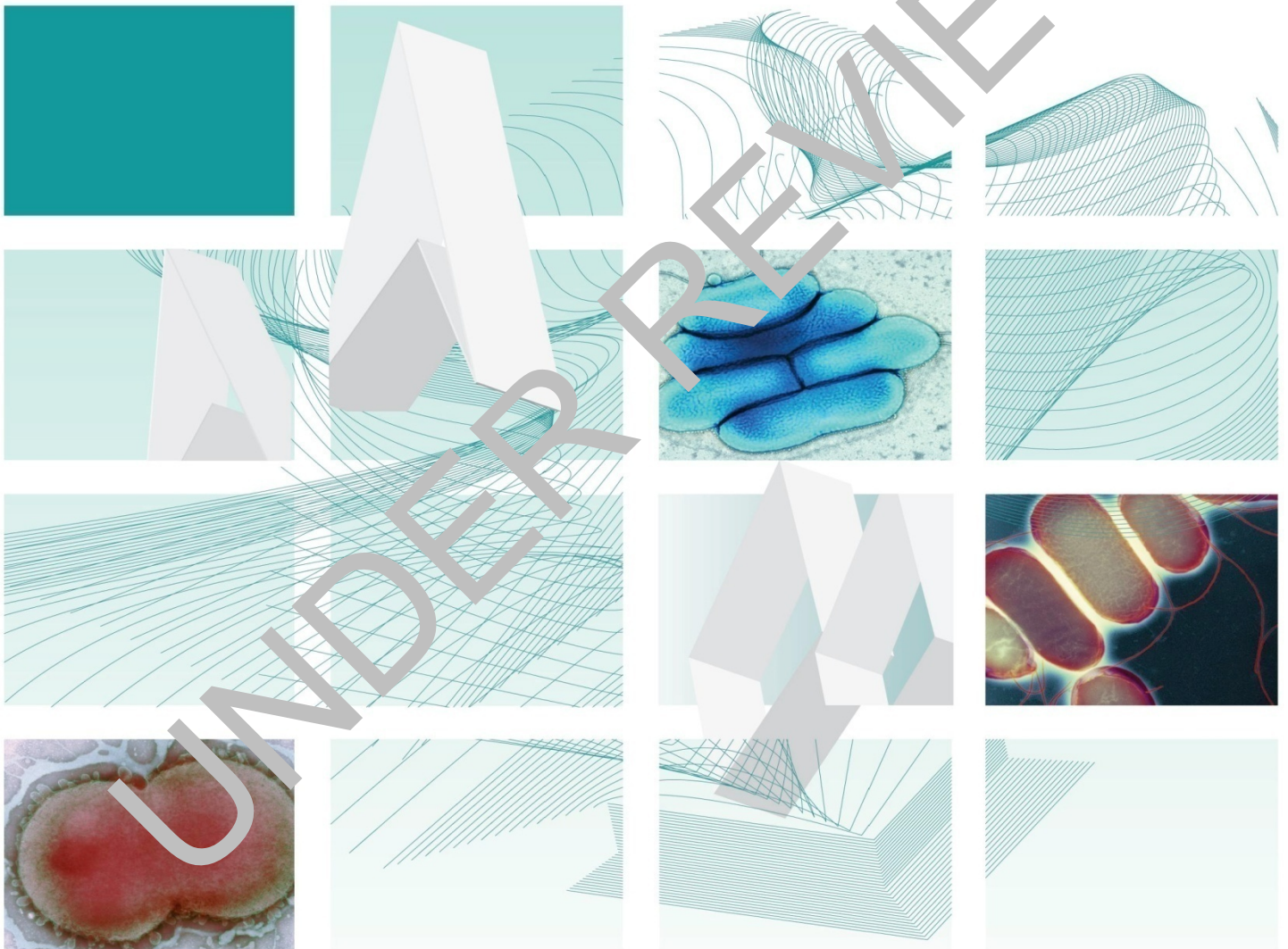




UK Standards for Microbiology Investigations

Investigation of Cerebrospinal Fluid



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>).

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

UK Standards for Microbiology Investigations are produced in association with:



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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 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.	8/15.04.14
Issue no. discarded.	5.1
Insert Issue no.	5.2
Section(s) involved	Amendment
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>

Amendment No/Date.	7/10.07.12
Issue no. discarded.	5
Insert Issue no.	5.1
Section(s) involved	Amendment
Whole document.	<p>Document presented in a new format.</p> <p>The term "CE marked leak proof container" replaces "sterile leak proof container" (where appropriate) and is referenced to specific text in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) and to Directive itself EC^{1,2}.</p> <p>Edited for clarity.</p> <p>Reorganisation of [some] text.</p> <p>Minor textual changes.</p>

Sections on specimen collection, transport, storage and processing.	Reorganised. Previous numbering changed.
References.	Some references updated.

UNDER REVIEW

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 <http://www.hpa.org.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 laboratories in the UK are expected to work. SMIs are NICE accredited and represent

[#] 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.

