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NATIONAL STANDARD METHOD

INVESTIGATION OF CEREBROSPINAL FLUID

BSOP 27

Issued by Standards Unit, Evaluations and Standards Laboratory
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AMENDMENT PROCEDURE

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

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

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
6/ 23/04/2008	4.1	5	1	**Front page**	Redesigned
			7	**Introduction**	Reworded
			16	**Supplementary media**	Paragraphs amended
			17	**4.5.2 Referrals**	Links to reference laboratory user manuals inserted.
			24	**References**	Reviewed and updated

INVESTIGATION OF CEREBROSPINAL FLUID

Type of specimen: Cerebrospinal fluid

SCOPE OF DOCUMENT

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

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 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, in order to optimise clinical management[3]. 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[2,4,5]. 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.

Viral (“aseptic”) meningitis[2]

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[6,7]. CSF glucose concentration is usually normal and protein concentration normal or slightly raised[5].

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)[8].

Chronic meningitis[9]

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[10,11]supplemented by molecular methods eg PCR are useful for rapid detection of acid-fast bacilli. Rapid tests are useful if positive, but culture remains the gold standard for diagnosis[12].

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 and produces a raised protein concentration and leucocyte count together with lesions on the meninges seen on magnetic resonance imaging[13].

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. 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 serologically[14].

Organisms causing meningitis

Species isolated tend to be characteristically, but not exclusively, associated with the age or predisposing status of the patient[2,4,9,15].

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 meningitis[16].

Other causes

Many other organisms have been documented to cause meningitis[2,17-19]. 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 National Standard Methods.

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 meningitis[20]. *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[17]. The raccoon roundworm *Baylisascaris procyonis* is a rare cause of neural larva migrans in the USA in infants and children[21].

Mixed infections

These are rare but may account for up to 1% of cases of meningitis[4]. They are associated with trauma, tumours or infections such as acute paranasal sinusitis that may extend directly to the meninges[18]. 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 streptococci[22].

Nocardia species are rare causes of meningitis, but predisposing factors such as immunosuppression, head trauma or surgery can be identified in the majority of patients[23].

Patients with intracranial prosthetic material such as CSF shunts (see BSOP 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[5,24].

PCR tests are available as a diagnostic procedure for some organisms[8] (see Sections 4.1 and 4.5.2). *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae* can be detected simultaneously from clinical samples by PCR[25]. A broad-range bacterial PCR primer set has recently been developed. This detects organisms that are found less frequently or that are unknown causative agents for bacterial meningitis[26]. PCR assays are also available for agents involved in aseptic meningitis[27,28]. 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 disease[29]. 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 PCR[30]. For meningococcal disease the combination of ultrasound-enhanced LAT and PCR may increase the diagnostic capabilities and acquisition of epidemiological data[41]. 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 [2,5,9,24,31]:

- 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 cytocentrifugation (eg Cytospin) is the most accurate method of cell differentiation but may not be routinely available.

Normal CSF values[5,20,32,33]

Leucocytes	Neonates 1-4yr old 5yr-puberty Adults	0 - 30 cells x 10^6/L 0 - 20 cells x 10^6/L 0 - 10 cells x 10^6/L 0 - 5 cells x 10^6/L
Erythrocytes	Newborn Adults	0 - 675 cells x 10^6/L 0 - 10 cells x 10^6/L
Protein	Neonates ≤6d Others	0.7 g/L 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[2,5,20,34];

- 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

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 meningitis[35].

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 x 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[36] to seek macroscopically invisible haematin or bilirubin, which, if present, will confirm pre-tap intracranial haemorrhage.

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 ≥1.5 g/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[35].

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 x 10^6 per litre[37]. 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[6,7]. 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[38,39] (most commonly coagulase-negative staphylococci). 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 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.

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 poor[40]. It has been shown that ultrasonic enhancement increased the sensitivity of the LAT up to 600-fold[41,42]. 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 hours[40]. 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 NSM.

