National Department of Health

CSF Microscopy and Culture

G_90_SOP_19_A

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1. Purpose & Scope

This Standard Operating Procedure (SOP) describes the procedure for processing cerebral spinal fluid (CSF) including result interpretation. The procedure applies to all personnel responsible for all stages of analysis and reporting.

2. Principle & Clinical application

Cerebrospinal fluid examinations are requested when patients present with symptoms including severe headache, neck stiffness, fever, altered senses (consciousness, mental state, photophobia), vomiting, and rash. A lumbar puncture is performed, the fluid collected under sterile conditions and dispatched to the laboratory without delay. Early intervention is vital to patient survival if infection of the meninges is evident.

The spinal fluid of healthy individuals is usually sterile. Positive cultures obtained from cerebrospinal fluids reflect a variety of conditions besides meningitis, including trauma, infectious complication of

surgery, cranial/subdural/spinal epidural/brain abscesses and septic thrombophlebitis of the venous sinuses. Contamination during collection or processing can result in false positive cultures.

Possible pathogens include bacteria (*Streptococcus pneumoniae, Streptococcus agalactiae, Listeria monocytogenes, Streptococcus suis, Neisseia meningitidis, Haemophilus influenzae* Type B*, E. coli, Mycobacterium tuberculosis* and *Treponema pallidum*), viruses (particularly Enterovirus), fungi (*Cryptococcus neoformans*) and parasites.

To determine if a patient has meningitis, the CSF usually has the following tests performed:

- CSF cell count (include WBC differential if leucocytes present)
- Gram stain
- Protein and glucose
- India ink examination
- Bacterial and fungal culture

3. Responsibilities

Role	Responsibility
Lab bench scientist/ technician	Specimen reception and registration in LIMS. Microscopy and set up of cultures Record culture results Identify bacteria, conduct AST, interpret, report Document AMR phenotypes that require storage/ referral
Senior scientist	Checking of culture and AST results, cross correlating with request and microscopy Addition of necessary interpretative comments on the LIMS prior to verification (validation) of the report Check that AMR phenotypes that require storage/ referral have been stored Supervision and sign off of bench scientist/technician competency and media/reagent QC compliance/results

4. Specimens

CSF specimen from lumbar puncture/spinal tap and CSF drains. Specimen, required volume 2 mL (minimum 0.5 mL), is collected sequentially into 2 or 3 (optimal) numbered sterile tubes. Low volume specimen may yield negative results.

CSF samples are considered precious specimens. Every effort must be made to process the sample promptly and provide feedback to the clinician ASAP as soon as the results are available, even if they are normal.

Rapid transportation (<15 minutes) at room temperature is essential for reliable laboratory diagnosis, e.g., fastidious organisms.

Do not refrigerate CSF specimen for culture (Refrigeration may prevent isolation of meningococcus, Haemophilus, and also Strep. pneumoniae).. If the specimen cannot be processed immediately, it should be kept at room temperature or placed in an incubator. CSF for cell count only can be refrigerated for up to 4 hours.

As per G_10_LQM_Ap_23, all CSF specimens are refrigerated for 1 month before being discarded.

5. Safety

For safety aspects, please review this document G_10_Info_3 Laboratory Biosafety.

6. Equipment, Materials and Reagents

- Blood Agar Plate (BA)
- Chocolate Agar (CA)
- MacConkey Agar for neonatal or neurosurgical (ventricular drain) specimens
- Mueller Hinton (MH) and Mueller Hinton with 5% human blood (MHF)
- Oxygen (O_2) Incubator and Carbon dioxide (CO_2) incubator or candle jar
- Glass slides
- Gram stain reagents
- Optochin disc
- XV discs
- Sterile loops
- Antibiotic discs
- Physiological saline
- Swab sticks
- Timer

7. Procedure

7.1 Check patient name, MRN and DOB/age matches with specimen and laboratory request form

7.2 Register the specimen on the LIMS and generate labels for the CSF sample containers and request form

7.3 If the CSF has been collected into 2-3 pre-numbered tubes, prioritise the last tube for cell count, culture and tube 2 for biochemistry.

7.4 **Macroscopic examination**

7.4.1 Record the appearance of the CSF sample and record in LIMS. Report as turbid, clear, bloodstained, colourless, xanthochromic (yellow) with or without a clot. If the CSF was not clear and colourless, also record the appearance of the supernatant once the specimen has been centrifuged.

7.4.2 Centrifuge the 2nd or last tube in the series – 3000 rpm for 10 minutes. Aseptically transfer supernatant into biochemistry sample cup for protein/glucose analysis. If relevant prepare Gram stain smear of the deposit.