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/HPAwebFiles/HPAwebContent/317133470313>.

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

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Scope of Document

Type of Specimen

Cerebrospinal fluid

Scope

This SMI describes the examination of cerebrospinal fluid (CSF) for the detection and recovery of the causative organisms of meningitis.

This SMI should be used in conjunction with other SMIs.

Introduction

Meningitis

Meningitis is defined as inflammation of the meninges. This process may be acute or chronic, and infective or non-infective. Many infective agents have been shown to cause meningitis, including viruses, bacteria, fungi and parasites.

Acute Bacterial Meningitis³

Acute bacterial meningitis is a medical emergency. Symptoms and signs of meningitis may evolve over a few days, or have a rapid onset and fulminant course over a few hours. The clinical picture may be dominated by accompanying septicaemia, as with meningococcal infection. Untreated, mortality is high. It is imperative that any specimen taken from a patient is processed as rapidly as possible, in order to optimise clinical management⁴. Typically the CSF becomes infiltrated with neutrophil leucocytes and has raised protein and reduced glucose concentrations.

A number of conditions predispose individual patients to develop meningitis^{3,5,6}. Abnormal post-surgical and traumatic communications between the subarachnoid space and colonised sites (eg the nose and paranasal sinuses following basilar skull fracture), presence of CSF shunts, presence of cochlear implants, meningomyelocele and other congenital malformations, infections of contiguous sites (eg the middle ear cavity or paranasal sinuses) and tumours in close proximity to the central nervous system are some examples. As well as direct spread, meningeal infection may occur as a result of blood-borne seeding from a distant site. Patients with immune dysfunction (such as complement deficiency syndromes, or hypogammaglobulinaemia) or who are receiving immunosuppressive treatment are at increased risk of meningitis.

Viral ('aseptic') Meningitis³

Viral ('aseptic') meningitis is relatively common in Britain. The condition is usually benign and complications are rare. The course is often subacute, evolving over two or three days. The major cause is enteroviral infection, especially in the summer and autumn months. Lymphocyte predominance in the CSF is typical but it must be remembered that early in the course of the disease, both neutrophils and lymphocytes (sometimes with neutrophil predominance) may be seen^{7,8}. CSF glucose concentration is usually normal and protein concentration normal or slightly raised⁶.

Herpes viruses may also cause this condition either as a complication of primary infection (eg genital herpes) or in recurrent disease (eg herpes zoster ophthalmicus). The diagnosis is usually obvious, but not necessarily so. Some patients develop recurrent attacks of lymphocytic meningitis (Mollaret's meningitis) that may be due to reactivation of herpes simplex 1 virus.

Aseptic meningitis complicates 10-30% of mumps virus infections and mumps is one of the commonest causes of meningitis in unimmunised populations.

It is important to distinguish between the relatively benign syndromes described above and the meningeal inflammation that may accompany viral meningoencephalitis. In the UK, herpes simplex virus is the commonest cause of encephalitis and requires prompt and vigorous antiviral treatment.

Arthropod borne viral infections are a major cause of meningoencephalitis syndromes overseas and may be imported to the UK (eg Japanese B encephalitis virus, St Louis encephalitis, West Nile virus).

It is important to remember that many conditions may cause "aseptic meningitis". The demonstration of a lymphocytic pleocytosis in the CSF is an indication towards a differential diagnosis, not a diagnosis in itself. The whole case history must be taken into account when assessing the individual problem. Many causes of viral meningitis, including enteroviruses, can be detected by polymerase chain reaction (PCR)⁹.

Chronic Meningitis¹⁰

Chronic meningitis is said to be present when signs and symptoms of meningeal inflammation (including abnormalities in the CSF) have been present for a month or more. A principal infective cause of this condition is tuberculous meningitis. In an established case the CSF may be infiltrated with lymphocytic cells.

Tuberculous meningitis has insidious and protean clinical manifestations. It is generally rare in the UK but the diagnosis should be considered in patients from areas of high TB prevalence and in high risk groups. Examination for alcohol and acid-fast bacilli or for the mycobacterial genome is usually only performed if there is a specific indication. Auramine-phenol staining supplemented by molecular methods, eg PCR are useful for rapid detection of acid-fast bacilli^{11,12}. Rapid tests are useful if positive, but culture remains the gold standard for diagnosis¹³.

Sarcoidosis is a multi-organ disease where the cause is unknown, although it has been postulated that it may be a result of the exposure of genetically susceptible individuals to infectious agents. Sarcoid meningitis is very rare, produces a raised protein concentration and leucocyte count together with lesions on the meninges seen on magnetic resonance imaging¹⁴.

Carcinomatous meningitis arises from metastasis from a primary site to the meninges and diagnosis usually rests on the presence of cranial nerve lesion symptoms eg deafness, and by use of magnetic resonance imaging and cytological examination of the CSF for signet cells. It is also important to distinguish between true infection and the result of the malignancy because the two may co-exist. Leukaemic meningitis is also very rare and its treatment exposes the patient to infection due to immunosuppression. Pathogens commonly isolated from CSF are *Listeria monocytogenes*, *Cryptococcus neoformans* and *Toxoplasma gondii* which may be diagnosed by brain biopsy or serologically¹⁵.

