UnityPoint Health Pekin
Department of Pathology
Pekin, IL 61554

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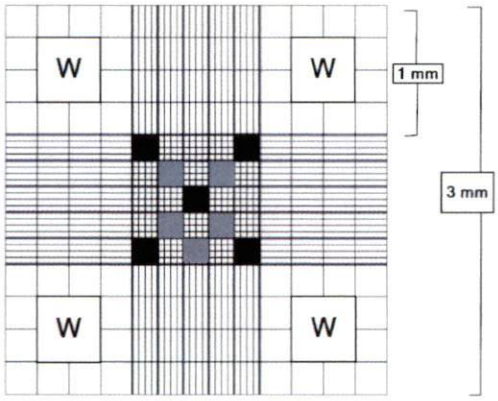
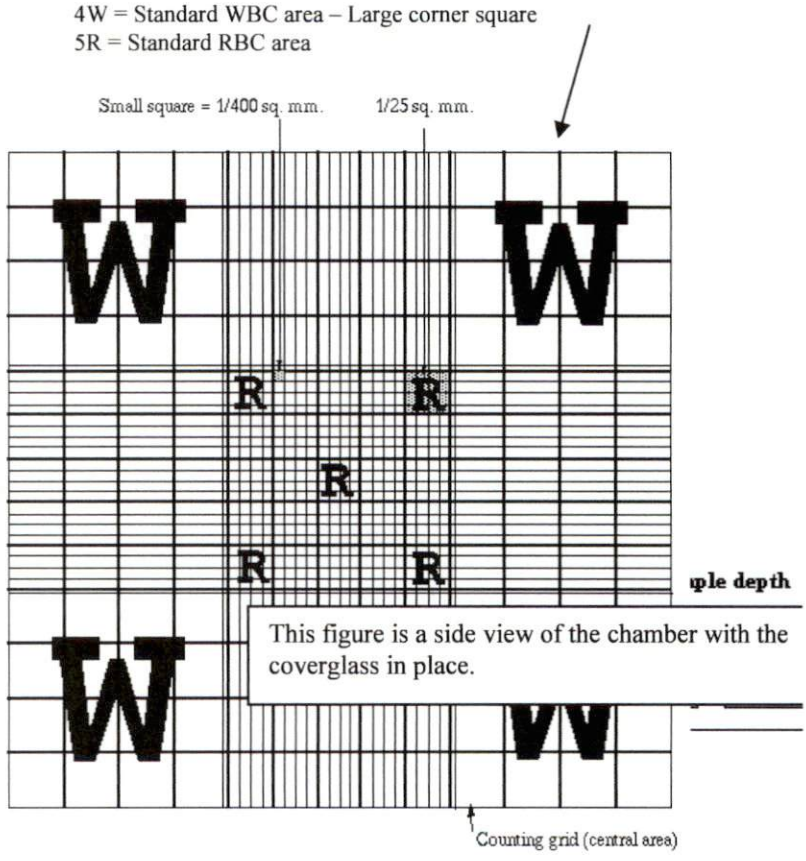
HEMACYTOMETER
MANUAL COUNTS

I. PRINCIPLE:

Under certain conditions or with unusual specimens a cell count cannot be done on an automatic instrument. A manual count may be done on an appropriately diluted specimen of blood or other fluid using an Improved Neubauer Ruled Hemacytometer. The counting chamber is etched into a 3.0 mm square with divisions at 1.0 mm, creating nine squares each with 1.0 sq mm area. The four outer corner squares are again ruled into fourths on each side, creating sixteen smaller areas, to facilitate order while counting particles through a microscope. The center square is ruled into fifths, creating 25 smaller areas and these are ruled again into 16 smaller areas. The chamber is 0.1 mm deep. From these dimensions and the dilution factor an accurate count per volume may be calculated.



The upper figure is a diagram of the improved Neubauer ruling:
This is etched on the surface of each side of the Hemacytometer.
The large corner squares, "W" are used for leukocyte counts.
The five black squares in the center are used for red cell counts or for platelet counts.
The 10 black plus shaded squares for platelet counts.



