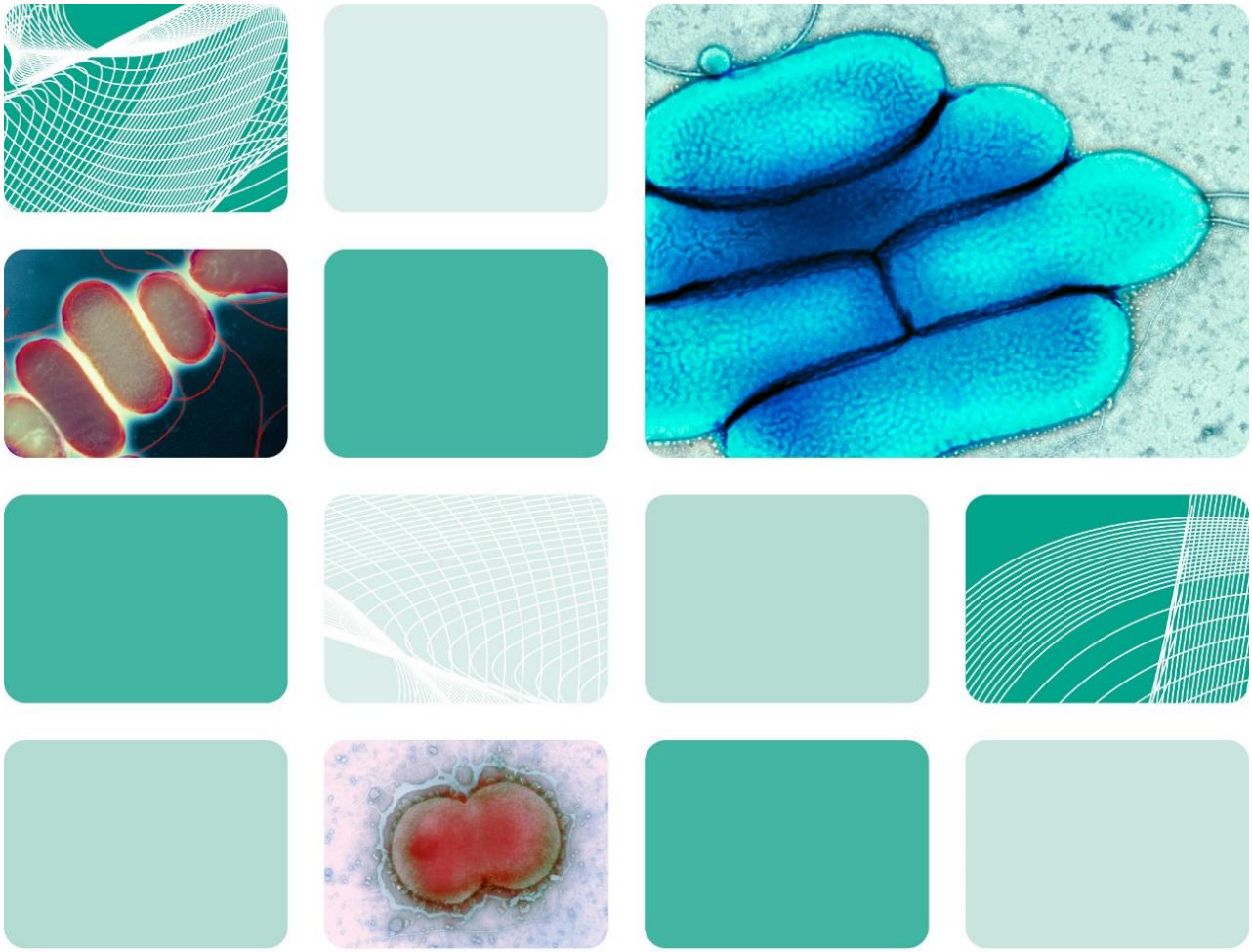
UK Health Security Agency

UK Standards for Microbiology Investigations

Investigation of cerebrospinal fluid



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Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee.