Organisms Causing Meningitis

Species isolated tend to be characteristically, but not exclusively, associated with the age or predisposing status of the patient^{3,5,10,16}.

From neonates and babies up to two months of age: Lancefield group B streptococci, *Escherichia coli*, *Listeria monocytogenes*, herpes simplex virus and *Neisseria meningitidis*. Premature neonates requiring intensive care are at risk of *Candida* species meningitis as a result of candidaemia.

From children older than two months to young adults: *N. meningitidis*, *Streptococcus pneumoniae*, viruses (in particular enteroviruses) and *Haemophilus influenzae* type b. The incidence of *H. influenzae* type b meningitis in the UK has been greatly reduced by routine Hib immunisation, although infections are increasing.

From adults: *S. pneumoniae*, *N. meningitidis*, viruses and occasionally non-group b *H. influenzae*. Patients older than 60 years without other predisposing factors may develop *Listeria monocytogenes* infection.

Fungi have also been found to cause meningitis¹⁷.

Other Causes

Many other organisms have been documented to cause meningitis^{3,18-20}. Bacteria such as *Salmonella* species, *Brucella* species and *Staphylococcus aureus* may seed the meninges as part of a bacteraemic infection. Patients with the strongyloidiasis hyperinfection syndrome are at risk of meningitis with Enterobacteriaceae as a result of recurrent bacteraemia or penetration of the CSF by larvae.

Spirochaetes such as *Treponema pallidum*, *Borrelia* and *Leptospira* species may cause meningitis as part of a generalised infection. Examinations for these organisms are described in separate UK Standards for Microbiology Investigations.

Fungi such as *Histoplasma capsulatum* and *Coccidioides immitis* may infect the meninges in disseminated infection.

Parasites (such as the amoebae *Acanthamoeba* species and *Naegleria species*) occasionally cause meningitis²¹. *Naegleria fowleri* invades the meninges via the cribriform plate in freshwater swimmers who inhale small quantities of water, giving rise to florid meningoencephalitis with a high fatality rate.

The nematode *Angiostrongylus cantonensis*, which has a distribution mainly in South East Asia, and has also reported from the Dominican Republic, may cause eosinophilic meningitis in infected persons¹⁸. The raccoon roundworm *Baylisascaris procyonis* is a rare cause of neural larva migrans in the USA in infants and children²².

Mixed Infections

These are rare but may account for up to 1% of cases of meningitis⁵. They are associated with trauma, tumours or infections such as acute paranasal sinusitis that may extend directly to the meninges¹⁹. Mixed infections may also arise by direct entry of organisms via fistulae or as a result of a ruptured brain abscess.

Special Risk Groups

Patients who are immunosuppressed are additionally susceptible to meningitis caused by organisms such as *Listeria monocytogenes*, *Cryptococcus neoformans* and *Toxoplasma gondii*.

After neurosurgery, patients are at risk of infection with *Staphylococcus aureus*, Enterobacteriaceae and pseudomonads. Patients with skull fractures involving the middle ear or paranasal sinuses are at risk of meningitis caused by *S. pneumoniae*, *H. influenzae* and other organisms which colonise these sites. Cochlear implant surgery has also been implicated in rare cases of meningitis due to infection with *S. pneumoniae* and Group A streptococci²³.

Nocardia species are rare causes of meningitis, but predisposing factors such as immunosuppression, head trauma or surgery can be identified in the majority of patients²⁴.

Patients with intracranial prosthetic material such as CSF shunts (see [B 22 - Investigation of Cerebrospinal Fluid Shunts](#)) are susceptible to infection caused by *Staphylococcus aureus*, coagulase negative staphylococci, *Corynebacterium* species, *Propionibacterium* species, *Candida* species and Enterobacteriaceae.

Diagnosis of Meningitis

Diagnosis of meningitis is best established by laboratory examination of the CSF. This is usually obtained by lumbar puncture, although ventricular, cisternal or fontanelle taps may also be used. Lumbar puncture may cause cerebral herniation, therefore in patients where there is a risk of increased intracranial pressure CT scanning is advised prior to the procedure. In some cases the patient is too unstable or has a bleeding diathesis as a result of sepsis syndrome and so cannot undergo immediate lumbar puncture.

In patients for whom lumbar puncture is contraindicated, every effort must be made to establish a microbiological diagnosis by other means. This is desirable both for epidemiological purposes and for the appropriate management of contacts of cases.

Therapy should not be delayed pending CSF microscopy or culture. It is important to initiate effective antimicrobial therapy quickly, and this may even commence before the examination of the CSF. CSF culture requires at least 24 hours incubation. Early management decisions therefore, must be based largely on the immediate examination of the sample by cell count and Gram stain^{6,25}.

