# Aim

To describe the processing of cerebrospinal fluid to identify bacterial meningitis.

# Principle

Cerebrospinal fluid (CSF) is fluid that surrounds the brain and spinal cord. CSF is taken when meningitis, encephalitis or other neurological infections are suspected. Meningitis is inflammation of the meninges. This may be acute, chronic, infective or non-infective. Many infective agents have been shown to cause meningitis including viruses, bacteria, fungi and parasites. Cell counts, CSF staining and culture can all aid its diagnosis.

Normal CSF contains zero to very few cells and the presence, number and type of cells can indicate whether bacterial, viral, parasitic or non-infectious aetiologies are likely (see Appendix 1).

Abnormalities associated with bacterial meningitis include:

* Higher white cell count (especially if mostly polymorphs)
* High intracranial pressure when taking the CSF
* Reduced glucose concentration (<60% blood glucose)
* Elevated protein concentration

Common bacterial causes of meningitis include:

* Neonates and young infants (< 2 months): Group B Streptococci; *E. coli*  and other coliforms; *Listeria monocytogenes*
* Infants and Children (> 2 months): *Haemophilus influenzae* type B*; Neisseria meningitidis; Streptococcus pneumoniae*
* Adults (> 60 years): *Listeria monocytogenes*
* Immunocompromised: *Cryptococcus neoformans*
* Others: *Mycobacterium tuberculosis; Streptococcus suis*

# Method

## Specimen collection

CSF should be collected in an aseptic manner into labelled sterile specimen tubes (e.g. universal containers). Conventionally, three tubes are submitted for investigation. A minimum of 1ml is required for complete examination and 10ml is desirable for investigation of suspected *Mycobacterium tuberculosis* infection.

## Specimen transport and storage

After collection, CSF specimens should be transported to the microbiology laboratory without delay in a sealed plastic bag or rigid specimen container. If a delay in processing is unavoidable, then the specimen should be maintained at ambient temperature (do not refrigerate).

## Specimen processing

### Reception

Log the specimen in the appropriate specimen book and assign a specimen number.

Process the specimen immediately. All manipulations/pipetting of CSF should be done in the class II biosafety cabinet.

Send CSF tube 1 to the main laboratory for protein and glucose estimation.

### Macroscopic appearance

In good lighting conditions describe the appearance of the CSF and record this on the specimen request form. Descriptions include:

* Clear
* Cloudy
* Blood stained (pink in colour)
* Purulent (looks like pus)
* Clot present
* ‘Spider web’ clot present (this is rare but suggestive of *M. tuberculosis*)

Estimate the volume and record this on the request form.

Note: A normal CSF is clear, bright and colourless.

### Cell counts

Perform a cell count on CSF samples using a Fuchs-Rosenthal counting chamber, unless the sample is clotted.

Always use CSF that has NOTbeen centrifuged.

If multiple tubes of CSF have been received from one patient, perform the cell count on the last sample taken (usually tube 2 at AHC).

Make sure the counting chamber and cover glass are completely clean. They can be cleaned with a soft tissue using microscope lens cleaning solution e.g. 30% ethanol: 70% ether.

Assemble the counting chamber.

Gently mix the specimen in the Class II biosafety cabinet.

Pipette the CSF into the counting chamber using a fine Pasteur pipette or pipette e.g. Gilson P20. Make sure the CSFdoes notoverflow into the channels on each side of the chamber. If the CSF overflows clean the chamber and the cover glass and repeat filling the chamber.

Wait for two minutes for the cells in the CSF to settle.

Count the number of WBCs and RBCs microscopically as described below: WBCs will have nuclei and RBCs will be smaller cells without nuclei.

#### White blood cell count

Count the WBCs (cells with a nucleus) microscopically using the x10 objective.

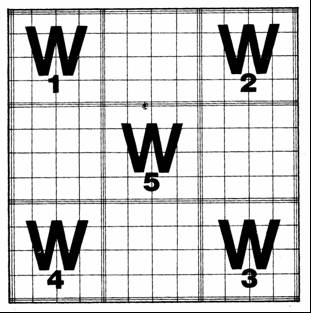
Focus on the WBCs and rulings with the condenser down and the diaphragm closed sufficiently to give good contrast.

Count the white cells in five of the large squares (W1, W2, W3, W4 & W5) as shown in Figure 1. This equals the cell count per mm3 (µl). If no WBCs are seen, report the count as zero.

