HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER
MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank (Microbiology)		
Document:	Internal Policy and Procedures		
Title:	Cerebrospinal Fluid Culture		
Applies To:	All Laboratory Staff		
Preparation Date:	January 06, 2025	**Index No:**	LB-IPP-120
Approval Date:	January 20, 2025	**Version :**	2
Effective Date:	February 20, 2025	**Replacement No.:**	LB-IPP-120(1)
Review Date:	February 20, 2028	**No. of Pages:**	05

1. PURPOSE:

1.1 To establish system and set responsibilities for processing Cerebrospinal Fluid (CSF) culture.

2. DEFINITONS:

2.1 **Meningitis** is an inflammation of the protective membranes covering the brain and spinal cord, known collectively as the meninges. The inflammation may be caused by infection with viruses, bacteria, or other microorganisms, and less commonly by certain drugs. Meningitis can be life-threatening because of the inflammation's proximity to the brain and spinal cord; therefore the condition is classified as a medical emergency.

2.2 **Acute Bacterial meningitis is medical emergency.** Identification of the infecting agents is one of the most important functions of the diagnostic microbiology laboratory. Aerobic bacteria commonly cause bacterial meningitis, but anaerobes may be present in CSF when a meningeal abscess or a similar infectious process is adjacent to the meninges. These include traumatic head injury or prostheses, such as metal cranial plates and shunt drains. So, inoculation of anaerobic media is not recommended for diagnosis of community- acquired meningitis.

2.3 **Cerebrospinal Fluid (CSF)** is obtained by trans-cutaneous lumbar puncture and aspiration is a sterile body fluid and therefore, all organisms recovered from the culture are considered potential pathogens and must be reported immediately. Because the number of organisms in the CSF can be quite low, concentration is important for rapid diagnosis.

2.4 **Shunt** is a surgically implanted device that diverts cerebrospinal fluid (CSF) in a controlled manner away from the central nervous system (CNS) fluid compartments (the ventricles or fluid space near the spine) to an internal delivery site, such as the abdomen or heart.

3. POLICY:

3.1 The specimen is better to be collected into Blood culture bottles. If not available, collect into a clean, sterile, leak-proof containers.

3.2 Specimen must be submitted to laboratory as soon as possible and **alert laboratory that specimen is in transit (better to inform lab. before sample extraction).**

3.3 If a delay in transport or processing is anticipated, the specimen should be incubated at 35+2 °C (**Don't refrigerate**).

3.4 All CSF specimens are processed immediately upon receipt in the microbiology lab.

3.5 Gram stain result is reported within **one hour** (STAT) after receiving specimens in microbiology Lab.

3.6 All positive Gram stains are immediately informed to ordering physician or ward and recorded in critical results log sheet.

3.7 Any discrepancies related to quantity not sufficient or leaking specimen are clarified by calling ordering physician.

3.8 Final culture reports, if negative, are released after 48 hrs. incubation.

3.9 All positive plates are kept for 03 days and All specimens are kept for 7 days.

4. PROCEDURE:

4.1 Examine the fluid for colour, turbidity and the presence of visible blood or clots.
4.2 Record the observations in the register.
4.3 If >1 ml of specimen is received, centrifuge the specimen at 2000 rpm x 15 minutes.
4.4 Transfer the supernatant to a sterile tube which used for latex agglutination test.
4.5 Use the sediment to inoculate the culture media listed below & to prepare Gram stain.
4.6 If <1 ml of specimen is received, do NOT centrifuge the specimen.
4.7 The remaining sample is inoculated in blood culture bottle & processed in Bactec Alert/ Bactec 9120.
4.8 If the sample was received in sterile container, directly inoculate the culture media listed below and then prepare a Gram smear using 1-2 drops of CSF without spreading it.

Media:	Incubation:
Blood Agar (BA)	O2, 35+2 °C x 48 hours
MacConkey's Agar (MAC)	O2, 35+2 °C x 48 hours
Chocolate Agar (CHOC)	CO2, 35+2 °C x 48 hours

Note: N.B. inoculation of anaerobic media is not recommended for diagnosis of community- acquired meningitis.

4.9 If bacterial antigens (latex test) are requested, follow the procedure for antigen detection in CSF (Appendix 7.1).
4.10 Gram stain: Note the presence of organisms and WBCs (Appendix 7.3).
4.11 **Interpretation of Cultures:**

4.11.1 Examine the BA, MAC and CHOC plates after 24- and 48-hrs incubation. Any isolate is to be identified by different identification tests & tested for antibiotic susceptibility.
4.11.2 In case of N. Meningitides isolation, perform serotyping (Appendix 7.2).
4.11.3 Susceptibility Testing: Refer to Susceptibility Testing policy.

4.12 **Reporting Results:**

4.12.1 Gram stain:
4.12.1.1 Prepare Gram stain smear on an alcohol washed slide.
4.12.1.2 Report the presence of organisms and WBCs (Do not quantitate).
4.12.1.3 Interpret all CSF gram stains immediately (within 1 h of receipt).

4.12.2 Culture:
4.12.2.1 Negative Report: "No growth after 48 hours incubation".
4.12.2.2 Positive Report: Report all isolates with appropriate sensitivities. Do not quantitate.
4.12.2.3 Notify results of a positive Gram stain and all positive cultures to the ward / ordering physician and the Infectious Disease Department.

4.12.3 Interpretation of results:
4.12.3.1 Generally, a positive culture indicates infection with the organism.
4.12.3.2 Lack of WBCs in CSF does not rule out infection, especially in listeriosis.
4.12.3.3 The most common cause of community-acquired bacterial meningitis is S. pneumoniae.