PCR tests are available as a diagnostic procedure for some organisms⁹. *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae* can be detected simultaneously from clinical samples by PCR²⁶. A broad-range bacterial PCR primer set has recently been developed. This detects organisms that are found less frequently or those of unknown causative agents for bacterial meningitis²⁷. PCR assays are also available for agents involved in aseptic meningitis^{28,29}. To overcome contraindications and possible detrimental effects of lumbar puncture, PCR of blood buffy coat has been shown to be successful for the diagnosis of meningococcal disease³⁰. However, the mobile nature of the target (insertion sequence IS1106) has led to the recording of false-positives, reflecting one of the problems of using PCR³¹. For meningococcal disease the combination of ultrasound-enhanced LAT and PCR may increase the diagnostic capabilities and acquisition of epidemiological data⁴¹. PCR may be particularly useful in situations where culture is negative because of chemotherapy, and serology may also be helpful retrospectively in patients who survive.

Examination of CSF

The diagnosis of meningitis from the examination of CSF involves the following^{3,6,10,25,32}:

- Complete cell count
- Differential leucocyte count
- Examination of Gram stained smear
- Culture
- Determination of glucose and protein concentrations (usually performed by chemical pathology departments)
- PCR where appropriate
- Antigen testing

Examination of the deposit by cyto-centrifugation (eg Cytospin) is the most accurate method of cell differentiation but may not be routinely available.

Normal CSF values^{6,21,33,34}

Leucocytes	Neonates	0 - 30 cells x 10 ⁶ /L
	1-4yr old	0 - 20 cells x 10 ⁶ /L
	5yr-puberty	0 - 10 cells x 10 ⁶ /L
	Adults	0 - 5 cells x 10 ⁶ /L
Erythrocytes	Newborn	0 - 675 cells x 10 ⁶ /L
	Adults	0 - 10 cells x 10 ⁶ /L
Protein	Neonates ≤6d	0.7 g/L
	Others	0.2-0.4g/L (<1% of serum protein concentration)
Glucose		≥60% of simultaneously determined plasma concentration (CSF: serum ratio ≥0.6)

These values represent the upper and lower limits of normality. Bacterial or viral infection may still need to be considered where leucocyte counts are near the upper normal limits in neonates and young children.

Abnormalities Associated with Bacterial Meningitis^{3,6,21,35}:

- Reduced glucose concentration: <60% blood glucose (CSF: serum ratio <0.6)
- Elevated protein concentration
- Raised white blood cell (WBC) count: 10¹ - 10⁴ predominantly polymorphs
- Elevated intracranial pressure

Presence of Red Blood Cells (RBCs)

The presence of RBCs in CSF can result from an intra-cerebral or sub-arachnoid haemorrhage or from a traumatic lumbar puncture (LP) in which peripheral blood contaminates the CSF. The presence of this contaminating blood may make interpretation of the CSF analysis more difficult but rarely obscures CSF abnormalities associated with bacterial meningitis³⁶.

Sequential samples 1 and 3, from one lumbar puncture, are examined. Uniform bloodstaining of all samples suggests previous haemorrhage into the sub-arachnoid space, whereas reducing counts in sequentially obtained samples suggest bleeding induced by the tap procedure.

A WBC: RBC ratio of 1:500 to 1:1000 is generally regarded as not indicative of infection. CSF obtained more than 12 hours post intra-cranial haemorrhage may show raised WBC counts of up to $500 \times 10^6/L$ as a result of an inflammatory response.

Xanthochromia

Because visual determination is unreliable, xanthochromia should be determined by examination of the supernatant of centrifuged CSF by spectrophotometry to seek macroscopically invisible haematin or bilirubin, which, if present, will confirm pre-tap intracranial haemorrhage³⁷.

Xanthochromia is yellow colouration of the supernatant of centrifuged CSF. It can result from the metabolism of products of RBC breakdown, increased CSF protein concentration, or bilirubin staining. RBC breakdown in CSF commences approximately 1-2 hours post haemorrhage. The supernatant may initially be pink in colour due to the presence of oxyhaemoglobin. After 24 hours, the supernatant begins to show increasing xanthochromia caused by the degradation of oxyhaemoglobin to bilirubin. This usually peaks at 36-48 hours.

In sub-arachnoid haemorrhage xanthochromia is associated with a ten-fold increase in protein to $\geq 1.5g/L$ which peaks at 2-10 days post onset and then declines. In a fresh, traumatic lumbar puncture the CSF supernatant is usually clear and colourless, although other factors may contribute to its appearance³⁶.

Presence of Polymorphs and Lymphocytes

Although patients with untreated acute bacterial meningitis usually have high CSF polymorph counts, the CSF polymorph: lymphocyte ratio is unreliable as a pointer to the cause of meningitis. This is particularly so in neonates or when total leucocyte counts are less than 1000×10^6 per litre³⁸. Viral meningitis is classically described as being associated with a lymphocytic CSF, but it must be remembered that neutrophils may predominate, especially early in the illness^{7,8}. Tuberculous meningitis may also be associated with a neutrophil rather than a lymphocytic infiltrate early in the infection.

