Ministry of Health

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Document Author	Dr. Hanaa Al Auraimi
Designation	Consultant medical microbiologist
Document Reviewer	Microbiology documents development team
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Validated by		**Approved by**	
Name	Dr. Muna Habib	Name	Dr.Badryah Al Rashidi
Designation	Director Department Development & Conterol (DGQAC)	Designation	Director General of Primary Health Care
Signature		Signature	
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Member name	**Institution**	**Designation**
Dr.Mahmoud Al Subhi	Rustaq Hospital	Team Leader Consultant medical microbiologist
Ms. Zainab Al Hadhrami	Directorate General of Specialized Medical Care	Team Coordinator Senior technologist specialist A
Ms. Saleh Al Shukairi	Ibra Hospital	Senior technologist specialist A
Dr. Hanaa Al Auraimi	Royal Police of Oman Hospital	Consultant medical microbiologist
Dr. Nawal AL Kindi	Khoula Hospital	Consultant medical microbiologist
Dr. Al Warith Al Kharusi	Nizwa Hospital	Consultant medical microbiologist
Dr. Abdulrahman Al Mahrouqi	Ibri Hospital	Specialist microbiologist pathologist
Dr. Nada Al Tamimi	Al Massara Hospital	Consultant medical microbiologist
Dr. Wafaa Al Tamtami	Armed Forces Hospital	Consultant medical microbiologist

Contributors:

- Ms. Hanaa Al Shaqsi, Rustaq Hospital, Senior technologist specialist A

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Acronyms:

BA	Blood agar
CA	Chocolate agar
CSF	Cerebral Spinal Fluid
MAC	MacConkey
ATCC	American Type Culture Collection
H&S	Health and Safety
ID	Identification
IQC	Internal Quality Control
MDRO	Multidrug Resistant Organism
MRSA	Methicillin Resistant *Staphylococcus aureus*
SOP	Standard operating procedure
TAT	Turnaround time
WHO	World Health Organization
PMN	polymorphonuclear leukocyte.
PCR	polymerase chain reaction;

1. **Purpose**

   This standard operating procedure provides instruction on cerebral spinal fluid (CSF) investigations. The document will not include the CSF shunt fluid.

2. **Scope**

   This document is applicable for all medical laboratories under MOH and other collaborative governmental and non-governmental health institutions.

3. **Definitions**

   3.1. Meningitis is defined as inflammation of the protective membrane covering the brain or spinal cord (meninges).

   3.2. Xanthochromic : is described as the yellowish appearance of CSF that occurs after several hours of bleeding into the subarachnoid space.

   3.3. Newborn infant (neonate): is a child under 28 days of age.

   3.4. Infant: child under the age of one year.

4. **Procedure:**

   4.1. **Clinical background**

   4.1.1. 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.

   4.1.2. Cerebrospinal fluid (CSF) is a fluid that surrounds the brain and spinal cord. CSF is taken when meningitis, encephalitis, brain abscess or other neurological infections are suspected.

   4.1.3. 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(refer to table1 for normal reference ranges)

   4.1.4. For the commonly isolated organisms, refer to the following table.

Table 1: Causes of Bacterial Meningitis (modified from; Mims at al., 2004)

Age group	**Pathogen**
New born infants	group B streptococci (early onset <7 days)
	L. monocytogenes (early onset)
	E. coli and other Gram negative enteric bacilli (early +late onset)
Infants and Children	*S. pneumoniae*
	N. meningitides
	H. influenzae type b
Children > 5years & **Adults**	*S. pneumoniae*
	N. meningitidis
Elderly	*S. pneumoniae*
	N. meningitidis
	L. monocytogenes, aerobic gram –ve Bacilli
With risk factors	*S. pneumoniae* *L. monocytogenes* *H. influenzae*
Post trauma **and Post Surgery**	Staphylococci and Streptococci
	S. pneumoniae
	Coliforms
	Pseudomonas aeruginosa
	Anaerobes

Other	*Mycobacterium tuberculosis* *Salmonella spp.* *Brucella* Spirochaetes

4.2. Principle

Cerebrospinal fluid (CSF) is a key tool in the diagnosis of meningitis. Analysis of the CSF abnormalities produced by bacterial, viral, mycobacterial, and fungal infections may greatly facilitate diagnosis and direct initial therapy.