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UK SMIs are produced in association with:

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Contents

Amendment table

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

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	11/31.10.25
Issue number discarded	6.1
Insert issue number	6.2
Section(s) involved	**Amendment**
Whole document.	**This is an administrative point change.** **The content of this UK SMI document has not changed.** **The last scientific and clinical review was conducted on 31/05/2017.** Hyperlinks throughout document updated to Royal College of Pathologists website. Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms Partner organisation logos updated. Broken links to devolved administrations replaced. References to NICE accreditation removed. Scope and Purpose replaced with General and Scientific information to align with current UK SMI template. ‘Public health responsibilities of diagnostic laboratories’ section added.

Amendment No/Date.	10/31.05.17
Issue no. discarded.	6
Insert Issue no.	6.1
Section(s) involved	**Amendment**

Diagnosis of meningitis.	Table referring to normal values of CSF has been updated to include a wider population: neonates, infants and elderly.

Amendment No/Date.	9/24.02.15
Issue no. discarded.	5.2
Insert Issue no.	6
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Scope.	Cross reference to G 4 inserted.
Introduction.	Restructured so that organisms causing meningitis are at the beginning followed by clinical presentations. Normal CSF values table amended to include the ages and the supporting text underneath has been strengthened.
2.3 Adequate quantity and appropriate number of specimens.	Section clarified to describe how many and what kind of samples should be taken.
4.3.1 Culture Media.	Slopes added for long term culture of fungi. Culture recommendations for anaerobes have been strengthened.
4.5.2 Specimen Processing.	The use of 16S PCR and MALDI TOF inserted.
5.1 Microscopy reporting time.	Guidelines for reporting of cell counts have been given.
References.	References reviewed and updated.

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

Type of Specimen

Cerebrospinal fluid

3.1 Scope

This UK SMI describes the examination of cerebrospinal fluid (CSF) for the detection and recovery of the causative bacterial or fungal organisms of meningitis. Viruses and other causes of meningitis are mentioned only briefly. For more information on viral meningitis refer to UK SMI V 43 – Investigation of Viral Encephalitis.

This UK SMI should be used in conjunction with other UK SMIs.

4 Introduction

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.

4.1 Organisms Causing Meningitis

Species isolated tend to be characteristically, but not exclusively, associated with the age or predisposing status of the patient[1,2].

From neonates and babies up to 2 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[2].

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 such as *Histoplasma capsulatum, Cryptococcus* species and *Coccidioides immitis* may infect the meninges in disseminated infection[3].

Spirochetes such as *Treponema pallidum*, *Borrelia* and *Leptospira* species may cause meningitis as part of a generalised infection.

Parasites (such as the amoebae *Acanthamoeba* species and *Naegleria species*) occasionally cause meningitis. *Naegleria fowlerii* 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 persons[4].

Many other organisms have been documented to cause meningitis and cannot all be covered in this document.

4.2 Acute Bacterial Meningitis[2]

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, to optimise clinical management. 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[1]. 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, meningomyelocoele 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.

Mixed infections are rare but can occur with certain predisposing conditions[5,6]. They are associated with trauma, tumours or infections such as acute paranasal sinusitis that may extend directly to the meninges. Mixed infections may also arise by direct entry of organisms via fistulae or as a result of a ruptured brain abscess.

4.3 Viral Meningitis[7]

Viral meningitis 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. CSF glucose concentration is usually normal and protein concentration normal or slightly raised[1].

4.4 Chronic Meningitis[8]

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. For further information see UK SMI B 40 - Investigation of Specimens for Mycobacterium species.

4.5 Other Types of Meningitis[8]

Sarcoid meningitis is very rare and produces a raised protein concentration and leucocyte count together with lesions on the meninges seen on magnetic resonance imaging. 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.

Carcinogenous 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.

4.6 Special Risk Groups

Patients who are immunosuppressed are additionally susceptible to meningitis caused by organisms such as *Listeria monocytogenes*, *Cryptococcus neoformans, Norcardia* and *Toxoplasma gondii*[9].

Patients with intracranial prosthetic material such as CSF shunts (see UK SMI 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*.