Antigen testing may be useful in[40]:

- 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

N/A

1 SAFETY CONSIDERATIONS[43-55]

1.1 SPECIMEN COLLECTION

N/A

1.2 SPECIMEN TRANSPORT AND STORAGE

Sterile leakproof container in a sealed plastic bag.

1.3 SPECIMEN PROCESSING

Containment Level 2 unless infection with a Hazard group 3 organism is suspected, in which case work should be performed in a microbiological safety cabinet under Containment level 3 conditions.

Although *N. meningitidis* is in Hazard group 2, local policy may dictate that suspected isolates of *N. meningitidis* should always be handled in a microbiological safety cabinet. Sometimes the nature of the work may dictate full containment level 3 conditions should be used eg for research work with *N. meningitidis* in order to comply with COSHH 2002 Schedule 1.5 (5e).

All work on suspected *N. meningitidis* isolates which is likely to generate aerosols must be performed in a microbiological safety cabinet[56].

Laboratories must take suitable safety precautions when handling CSF specimens. Laboratory policies that take into account the local risk assessments may dictate the use of a microbiological safety cabinet when dispensing the specimen. Refer to Guidance Note QSOP 42 - Microbiological examination of CSFs that may contain agents of spongiform encephalopathies.

"All clinical specimens from known, suspected or at risk patients should be handled at Containment Level 3. However, the option to derogation does apply and, based on local risk assessment [such as the source of the CSF whether from a neurological unit, diagnosis, likelihood with regard to age etc], certain Containment Level 3 precautions can be dispensed with"[53].

Where *Mycobacterium* species are suspected, all specimens must be processed in a microbiological safety cabinet in a Containment Level 3 room.

Prior to staining, fix smeared material by placing the slide on an electric hotplate (65 to 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[57]. 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 NSM.

The above guidance should be supplemented with local COSHH and risk assessments.
Compliance with postal and transport regulations is essential.

2 SPECIMEN COLLECTION

2.1 OPTIMAL TIME OF COLLECTION

Preferably before antimicrobial therapy is started, but this must not be delayed unnecessarily pending lumbar puncture and CSF culture.

2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION

Specialist collection according to local protocols.

2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

Ideally a minimum volume of 1 mL. For *Mycobacterium* species*,* at least 10 mL where possible.

CSF is normally collected sequentially into three or more separate containers which should be numbered consecutively. Disposable sterile screw-capped plastic containers should be used.

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

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens should be transported and processed as soon as possible.

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

Specimens should be cultured as soon as possible after receipt[2,5,32], ideally within 10 minutes and within a maximum of two hours. Cells disintegrate and a delay may produce a cell count that does not reflect the clinical situation of the patient.

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

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 (BSOP 40 - Investigation of specimens for Mycobacterium species), examination for parasites (BSOP 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 2 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[36] if requested or if clarification of the source of RBCs in the CSF is required. This is often carried out by Clinical Biochemistry departments as are protein and glucose determinations.

4.3 MICROSCOPY

4.3.1 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 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. 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 BSOPTP 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 - 10 min.

Note: If investigation for *Mycobacterium* species is also requested, the centrifugation time may be increased to 15-20 min at 3000 xg[58] (see BSOP 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.

4.3.2 SUPPLEMENTARY

Examination for *M. tuberculosis*

The "build up" technique for films as described above is recommended for the examination for *Mycobacterium* species (see BSOP 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 BSOPTP 39 - Staining Procedures)

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.

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 (BSOP 31 - Investigation of specimens other than blood for parasites).

4.4 CULTURE AND INVESTIGATION

4.4.1 PRE TREATMENT

Standard

Centrifuge specimen (already performed for microscopy - see 4.3.1).

Supplementary

Mycobacterium species (BSOP 40 - Investigation of specimens for Mycobacterium species) and parasites (see BSOP 31 - Investigation of specimens other than blood for parasites).

4.4.2 SPECIMEN PROCESSING

Standard

For all CSF

- With a sterile pipette inoculate each agar with the centrifuged deposit (see QSOP 52 - Inoculation of culture media)
- 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[59,60].