4.3. Important note:

4.3.1 CSF is an urgent specimen that should be analyzed within one (1) hour from time of collection. If there is a delay in processing, the fluid should be kept at room temperature.

4.3.2 If more than one hour has passed since collection, the specimen will be processed with the comment "Results may be erroneous due to a delay in transit/processing".

4.3.3 Notify all high cell count, positive gram stain or cultures to microbiologist / staff nurse or physician.

4.4. Pre- analytical stage:

4.4.1 Sample

4.4.4.1 Cerebral spinal fluid must be collected aseptically into sterile labelled universal containers. It should be delivered to the laboratory immediately after collection and transported at room temperature.

4.4.4.2 If possible, it is better to notify laboratory personnel before specimen collection to ensure staff is ready for testing immediately after collection.

4.4.4.3 Specimens should be always delivered to laboratory personnel by hand - never drop specimens off or, leave unattended. Don't send CSF through pneumatic tube.

4.4.4.4 CSF is normally collected sequentially into three or more separate containers (1-5 ml per tube) which should be labelled and numbered consecutively .10ml is desirable for investigation of suspected Mycobacterium tuberculosis infection.

4.4.4.5 All tubes must be labelled properly and delivered immediately to the following sections (refer to appendix 1: algorithm of CSF processing):

- Tube #1: for protein and glucose or serology study.
- Tube #2: for culture and gram stain.
- Tube #3: for cell count and differential
- Tube #4: Other tests as required – cytology, virology

4.4.4.6 If only one tube is collected, Microbiology (culture and gram stain) tests are performed first, then cell count and finally chemistry, to preserve specimen and avoid contamination.

4.4.4.7 If £ 1 ml is received, do not centrifuge. Inoculate the media directly and prepare the Gram stain, cell count and differential cell count.

4.4.4.8 If the volume is > 1 ml the specimen should be centrifuged prior to inoculating media and preparing the Gram and Giemsa for cell differential.

4.4.4.9 If several tests are requested and there is limited volume of fluid, contact the physician regarding the priorities of testing and process accordingly.

4.4.4.10 Clotted specimens are not satisfactory for testing. If the specimen is clotted, the clot should be broken up with a sterile pipette and a portion used to make a smear for Gram stain. The

cell count cannot be performed. Notify the physician immediately.

4.4.2 Materials

Reagents	Consumables/Supplies	Equipment
Normal saline Blood Agar Chocolate Agar India ink stain Gram stain Geimsa stain Saline / WBC diluting fluid	Slides Cover slip Neubauer Chamber capillary tube 15 µL sterile pipette	Slide dryer Microscope Centrifuge / Cytospin Incubators, including CO2, Aerobic and 30°C.

4.4.3 Safety Precautions

4.3.1.1. Standard procedures for handling of biohazard material must be always followed.

4.3.1.2. CSF specimen should be collected in sterile leak proof container and transported in a sealed plastic bag.

4.3.1.3. The processing of most diagnostic work can be carried out at Containment Level 2 unless infection with N. meningitidis, or hazard group 3 organism is suspected.

4.3.1.4. Due to the severity of the disease and the risks associated with generating aerosols of N. meningitidis , any manipulation of suspected isolates of should always be undertaken in a microbiological safety cabinet until N. meningitidis has been ruled out .

4.3.1.5. Where Hazard Group 3 Mycobacterium species are suspected, all specimens must be processed in a microbiological safety cabinet under full containment level 3 conditions

4.4.4 Quality Control

4.4.4.1 Check the expiry dates of all media, reagents and stains before use.

4.4.4.2 All media, reagents, kits, and stains **MUST** be quality controlled before use.

4.4.4.3 Identification tests should be run with appropriate controls.

4.4.4.4 Record the quality control results in the appropriate QC sheet.

4.5. Analytical stage:

4.4.5 Macroscopic examination:

4.4.5.1 Estimate the volume and record it.

4.4.5.2 In good lighting conditions describe the appearance of the CSF.

4.4.5.3 Descriptions include turbidity, Xanthochromic (yellow stained), and if blood stained or Clot present. Spider web' clot is rare but suggestive of M. tuberculosis.