4.7 Diagnosis of Meningitis[1,2]

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 cannot undergo immediate lumbar puncture. 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.

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.

The diagnosis of meningitis from the examination of CSF includes the following[1]:

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

Therapy should not be delayed pending CSF microscopy or culture. It is important to initiate effective antimicrobial therapy quickly, and this may commence before the examination of the CSF. Further early management decisions therefore, should be based on the immediate examination of the sample by cell count and Gram stain.· Examination of the deposit by cytocentrifugation (eg Cytospin) is the most accurate method of cell differentiation but may not be routinely available.

PCR tests are available as a diagnostic procedure for viruses and some other microorganisms although these techniques remain expensive and show differences in sensitivity and specificity between primer sets and laboratory set ups[2,10,11]. A broad-range bacterial PCR primer set has been established and this detects organisms that are found less frequently or that are unknown causative agents for bacterial meningitis[12]. It may be particularly useful in situations where culture is negative because of chemotherapy, and serology may also be helpful retrospectively in patients who survive. However accuracy of the 16S rDNA PCR approach differs depending on the sample, the microorganisms involved, the expected bacterial load and the presence of bacterial DNA other than that from the pathogen implied in the infectious disease[13].

The bacteria commonly causing meningitis carry specific polysaccharide surface antigens that can be detected by Latex Agglutination Test (LAT). LATs are expensive, reliability is disputed and sensitivity is poor[14]. LAT should not be used on CSF unless the cell count is abnormal, Gram stained film is negative and CSF and blood cultures remain negative after 48hr[14]. 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.

CSF cryptococcal antigen testing should be carried out in all cases of suspected cryptococcal meningitis, and all cases of meningitis in immunocompromised patients in which there is an elevated CSF white cell count and no alternative diagnosis has been made[15]. In these cases serum should also be tested for cryptococcal antigen (CRAG).

Normal CSF values[16-25]

Leucocytes	Neonates	less 28 days	0-30 cells x 10^6/L
	Infants	1 to 12 months	0-15 cells x 10^6/L
	Children/Adults	1 year +	0-5 cells x 10^6/L
Erythrocytes	No RBCs should be present in normal CSF		
Glucose	Neonates	less 28 days	1.94-5.55 mmol/L

	Infants	29 to 58 days	1.55-5.55 mmol/L
		2-12 months	1.94-5.0 mmol/L
	Children/Adults	1 year +	2.22-4.44 mmol/L
Proteins	Neonates	less 28 days	0.65-1.5 g/L
	Infants	29-56 days	0.5-0.9 g/L
	Children	2 months to 18 years	0.05- 0.35 g/L
	Adults	over 60	0.15-0.6 g/L
		18 to 60	0.15-0.45 g/L

These values represent the approximate upper and lower limits of normality and are for guidance only.

Abnormalities associated with bacterial meningitis[1]

- Reduced glucose concentration: <60% blood glucose (CSF: serum ratio <0.6)
- Elevated protein concentration
- Raised white blood cell (WBC) count: 10^1 - 10^4 predominantly polymorphs
- Elevated intracranial pressure

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 meningitis[26].

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 12hr post intra-cranial haemorrhage may show raised WBC counts of up to 500 x 10^6/L as a result of an inflammatory response.

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 x 10^6/L[2]. Viral meningitis is classically described as being associated with a lymphocytic CSF but neutrophils may predominate, especially early in the illness[27,28]. Tuberculosis meningitis may also be associated with a neutrophil rather than a lymphocytic infiltrate early in the infection[8]. Neutropenic patients may not produce reliable or characteristic polymorph or neutrophil responses in the CSF.

Occasionally examination of a wet preparation or performance of an India 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, this should be confirmed by latex agglutination[14].

4.8 Xanthochromia

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 ≥1.5g/L which peaks at 8-10 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 appearance[26].

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 haemorrhage[29].

5 Technical Information/Limitations

Limitations of UK SMIs

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

Selective Media in Screening Procedures

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

Specimen Containers[30,31]

UK 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”.