4.4.3 CULTURE MEDIA, CONDITIONS AND ORGANISMS FOR ALL SPECIMENS:

Clinical details/conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
Meningitis Post neurosurgery Reservoirs Ventriculitis Immunocompromised	Chocolate agar	35 - 37	5 - 10% CO_2	40-48 h	daily	Any organism
	Blood agar	35 - 37	5 - 10% CO_2	40-48 h	daily	
For these situations, add the following:						
Clinical details/conditions	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
		Temp °C	**Atmos**	**Time**		
Immunocompromised patients	Sabouraud plate	35 - 37	air	2 – 5 d	≥ 40 h: up to 8 weeks	Fungi
Brain abscess Ventriculitis Reservoirs Post neurosurgery Post otitis media with complications	Fastidious anaerobe agar	35-37	anaerobic	7 - 14 d	40 h and at 5d	Anaerobes
If mixed infection suggested by Gram-stained film	Neomycin fastidious anaerobe agar	35-37	anaerobic	7 - 14 d	≥40 h and at 5 d	
Other organisms for consideration - *Mycobacterium* species and parasites, *T. pallidum* and viruses can be found in relevant NSMs						

4.5 IDENTIFICATION

4.5.1 MINIMUM LEVEL OF IDENTIFICATION IN THE LABORATORY

Anaerobes	species level BSOPID 14 - Identification of non-sporing, non branching anaerobes BSOPID 25 - Identification of anaerobic Gram-negative rods
Actinomyces	species level
β-haemolytic streptococci	Lancefield group level
All other organisms	species level
Mycobacterium	BSOP 40 - Investigation of specimens for Mycobacterium species
Parasites	BSOP 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 characterised if clinically or epidemiologically indicated.

4.5.2 REFERRAL TO REFERENCE LABORATORIES

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

β-haemolytic streptococci	Serotyping
S. pneumoniae	Serotyping
H. influenzae	Serotyping
Listeria species	Serotyping
N. meningitidis	Strain characterisation, antimicrobial susceptibility testing
Fungi	Identification and/or susceptibility testing

Mycobacterium species (BSOP 40 - Investigation of specimens for *Mycobacterium* species)

Isolates associated with outbreaks, where epidemiologically indicated and 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.

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem or anomaly that requires elucidation.

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.

4.6 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Refer to BSOP 45 - Susceptibility testing

5 REPORTING PROCEDURE

5.1 APPEARANCE

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

5.2 MICROSCOPY

Cell count

Report numbers of RBCs x 10^6 per litre and

Report numbers of PMNs and lymphocytes x 10^6 per litre 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.

India ink or nigrosin.

Report on encapsulated yeasts detected.

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

5.2.1 MICROSCOPY REPORTING TIME

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

Written report, 16 – 72 h.

5.3 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 h stating, if appropriate, that a further report will be issued.

Molecular testing results (if applicable).

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

5.4 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Report susceptibilities as clinically indicated.

6 REPORTING TO THE HPA[61] (LOCAL AND REGIONAL SERVICES AND CENTRE FOR INFECTIONS)

Clinically significant isolates from CSF should be reported to the Regional CDSC and Local CCDC.

Refer to the following:

Individual NSMs on organism identification

Health Protection Agency publications "Laboratory Reporting to the Health Protection Agency. Guide for diagnostic laboratories"

Local Memorandum of Understanding

Current guidelines on CDSC and COSURV reporting

In cases of suspected meningococcal disease and contacts the isolation of *N. meningitidis* should be reported to the CCDC immediately.

Report all isolates of the following: *Mycobacterium* species.