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

4.4.6 Total cell count

4.4.6.1 Cell counts should not be performed on specimens containing a clot (which invalidates the result).

4.4.6.2 Perform total WBC and RBC counts on the un-centrifuged specimen in a Neubauer Chamber.

4.4.6.3 Mix the specimen and estimate (based on turbidity) if the specimen can be counted diluted or undiluted.

4.4.6.4 Prepare and charge Neubauer counter chamber.

4.4.6.5 Draw up well mixed specimen using capillary tube or 15 µL pipette.

4.4.6.6 Place the end of the capillary tube against Neubauer counter chamber and charge both sides with the fluid. Very little pressure is needed - the counter chamber should fill by capillary action.

4.4.6.7 Be careful not to over or under-fill and do not bump the cover slip or the count will be inaccurate.

4.4.6.8 Wait for 2 minutes for the cells in the CSF to settle and then perform cell count

4.4.6.9 Perform total WBC and RBC counts on the un-centrifuged specimen in a Neubauer Chamber.

- Before starting the count, use the 40 objective to check that the cells are WBC.
- Count cells in 5 of the large squares (W1, W2, W3, W4, W5) using the 10× objective as shown in Figure 1.
- If no WBCs are seen, report the count as zero.
- Calculate total number of cell according to the following formula to get the number of cells/ cu mm

$$\frac{\text{Number of cells counted) x (dilution factor)}}{\text{(Number of squares counted) x (volume of 1 square)}}$$

- If cells are present in large numbers, count square 5 only.
- If there are too many cells to be counted, repeat the whole procedure with dilution.

Note 1: Cells touching the upper and left limits should be counted, unlike cells touching the lower and right limits, which should not be taken into account. Refer to figure 2.

Note 2: It will become very easy to get lost when counting the cells. In this case use the zigzag counting technique as in figure 3.

- Dilutions must be prepared in sterile specimen tubes and labeled accordingly. The most commonly used dilutions are prepared as follows:
    - 1:10 - 0.1 mL sample to 0.9 mL of 0.85% Saline(normal)/ WBC diluting fluid. Multiply the count by 10 (dilution factor)

- 1:100 - 0.1 mL of the 1:10 dilution to 0.9 mL of 0.85% Saline(normal) / WBC diluting fluid.
- Use only calibrated pipettes to perform dilutions. Multiply the count by 100 (dilution factor).

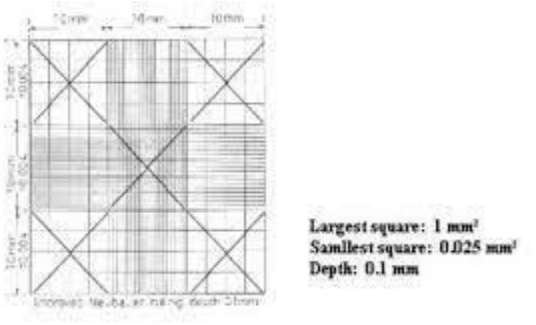


Fig. 8 - Ruled area of the Improved Neubauer counting chamber

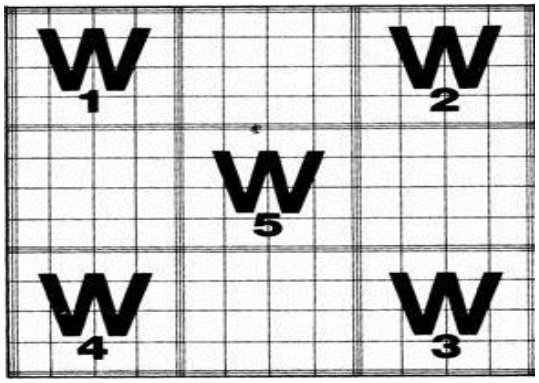


Figure 1: Nebular counting chamber

- Fig 1: The grid consists of 9 large squares, each measuring 1 x 1 mm, and a chamber depth of 0.1 mm. **Each square has a total volume of 0.1 mm3** (i.e.0.1 µl)**.**
- Each large square (except the middle square) is divided into 16 smaller squares, each with an area of 0.0625 mm2.
- c) The large square in the middle is divided into 25 squares, each with an area of 0.04 mm2 which are in turn are divided into 16 smaller squares, each with an area of 0.0025 mm2.

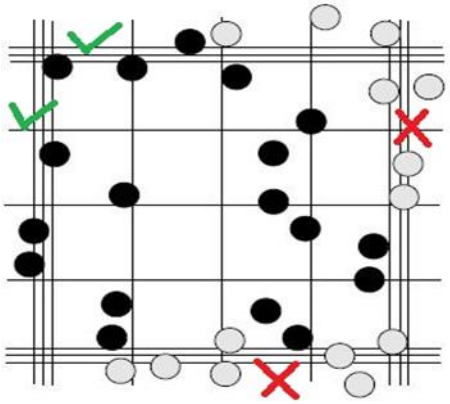


Figure 2: method of counting the cells near the upper and lower limits.