There is a disputed syndrome in neonates of systemic sepsis with normal CSF leucocyte counts but positive CSF cultures (most commonly coagulase negative staphylococci)^{39,40}. This causes difficulty diagnostically because coagulase negative staphylococci are frequently encountered as contaminants, but undoubtedly may cause sepsis in compromised neonates.

Neutropenic patients may not produce reliable or characteristic polymorph or neutrophil responses in the CSF.

Occasionally prolonged examination of a wet preparation, or performance of an Indian ink preparation, will be indicated for the detection of amoebae and *C. neoformans* respectively. The latter is essential if cryptococcal infection is suspected in a patient who is immunocompromised.

Latex Agglutination Test (LAT)

The bacteria commonly causing meningitis carry specific polysaccharide surface antigens that can be detected by LAT. LATs are expensive, reliability is disputed and sensitivity is poor⁴¹. It has been shown that ultrasonic enhancement increased the sensitivity of the LAT up to 600-fold^{42,43}. It was recommended that LAT should not be used on CSF unless an abnormal cell count, a negative Gram stained film and CSF and blood cultures remain negative after 48 hours⁴¹. The clinician should be informed that, although a positive LAT indicates the presence of an infectious agent, a negative result is not definitive. The routine use of LAT is not recommended in this SMI.

Antigen testing may be useful in⁴¹:

- Patients with certain types of immunodeficiency who may fail to produce a WBC response even in the presence of infection
- Patients partially treated before examination of the CSF which may have a negative Gram stain and/or culture

Antigen testing may yield a positive result under these circumstances. It may be the only indication of the responsible organism.

Blood cultures and pharyngeal swabs may be useful in addition to CSF examination in the diagnosis of meningococcal meningitis and serology may allow retrospective diagnosis on acute and convalescent sera.

Current recommendations require that patients should be treated with antibiotics for suspected invasive meningococcal disease prior to admission to hospital, and CSF may be culture negative as a result. However, pharyngeal swabs and smears of fluid expressed from suspected meningococcal petechiae may yield a positive Gram stained film and/or culture results. This may confirm the meningococcal aetiology of cases with blood, and CSF cultures rendered sterile by prior antibiotic therapy.

Cryptococcal antigen may also be detected by LAT, although testing of serum is more sensitive than testing CSF alone. This should be performed in addition to microscopy on all suspected cases of cryptococcal meningitis.

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.

Specimen Containers^{1,2}

SIMs 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^{1,2,44-58}

1.1 Specimen Collection, Transport and Storage^{1,2,44-47}

Use aseptic technique.

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

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing^{1,2,44-58}

Containment Level 2 unless infection with a) *N. meningitidis*, b) a Hazard Group 3 organism or c) TSE is suspected.

a) Although *N. meningitidis* is in Hazard group 2, suspected and known isolates of *N. meningitidis* 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 e.g. for the propagation of *N. meningitidis* in order to comply with COSHH 2004 Schedule 3 (4e).

b) Where Hazard Group 3 *Mycobacterium* species are suspected, all specimens must be processed in a microbiological safety cabinet under full containment level 3 conditions.

c) Laboratory policies that take into account the local risk assessments may dictate that the use of a microbiological safety cabinet should be used when dispensing the specimen.

“Although TSE agents are formally classified as HG3 ...the containment measures required when working with them may not necessarily fully meet Containment Level 3 because of the agent’s unique features.” Refer to current ACDP guidance.

Prior to staining, fix smeared material by placing the slide on an electric hotplate (65-75°C), under the hood, until dry. Then place in a rack or other suitable holder.

Note: Heat-fixing may not kill all *Mycobacterium* species⁵⁹. Slides should be handled carefully.

Centrifugation must be carried out in sealed buckets which are subsequently opened in a microbiological safety cabinet.

Specimen containers must also be placed in a suitable holder.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet⁵⁰.

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

Cerebrospinal fluid

2.2 Optimal Time and Method of Collection⁶⁰

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible, but this must not be delayed unnecessarily pending lumbar puncture and CSF culture⁶⁰.

Collect specimens into appropriate CE marked leak proof containers and place in sealed plastic bag.

Specialist collection according to local protocols.

Specimens should be cultured as soon as possible after receipt, ideally within 10 minutes, or else a maximum of two hours^{3,6,33}. Cells disintegrate and a delay may produce a cell count that does not reflect the clinical situation of the patient.

2.3 Adequate Quantity and Appropriate Number of Specimens⁶⁰

Ideally a minimum volume of 1mL.

For *Mycobacterium* species, at least 10mL where possible.

CSF is normally collected sequentially into three or more separate containers which should be numbered consecutively.

Collection of an additional sample in a container with fluoride for glucose estimation is also recommended, although such tubes should be filled last because they may contain environmental bacteria which might otherwise contaminate samples for culture.

Common practice is to send the first and last specimens taken for microbiological examination and the second specimen for protein.

3 Specimen Transport and Storage^{1,2}

3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible⁶⁰.