If there are too many cells to be counted, repeat the whole procedure with a 1:10 dilution of the CSF (2μl CSF + 18μl saline) using a clean chamber. Then multiply the WBC counted by 10 to give the number of WBC per mm3. For example if you counted 64 white cells in five squares, then 64 x 10 = 640 WBC/mm3 CSF.

[For conversion of the counted number of cells to the number of WBCs per litre of CSF, multiply by 106. For example if you counted 64 white cells in five squares (1μl / 1mm3), then this is 64 (x106 WBC/L).]

**Figure 1.** Fuchs-Rosenthal counting chamber



#### Red blood cell count

Count the RBCs microscopically using the x40 objective.

Focus on the RBCs with the condenser half down and the diaphragm half closed to give good contrast.

Count the RBCs in five of the large squares of the counting chamber (W1, W2, W3, W4 & W5) as shown above.

Report the number of RBCs per litre of CSF following the same calculations used for the WBCs.

### CSF concentration and further microscopic examination

If there is sufficient CSF (≥1ml), concentrate it by centrifugation.

Place the CSF sample in a sealed bucket in a centrifuge, ensuring that the centrifuge is balanced.

For routine samples centrifuge for 3,000g for 10 minutes and increase to 3,000 g for 20 min if *M. tuberculosis* is suspected.

Remove the sealed buckets and open in the class II biosafety cabinet and take out the CSF sample (take care not to disturb the pellet).

Note the colour of the supernatant (yellow colouration is xanthochromia resulting from the degradation of RBC, increased protein or bilirubin).

With a sterile Pasteur pipette carefully remove some of the supernatant and discard, leaving behind the pellet and approximately 500μl of supernatant.

Resuspend the pellet with the remaining supernatant using the Pasteur pipette.

#### Giemsa stain to estimate WBC proportions

If the CSF WBC count is above the upper limit of normal for age (see Appendix 1) prepare a smear of concentrated CSF, air dry, and stain with Giemsa stain (in the AHC main lab).

Estimate the percentage of each WBC type: polymorphonuclear neutrophils (PMNs) have lobed nuclei and lymphocytes have a single round nucleus.

#### Gram stain

Pipette one drop of the concentrated CSF onto a clean, labelled glass microscope slide and allow to dry. Then add another drop on top and allow to dry (this improves the detection of bacteria).

If the sample is clotted, break up the clot as much as possible.

Perform a Gram stain (see SOP MID-001).

Examine the Gram film using x40 and x100 for at least 10 minutes or until bacteria are seen.

Record the presence of WBCs, bacteria and yeasts.

If organisms are seen record if they are Gram negative or Gram positive and their shape, bearing in mind possible pathogens (Table 1).

**Table 1.** Possible identity of organisms seen on CSF Gram film

|  |  |
| --- | --- |
| Gram result | Possibility of: |
| Gram negative diplococci | *N. meningitidis* (may look like coffee beans) |
| Gram positive diplococci | *S. pneumoniae* |
| Gram negative rods | *H. influenzae* (may be pleomorphic including GNB/GNCB)  *E. coli* and other coliforms |
| Yeast cells | *C. neoformans* (some may be budding) |

#### India ink stain

If cryptococcal meningitis is suspected, perform an India ink preparation.

In a BSC place one drop of the concentrated CSF on a labelled clean glass slide.

Add one drop of India ink.

Mix well.

Place a coverslip over the top.

Examine microscopically using x10 and x40 objectives.

Record if yeast cells with a capsule are present (yeast-like organisms surrounded by a clear zone indicate *Cryptococcus neoformans*).

#### Additional stains

If *M. tuberculosis* is suspected perform a Ziehl-Neelsen stain following SOP MID-001. If TB is suspected and the WBC count is above the upper limit of normal for age (see Appendix 1), also send the specimen for molecular detection of *Mycobacterium tuberculosis* (GeneXpert, SOP MOL-002).

Note: If only a small volume of CSF is obtained then the Gram stain can be over-stained with Giemsa and Ziehl-Neelsen.

### Culture

Inoculate the following media with a drop of concentrated CSF and incubate as documented in Table 2.

Read plates daily and follow up all growth.