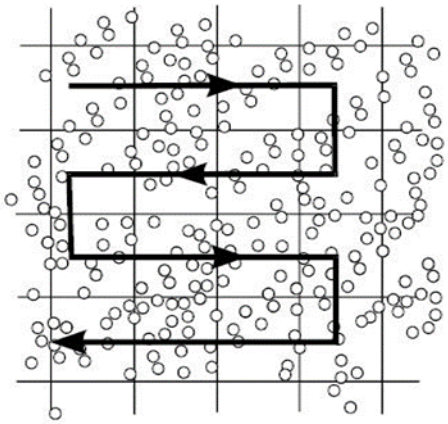


Figure 3: zigzag counting technique

4.4.7 Gram stain of the CSF specimen:

4.4.7.1 Label a clean microscopic slide with the laboratory specimen number.

4.4.7.2 Pipette one drop of the cyto-centrifuged deposit CSF onto the slide and allow heat dry.

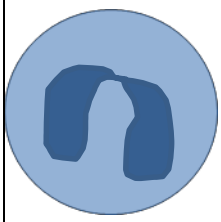
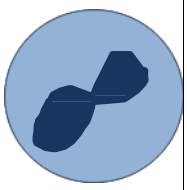
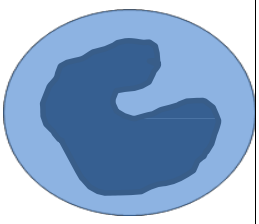
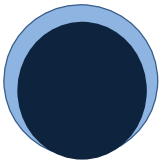
4.4.7.3 Then add another drop on top and allow to dry (this improves the detection of bacteria).

- If the sample is clotted, break up the clot as much as possible using sterile pipette.
- Record the presence of RBCs, WBCs, bacteria and yeast.

4.4.8 Differential Leukocyte count Using Giemsa Stain:

4.4.8.1 If the CSF WBC count is above the upper limit of normal for age, prepare a smear of concentrated (centrifuged) CSF, air dry, and stain with Giemsa stain.

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

				
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

4.4.9 Other test :

4.4.9.1 Those tests are not performed as a routine unless they are requested by the physician or if clinically indicated. Those tests includes the following:

4.4.10 Examination for *C. neoformans*

4.4.10.1 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 cover slip.

4.4.10.2 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*

4.4.11 Examination for amoebae :

4.4.11.1 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

4.4.11.2 If culture negative result from clinically ill patient consider other non-culture methods for diagnosis eg 16S PCR, MALDI TOF, etc.

4.4.12 Examination for Cryptococcal antigen test :

It is performed if requested or if an India ink stain is requested. CSF cryptococcal antigen testing should be carried out in all cases of suspected cryptococcal meningitis, and all cases of meningitis in immunocompromised patients in which there is an elevated CSF white cell count and no alternative diagnosis has been made.

4.4.13 Examination for Bacterial antigen screen panel: refer to the kit insert.

The routine use of Latex Agglutination Test is not recommended due to poor sensitivity and specificity.

4.4.14 PCR tests :

PCR can be used for diagnosis of viral, culture negative bacterial or TB meningitis.

4.4.15 Culture set up:

4.4.15.1 With a sterile pipette inoculate each agar with the cyto-centrifuged deposit. If the specimen is clotted inoculate the clot fragments to each agar plate.

4.4.15.2 Culture the centrifuged specimen into the following media:

Media	**Clinical indication**	**Incubation temperature**	**Incubator**	**Length of incubation**
Blood agar (BA)	All CSF samples	35±2°C	CO2	48 hrs
Chocolate agar (CA)		35±2°C	CO2	48 hrs
MacConkey		35±2°C	Aerobic	48 hrs
Sabouraud agar (SAB)*	If cryptococcal antigen or India ink requested Or yeasts seen Or known immunocompromised	35±2°C	Aerobic	2-5 days
Blood Agar Plate with MTZ disc	Brain abscess Ventriculitis Reservoirs Post neurosurgery Post otitis media with complications	35±2°C	anaerobic	