Do not refrigerate specimen until after microscopy and bacterial culture have been performed. The specimen should then be refrigerated pending further investigation.

4 Specimen Processing/Procedure^{1,2}

4.1 Test Selection

Specimens taken after routine neurological examination (eg myelogram, multiple sclerosis) do not require Gram film or culture unless the leucocyte count is raised, or these tests are clinically indicated or specified in local protocols.

Divide specimen, if multiple samples are not taken after performing microscopy and bacterial culture, for appropriate procedures such as protein estimation, culture for *Mycobacterium* species ([B 40 - Investigation of Specimens for *Mycobacterium* species](#)), examination for parasites ([B 31 - Investigation of Specimens other than Blood for Parasites](#)), screening for cryptococcal antigen or virology as may be appropriate in view of clinical details, tests requested or microscopy results.

Note: If there is an insufficient volume of sample for all investigations, these should be prioritised following medical microbiological advice.

Rapid screening for antigens in CSF from cases of bacterial meningitis is not recommended routinely. However, it may be useful for example when deciding if two or more cases of the same type have occurred in a school (to guide mass prophylaxis or vaccination).

PCR is available as a diagnostic procedure for some organisms. An unopened sample, if available, is preferred for PCR.

4.2 Appearance

Describe turbidity and whether a clot is present (which would invalidate the cell count).

In extreme cases of TB meningitis, a typical 'spider-web' clot may be present. Although rarely seen, its presence should be noted.

Record if the estimated specimen volume is insufficient for all investigations to be performed and obtain medical microbiological advice about prioritisation if appropriate.

Describe colour of supernatant after centrifugation.

Confirmation of xanthochromia should be performed by spectrophotometry if requested, or if clarification of the source of RBCs in the CSF is required³⁷. This is often carried out by Clinical Biochemistry departments, as are protein and glucose determinations.

4.3 Sample Preparation

For safety considerations refer to Section 1.2.

4.4 Microscopy

4.4.1 Standard total cell count

Perform total WBC and RBC counts on the uncentrifuged specimen, preferably the last specimen taken, in a counting chamber.

Cell counts should not be performed on specimens containing a clot (which would invalidate the result).

Differential leucocyte count

1. Counting chamber method (recommended for lower WBC counts).

a) Non- or lightly-bloodstained specimens

Stain the unspun CSF with 0.1% stain solution such as toluidine, methylene or Nile blue. These stain the leucocyte nuclei, aiding differentiation of the cells. If the CSF is diluted when adding the stain, remember to take the dilution factor into account when calculating the final cell count.

Count and record the actual numbers of each leucocyte type. Express the leucocyte count as number of cells per litre.

b) Heavily bloodstained specimens

Dilute specimen with WBC diluting fluid and leave for 5min, before loading the counting chamber. This will lyse the RBCs and stain the leucocyte nuclei for differentiation.

Count and record the actual numbers of each leucocyte type. Taking the dilution factor into account, express as number of cells per litre.

2. Stained method (recommended for very high WBC counts where differentiation in the counting chamber is difficult) in the counting chamber is difficult)

Prepare a slide from the CSF centrifuged deposit as for the Gram stain, but allow to air dry. Fix in alcohol and stain with a stain suitable for WBC morphology.

Note 1: Heat fixation distorts cellular morphology. Count and record the actual numbers of each leucocyte type. Taking the dilution factor into account, express as number of cells per litre.

Note 2: A cytocentrifugation deposit (eg Cytospin) permits the most accurate cell differentiation. Care should be taken to use a sterile tube if this deposit is to be used for Gram stain examination.

Total red cell count

If haemorrhage is suspected, perform a total RBC count on a minimum of two specimens from the same lumbar puncture to assess uniformity of bloodstaining. Isotonic or phosphate buffered saline should be used for any dilutions required.

Gram stain (refer to [TP 39 - Staining Procedures](#))

Perform Gram stain on all specimens except:

- Clotted specimens (see below)
- Routine neurological specimens unless leucocyte counts are raised

Centrifuge in a sterile, capped, conical-bottomed container at 1200 xg for 5-10min.

Note: If investigation for *Mycobacterium* species is also requested, the centrifugation time may be increased to 15-20 min at 5000 xg (see [B 40 - Investigation of Specimens for Mycobacterium species](#)) and the same deposit used for this as well as routine microscopy and culture.

Transfer all but the last 0.5 mL of the supernatant with a sterile pipette to another sterile container for additional testing if required (eg protein, virology).

Resuspend the deposit in the remaining fluid.

Place one drop of centrifuged deposit with a sterile pipette on a clean microscope slide.

Spread this with a sterile loop to make a thin smear for Gram staining.

The sensitivity of the Gram stain may be improved by serial drops being 'built up' on the slide after each drop has dried, to maximise the amount examined. Care should be taken to ensure that the smear does not wash off during staining.

Clotted specimens

If possible the clot should be broken up with a sterile pipette and a portion used to make a smear for Gram staining.