**Table 2.** Culture plates and conditions

|  |  |  |  |
| --- | --- | --- | --- |
| Media | Incubation temperature (°C) | Atmosphere | Length of incubation |
| Blood agar (BA) | 35 – 37 | 5 – 10% CO2 | 48h |
| Chocolate agar (CA) | 35 – 37 | 5 – 10% CO2 | 48h |
| MacConkey (Mac) | 35 – 37 | Aerobic | 48h |
| Sabouraud agar (SAB) | 28 – 30 | Aerobic | 48h |

# Interpretation

## Minimum level of identification in the laboratory

Identify all growth according to SOP MID-003. Potential pathogens should be identified to the species level wherever possible. Organisms likely to represent contamination do not require full species level identification (e.g. coagulase negative staphylococci from community acquired meningitis).

Store significant isolates at -80°C in a cryovial containing 1ml STGG and record the isolate details in the freezer logbook/database.

## Antimicrobial susceptibility testing

All potential pathogens should have antimicrobial susceptibilities performed according to SOP MIC-001.

## Reporting

Cell count: report the number of RBC and WBC per mm3, including percentages for PMNs and lymphocytes.

Gram, ZN, and India ink stains: report organism detected (by telephone / urgent ward review + hard copy).

Culture: report either absence (“No growth at 48 hours”) or presence of growth (“Growth of [organism name]”). Do not report CSF cultures as “No significant growth”.

Antimicrobial susceptibilities: report for all significant isolates.

# Quality assurance

Media and identification tests should be quality controlled according to the relevant MOPSOP.

# Limitations

Exposure to antimicrobial agents prior to blood sampling may result in a false negative culture.

A negative CSF microscopy and culture does not fully exclude the possibility of bacterial meningitis.

# References

1. Health Protection Agency, UK SOP B27: Investigation of Cerebrospinal Fluid (Issue 5.1; July 2012).
2. Cheesbrough, M. District Laboratory Practice in Tropical Countries, Part 2. 2nd Edition Update (2006). Cambridge University Press.
3. Standard Operating Procedures from LOMWRU, SMRU and AHC.

# Synopsis / Bench aid



# Risk assessment

|  |  |
| --- | --- |
| **COSHH risk assessment - University of Oxford COSHH Assessment Form** | |
| **Description of procedure**  Culture of cerebrospinal fluid to identify bacterial and fungal pathogens | **Substances used**  Variable, depending on organism cultured (may include Gram stain reagents; Giemsa reagents; 3% hydrogen peroxide (catalase test); N,N,N',N'-tetramethyl-1,4-phenylenediamine (oxidase test); sodium deoxycholate (bile solubility test); bioMerieux API reagents) |
| **Quantities of chemicals used**  Small | **Frequency of SOP use**  Daily |
| **Hazards identified**  1. Autoclaved liquid  2. Potentially infectious material in sample  3. Potentially pathogenic bacteria  4. Chemical exposure form bacterial identification tests | **Could a less hazardous substance be used instead?**  No |
| **What measures have you taken to control risk?**  1. Training in good laboratory practices (GLP)  2. Appropriate PPE (lab coat, gloves, eye protection)  3. Use of biosafety cabinet for preparation of reading of plates until known not to be growing an HG3 organism / preparation of organism suspensions / follow-up of BSL-3 organisms (e.g. *B. pseudomallei*) | |
| **Checks on control measures**  Observation and supervision by senior staff | |
| **Is health surveillance required?**  No | **Training requirements:**  GLP |
| **Emergency procedures**:  1. Report all incidents to Safety Adviser  2. Use eyewash for splashes  3. Clean up spills using 1% Virkon or chemical spill kit | **Waste disposal procedures**:  1. Sharps discarded into appropriate rigid containers for incineration  2. Infectious waste discarded into autoclave bags or 1% Virkon solution prior to autoclaving and subsequent incineration  3. Chemical waste disposed of according to manufacturer’s instructions  4. Blood culture bottles are disposed of by autoclaving and subsequent incineration |

# Appendix 1. CSF cell counts

The appearance of the CSF can give clues to the aetiology of disease. It is normally a clear colourless fluid with a consistency similar to water. If cloudy this can indicate increased white blood cells (WBC), organisms and/or protein. If pink this indicates blood. Red blood cells (RBC) can be present due to an intra-cerebral or sub-arachnoid haemorrhage (bleeding into the brain) or during a traumatic lumbar puncture (contamination of CSF from peripheral blood when it is being taken). If two samples are taken and both contain similar RBC counts then this indicates previous haemorrhage, however, if the numbers are higher in the first sample this suggests a traumatic lumbar puncture. The presence and timing of xanthochromia (yellow, orange or pink colour) can be helpful. If haemorrhage has occurred, 2-4 hours post haemorrhage the RBCs lyse and release oxyhaemoglobin giving a pink/orange colour and after 24 hours haemoglobin is metabolized to bilirubin giving a yellow colour. Therefore, if the CSF has been processed rapidly in the laboratory and the centrifuged CSF supernatant is yellow in colour, this indicates pathological bleeding.