7 ACKNOWLEDGEMENTS AND CONTACTS

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The National Standard Methods are issued by Standards Unit, Evaluations and Standards Laboratory, Centre for Infections, Health Protection Agency, London.
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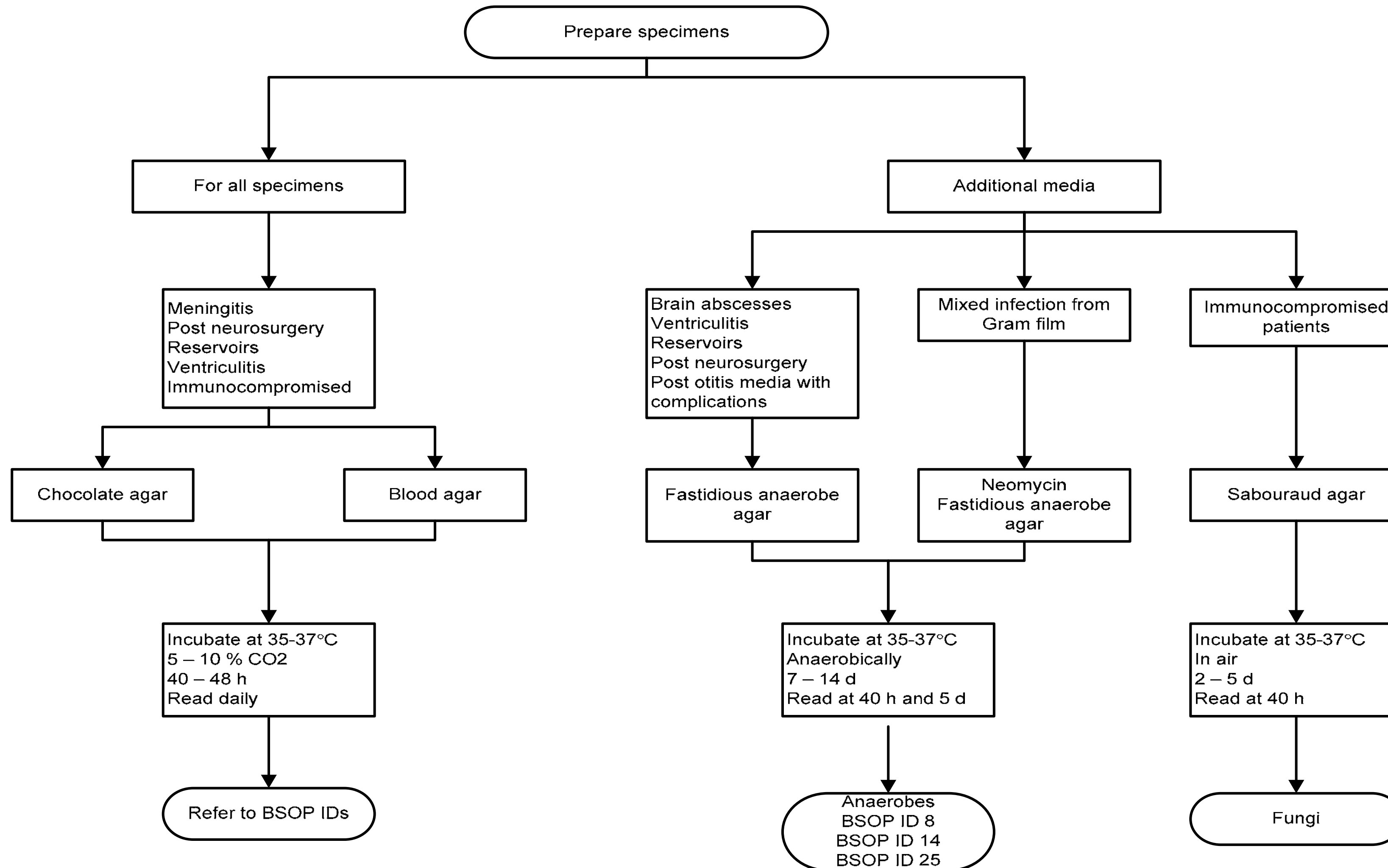
APPENDIX



REFERENCES

1. Department of Health NHS Executive: The Caldicott Committee. Report on the review of patient-identifiable information. London. December 1997.