4.4.2 Supplementary

Examination for *M. tuberculosis*

The 'build up' technique for films, as described above, is recommended for the examination for *Mycobacterium* species (see [B 40 - Investigation of Specimens for *Mycobacterium* species](#)). If a 'spider-web' clot is present this should be included in the portion of the specimen examined by microscopy and culture.

Examination for *C. neoformans* (see [TP 39 - Staining Procedures](#)).

Mix a drop of the centrifuged deposit with a drop of 50% aqueous Indian ink or nigrosin on a clean microscope slide and cover with a coverslip.

Examine for the presence of round or oval yeasts with a clear halo around the cell, indicating the presence of a capsule. The presence of a capsule permits a presumptive identification of *C. neoformans*.

Examination for amoebae

Examine both uncentrifuged and centrifuged deposits as wet preparations. Place a drop of specimen on a clean microscope slide, cover with a coverslip and examine for amoebic trophozoites ([B 31 - Investigation of Specimens other than Blood for Parasites](#)).

4.5 Culture and Investigation

4.5.1 Pre treatment

Standard

Centrifuge specimen (already performed for microscopy - see 4.4.1).

Supplementary

Mycobacterium species ([B 40 - Investigation of Specimens for *Mycobacterium* species](#)) and parasites (see [B 31 - Investigation of Specimens other than Blood for Parasites](#)).

4.5.2 Specimen processing

Standard

For all CSF:

- With a sterile pipette inoculate each agar with the centrifuged deposit (see [G 5 - Inoculation of Culture Media for Bacteriology](#))
- Allow inoculum to dry before spreading to minimise any antibiotic effect which may be present
- For the isolation of individual colonies, spread inoculum with a sterile loop

Clotted specimens

Inoculate the clot fragments to each agar plate.

If the specimen contains only a small clot, this should be included in the inoculum applied to the chocolate agar plate. The unclotted portion of the CSF should be cultured in the normal way as described above.

Supplementary

Broth cultures are not recommended as a significant positive yield is rarely achieved and contamination is frequent^{62,63}.

4.5.3 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Meningitis Post neurosurgery Reservoirs Ventriculitis Immunocompromised	Cerebrospinal fluid	Chocolate agar	35-37	5-10% CO ₂	40- 48hr	daily	Any organism
		Blood agar	35-37	5-10% CO ₂	40- 48hr	daily	
For these situations, add the following:							
Clinical details/ conditions	Specimen	Supplement ary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Immunocompromised patients	Cerebrospinal fluid	Sabouraud plate	35-37	air	2-5d	≥ 40hr: up to 8 weeks	Fungi
Brain abscess Ventriculitis Reservoirs Post neurosurgery Post otitis media with complications	Cerebrospinal fluid	Fastidious anaerobe agar	35-37	anaerobic	7-14d	40hr and at 5 d	Anaerobes
If mixed infection suggested by Gram stained film	Cerebrospinal fluid	Neomycin fastidious anaerobe agar	35-37	anaerobic	7-14d	≥40hr and at 5 d	
Other organisms for consideration: <i>Mycobacterium</i> species and parasites, <i>T. pallidum</i> and viruses can be found in relevant SMIs.							

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Anaerobes	species level ID 14 - Identification of Anaerobic Cocci ID 25 - Identification of Anaerobic Gram Negative Rods
<i>Actinomyces</i>	species level
β-haemolytic streptococci	Lancefield group level
All other organisms	species level

<i>Mycobacterium</i>	B 40 - Investigation of Specimens for <i>Mycobacterium</i> species
Parasites	B 31 - Investigation of Specimens other than Blood for Parasites

Note: Any organism considered to be a contaminant may not require identification to species 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](#).

β -haemolytic streptococci	Serotyping
<i>S. pneumoniae</i>	Serotyping
<i>H. influenzae</i>	Serotyping
<i>Listeria</i> species	Serotyping
<i>N. meningitidis</i>	Strain characterisation, antimicrobial susceptibility testing
Fungi	Identification and/or susceptibility testing
<i>Mycobacterium</i> species	B 40 - Investigation of Specimens for <i>Mycobacterium</i> species

Isolates associated with outbreaks, where epidemiologically indicated.

CSF, EDTA blood and paired serum samples may be sent to the Meningococcal Reference Unit (MRU) for examination using molecular methods and serological examination if culture is negative and meningococcal infection suspected.

Specimens for molecular testing for other organisms may be sent to appropriate laboratories if clinically indicated.

All CSF specimens from cases of neuroparalytic disease.

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/webw/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

Cell count

Report numbers of RBCs x 10⁶ per litre and

Report numbers of PMNs and lymphocytes x 10⁶ per litre or

Report PMNs and lymphocytes as percentages of the total WBC (which is reported as x 10⁶).

In certain cases referral to cytology for identification of mononuclear and other cells may be indicated.

Gram stain

Report on organisms detected and presence or absence of pus cells.

Indian ink or nigrosin.

Report on encapsulated yeasts detected.

