LABORATORY 8: Body Fluid Cell Count

Overview
This lab is a review of the manual cell counting procedure of body fluids.

Objectives
Upon completion of this laboratory exercise, the student should be able to:
1. Correctly classify color and transparency of body fluids such as CSF, pleural, peritoneal, and synovial fluid.
2. Perform WBC and RBC cell counts on two body fluid specimens within ± 20% accuracy using the hemacytometer.
3. Perform calculations for WBC and RBC counts from the hemacytometer.
4. Report results in the proper format.

Equipment and Supplies
1. Two body fluid specimens
2. Capillary pipets or 10uL automatic pipette
3. Kimwipes
4. Hemacytometer with coverslip or disposable hemacytometer
5. Lens cleaner, lens paper, and alcohol prep pads
6. Microscope
7. Cell counter
8. Petri dish with cover and dampened cotton ball (if using glass hemacytometer)

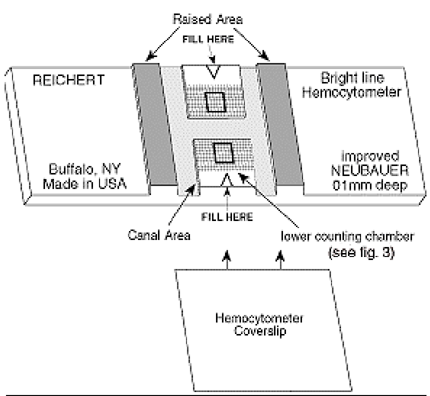
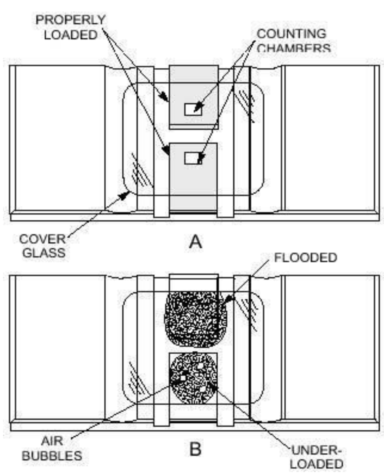
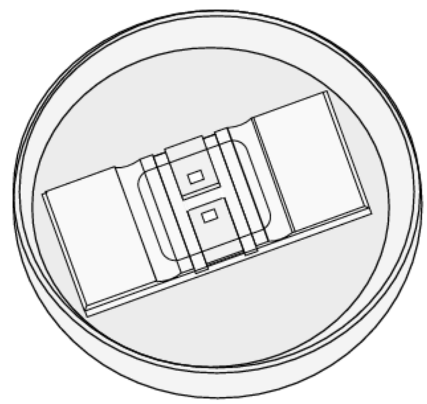


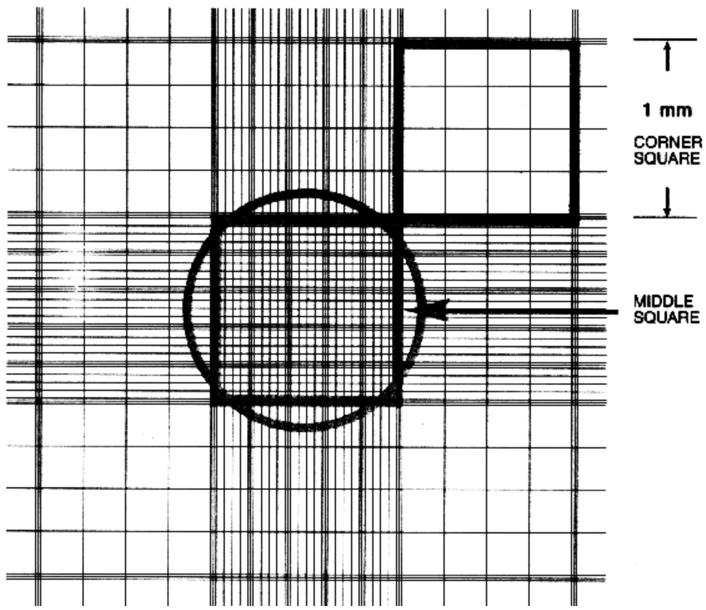
Fig. 2 Hemocytometer, showing counting chambers and fill points.