2. Tunkel AR, Scheld WM. Acute Meningitis. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 5th ed. Vol 1. Edinburgh: Churchill Livingstone; 2000. p. 959-97.

3. Pezzlo M. Processing and interpretation of cerebrospinal fluid. In: Isenberg HD, editor. Essential Procedures for Clinical Microbiology. Washington D.C: American Society for Microbiology; 1998. p. 67-72.

4. Downs NJ, Hodges GR, Taylor SA. Mixed bacterial meningitis. [Review] [76 refs]. Reviews of Infectious Diseases 1987;9:693-703.

5. Gray LD, Fedorko DP. Laboratory diagnosis of bacterial meningitis. Clin Microbiol Rev 1992;5 :130-45.

6. Negrini B, Kelleher KJ, Wald ER. Cerebrospinal fluid findings in aseptic versus bacterial meningitis. Pediatrics 2000;105:316-9.

7. Spanos A, Harrell FE, Jr., Durack DT. Differential diagnosis of acute meningitis. An analysis of the predictive value of initial observations. JAMA 1989;262:2700-7.

8. Espy MJ, Uhl JR, Sloan LM, Buckwalter SP, Jones MF, Vetter EA, et al. Real-time PCR in clinical microbiology: applications for routine laboratory testing. Clin Microbiol Rev 2006;19:165-256.

9. Gripshover BM, Ellner JJ. Chronic meningitis. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 5th ed. Vol 1. Edinburgh: Churchill Livingstone; 2000. p. 997-1009.

10. Freeman R, Magee JG, Watt B, Rayner AB. Being positive about the smear. Thorax 2001;56:417.

11. Ba F, Rieder HL. A comparison of fluorescence microscopy with the Ziehl-Neelsen technique in the examination of sputum for acid-fast bacilli. Int J Tuberc Lung Dis 1999;3:1101-5.

12. Thwaites G, Chau TT, Mai NT, Drobniewski F, McAdam K, Farrar J. Tuberculous meningitis.[erratum appears in J Neurol Neurosurg Psychiatry 2000 Jun;68(6):802]. [Review] [123 refs]. Journal of Neurology, Neurosurgery & Psychiatry 2000;68:289-99.

13. Zajicek JP. Neurosarcoidosis. [Review] [22 refs]. Current Opinion in Neurology 2000;13:323-5.

14. Pauw B, Meunier F. Infections in patients with acute leukaemia and lymphoma. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 5th ed. Vol 1. Edinburgh: Churchill Livingstone; 2000. p. 3090-102.

15. Bingen E, Lambert-Zechovsky N, Mariani-Kurkdjian P, Doit C, Aujard Y, Fournerie F, et al. Bacterial counts in cerebrospinal fluid of children with meningitis. Eur J Clin Microbiol Infect Dis 1990;9:278-81.

16. Salaki JS, Louria DB, Chmel H. Fungal and yeast infections of the central nervous system. A clinical review. Medicine (Baltimore) 1984;63:108-32.

17. Furugen M, Yamashiro S, Tamayose M, Naha Y, Miyagi K, Nakasone C, et al. Elsberg syndrome with eosinophilic meningoencephalitis caused by Angiostrongylus cantonensis. Intern Med 2006;45:1333-6.

18. Martinez E, Domingo P, Cadafalch J, Coll P. Mixed bacterial meningitis associated with CSF leak. Clin Infect Dis 1992;14:1263.

19. Waites KB, Rudd PT, Crouse DT, Canupp KC, Nelson KG, Ramsey C, et al. Chronic Ureaplasma urealyticum and Mycoplasma hominis infections of central nervous system in preterm infants. Lancet 1988;1:17-21.

20. Ray CG, Smith JA, Wasilauskas BL, Zabransky R, editors. Cumitech 14A. Laboratory Diagnosis of Central Nervous System Infections. Washington D.C: American Society for Microbiology; 1993. p. 1-16

21. Gavin PJ, Kazacos KR, Tan TQ, Brinkman WB, Byrd SE, Davis AT, et al. Neural larva migrans caused by the raccoon roundworm Baylisascaris procyonis. Pediatr Infect Dis J 2002;21:971-5.