4.4.15.3 Send an aliquot to the TB section to the PHL if TB is requested.

4.4.15.4 Send an aliquot to virology if viral investigation is requested.

4.4.15.5 If PCR or HSV (Herpes simplex virus) is requested, to be approved by microbiologist / pathologist.

4.4.16 Isolation and identification:

4.4.16.1 Identify all isolates growing on the agar plates.

4.4.16.2 For positive culture, do further identification at species level and antibiotic sensitivity testing (AST).

4.4.16.3 Report final identification of organism and susceptibility as appropriate

4.4.17 Susceptibility testing:

4.4.17.1 Report susceptibilities as clinically indicated.

4.4.17.2 Prudent use of antimicrobials according to local and national protocols is recommendedd

4.4.17.3 Do not report antibiotics that cannot cross the blood brain barrier: e.g. Cefazolin, cefuroxime, erythromycin, clindamycin, gentamicin, tobramycin, amikacin, and ciprofloxacin

4.4.18 Interpretations:

4.4.18.1 **Macroscopic examination interpretation :**

- Normal CSF: is normally clear, colorless, and hypocellular. Any turbidity or color presence is abnormal.
- To differentiate a traumatic tap from subarachnoid hemorrhage:
- Traumatic tap - staining of the (3) tubes of CSF is uneven, being greatest in the first tube, and least in the last tube. After centrifugation, the supernatant is colorless and the specimen tends to clot.
- Subarachnoid hemorrhage - the blood is evenly mixed, the supernatant becomes yellowish within a few hours after the hemorrhage, and the fluid will not clot.
- Pink color - indicates RBC lysis and hemoglobin release. It can be seen 4 to 10 hours after a subarachnoid hemorrhage.
- Yellow or xanthochromic - indicates pathologic bleeding resulting from hemoglobin breakdown to bilirubin in the subarachnoid space. Xanthochromia persists for 2 to 3 weeks after

hemorrhage. It is also caused by a very high protein concentration in the CSF or by liver disease.

- Brown - indicates the presence of methemoglobin, which forms after a subdural or intracerebral hematoma.

4.4.18.2 **CSF characteristics by Infection Type (cell count, biochemical, culture ….).**

Infection type	**differential**	**Glucose level**	**Opening pressure**	**Protein level**	**White blood cell count**	**Other studies**
Bacterial (typical)*	Usually 80% to 90% PMNs: > 50% lymphocytes possible	< 40 mg per dL (2.22mmol per L) in 50% to 60% of cases; CSF: serum glucose ratio <0.4 is 80% sensitive and 98% specific	Adult and children 8 years and older: 200 to 500 mm H_2O Children younger than 8 years can have lower pressures	Almost always elevated	Usually 1,000 to 5,000 per μL 99% of children have > 100 per μL	Gram stain, CSF culture, CSF lactate (> 35.1mg per dL [3.9mmol per L]), PCR testing: if Gram stain is negative and antibiotics were given before lumbar puncture
Cryptococcal	Lymphocyte predominance	Usually > 40 mg per dL	>250 mm H_2O in severe cases: serial lumber punctures or ventriculoperitoneal shunt required to drain CSF in pressure persistently > 250	Usually < 40 mg per dL (400mg per L)	Usually mildly elevated; normal count possible, especially in patients with	CSF culture, CSF Cryptococcal antigen test, India ink capsule stain, latex agglutination, enzyme immunoassay, lateral flow assay, HIV test

			mm H_2O		HIV infection	
Fungal (Excluding cryptococcal)	Possible early PMNs progressing to lymphocyte predominance. Eosinophils possible	Significant decrease possible	Variable	50 – 250 mg per dL (500 – 2500 mg per L)	Usually elevated, up to several hundred per µL	CSF (1-3)-beta-D-glucan (elevated level is 95% to 100% sensitive and 83% to 99% specific). CSF fungal culture, Gram stain (hyphae), PCR test is only 29% sensitive
Neurosyphilis	Variable	Possibly decreased	Usually elevated in immunocompetent patients: may not be elevated in Immunocompromised patients	>45 mg per Dl (450mg per L)	Early stage: 10 to 400 per µL Late stage: 5 to 100 per µL Declines over decades	HIV test, CSF VDRL test (30% to 75% sensitive and 100% specific†), CSF fluorescent and treponemal antibody absorption test (100% sensitive and 50% to 70% specific)

