HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank		
Document:	Internal Policy and Procedure		
Title:	Cerebrospinal Fluid Cell Count		
Applies To:	All Hematology Staff		
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1. PURPOSE:

1.1 Evaluate Cerebrospinal Fluid for presence of RBC and WBC.
1.2 As counting chamber is flooded with CSR and a cell count is performed. Cell count of the CSF may help in the detection.
1.3 Confirmation and monitoring of diseases involving the CSF.
1.4 To establish system & set responsibilities for work

2. DEFINITIONS:

N/A

3. POLICY:

3.1 The proper processing for the CSF total count and differential.

4. PROCEDURE:

4.1 **Specimen Requirement:**
- 4.1.1 Specimen Type :
  - 4.1.1.1 CSF: Tube # 3 should be used for cell count. The cell count should be performed as soon as possible.
- 4.1.2 Tube Type : Red tube and sterile container
- 4.1.3 Amount Required : 1 ml for CSF
- 4.1.4 Delivery Arrangement: As soon as possible and should not exceed 30 minutes from collection time.
- 4.1.5 Temperature Restriction: 37 °C
- 4.1.6 Time of the test : Anytime for CSF; Daily morning for others
- 4.1.7 Stability: 2 hours at RT. Do not refrigerate
- 4.1.8 Unacceptable SP:
  - 4.1.8.1 Clerical Error
  - 4.1.8.2 Clotted
  - 4.1.8.3 Refrigerated
  - 4.1.8.4 Contaminated with Bone Marrow

4.2 **Handling instruction:**
- 4.2.1 If sample contains small clots:
  - 4.2.1.1 Report the presence of clots under appearance.
  - 4.2.1.2 Perform cell counts on well-mixed, unclotted portion of fluid.
  - 4.2.1.3 Report cell counts with the following comment: "cell counts are inaccurate due to the presence of clots".
- 4.2.2 If entire sample is clotted:
  - 4.2.2.1 Report sample as unsuitable for cell count and differential.

4.3 **Steps:**
- 4.3.1 Macroscopic examination of the specimen at the time it is submitted to the laboratory

4.3.1.1 Colorless, yellow or xanthochromic.
4.3.1.2 If the specimen is bloody, centrifuge and note degree of the color (slight, moderate and marked)and note the color of the supernatant

4.3.2 Counting:
4.3.2.1 Determination of total cell count by the cell counter machine if there is numerous count
4.3.2.2 Hemocytometer Method: (Perform the count as quickly as possible)
4.3.2.3 Transfer well- mixed fluid to the hemocytometer chamber
4.3.2.4 Place inside the Petri dish and wait for 5 minutes to allow the cells to settle.
4.3.2.5 Count all WBC or RBC in 4 large square.
4.3.2.6 If cells are too crowded make a dilution to count accurately
4.3.2.7 Take the Average of the cell count (Total number of cells in 4 squares divided by 4).
4.3.2.8 Multiple the result by 10.

4.3.3 Calculation:

If cells are counted in 4 corner squares:

$$\text{WBC/ul} = \frac{\text{No of WBC counted} \times 10}{4}$$

The results will be cells per uL .

4.3.4. Notes :

For cloudy CSF: dilute 1 volume CSF in 9 volumes Saline or CSF diluting fluid. Then multiply results by 10.

****Automated machine is recommended only if the count is very high (Severely cloudy or bloody CSF).***

****Determine a true WBC count if a traumatic tap has been performed :***

True WBC Count = (WBC count in CSF) – Z

$$Z = \frac{\text{WBC(blood)} \times \text{RBC(CSF)}}{\text{RBC(Blood)}}$$

Another Procedure: Subtract one WBC for every 750 RBC present in CSF

4.3.5 If there are more than 10 WBC/ul perform differential count must be done.

4.4 **Differential Count of CSF:**
4.4.1 Centrifuge CSF Speed at 1000 rpm for 5 minutes
4.4.2 Remove supernatant.
4.4.3 Re-suspend button of cells in the residual fluid.
4.4.4 Air dry and stain with Giemsa- Wright Stain.
4.4.5 The smear then introduced to the hematologist for microscopic examination and reports the result report form by Count and evaluate 100 cells.

4.5 **Reference range:**
4.5.1 Adult = 5/ul, Newborn= 0-20/ul
Microscopically:

Test	Age	Reference Range	Unit
CSF WBC	Adults	0-5	cell /uL
CSF WBC	Newborn	0 – 30	cell /uL
CSF RBC	All	0	cell /uL

4.6 **Source of error:**
4.6.1 Delayed counting
4.6.2 Presence of clot
4.6.3 Contamination by peripheral blood due to traumatic tap
4.6.4 Inability to distinguish between RBC and WBC.
4.6.5 High speed centrifugation that may damage the cells

5. MATERIALS AND EQUIPMENT:

5.1 Hemacytometer
5.2 Microscope
5.3 12 x 75 test tube
5.4 Manual counter
5.5 Petri dish with moist gauze
5.6 Reagents
  5.6.1 Isotonic diluents – use cell dyne diluents (ready to use)
  5.6.2 Methylene blue: Dissolve 0.3 g of methylene blue in 100ml of 95% ethanol, Store the solution at room temperature, Note the expiry date on the label, it is stable for one year.

6. RESPONSIBILITIES:

6.1 Most Responsible Physician

7. APPENDICES:

N/A

8. REFERENCES:

8.1 CRC Handbook Series in Clinical Laboratory, Science, Section 1: Hematology Volume III, 1980. CRC Press, Inc. Boca Raton, Florida.

9. APPROVALS:

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