Principles & Related Information
A hemacytometer is a thick glass microscope slide with a laser-etched grid of perpendicular lines.
The typical Neubauer-type hemacytometer consists of two identical ruled counting areas composed of laser-etched lines that define squares of specific dimensions. When a cover glass of specified uniform thickness is positioned over the ruled areas of the hemacytometer, a chamber with a depth of 0.1mm is created. By counting the cells within a specified part of the grid, it is possible to calculate the number of cells in a volume of the fluid.

STANDARD HEMOCYTOMETER CHAMBER



To the left is a drawing representative of how the entire grid area of one of the hemacytometer chambers would appear under the microscope. The total dimension of the grid area is 3mm X 3mm.

There are nine (9) larger squares contained within the grid; each of which measures 1 mm x 1mm. Although their internal appearance varies, each of these 9 larger squares has a total volume of 0.1 mm^3.
» The volume of a large square = 0.1 mm^3.

The large square in the middle of the hemacytometer grid has been subdivided into twenty-five (25) smaller squares. Each of these smaller squares has a volume of 0.004 mm^3.

» The volume of a small square is 0.004 mm^3.

Body fluid specimens are collected by way of a minor surgical procedure.

Fluid	Collection procedure
cerebral spinal fluid (CSF)	Spinal tap/lumbar puncture (needle inserted between the 3rd and 4th OR 4th and 5th lumbar vertebra)
plural fluid	Thoracentesis (needle aspiration of fluid in lung cavity)
pericardial fluid	Pericardiocentesis (needle aspiration of fluid surrounding the heart)
synovial fluid	Arthrocentesis (needle aspiration of joint fluid)

Normally, the volumes of these fluids (with the exception of CSF) are too low to be easily obtained. It is only in conditions of disease or trauma would there be justifiable reason and volume for testing. The exact number and types of body fluid specimen tubes drawn depends on the fluid type and volume, the reason for the procedure, and the test procedures being ordered. Body fluid testing must be performed ASAP, as the deterioration of specimen components occurs quickly.

The appearance of body fluids can provide valuable diagnostic information about a patient's condition. Both color and clarity must be recorded on the report form. An abnormal color or clarity in a body fluid can provide clues to suspected conditions or disease. A milky appearance may indicate involvement by the lymphatic system and a reddish coloring often indicates the presence of blood. The cloudy thick purulent fluid is often the result of microorganisms and WBCs.

White and red blood cell counts provide important information for the diagnosis and treatment of diseases involving CSF, serous, and synovial cavities. Under normal circumstances, these fluids have very low counts. Increased numbers of cells can be seen as the result of trauma or a disease process.

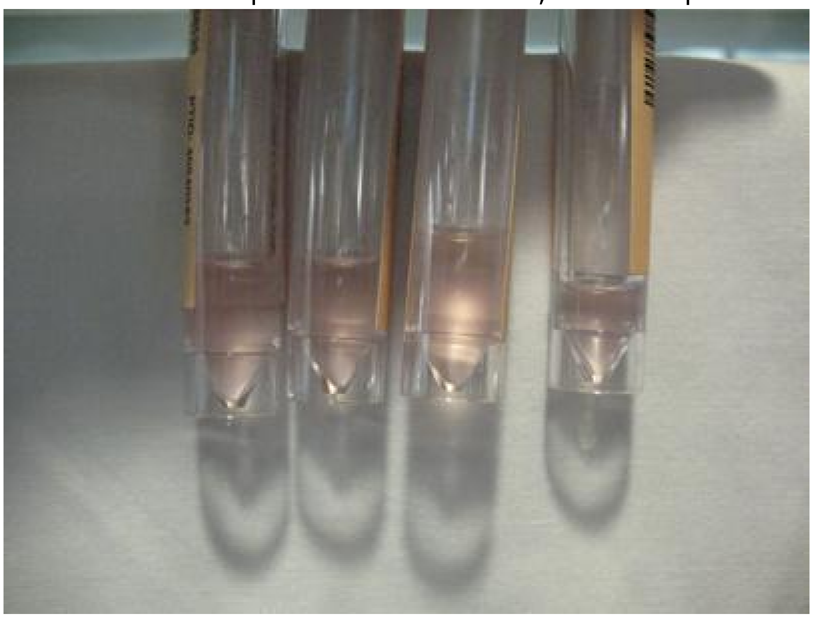
Cerebral spinal fluid (CSF)
CSF is a clear colorless fluid produced by the choroid plexus of the brain at a rate of approximately 20 mL/hr. in the adult patient. The fluid flows through the subarachnoid space where it serves to cushion, protect and nourish the brain cortex before reabsorption by the arachnoid villus at about the same rate as its production.