II. EQUIPMENT/SUPPLIES:

A. Improved Neubauer Hemacytometer, clean and free of lint or scratches.
B. Hemacytometer coverslip, clean and free of lint or scratches.

C. Humidity chamber - Petri dish with moist pad.
D. Microscope.
E. Saline or other appropriate diluent.
F. Hemocytometry controls, Levels 1 and 2, as specified by QC policy.

III. SPECIMEN:

Any body fluid may be processed for a manual cell count provided it is not clotted or too highly viscous or mucoid to mix well. Higher cellularity fluids may be run on the Hematology analyzers. See separate procedure.

A. Cerebrospinal Fluid Cell Counts for WBC and/or RBC
B. Other Body Fluids Cell Counts for WBC and/or RBC
    1. Synovial
    2. Peritoneal
    3. Pericardial
    4. Thoracentesis

IV. DILUTIONS USED:

A. Dilutions can be made using Cell Pack or Nerl saline.
    a. Perform a background check with just the diluting solution to confirm that no cells or debris is seen prior to making dilution.
    b. If cells or debris is seen, get a new aliquot or container of diluting solution
B. Straight - No dilution is used with clear fluids.

V. STANDARD SELECTION OF SQUARES FOR AREAS TO COUNT:

A. WBC area = four large corner squares = 0.4 cmm
B. RBC area = four corner and one center small squares (of the 25 on the central large squares grid) = 0.02 cmm (see diagram on first page).
C. Total Grid Area = All nine large squares = 0.9 cmm (see diagram on first page)
D. CSF specimens require all 9 squares counted for WBC and RBC.

VI. CALIBRATION

The hemacytometer is checked for accuracy by manufacturer.

VII. PROCEDURE:

A. Prepare an appropriate dilution of the body fluid to be examined. Some fluids such as clear CSF may not need dilution.
    1. CRITICAL STEP- Mix the cells thoroughly to disperse clumps and produce a uniform suspension.
    2. Be certain the fluid is well mixed before sampling for the dilution.
    3. Mix the dilutions thoroughly prior to plating.

B. Plate the dilution.
    1. Label Hemacytometer using a small LIS label or label with the last four numbers of patients accession number with a pencil.

2. Thoroughly clean a hemacytometer and coverslip. Assemble the counting chamber and prepare a humidity chamber (petri dish with moist gauze and supportive applicator stick pieces).
3. Expel one to two droplets of well-mixed dilution from a disposable pipette onto a waste gauze. Carefully touch the edge of the cover-slip with the pipette tip and allowing the chamber to fill by capillary action.
    a. CRITICAL STEP- Do not over fill or underfill the chamber. Improper fill of the chamber can alter the distance between the hemocytometer surface and the coverslip, thus altering the volume and calculated concentration.
4. Place the hemacytometer in the humidity chamber. Let the liquid wait for at least 10 minutes for cells to settle into an even focal plane (15 minutes for platelets). Read within 30 minutes.
5. View the slide at 100X magnification (10X ocular with 10X objective). The central area of the grid should occupy the center of the microscope field and cells should be evenly distributed and without clumps.
    a. If the cells are not evenly distributed or clumped, clean the hemocytometer and coverslip. Thoroughly mix the specimen and recharge the hemocytometer.

C. Count
1. Use a counter to record the number of cells in the grid. Repeat the count using the other chamber of the hemacytometer.
    a. Standard Selection of squares
        1) WBC area = four large corner squares = 0.4 cmm
        2) RBC area = four corner and one center small squares (of the 25 on the central large squares grid) = 0.02 cmm (see diagram on first page).
        3) Total Grid Area = All nine large squares on a side = 0.9 cmm (see diagram on first page)
        4) CSF specimens require all 9 squares counted for WBC and RBC.
    b. Certain types of cells or specimens may require more or less area to be counted.
        1) A statistically significant count requires counting 100 total cells.
        2) The more squares and cells, the better the precision.
    c. Count the cells toughing the middle line of the triple line on the top and left of the grid. Do not count cells touching the lines on the bottom or right.