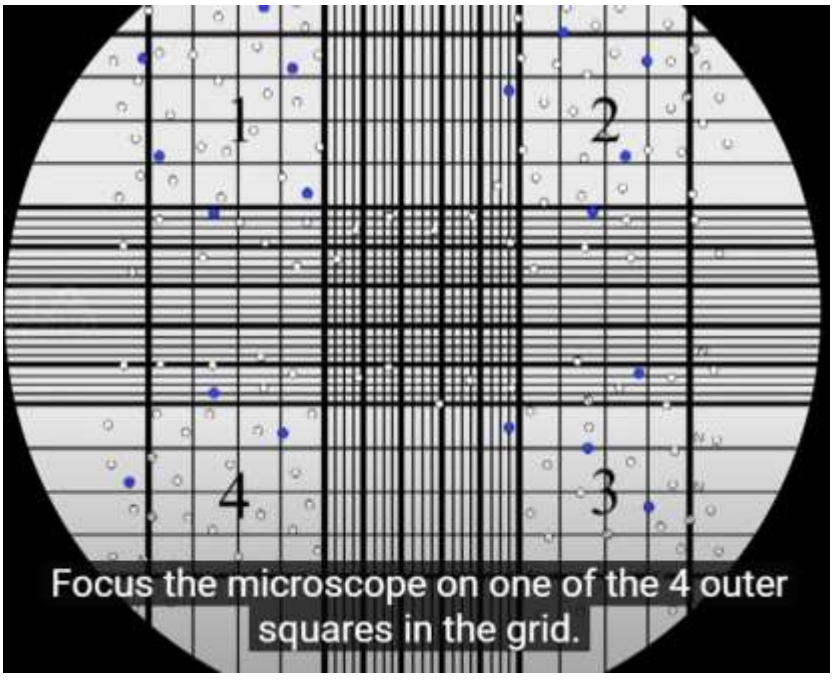
7.5 Microsopic examination

7.5.1 For turbid (purulent) CSF, immediately perform Gram stain for bacteria and yeasts on the centrifuged deposit and issue the report without delay. Ensure a gram control slide is stained simultaneously and checked.

7.5.2 All CSFs, whether clear or not, have a cell count and india ink examination or the more sensitive CNAG test performed on the unspun specimen. Note whether there is an increase in WBC and whether the cells are predominantly neutrophils or lymphocytes.

7.5.3 Cell count with the Neubauer Counting Chamber

- Clean the chamber with 70% alcohol
- Apply coverslip
- Mix the CSF for the even distribution of the cells
- Touch one side of the coverslip with the CSF-filled capillary tube so that the fluid flows slowly and fills the chamber. Do not overfill.
- Focus with 10X objective and then to 40X.
- With the 40X objective, count the number of white and red cells in one large square (labeled 1 to 4 in figure below). Note any presence of yeast (Cryptococcus).
- If the cell count is markedly raised, a 1 in10 dilution with saline can be performed (don't forget to multiply cell count by dilution factor).



- Read and calculate the cell count as per this approach:

If cells are <10 in one square (WCC)	count 5 squares and multiply by 2 to obtain the count per cubic mm (cmm = x 10^6 per litre)
If cell are >10 or <20 in one square	count 2 squares x 5/cmm
If cells are >20 in one square	count 1 square x 10/cmm
If cells are >100 in one square	report as >100/cmm.

No cells	report as < 1
** Record presence of bacteria and yeasts as +, ++ or +++	

- If > 30 WBCs/cmm present, perform WBC differential. Prepare and stain a slide with Wrights/Giemsa stain and read.

7.5.4 Expected normal cell count values:

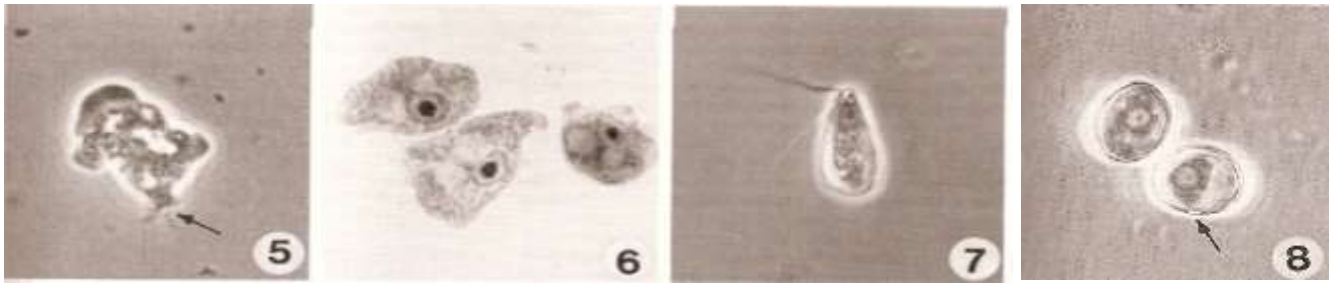
Sample type	WBC/ cmm
CSF – neonate	<30
CSF – infants	<10
CSF – adults	<5

The presence of RBC in CSF may represent blood contamination of the sample during collection. That contamination is maximal in the first tube which is why a later collected tube should be used for microcscopy. A ratio of 1 WBC:500 RBC is considered normal (reflects the proportion seen in whole blood). A lower ratio (e.g. 1: 100) implies that the CSF WBC count is elevated; however this remains imprecise and a obtaining better sample is best.

