

## Examination of cerebrospinal fluid (c.s.f.)

### Possible pathogens

#### \_ BACTERIA

##### **Gram positive**

*Streptococcus pneumoniae*

*Streptococcus agalactiae*

*Listeria monocytogenes*\*

##### **Gram negative**

*Neisseria meningitidis*

*Haemophilus influenzae* type b

*Escherichia coli*\*

*Pseudomonas aeruginosa*\*

*Proteus* species\*

*Salmonella* serovars

*Flavobacterium*

*Meningosepticum*

\*Mainly isolated from neonates (see also *Notes on pathogens*).

Also *Mycobacterium tuberculosis* and *Treponema pallidum*.

*Note:* Bacteria may also be found in the c.s.f. when there is a brain abscess, e.g. *Bacteroides* species and other anaerobes.

**Commensals:** Cerebrospinal fluid has no normal microbial flora.

### COLLECTION AND TRANSPORT OF CSF

Cerebrospinal fluid *must be* collected by an experienced medical officer or health worker. It must be collected *aseptically* to prevent organisms being introduced into the central nervous system. The fluid is usually collected from the arachnoid space. A sterile wide-bore needle is inserted between the fourth and fifth lumbar vertebrae and the c.s.f. is allowed to drip into a dry sterile container. A ventricular puncture is sometimes performed to collect c.s.f. from infants.

- 1- Take two sterile, dry, screw-capped containers and label one No. 1 (first sample collected, to be used for culture), and the other No. 2 (second sample collected, to be used for other investigations).
- 2- Collect about 1 ml of c.s.f. in container No. 1 and about 2–3 ml in container No. 2.

3 - *Immediately* deliver the samples with a request form to the laboratory.

## LABORATORY EXAMINATION OF C.S.F.

### Day 1

#### 1- Report the appearance of the c.s.f.

As soon as the c.s.f. reaches the laboratory, note its appearance. Report whether the fluid:

- is clear, slightly turbid, cloudy or definitely purulent (looking like pus),
- contains blood,
- contains clots.

*Normal c.s.f.* Appears clear and colourless.

#### 2- Test the c.s.f.

Depending on the appearance of the c.s.f., proceed as follows:

##### ***Purulent or cloudy c.s.f.***

Suspect pyogenic meningitis and test the c.s.f. as follows:

- \_ *Immediately* make and examine a Gram stained smear for bacteria and polymorphonuclear neutrophils (pus cells). Issue the report without delay.
- \_ Culture the c.s.f.

##### ***Slightly cloudy or clear c.s.f.***

Test the c.s.f. as follows:

- \_ Perform a cell count and note whether there is an increase in white cells and whether the cells are mainly pus cells or lymphocytes.
- \_ *When cells predominantly pus cells:*
  - Examine a Gram stained smear for bacteria.
  - Culture the c.s.f.
- \_ *When cells predominantly lymphocytes:* This could indicate viral meningitis, tuberculous meningitis .

#### 3-Examining a c.s.f. Gram smear

Examine the smear microscopically for pus cells and bacteria using the 40\_ and 100\_ objectives.

***Pus cells:*** Report as many, moderate number, or few. Pus cells will be found mainly in pyogenic bacterial meningitis and in amoebic-meningoencephalitis (rare).

**Bacteria:** Look in well stained (not too thick) areas for:

- \_ Gram negative intracellular diplococci that could be *N. meningitidis* .
- \_ Gram positive diplococci or short streptococci, that could be *S. pneumoniae*. It is often possible to see the capsules as unstained areas around the bacteria.
- \_ Gram negative rods, possibly *H. influenzae*, especially if filamentous or other polymorphic forms are seen. Gram negative rods could also be *E. coli* or other coliforms, especially when the c.s.f. is from a newborn infant.

#### **4- Ziehl-Neelsen smear when tuberculous meningitis is suspected**

#### **5- CULTURING C.S.F.**

##### ***Chocolate (heated blood) agar and blood agar***

- Inoculate the specimen on chocolate agar and blood agar. When Gram positive diplococci are seen in the Gram smear, add an optochin disc to the blood agar plate to assist in the identification of *S. pneumoniae*.
- Incubate both plates in a carbon dioxide enriched atmosphere at 35–37 °C for up to 48 hours, checking for growth after overnight incubation.

### **Day 2 and Onwards**

#### **Examine and report the cultures *Chocolate agar and blood agar cultures***

Look especially for colonies that could be:

- \_ *Neisseria meningitidis* (growing on chocolate agar and blood agar, oxidase positive)
- \_ *Streptococcus pneumoniae* (sensitive to optochin)
- \_ *Haemophilus influenzae* (growing only on chocolate agar)
- \_ *Cryptococcus neoformans* (Gram stain the colonies).

##### ***MacConkey agar culture***

Look especially for colonies that could be:

- \_ *Escherichia coli* or other coliform.
- \_ *Streptococcus agalactiae*.
- \_ *Listeria monocytogenes*.



**7- Antimicrobial susceptibility testing**

Test isolates of *S. pneumoniae* for susceptibility to chloramphenicol and penicillin (use 1\_g oxacillin disc). Test *H. influenzae* for *beta*-lactamase production and susceptibility to chloramphenicol. Perform susceptibility testing on Gram negative rods as indicated.

### Summary of the Examination of C.S.F.

#### Day 1

#### 1 Report Appearance

- Describe whether c.s.f.
  - Clear, slightly turbid, cloudy, purulent
  - Contains blood
  - Contains clots

#### 2 Test c.s.f.

##### Purulent or cloudy c.s.f.

*Suspect pyogenic bacterial meningitis*

- **Gram smear**  
Report:
  - Number of pus cells
  - Bacteria
- **Culture c.s.f.**
  - Blood agar and chocolate agar. Incubate in CO<sub>2</sub>
  - If neonate: Also MacConkey agar. Incubate aerobically

##### Slightly turbid or clear c.s.f.

- Perform cell count  
Note whether pus cells or lymphs
- |  |  |
|--|--|
| <p style="text-align: center;">PUS CELLS</p> <ul style="list-style-type: none"> <li>■ <b>Gram smear</b><br/>See opposite</li> <li>■ <b>Culture c.s.f.</b><br/>See opposite</li> </ul> <p style="text-align: center;">ADDITIONAL TESTS</p> <ul style="list-style-type: none"> <li>■ <b>Wet preparation:</b><br/>For motile amoebae</li> </ul> | <p style="text-align: center;">LYMPHS</p> <ul style="list-style-type: none"> <li>■ <b>Measure protein</b></li> <li>■ <b>Measure glucose</b></li> <li>■ <b>Zn:</b> For AFB</li> <li>■ <b>India Ink:</b> For encapsulated yeasts</li> </ul> <p style="text-align: center;">ADDITIONAL TESTS</p> <ul style="list-style-type: none"> <li>■ <b>Wet preparation and Giemsa smear:</b><br/>For trypanosomes and morula cells</li> </ul> |
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#### Day 2 and Onwards

#### 3 Examine and Report Cultures

- **Chocolate agar and blood agar cultures**  
Look particularly for:  
*N. meningitidis*  
*S. pneumoniae*  
*H. influenzae* (chocolate agar)
  - **MacConkey agar culture**  
Look especially for bacteria that cause neonatal meningitis
- ADDITIONAL
- Perform antimicrobial susceptibility tests as indicated
  - **Beta-lactamase test.**  
*H. influenzae* isolates