D. Disinfect and clean the hemacytometer.
1. Cover hemacytometer and coverslip with alcohol.
2. Soak two to five minutes.
3. Rinse with tap water and alcohol and dry.
4. Air dry or polish with lint-free material.
5. Before reusing, use alcohol and lens paper to polish surfaces ~~to perfection.~~

VII. CALCULATIONS:

A. Background:
1. The volume of each area on the grid of the hemacytometer is a known number.
    - The volume of the grid (all nine large squares) is 0.9 cmm

(3 mm x 3 mm x 0.1 mm).

- The volume of one large square is 0.1 cmm (1 mm x 1 mm x 0.1 mm).
- The volume of one small square is 0.004 cmm (0.2 mm x 0.2 mm x 0.1 mm).
- The volume of five small squares is 0.02 cmm (0.004 x 5).

2. The formula to calculate the # of cells/cmm is:

$$\frac{\text{Avg \# Cells counted}}{\text{Volume (cmm) of the squares counted*}} \times \text{dilution factor} = \text{\#cells/cmm}$$

3. Count cells on both sides of the hemacytometer.
   a. Hemocytometer Rule:
      1) The two sides must have counts that agree within ± 20% of the higher count or within four cells if the count is very low.
      2) If the sides do not match, the specimen must be replated and recounted.
      3) Example:

         Side A = 243
         Side B = 230

         20% of 243 = 48.6
         Side B must be within 243 ± 48.63 (195 – 291).
         The sides must match; therefore, the counts are good and may be averaged.

$$\frac{243 + 230}{2} = \frac{473}{2} = 237$$

         This average of 237 is then considered the cell count average and is used in calculations.

4. Dilution Factor:
   a. The dilution factor is one (1) if no dilution was performed. If a dilution was performed, the dilution is the bottom number (1/20 dilution has a dilution factor of 20 and a 1/10 dilution has a dilution factor of 10)

B. Examples:
   1. If **5 small squares** (four corner and one center of the center 25 small squares)

      a. $\frac{\text{Avg \# Cells counted}}{\text{0.004 cmm x 5 squares}} \times \text{dilution factor} = \text{\# cells/ccmm}$

b. This is the same as:

Avg # Cells counted x dilution factor = # cells/ccmm
0.02 cmm

c. This is the same as:

 # Cells counted x dilution factor x 50/cmm = # cells/cmm

d. Example: Average of two sides is 22. Dilution is 1:10, factor is 10.

22 x 10 = 11,000 cells/cmm ***OR*** **22 x 10 x 50 = 11,000 cells/cmm**
.02

2. If **25 small squares are counted:**
   a. Avg # Cells counted x dilution factor = # cells/cmm
   0.004 cmm x 25 squares
   b. This is the same as:
   Avg # Cells counted x dilution factor = # cells/cmm
   0.1 cmm
   c. This is the same as:
   Avg # Cells counted x dilution factor x 10/cmm = # cells/cmm
   d. Example: Average of two sides is 24. Dilution is 1:5, factor is 5.

   24 x 5 = 1,200 cells/cmm ***OR*** **24 x 5 x 10 = 1,200 cells/cmm**
   .1

3. If the **whole grid** is counted:
   a. Avg # Cells counted x dilution factor = #cells/cmm
   0.9 cmm
   b. This is the same as:
   # Cells counted x dilution factor x 1.1/cmm = # cells/cmm
   c. Example: Average of 2 sides is 266. Dilution was not done, so factor is one (1).

   266 x 1 = 295.55 = 296 cells/cmm ***OR*** **266 X 1.1 = 293 cells/cmm**
   0.9

   *Note a slight difference in results will show up when using high numbers because 1.1 is rounded off from 1.111111 etc.; this is clinically insignificant.