22. Pettersen G, Ovetchkine P, Tapiero B. Group A streptococcal meningitis in a pediatric patient following cochlear implantation: report of the first case and review of the literature. J Clin Microbiol 2005;43:5816-8.

23. Bross JE, Gordon G. Nocardial meningitis: case reports and review. Rev Infect Dis 1991;13:160-5.

24. Bonadio WA. The cerebrospinal fluid: physiologic aspects and alterations associated with bacterial menigitis. Pediatr Infect Dis J 1992;11 :423-32.

25. Corless CE, Guiver M, Borrow R, Edwards-Jones V, Fox AJ, Kaczmarski EB. Simultaneous detection of Neisseria meningitidis, Haemophilus influenzae, and Streptococcus pneumoniae in suspected cases of meningitis and septicemia using real-time PCR. J Clin Microbiol 2001;39:1553-8.

26. Schuurman T, de Boer RF, Kooistra-Smid AM, van Zwet AA. Prospective study of use of PCR amplification and sequencing of 16S ribosomal DNA from cerebrospinal fluid for diagnosis of bacterial meningitis in a clinical setting. J Clin Microbiol 2004;42:734-40.

27. Petitjean J, Vabret A, Dina J, Gouarin S, Freymuth F. Development and evaluation of a real-time RT-PCR assay on the LightCycler for the rapid detection of enterovirus in cerebrospinal fluid specimens. J Clin Virol 2006;35:278-84.

28. Read SJ, Jeffery KJ, Bangham CR. Aseptic meningitis and encephalitis: the role of PCR in the diagnostic laboratory. J Clin Microbiol 1997;35:691-6.

29. Newcombe J, Cartwright K, Palmer WH, McFadden J. PCR of peripheral blood for diagnosis of meningococcal disease. J Clin Microbiol 1996;34:1637-40.

30. Borrow R, Guiver M, Sadler F, Kaczmarski EB, Fox AJ. False positive diagnosis of meningococcal infection by the IS1106 PCR ELISA. FEMS Microbiol Lett 1998;162:215-8.

31. Rodewald LE, Woodin KA, Szilagyi PG, Arvan DA, Raubertas RF, Powell KR. Relevance of common tests of cerebrospinal fluid in screening for bacterial meningitis. J Pediatr 1991;119:363-9.

32. Bauer JD. Examination of biologic fluids, sputum and pus. Clinical Laboratory Methods. 9th ed. St Louis: CV Mosby Company; 1982. p. 750-79.

33. Collee JG, Duguid JP, Fraser AG, et al. Laboratory strategy in the diagnosis of infective syndromes: Meningitis. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie & McCartney Practical Medical Microbiology. 14th ed. Edinburgh: Churchill Livingstone; 1996. p. 77-80.

34. Begg N, Cartwright KA, Cohen J, Kaczmarski EB, Innes JA, Leen CL, et al. Consensus statement on diagnosis, investigation, treatment and prevention of acute bacterial meningitis in immunocompetent adults. British Infection Society Working Party. J Infect 1999;39:1-15.

35. Bonadio WA, Smith DS, Goddard S, Burroughs J, Khaja G. Distinguishing cerebrospinal fluid abnormalities in children with bacterial meningitis and traumatic lumbar puncture. J Infect Dis 1990;162:251-4.

36. A working group of UK NEQAS for immunochemistry. National Guidelines for analysis of cerebrospinal fluid for bilirubin in subarachnoid haemorrhage. Ann Clin Biochem 2003;40:481-8.

37. Powers WJ. Cerebrospinal fluid lymphocytosis in acute bacterial meningitis. Am J Med 1985;79:216-20.

38. Gruskay J, Harris MC, Costarino AT, Polin RA, Baumgart S. Neonatal Staphylococcus epidermidis meningitis with unremarkable CSF examination results. Am J Dis Child 1989;143:580-2.

