HEMOCYTOMETER COUNTS

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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

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
- Coulter Clenz cleansing agent, DI water, methanol, lens paper and 10% fresh bleach

QUALITY CONTROL

1. The hematology analyzer diluting fluid has a background check performed daily at startup for identification of cellular and non-specimen particulates. Ensure that the background count on the analyzer says “pass” if using this fluid as a diluent and record on your laboratory worksheet or according to your site lab protocol. NOTE: If alternate diluents (i.e. Turks or CSF diluting fluid) are used, performance/documentation of a background count is required by examining under the microscope.
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.
5. A cytospin slide is submitted for pathologist review on all body fluid cell counts, except synovial fluids.

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 with a moist cloth for 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.
8. 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.
9. 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.
10. Calculate the cell concentration using the standard mathematical formula for improved-Neubauer Hemocytometers. Note: 1 LARGE square= 1 mm^2

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

11. The cell count results may be entered in the LIS for automatic calculation.
    CAUTION: Auto-calculated results are based on counting the LARGE squares. The mathematical formula is different if the small squares are counted. Do not enter the number of small squares in the LIS (see LIS Ordering and Resulting).
12. Document all results on a worksheet according to your site lab protocol (RQW or other worksheet). Required documentation: number of cells counted for each side of the chamber, dilution factors, number of squares counted (large or small), 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.
13. Clean the glass Hemocytometer Chamber, if used.
    - Rinse the chamber/ cover glass with Clenz and D.I. water and soak, if indicated in fresh 10% bleach. Soak for 1 hour if CJD Protocol is in use (see procedure R-W-HEM1437, Hematology CJD Protocol).
    - Rinse with methanol or carefully wipe with an alcohol wipe.
    - Allow to air dry. Remove any remaining debris using lens paper.

PROCEDURAL NOTES

The center square is often used for RBC/ platelet counts. 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.

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

Sources of errors include: poor distribution of cells in the chamber, improper dilutions for cells counted, improper counting techniques creating bias in the final count.

LIS ORDERING/RESULTING

DILU WBC: (enter 1 if undiluted, or the dilution factor)
WBC CTD: (total number of cells counted)
#SQ WBC: (total number of LARGE squares counted)

BF WBC CT: (LIS calculates the total count.)
NOTE: RBC'S are counted on spinal fluids only. It is not necessary to enter results in the fields for RBC's.

If only the SMALL squares have been used for counting, enter the following information at the appropriate prompt:

- Enter the dilution factor and number of cells counted.
- Enter SEE COM as result for #SQ Counted and add footnote how many small squares were counted. Note: The LIS will not auto-calculate a result due to the non-numeric entry.
- Manually enter the total cell count. Results must be calculated from the formula listed above in PROCEDURAL NOTES.

REFERENCES

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

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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.

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