4. If **5 Large squares** (4 large corners + Large center squares)
   a. Avg # Cells counted x dilution factor = #cells/cmm
   (0.1+ 0.1 + 0.1 + 0.1 + 0.1) cmm
   b. ***This is the same as:***
   Avg # Cells counted x dilution factor = #cells/cmm
   0.5 cmm

c. ***This is the same as*:**
 # Cells counted x dilution factor x 2.0 = # cells/cmm

d. **Example: Average of 2 sides is 84. Dilution was not done, so factor is one (1).**

$$\frac{84}{0.5} \times 1 = 168 \qquad OR \qquad 84 \times 1 \times 2.0 = 168 \text{ cells/cmm}$$

5. If **four large corner squares are counted**:
   a. $\frac{\text{Avg \# Cells counted}}{0.1 + 0.1 + 0.1 + 0.1 \text{ cmm}} \times \text{dilution factor} = \#\text{cells/cmm}$
   b. This is the same as:
   $\frac{\text{Avg \# Cells counted}}{0.4 \text{ cmm}} \times \text{dilution factor} = \#\text{cells/cmm}$
   c. This is the same as:
   # Cells counted x dilution factor x 2.5 = # cells/cmm

   d. Example: Average of 2 sides is 84. Dilution was not done, so factor is one (1).

$$\frac{84}{0.4} \times 1 = 210 \qquad OR \qquad 84 \times 1 \times 2.5 = 210 \text{ cells/cmm}$$

C. Count the cells in the prescribed areas. Include the cells touching the left side and top lines, but omit those touching the right side and bottom line. Be sure to count only the type of cell needed (e.g. WBC but not RBC or histiocytes). The two sides of the hemacytometer should agree within 20% or four cells in a very low count. If they do not agree, re-plate both sides and then recount. Average the sides. **Use the average in the calculations.**

D. In some specimens, distinguishing different types of cells may be difficults. To ensure accurate distinction of RBC from other cells, a phase microscope, located in urinalysis, may be used to differentiate cell type on the hemacytometer.

VIII. NOTES

A. Most errors occur by incorrect sampling and transfer of cells to the chamber.
B. CSF specimens require all 9 squares counted for WBC and RBC.
C. If tissue cells or unclassifiable cells are present, the specimen should be reviewed by a pathologist. Order a Path Review. Make a cytospin slide and stain.
D. If fibrin or other debris causes cell clusters repeat the count. Report the average with a comment that the cell count is questionable due to debris or interferences.

IX. TROUBLE SHOOTING

A. Re-plating of specimen is required for any of the following;
   1. Inconsistent results
   2. Poor precision
   3. Clumps
   4. Non-uniformed suspension
   5. Overfilling or underfilling

X. REPORTING RESULTS

A. Document the following on the HEMO-MANUAL WORKSHEET

1. Specimen type
2. Cell counts and Calculation
3. Manual diluent clarity checked (if used)

B. Result appropriately in LIS per test ordered. Refer to specific Body Fluid procedures.

XI. QUALITY CONTROL

A. Manual counts should agree with those from the Hematology analyzers.
B. Hemacytometer competency is checked with the CAP surveys, performance of quality control and specimens performed in duplicate.
C. Stained slide estimates should agree with the results of manual counts. Always make and scan a slide or do a differential as per procedures.
D. Both sides of the hemacytometer must give equivalent counts or both are rejected and the specimen must be re-plated.
E. Two levels of controls are performed each 8 hours of patient testing and by each performing technologist. WBC and RBC counts must be counted using all 9 large squares (UPPK HE-0101.01)
F. Record counts and calculations on the Hemacytometer Controls worksheet (UPPK HE-0101.02) Verify controls are within ranges stated.

XII. REFERENCES

A. Henry, John, M.D., Clinical Diagnosis and Management of Laboratory Methods. Volume 1, 17th Edition, pp 459-474. Philadelphia, 1984.
B. Kjeldsberg, C.R. and Knight, J.A., Body Fluids, ASCP 1982.

POLICY CREATION :		*Date*
Author:	*Karen Forbes, MT (ASCP)*	*03/01/1999*
Medical Director:	*Kathryn Kramer, MD*	*03/01/1999*

MEDICAL DIRECTOR		
DATE	NAME	SIGNATURE
11-20-18		Kathryn O. Kramer MD
SECTION MEDICAL DIRECTOR		

REVISION HISTORY (began tracking 2011)			
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Reviewed by:

Lead	Date	Coordinator/ Manager	Date	Medical Director	Date
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