

Department:	Laboratory and Blood Bank		
Document:	Internal Policy and Procedure		
Title:	Cerebrospinal Fluid Cell Count		
Applies To:	All Hematology Staff		
Preparation Date:	January 07, 2025	Index No:	LB-IPP-061
Approval Date:	January 21, 2025	Version :	2
Effective Date:	February 21, 2025	Replacement No.:	LB-IPP-061 (1)
Review Date:	February 21, 2028	No. of Pages:	03

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:

$$\frac{\text{WBC}/\mu\text{L} \quad \text{No of WBC counted} \times 10}{4}$$

The results will be cells per μL .

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/ μL 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/ μL , Newborn = 0-20/ μL

Microscopically:

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

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

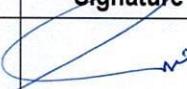
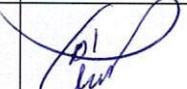
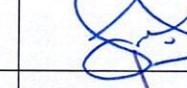
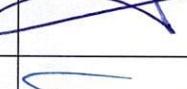
7. APPENDICES:

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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