4.12.4 Limitations:
4.12.4.1 False-positive results can result from contamination of the specimen or the culture with skin microbiota (N.Flora).
4.12.4.2 False-negative results can be caused by low numbers of organisms, prior anti-microbial treatment, or the fastidious nature of the infective organism.

5. MATERIAL AND EQUIPMENT:

5.1 Gram Stain Reagents
5.2 Routine Culture Media
5.3 Microscan Panels/ ID & AST cards of Vitek 2 system

5.4 Latex Agglutination Kit for CSF Antigens
5.5 N. meningitides anti- sera.

6. RESPONSIBILITIES:

6.1 Assigned Technician for Microbiology
6.2 Clinical Pathology Specialist/ Consultant

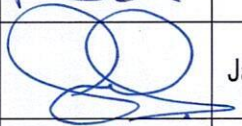
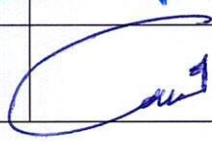
7. APPENDICES:

7.1 Latex test for detection of antigens in CSF
7.2 Serological testing of Neisseria meningitides
7.3 Gram stain of CSF

8. REFERENCES:

8.1 Clinical Microbiology Procedures Handbook, American Society of Microbiology, Washington DC, 2005
8.2 P.R. Murray, E.J. Baron, M.A. Pfaller, R.H. Yolken. 2003. Manual of Clinical Microbiology, 8th ed. ASMPress, Washington, D.C.
8.3 H.D. Isenberg. 2003. Blood Cultures-General Detection and Interpretation, p.3.4.1.1-3.4.1.19 In ClinicalMicrobiology Procedures Handbook, 2nd ed. Vol.1 ASM Press, Washington, D.C.

9. APPROVALS:

	Name	Title	Signature	Date
Prepared by:	Dr. Kawther M. Abdou	Consultant & Lab. Medical Director		January 06, 2025
Reviewed by:	Ms. Noora Melfi Alanizi	Laboratory & Blood Bank Director		January 08, 2025
Reviewed by:	Mr. Abdulelah Ayed Al Mutairi	QM&PS Director		January 12, 2025
Reviewed by:	Dr. Tamer Mohamed Naguib	Medical Director		January 13, 2025
Approved by:	Mr. Fahad Hazam Alshammari	Hospital Director		January 20, 2025

Appendix 7.1: Latex test for detection of antigens in CSF

LATEX AGGLUTINATION TEST FOR CSF

Principle:

1. The common bacterial organisms causing meningitis carry specific polysaccharide antigens that can be detected by latex agglutination. The most common agents associated with bacterial meningitis include:
   1.1 H. influenza, N. meningitides & S. pneumonia.
   1.2 Streptococcus group B (in neonatal meningitis), and
   1.3 E. coli (in neonatal meningitis).
2. Reagents provided in kit contain polystyrene latex particles coated with antibodies to these antigens. These latex particles agglutinate in the presence of sufficient quantity of homologous antigen.

Indication:

1. Perform latex on request.
2. Perform latex if suspected meningitis by low glucose, high protein & WBCS with predominant neutrophils.

Procedure:

1. If CSF is very turbid or contain RBCs, centrifuge it for 5 min at 1500 rpm.
2. Take 2-3 ml of CSF supernatant in a glass test tube.
3. Heat in a boiling water bath for 5 minutes at 100 C.
4. Cool the specimen to room temperature for 3 min.
5. Perform Centrifugation for 5 min at 3000 rpm.
6. Put one drop of latex reagent into the corresponding circle on the card provided
7. Add one drop of the supernatant next to each drop of latex. Mix with stick and cover the whole area of circle.
8. Rock the card slowly and observe agglutination up to 10 minutes. (N.B. wait for the end of those 10 min to conclude a negative result).

Interpretation:

1. Clear visible clumping or agglutination----------POSITIVE
2. Milky homogeneous or faint granulation----------NEGATIVE
3. Agglutination in more than one circle or in the control circle------NON-SPECIFIC REACTION

Appendix 7.2: Serological Testing of Neisseria Meningitides

SEROLOGICAL TESTING OF NEISSERIA MENINGITIDES

(Series of Slide Agglutination Tests from Primary culture)

STEP I:

1. Test for agglutination with N. meningitidis polyvalent antisera.
   - N.meningitis Polyvalent A-D
   - N.meningitis polyvalent XYZW135

STEP II:

1. If polyvalent A-D is positive, then do agglutination tests with the following anti-sera:
   - N. meningitis Group A
   - N. meningitis Group B
   - N. meningitis Group C
   - N. meningitis Group D
2. If polyvalent XYZW135 is positive, then do agglutination tests with the following anti-sera:
   - N. meningitis Group X
   - N. meningitis Group Y
   - N. meningitis Group Z
   - N. meningitis Group W135

INTERPRETATION:

1. Clear agglutination ---------- positive
2. No agglutination within 2 minutes-------- Negative
3. Agglutination with more than one anti-sera or control------- Non specific

Appendix 7.3: Gram stain of CSF

CSF GRAM STAIN

Procedure: (Refer to Gram stain policy)

- **Gram stain morphology of causative agent of bacterial meningitis:**

Neisseria meningitidis	Gram negative intracellular diplococci
Strept. pneumoniae	Gram positive capsulated diplococci
H. Influenza	Gram negative coccobacilli, pleomprphic
Streptococcus Group B	Gram positive cocci in chain
E.coli & Other coliforms	Gram negative bacilli