39. Michael M, Barrett DJ, Mehta P. Infants with meningitis without cerebrospinal fluid pleocytosis. Am J Dis Child 1986;140:851.

40. Perkins MD, Mirrett S, Reller LB. Rapid bacterial antigen detection is not clinically useful. J Clin Microbiol 1995;33:1486-91.

41. Barnes RA, Jenkins P, Coakley WT. Preliminary clinical evaluation of meningococcal disease and bacterial meningitis by ultrasonic enhancement. Arch Dis Child 1998;78:58-60.

42. Ellis RW, Sobanski MA. Diagnostic particle agglutination using ultrasound: a new technology to rejuvenate old microbiological methods. J Med Microbiol 2000;49:853-9.

43. Advisory Committee on Dangerous Pathogens. Categorisation of biological agents according to hazard and categories of containment. 4th ed. Suffolk: HSE Books; 1995. Supplements 1, 1998 and 2, 2000.

44. Health and Safety Executive, editor. Biological Agents: Managing the risks in laboratories and healthcare premises. 5 A.D.

45. Public Health Laboratory Service Standing Advisory Committee on Laboratory Safety. Safety Precautions: Notes for Guidance. 4th ed. London: Public Health Laboratory Service (PHLS); 1993.

46. Control of Substances Hazardous to Health Regulations 2002. General COSHH Approved Code of Practice and Guidance, L5. Suffolk: HSE Books; 2002.

47. Health and Safety Executive. 5 steps to risk assessment: a step by step guide to a safer and healthier workplace, IND (G) 163 (REVL). Suffolk: HSE Books; 2002.

48. Health and Safety Executive. A guide to risk assessment requirements: common provisions in health and safety law, IND (G) 218 (L). Suffolk: HSE Books; 2002.

49. Health Services Advisory Committee. Safety in Health Service laboratories. Safe working and the prevention of infection in clinical laboratories and similar facilities. 2nd ed. Suffolk: HSE Books; 2003.

50. BS EN 12469: 2000. Biotechnology - performance criteria for microbiological safety cabinets. London: British Standards Institution (BSI); 2000.

51. BS 5726: 1992. Microbiological safety cabinets. Part 2. Recommendations for information to be exchanged between purchaser, vendor and installer and recommendations for installation. London: British Standards Institution (BSI); 1992.

52. BS 5726: 1992. Microbiological safety cabinets. Part 4. Recommendations for selection, use and maintenance. London: British Standards Institution (BSI); 1992.

53. Advisory Committee on Dangerous Pathogens: Spongiform Encephalopathy Advisory Committee. Transmissible spongiform encephalopathy agents: safe working and the prevention of infection. 1st ed. London: The Stationary Office (TSO); 1998.

54. Health and Safety Executive, editor. Biological Agents: Managing the risks in laboratories and healthcare premises. 5 A.D.

55. NHS Estates. Health Building Note 15. Facilities for pathology services. 2nd ed. London: The Stationary Office; 2005.

56. Advisory Committee on Dangerous Pathogens. Categorisation of biological agents according to hazard and categories of containment. 4th ed. Suffolk: HSE Books; 1995. Supplements 1, 1998 and 2, 2000.

57. Allen BW. Survival of tubercle bacilli in heat-fixed sputum smears. J Clin Pathol 1981;34:719-22.

58. Ratnam S, March SB. Effect of relative centrifugal force and centrifugation time on sedimentation of mycobacteria in clinical specimens. J Clin Microbiol 1986;23:582-5.

59. Meredith FT, Phillips HK, Reller LB. Clinical utility of broth cultures of cerebrospinal fluid from patients at risk for shunt infections. J Clin Microbiol 1997;35:3109-11.

60. Dunbar SA, Eason RA, Musher DM, Clarridge JE, III. Microscopic examination and broth culture of cerebrospinal fluid in diagnosis of meningitis. J Clin Microbiol 1998;36:1617-20.

61. Health Protection Agency. Laboratory Reporting to the Health Protection Agency. Guide for diagnostic laboratories. February. 2007.