Normal CSF cell counts are shown in Table 3. Increases in different WBCs can be seen with various conditions (Tables 4 & 5). Figure 2 demonstrates the morphologies of the various WBC found in CSF specimens,

**Table 3.** CSF normal ranges

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Patient group** | **Normal range** |
| WBC | 0 – 28d (Neonates) | 0 – 30 x106/L |
| 29d – 4y old | 0 – 20 x106/L |
| 5 – 15y old | 0 – 10 x106/L |
| >15y (Adults) | 0 – 5 x106/L |
| RBC | Newborn | 0 – 675 x106/L |
| Others | 0 – 10 x106/L |
| Protein | Neonates ≤6d | 0.7 g/L |
| Others | 0.2 – 0.4g/L |
| Glucose | All ages | ≥60% of simultaneously determined plasma concentration |

**Table 4.** Interpretation of raised CSF WBC counts\*

|  |  |  |
| --- | --- | --- |
| **Increase in** | **Found in** | |
| Lymphocytes | Viral meningitis  Fungal meningitis  Parasitic meningitis  TB meningitis | Treated bacterial meningitis  Non-infectious causes |
| Monocytes | Viral meningitis  Fungal meningitis  Parasitic meningitis | Chronic bacterial meningitis  Treated bacterial meningitis  Non-infectious causes |
| Neutrophils | Bacterial meningitis  Very early viral meningitis  TB meningitis (early) | Brain abscess  Non-infectious causes |
| Eosinophils | Parasitic infection |  |

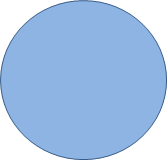
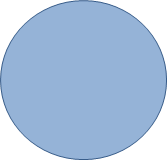
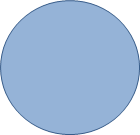
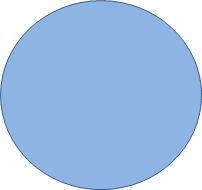
*\*Note:* If there is blood in the CSF due to traumatic tap the WBC will need to be adjusted.

**Table 5.** CSF values for different aetiologies of meningitis

(Adapted from <http://www.rch.org.au/clinicalguide/guideline_index/CSF_Interpretation/>)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Aetiology | Appearance | Neutrophils  (x 106 /L) | Lymphocytes  (x 106 /L) | Protein  (g/L) | Glucose (mmol/L)  (CSF:blood ratio) |
| Bacterial | Clear, cloudy or purulent | 100-10,000  (but may be normal) | Usually <20 | Raised >1.0 | Low <0.4  (but may be normal) |
| Viral | Clear, may have faint opalescence | Usually <100 | 10-1000  (raised, but may be normal) | 0.4-1  (slightly raised or normal) | Usually normal, can be low e.g. herpes or mumps |
| TB | Clear, opalescent or ground glass | Usually <100 | 50-1000  (raised but may be normal) | 1-5  (raised, but may be normal) | <0.3  (but may be normal) |

**Figure 2.** Differentiation of blood cells (illustration by Caoimhe Nic Fhogartaigh, adapted from ‘Tissues of the Human Body’ (McGraw Hill): <http://www.mhhe.com/biosci/ap/histology_mh/wbc1.html>)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Neutrophil polymorph** | **Eosinophil** | **Basophil** | **Monocyte** | **Lymphocyte** |
| Medium size\* (10-14µm);  highly variable dense (dark-staining) nucleus with >2 lobes connected by narrow nuclear strands | Medium size (10-14µm);  less dense, bi-lobed nucleus connected by a narrow band of nuclear material | Small (8-14 µm); bi-lobed nucleus | Largest cell (12-18µm;  less dense, large, horse-shoe shaped nucleus | Smallest cell (8-12µm); dense circular nucleus taking up most of the WBC |

\*Red blood cell (size 7-8 µm) can be used as comparison to estimate size