6 Safety Considerations[30-46]

6.1 Specimen Collection, Transport and Storage[30-35]

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.

6.2 Specimen Processing[30-46]

The processing of most diagnostic work can be carried out at Containment Level 2 unless infection with a) *N. meningitidis,* b) a Hazard group 3 organism or c) TSE is suspected.

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

N. meningitidis is a Hazard group 2 organism and the processing of diagnostic samples can be carried out at Containment Level 2.

Due to the severity of the disease and the risks associated with generating aerosols of the organism, any manipulation of suspected isolates of *N. meningitidis* should always be undertaken in a microbiological safety cabinet until *N. meningitidis* has been ruled out (as must any laboratory procedure giving rise to infectious aerosols).

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) Refer to https://www.gov.uk/government/groups/advisory-committee-on-dangerous-pathogens for guidance on TSE agents. 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.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[38].

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* species[47]. 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.

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.

7 Specimen Collection

7.1 Type of Specimens

CSF

7.2 Optimal Time and Method of Collection[48]

For safety considerations refer to Section 6.

Collect specimens preferably before antimicrobial therapy is started, but this must not be delayed unnecessarily pending lumbar puncture and CSF culture[48].

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

Specialist collection according to local protocols.

7.3 Adequate Quantity and Appropriate Number of Specimens[48]

CSF is normally collected sequentially into three or more separate containers which should be numbered consecutively. Collect specimens in appropriate CE marked leak proof containers and transport specimens in sealed plastic bags.

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. The fluoride sample should not be sent to Microbiology. Ideally testing should be carried out on the last sample with the first one reserved as a backup.

Ideally a minimum volume of 1mL for each tube 1 and 3 taken for microscopy (in adults). When sample volume is below this it is possible to pool samples.

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

Note: The larger the volume, the greater the cultural yield particularly in relation to *M. tuberculosis* investigations.

8 Specimen Transport and Storage[30,31]

8.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 6.

Time between collection to microscopy and culture should occur within a maximum of 2 hours[1,49]. Cells disintegrate and a delay may produce a cell count that does not reflect the clinical situation of the patient.

Specimens should be transported and processed as soon as possible[48].

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

9 Specimen Processing/Procedure[30,31]

9.1 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 (UK SMI B 40 - Investigation of specimens for Mycobacterium species), examination for parasites (UK SMI 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.

9.2 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 required[29]. This is often carried out by clinical biochemistry departments as are protein and glucose determinations.

9.3 Sample Preparation

For safety considerations refer to Section 6.

See Microscopy section.

9.4 Microscopy

9.4.1 Standard total cell count

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

Cell counts should not be performed on specimens containing a clot (which invalidates 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 5 min 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)**

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.

Note 2: Count and record the actual numbers of each leucocyte type. Taking the dilution factor into account, express as number of cells per litre.

Note 3: 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 UK SMI TP 39 - Staining Procedures

Perform Gram stain on all specimens except:

- Clotted specimens (see below)
- Routine neurological specimens unless leucocyte counts are raised
- PM specimens cell counts are unreliable but should be cultured

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

Note: If investigation for *Mycobacterium* species is also requested, the centrifugation time may be increased to 15-20 min at 3000 xg (see UK SMI B 40 - Investigation of specimens

for Mycobacterium species) and the same deposit used for this as well as routine microscopy and culture[50].

Transfer all but the last 0.5mL 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.

9.4.2 Supplementary

Examination for M. tuberculosis

The "build up" technique for films as described above is recommended for the examination for *Mycobacterium* species (see UK SMI 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*

Mix a drop of the centrifuged deposit with a drop of 50% aqueous India ink or nigrosin on a clean microscope slide and cover with a coverslip (see UK SMI TP 39 - Staining procedures).

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 (UK SMI B 31 - Investigation of Specimens other than Blood for Parasites).

9.5 Culture and Investigation

9.5.1 Pretreatment

Standard

Centrifuge specimen (already performed for microscopy - see 4.4).

