STANDARD OPERATING PROCEDURE
FOR CEREBROSPINAL FLUID (CSF) FOR THE DIAGNOSIS OF BACTERIAL MENINGITIS

SPECIMEN OPTIMAL SAMPLING

(This SOP does not cover neurosurgical infections (i.e. shunt/ external ventricular drain infection) or brain abscesses.)

Perform pre-analytic checks (refer to Specimen Optimal Sampling Checklist and Sample Acceptance guide.

CSF is an urgent, unrepeatable sample. If information is missing, investigate to confirm details by contacting the clinical team and continue to process the sample.

The macroscopic appearance of the CSF should be visualised by eye and noted in the results. Flocculant and/or clotted samples are unsuitable for a cell count. Note the volume of fluid received and refer to the Clinical Microbiologist or equivalent person to prioritise if insufficient for all tests requested.

Blood-stained CSF

If all 3 CSF samples are equally blood-stained by eye, bottles 1 and 3 will need a red cell count prior to referring bottle 1 to Biochemistry. If Bottle 1 is visually more bloodstained that bottle 3 there is no need to count the red blood cells of bottle 1.

Xanthochromic analysis can be performed by biochemistry if requested by clinician.

Using an individually wrapped sterile pastette, add a small amount of CSF (bottle 3 or 2 if bottle 3 is unavailable) to a counting chamber.
Do not return the same pastette back into the sample.

Centrifuge (1200 xg for 5-10 mins) the remaining sample and split off the supernatant (leaving 0.5ml) into a labelled, sterile universal for storage. Resuspend the deposit and inoculate media (blood agar and chocolate agar) incubated in 5-10% CO^2 at 35-37°C. If the patient is immunocompromised add a Sabouraud Dextrose Agar (SAB) plate if available and incubate for up to 14 days in air at 35-37°C.

Prepare a clean microscope slide for Gram staining. CSF should be sterile so any organisms grown should be identified and reported.

***Neisseria meningitidis* is a category 2 organism (defined by Advisory Committee on Dangerous Pathogens) but cultures of this organism should be handled in a class 1 microbiology safety cabinet.**

Microscopy:

Use a x10 lens on a light microscope to perform a cell count. Count both red blood cells (RBC) and white blood cells (WBC) and if able, differentiate between lymphocytes and neutrophils. 0.1% toluidine, methylene or Nile blue stain can help to differentiate. If the sample is heavily blood stained, Turk's fluid can be added to lyse the RBCs to visualise the WBCs. When adding stain to differentiate, remember to consider the dilution factor into the final count. Scan every field of the Gram stain for organisms. They may be scanty, intracellular and difficult to see.

Additional testing:

Cryptococcus sp: Microscopy using India ink or nigrosine can be performed by mixing a drop of centrifuged CSF with 50% stain. Cover with a coverslip and examine at x10 for the presence of a halo. Serology can be performed on the CSF for the presence of *Cryptococcal* antigen (CrAg)

Viral meningitis/encephalitis: target multiplex PCR should be performed, preferably on the unopened bottle 2. The supernatant of bottle 3 can be used if bottle 2 is not available.

Mycobacteria tuberculosis (TB): Prepare a microscope slide for a Zeihl Neeson stain. Multiple layers of CSF can be built up on the slide by adding a drop and allowing it to dry before adding another drop. Refer to local TB guidelines for processing CSF for *M.tuberculosis.* All samples from patients with a clinical suspicion of TB should be handled in a class or class 2 safety cabinet in a category 3 facility.

Amoebae: Add a drop of uncentrifuged CSF to a clean microscope slide. Repeat with the spun deposit. Examine both slides for the presence of trophozoites and refer to local guidelines for reporting.

References: UK Standards of Microbiology Investigations: Bacteriology: B27: Investigation of Cerebrospinal Fluid; Standards Unit, PHE.

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