HEMOCYTOMETER COUNT

☒ St. Joseph Medical Center, Tacoma, WA	☒ St. Anthony Hospital Gig Harbor, WA	☐ Harrison Medical Center, Bremerton, WA
☒ St. Francis Hospital, Federal Way, WA	☒ St. Elizabeth Hospital Enumclaw, WA	☐ Harrison Medical Center, Silverdale, WA
☒ St. Clare Hospital Lakewood, WA	☐ Highline Medical Center Burien, WA	☐ PSC

PURPOSE

To provide instruction for performing manual cell counts using the Hemocytometer.

BACKGROUND

The Hemocytometer is used to determine the concentration of cells in a fluid suspension. The chamber has two grids divided into 9 large squares equal to 1mm². Each large square has subdivisions to aid in counting. The depth of the chamber is 0.1 mm. By using these measurements the cell concentration can be calculated.

RELATED DOCUMENTS

R-W-HEM1401	Body Fluid Cell Count
R-W-HEM1400	Cerebrospinal Fluid Cell Count
R-W-HEM1402	Synovial Fluid Cell Count
R-PO-HEM0108	Pathologist Review of Blood and Body Fluids
R-W-HEM1437	Hematology CJD Protocol
R-W-HEM1436	Hematology Calculations

SPECIMEN REQUIREMENT

Any body fluid containing cellular elements

EQUIPMENT/SUPPLIES

- Hemocytometer (Improved-Neubauer) with cover glass or disposable “C-Chip” counting chamber.
- Capillary tubes, plastic petri dish with moist cloth
- Hematology analyzer diluting fluid, Turks or CSF diluting fluid, plastic tubes, certified diluting pipettes
- Microscope and Tally counter
- DI water, methanol, lens paper and 10% fresh bleach

QUALITY CONTROL

1. Performance/documentation of a background count is required by examining diluent (Hematology Analyzer, Turks, CSF diluting fluid or other diluent) under the microscope for cellular contamination or debris. Record on your laboratory worksheet or according to your site lab protocol.

2. Cell counts are performed in duplicate. Counts must agree within 20%. Document appropriately.

3. One manual body fluid cell count control specimen must be analyzed for each 8 hours of patient testing. Record QC results on laboratory worksheet or in LIS according to your site lab protocol.

4. Evaluate the integrity of the Hemocytometer and document appropriately. It should be free of scratches that may interfere with count.

5. A cytospin slide is submitted for pathologist review on all body fluid cell counts, except synovial fluids. If a cytocentrifuge is not available, a manual slide can be made from spun sediment if sample volume is sufficient. If an appropriate slide cannot be made without a cytocentrifuge, an aliquot of sample should be sent to pathology for cytospin prep. If sending sample for cytospin to another hospital, include a copy of the results with a note indicating what additional steps need to be completed.

INSTRUCTIONS

1. If using glass hemocytometer, prepare the chamber and cover glass by carefully cleaning the surface.

2. Charge both sides of the counting chamber by using separate capillary tubes and place a drop of specimen at the edge of the V-shaped wells.

3. Allow the chamber to fill by capillary action. Do not over-fill or under-fill.

4. Place the chamber in a covered petri dish to prevent drying out if count not performed within 3-5 minutes.

5. Place the chamber on the microscope stage and focus using the 10X objective.

6. Scan both sides and check for even cell distribution or cell clumps.

7. Determine if a dilution is indicated. Choose an appropriate dilution so that cells can be easily counted (between 50-450 cells on one grid). Separate dilutions may be indicated for counting different cell types. Dilutions are plated on both sides of a hemocytometer, counted in duplicate, and must agree within 20%. If not, mix tube thoroughly, replate, and recount.

8. Frequently used dilutions:

Ratio	Volume of fluid	Volume of diluent
1:2	100 µL	100 µL
1:5	100 µL	400 µL
1:10	100 µL	900 µL
1:20	50 µL	950 µL
1:50	20 µL	980 µL
1: 101	20 µL	2 mL

9. Pre-determine an appropriate number of large squares to count.
Note: For a statistically significant count, at least 100 cells should be counted. Include squares from both sides of the chamber. If less than 50 cells are present, all 18 large squares must be counted.

10. Determine the proper magnification to use and count the cells in the pre-determined squares. Decide on a specific counting pattern to avoid bias. For example: For cells that overlap a ruled line, count the cell as "in" if it overlaps the top or right ruling of the square, and "out" if it overlaps the bottom or left ruling of the square.

11. Calculate the cell concentration using the standard mathematical formula for improved-Neubauer Hemocytometers. Note: 1 LARGE square= 1 mm^2

$$\frac{\text{\# Cells counted}}{\text{\# Large squares}} \times \frac{\text{Dilution Factor}}{\text{0.1 (depth)}} = \text{Cells/mm}^3$$

12. The cell count results may be entered in the LIS for automatic calculation.
CAUTION: Auto-calculated results are based on counting the LARGE squares.

13. Document all results on a worksheet according to your site lab protocol. Required documentation: number of cells counted for each side of the chamber, dilution factors, number of squares counted (large), presence of clumps or clots, manual calculations, and Tech I.D. Also record quality control results (i.e.- background, cell count QC, etc) where appropriate.

14. Clean the glass Hemocytometer Chamber, if used.
    - Rinse the chamber/ cover glass with 10% bleach and let sit for 10 minutes. Soak for 1 hour if CJD Protocol is in use.
    - Rinse with methanol or carefully wipe with an alcohol wipe.
    - Allow to air dry. Remove any remaining debris using lens paper.

PROCEDURAL NOTES

RBC's are only counted on spinal fluids. If the sample is bloody, the center square could be used for the RBC count. Each small square is equal to 0.04 mm^2 or $1/25^{th}$ of the large square. If using the small squares for the count, use the following mathematical formula for calculations.

$$\frac{\text{\# cells counted}}{\text{\#small squares}} \times 25 \times \frac{\text{dilution factor}}{\text{0.1 depth}} = \text{cells/mm}^3$$

Example: if 100 RBC's were counted in only 5 of the small center squares with a 1:2 dilution

$$\frac{100}{5} \times 25 \times \frac{2}{0.1} = 10{,}000 \text{ RBC's/mm}^3$$

The LIS cannot auto-calculate for counts using only the small squares. Results would need to be manually calculated and entered in the computer.

LIS RESULTING

Dilution used for count: (enter 1 if undiluted, or the dilution factor)
WBC/RBC Count side 1 or 2: (number of cells counted, side 1 and side 2 entered separately)
#SQ counted: (total number of LARGE squares counted, add both sides together)
WBC/RBC Count: (LIS calculates the total count.)

REFERENCES

Henry, John B. Clinical Diagnosis and Management by Laboratory Methods, 19th ed. W. B. Saunders Co., 1996, pp. 556-559.

Hausser Scientific Co. Directions for Use: Bright Line Counting Chamber. (Package Insert.)

Caprette, David R. Using a Counting Chamber. Rice University, May 2000. www.Ruf.rice.edu/bioslab/methods/microscopy/cellcounting.html

McKenzie, Shirlyn B. Clinical Laboratory Hematology. Pearson, Prentice-Hall, 2004. Chapter 7, pp. 130-131

.