Parasitic	Eosinophilia (>10 eosinophils per μL or > 10% of total cells)	Usually low normal or normal	Variable but can be persistently elevated. Requiring CSF draining	Usually elevated	150 to 2,000 per μL	PCR test; enzyme-linked immunosorbent assay or Angiostrongylus cantonensis, Gnathostoma spinigerum and Baylisascaris procyonis
Tuberculosis	Early lymphocyte and PMN predominance progressing to lymphocyte predominance	Median: 40 mg per dL: lower in advanced stages	Variable depending on stage	Usually 100 to 200 mg per dL (1,000 to 2,000 mg per L)	Usually 5 to 300 per μL: 500 to 1,000 per μL in 20% of cases	Multiple culture with acid-fast stain; PCR test (50% sensitive and 98% specific), CSF adenosine deaminase (> 10 U per L [166.67 nkat per L}; "pellicle" appearance of CSF‡
Viral	lymphocyte predominance: possible PMN predominance in early infection	Usually normal; decreased in 25% of patients with mumps: mild decrease possible in patients with	Usually normal	Normal or mildly elevated	Usually 100 to 1,000 per μL: higher in patients with enterovirus infection (elevated red	PCR test performed, other tests include CSF lactate (low), Gram stain, CSF or serum immunoglobulin M antibodies for arboviruses, electroencephalography or other neuroimaging for suspected

		HIV infection			blood cell count possible in patients with herpes infection)	encephalitis

*-Most Commonly Streptococcus Pneumonia, Haemophilus influenzae, Listeria monocytogenes, and Neisseria meningitidis.

†-False-Positive results can occur if blood is present in the CSF.

‡ -Cobweb-like clot that forms after CSF is allowed to stand for a short time

4.6. Post – analytical stage:

4.6.1 Reporting of microscopy and culture results:

4.6.1.1 All results, cell count data and differential, will be reported in the LIS and results released to the physician within one hour of receipt in the laboratory.

4.6.1.2 Before results are released, compare results of microbiology and chemistry. If discrepancies are detected between results, testing must be repeated if sample volume permits.

4.6.1.3 If the problem cannot be resolved, notify the attending physician and document all actions taken.

4.6.2 Cell count reporting:

RBC	Report numbers of RBC cell/ mm3 (µL)
WBC	Report numbers of WBC cell/ mm3 (µL)
Polymorphs /Lymphocytes	Report PMNs and lymphocytes as percentages of the total WBC
Gram stain	Report any finding in the gram stain

4.6.3 Culture reporting:

4.6.3.1 Negative report: Send negative report after 48 hrs incubation. "No growth, after 48 hrs of incubation.

4.6.3.2 Positive report: Quantitate and report all isolates with appropriate susceptibilities.

4.6.3.3 Reference ranges of Normal CSF values:

Leucocytes	Neonates	less 28 days	0-30 cells x 10^6/L
	Infants	1 to 12 months	0-15 cells x 10^6/L
	Children/Adults	1 year +	0-5 cells x 10^6/L
Erythrocytes	No RBCs should be present in normal CSF		

Glucose	Neonates	less 28 days	1.94-5.55 mmol/L
	Infants	29 to 58 days	1.55-5.55 mmol/L
		2-12 months	1.94-5.0 mmol/L
	Children/Adults	1 year +	2.22-4.44 mmol/L
Proteins	Neonates	less 28 days	0.65-1.5 g/L
	Infants	29-56 days	0.5-0.9 g/L
	Children	2 months to 18 years	0.05- 0.35 g/L
	Adults	over 60	0.15-0.6 g/L
		18 to 60	0.15-0.45 /L

5. Responsibility

5.1. Responsible staff:

- To ensure the adherence to critical result communication procedure
- To facilitate the alternative channels once needed

5.2. Quality manager /officer

- To follow up the implementation of the procedure
- To monitor regularly communication of critical results and raise non-conformance with corrective action once needed.