7.5.5 Unidentifed cells on microscopy

It is essential that these cells be correctly identified. This may involve referral of the specimen (Cytology) or a review of the sample by a Senior Scientist or pathologist.

Non-motile cells could include malignant cells due to a CNS malignancy; alternatively, motile cells may be amoeba (i.e. *Naegleria fowleri* – patient may have a history of swimming in thermal pools or splashing in puddles of warm water). Amoeba are best visualised microscopically using a glass slide and cover slip.



(5) *Naegleri fowleri* trophozoite phase contrast (note the uroid and filaments at arrow); **(6)** trophozoite stained; **(7)** biflagellate phase contrast; **(8)** smooth walled cyst phase contrast (note the pore at the arrow).

7.6 Culture

- The remaining spun deposit is used for culture
- As per G_90_J_21, inoculate sediment onto BA and CA half plate. Add SAB plate if India ink positive. Add MAC plate for neonatal and external ventricular drain samples.
- Incubate in CO_2 / candle jar for 4 days, examining plates daily for growth
- Identify significant isolates as per the Bacterial Identification SOP
- Perform disc susceptibility testing as appropriate

8. Results Recording

The following elements are recorded on the sample record within the LIMS:

Analysis Profile:

Analyses:

Service
CSF Appearance
CSF Protein
CSF Glucose
Polymorphs (CSF)
Mononuclear (CSF)
Red Blood Cells
Microscope Organisms
India Ink
Cryptococcal Antigen (Strongstep)
Cryptococcal Antigen (Remel)
Culture (C,A,T)

9. Interpretation

Careful interpretation of abnormal microscopy and biochemistry results is required **– always consult with a laboratory round mentor and/or Dr Ak via WHATSAPP.**

In general, report only significant bacterial and fungal isolates (see below).

Do NOT report skin flora organisms or *Bacillus* species as these almost always represent contaminants. The exception are cultures from ventricular drainage devices or CSF shunts. **If in doubt, always discuss with a senior staff member or Dr Ak.**

10. Quality Control

- AST disc QC is to be done weekly
- Bench reagent QC is done according to SOP

11. Related documents

Quality control of media and reagents	G_90_SOP_4
Antibiotic disc susceptibility testing	G_90_SOP_6
Setup of disc antibiotic susceptibility tests	G_90_J_1
Gram stain procedure	G_90_J_15
Processing of Routine Primary Specimens	G_90_J_21
Cryptococcal antigen testing SOP	Pends
Bacterial identification SOP	Pends

12. References

- Pathology NSW Standard Operating Procedure for CSF 2022
- Barrow GI and Feltham R. Cowan and Steel's Identification of Medical bacteria. 6th Edition. 1993. Cambridge University Press.
- Murray P *et al*. Manual of Clinical Microbiology. 8th Edition. 2003. American Society for Microbiology Press.

Appendix: CSF parameters associated with different conditions

Condition	Supernatant	Cells x 10^6/L (equivalent to / cmm)	Protein g/L	Glucose mmol/L[1]
Normal	Clear, Colourless	0-5 mononuclear (lymphocytes)	0.15 – 0.45	2.2-4.4 (> 60% of the serum BSL value)
Bacterial meningitis	Turbid, Colourless	> 500 polymorphs	Increased markedly	Decreased (< 50% serum BSL)
Viral (aseptic) meningitis	Clear to Opalescent, Colourless	10-300 mononuclear, but some polymorphs early in disease	Normal/ Slightly increased	Normal
TB meningitis	Opalescent, Colourless, Small clot	Usually 100-500 mononuclear	Increased	Decreased Chloride low
Cryptococcal meningitis	Clear, Colourless	10-200	Moderately Increased	Normal / Decreased
Viral encephalitis	Clear, Colourless	< 5-30 mononuclear +/- some neutrophils and RBC	Normal	Normal
Amoebic meningo-encephalitis	Opalescent, Colourless	Moderate number mononuclear and polymorphs	Normal/ Moderately Increased	Normal/ Decreased
Traumatic tap	Blood and serum stain	WBC/RBC=1/500-1/100	Increased	Normal
Subarachnoid haemorrhage	Blood stained, Xanthochromic supernatant	Same as for blood ratio WBC: RBC from FBC or 1:500	Increased	Normal

[1] Note that best practice is to measure a serum BSL from a sample collected at the same time as the CSF. The CSF/Serum ratio discriminates between viral and bacterial meningitis with a higher reliability that just a consideration of the CSF value by itself.