Microscopy for *Mycobacterium* species ([B 30 - Investigation of Specimens for *Mycobacterium* species](#)) and parasites ([B 31 - Investigation of Specimens other than Blood for Parasites](#)).

5.1.1 Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically when available.

Written report, 16–72hr

5.2 Culture

Report the organism isolated or

Report absence of growth.

Also, report results of supplementary investigations.

Culture reporting time

Clinically urgent culture results to be telephoned or sent electronically when available.

Interim / final written report, 16–72hr stating, if appropriate, that a further report will be issued.

Molecular testing results (if applicable).

Supplementary investigations: *Mycobacterium* species ([B 40 - Investigation of Specimens for *Mycobacterium* species](#)) fungi ([B 39 - Investigation of Dermatological Specimens for Superficial Mycoses](#)) and parasites ([B 31 - Investigation of Specimens other than Blood for Parasites](#)).

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^{64,65} or Equivalent in the Devolved Administrations⁶⁶⁻⁶⁹

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.

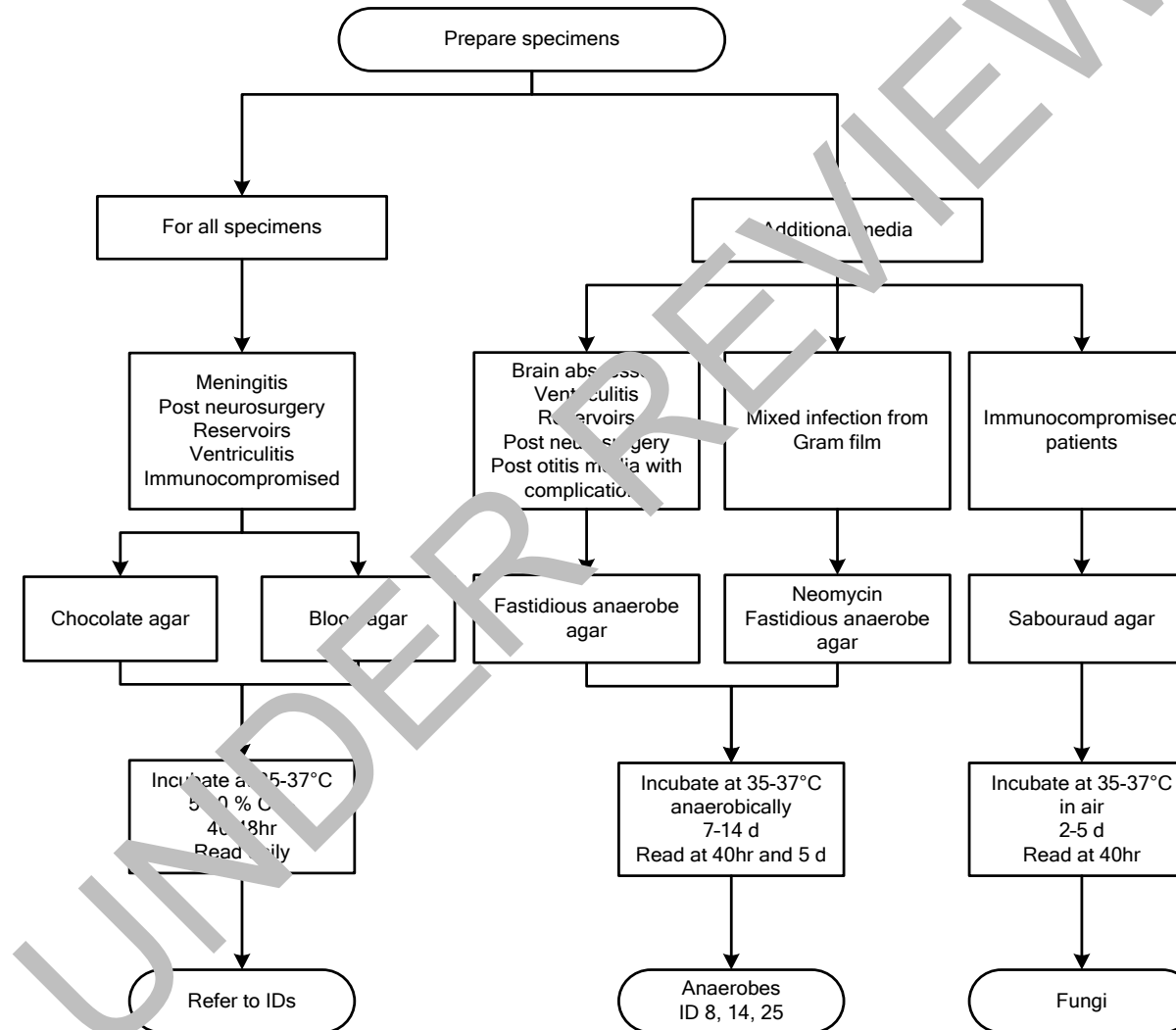
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health Protection Teams have agreements with local 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](#)^{66,67}, [Wales](#)⁶⁸ and [Northern Ireland](#)⁶⁹.

Appendix: Investigation of Cerebrospinal Fluid



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