5.3. All lab staff:

- To adhere to the procedure.
- To document record and release results as recommended
- To report test failures or incident

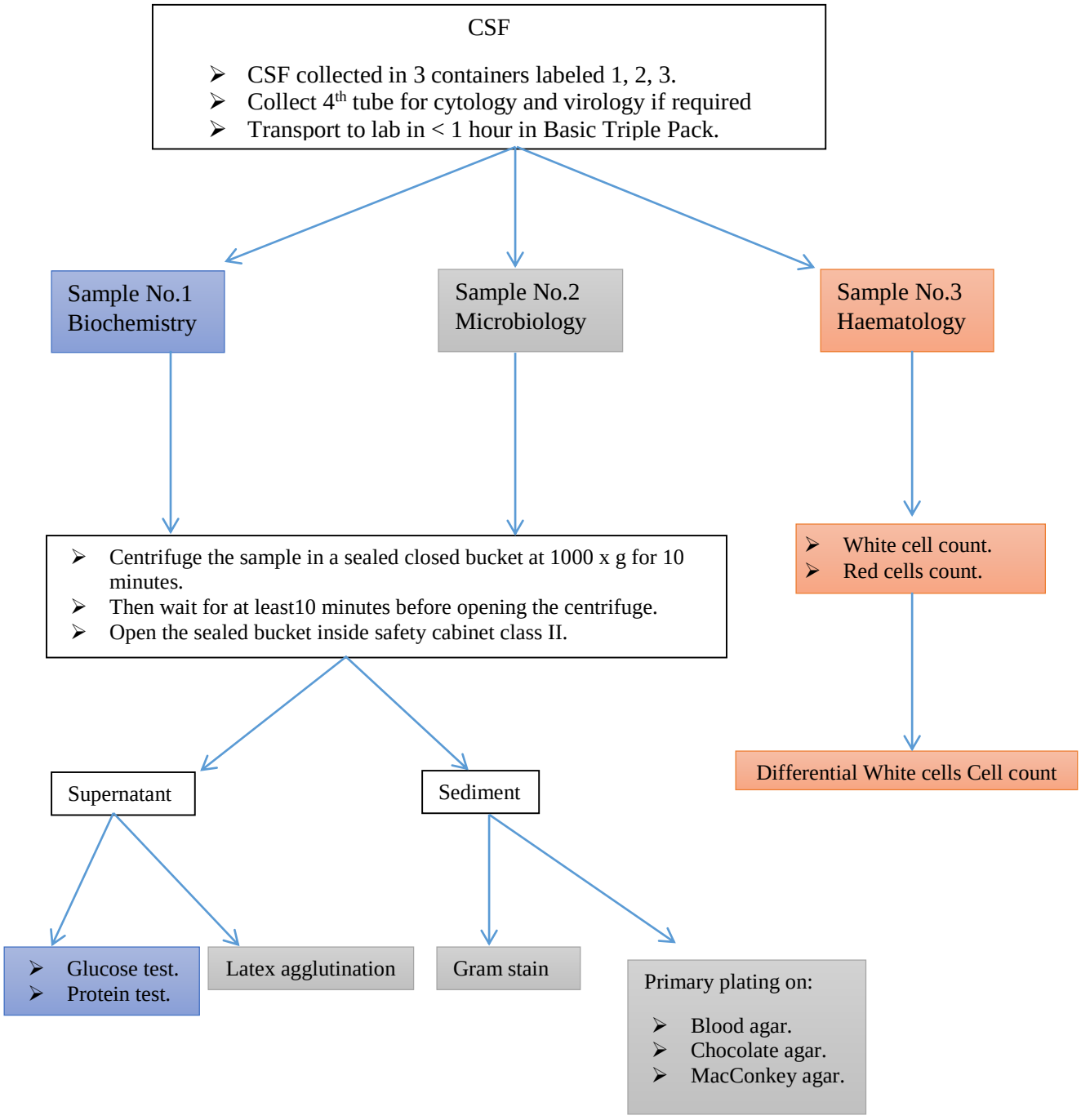
6. Document History and Version Control

Version	Description	Review Date
1	Initial Release	May 2026

7. References

Title of book/ journal/ articles/ Website	Author	Year of publication	Page
investigation of cerebrospinal fluid bacteriology, uk standards for microbiology investigations	the standards unit, public health England	31.05.17	page 6
District laboratory practice in tropical countries part 2second edition.	monica cheesbrough	-	-
Pro6.4-e-04 cerebrospinal fluid sop.doc	johnshopkins university baltimor.	-	-
Microbiology standard operating procedure.	global health network	-	-
Cerebrospinal Fluid Analysis Am Fam Physician. ;	BRIAN SHAHAN, MD, EDWIN Y. CHOI, MD, MS, AND GILBERTO NIEVES, MD	2021	103 (7):422-428

8. Annexes: Processing of CSF algorithm.



Note: - Save the remaining CSF in micro-tube inside double specimen bag inside the fridge at 4°C.