Supplementary

Mycobacterium species (UK SMI B 40 - Investigation of Specimens for Mycobacterium species) and parasites (see UK SMI B 31 - Investigation of Specimens other than Blood for Parasites).

9.5.2 Specimen processing

Standard

For all CSF

- With a sterile pipette inoculate each agar plate with the centrifuged deposit (see UK SMI Q 5 - Inoculation of Culture Media for Bacteriology)
- Allow inoculum to dry before spreading to minimise any antibiotic effect which may be present
- Spread inoculum with a sterile loop for the isolation of individual colonies

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

If culture negative result from clinically ill patient consider other non-culture methods for diagnosis eg 16S PCR, MALDI TOF MS, etc.

Broth cultures are not recommended as a significant positive yield is rarely achieved and contamination is frequent, unless dealing with shunt infections where they may add value[51-53].

9.5.3 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	CSF	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**	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
Immunocompromised patients	CSF	Sabouraud plate	35 - 37	air	14d*	≥ 40hr:	Fungi
Brain abscess Ventriculitis Reservoirs Post neurosurgery Post otitis media with complications	CSF	Fastidious anaerobe agar	35-37	anaerobic	10d	≥40hr, 5d and at 10 days if you have an anaerobic cabinet otherwise at 10days.	Anaerobes
If mixed infection suggested by Gram stained film	CSF	Neomycin fastidious anaerobe agar	35-37	anaerobic	10d	≥40hr, 5d and at 10 days if you have an anaerobic cabinet otherwise at 10 days	
* If longer culture times are likely to be required a sabouraud slope should be put up in addition to the plate. Enrichment broths may add value when diagnosing shunt infections (see page 18). Other organisms for consideration - *Mycobacterium* species and parasites as described in supplementary testing, *T. pallidum* and viruses can be found in relevant SMIs.							

9.6 Identification

Refer to individual UK SMIs for organism identification.

9.6.1 Minimum level of identification in the laboratory

Anaerobes	species level
Actinomyces	species level
β-haemolytic streptococci	Lancefield group level
All other organisms	species level
Mycobacterium	UK SMI B 40 - Investigation of Specimens for Mycobacterium species
Parasites	UK SMI 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.

9.7 Antimicrobial Susceptibility Testing

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

9.8 Referral for Outbreak Investigations

N/A

10 Referral to Reference Laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory see user manuals and request forms

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

England

Wales

Scotland

Northern Ireland

Note: In case of sending away to laboratories for processing, ensure that specimen is placed in appropriate package and transported accordingly.

11 Reporting Procedure

11.1 Appearance

Report the appearance of the CSF and the presence of a clot if applicable.

11.2 Microscopy

Cell count

Report numbers of RBCs x 10^6 per litre and

Report numbers of PMNs and lymphocytes x 10^6/L or

Report PMNs and lymphocytes as percentages of the total WBC (which is reported as x 10^6).

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.

Supplementary

India ink or nigrosin.

Report on encapsulated yeasts detected.

Microscopy for *Mycobacterium* species (UK SMI B 40 - Investigation of specimens for Mycobacterium species) and parasites (UK SMI B 31 - Investigation of specimens other than blood for parasites).

11.2.1 Microscopy reporting time

Results of cell counts and stains should be communicated immediately, within two hours of receiving the specimen and made available on the clinical users' results viewing system. Where such facilities are not available, written or computer generated reports should follow preliminary/verbal reports within 24 hours.

11.3 Culture

Report the organisms 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, 16–72 hours stating, if appropriate, that a further report will be issued.

Molecular testing results (if applicable).

Supplementary investigations: *Mycobacterium* species (UK SMI B 40 - Investigation of specimens for Mycobacterium species) fungi (UK SMI B 39 - Investigation of dermatological specimens for superficial mycoses) and parasites (UK SMI B 31 - Investigation of specimens other than blood for parasites).

11.4 Antimicrobial Susceptibility Testing

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

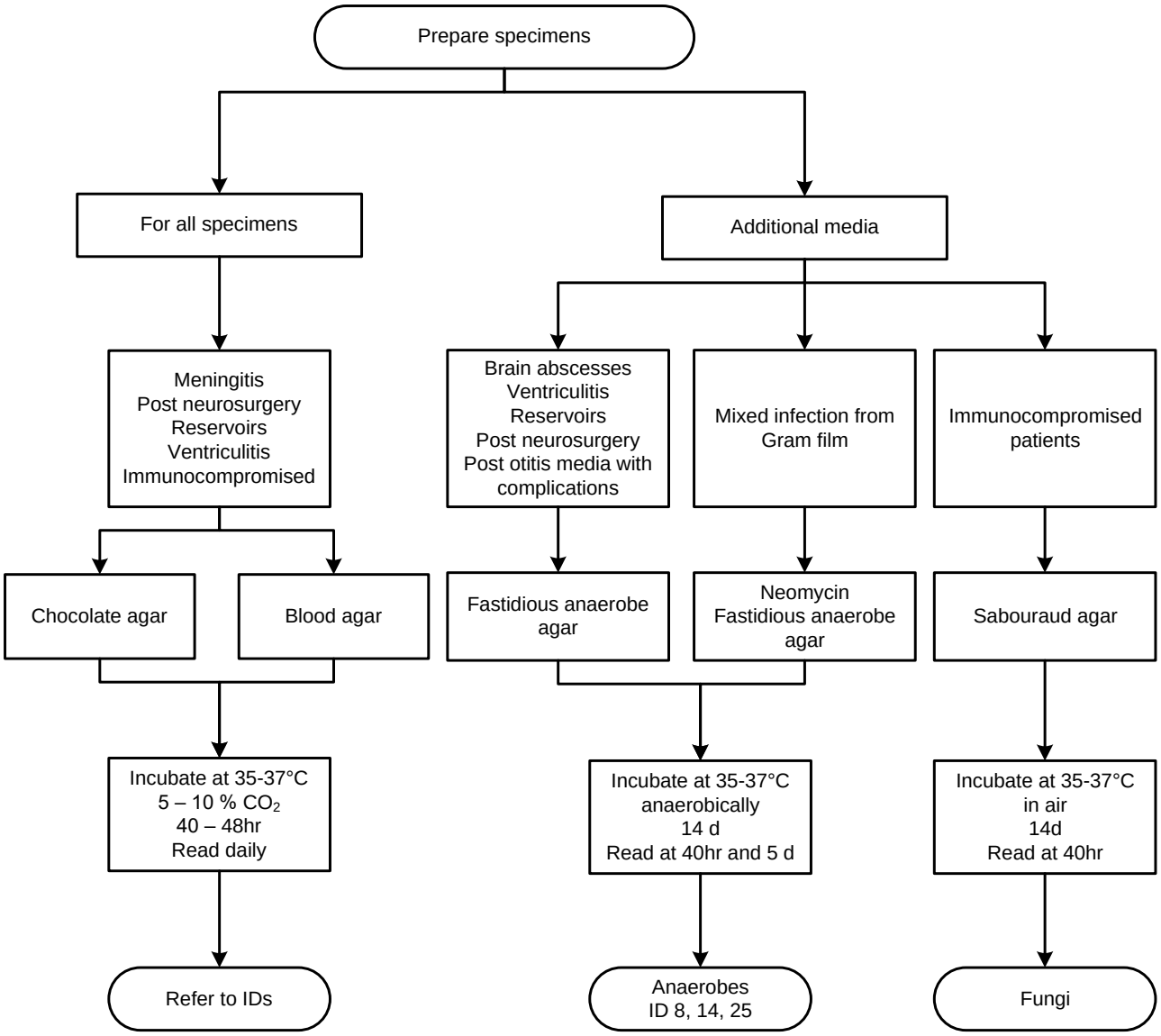
12 Public health responsibilities of diagnostic laboratories

Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral, to further characterise the organism, as required, primarily for public health purposes e.g. routine cryptosporidium detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance, forming an essential component of healthcare. It is recognised that additional testing and referral of samples may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

Algorithm: Investigation of Cerebrospinal Fluid



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An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

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