The CSF specimen is collected by lumbar puncture between the 3rd and 4th or 4th and 5th lumbar vertebrae and placed in three sterile tubes. The tubes are labeled 1, 2, and 3 in the order in which they are drawn. Tube 1 is used for chemical and serological tests. Tube 2 is used for microbiological tests. Tube 3 is used for cell counts and differentials.

The analysis of cerebral spinal fluid (CSF) provides physicians a means of evaluating the central nervous system. Spinal tap and CSF analysis is performed on patients suspected of having meningitis and encephalitis (due to bacterial, viral, fungal or parasitic organisms); neurosyphilis, degenerative nerve diseases such as multiple sclerosis and Guillain-Barre syndrome; subarachnoid hemorrhages; and metastatic tumors including leukemias.

The CSF is normally colorless and clear in appearance. Yellow, pink or orange are abnormal colors. Yellow color is referred to as xanthochromic. The normal CSF WBC count is 0-5 WBC per uL in an adult patient (0-3/uL newborns) while the RBC count is normally 0/uL.

If the CSF sample contains equal amounts of blood in each tube, the patient likely has suffered a cerebral hemorrhage. (see picture below). If tube 1 contains blood, but tubes 2 and 3 contain decreasing amounts, a traumatic tap can be suspected. If there is a lot of blood, it is possible for a small clot to form. A cell count on these tubes will NOT produce accurate results, due to the presence of the clot(s).



Serous fluids

Serous fluids are the small amounts of fluids within closed cavities of the body. They primarily function to provide lubrication between the organs and the tissues lining the body cavity. Serous fluids are ultra filtrates of plasma. These fluids are usually pale yellow to yellow in color and clear. They are normally produced and reabsorbed at constant and balanced rate. An imbalance in the production and/or reabsorption results in an effusion or increased volume. An effusion resulting from a systemic problem would likely be classified as a transudate and one resulting from a local inflammatory response would be an exudate.

Serous fluids are usually collected in sterile tubes and sent to the laboratory for microbiology culture, cell counts, differential, cytology, and chemical testing.

The serous fluids commonly discussed are the pleural fluid (lung cavity) and the pericardial fluid (surrounding the heart). In addition, the peritoneal fluid, also known as the ascites fluid, is found in the abdominal cavity.

Synovial fluid, from the joint cavities, is also an ultrafiltrate of plasma, but contains hyaluronate which makes it much more viscous than plasma. Normally pale yellow and clear, but may have some slight cloudiness due to the presence of synovial cell debris and fibrin.

The Body Fluid Cell Count

Traditionally, body fluid white and red blood cells have been done by manual methods using a hemacytometer counting chamber. The average number of cells (individually) counted on both sides of the chamber is placed into a formula that calculates the number of cells per unit of volume.

The basic formula to calculate manual cell counts:

Average number of cells counted X the Dilution Factor

$$\frac{\text{Average number of cells counted X the Dilution Factor}}{\text{Number of squares counted X Volume of each square}} = = \text{\# Cells/uL}$$

Notes:

1. Since the body fluids do not normally contain many cells, they *rarely need to be diluted* prior to plating on the hemacytometer. When the specimen is NOT diluted, the dilution factor (the number that is put in the calculation formula stated above) is "1".

On the rare occasions when a dilution is required, *normal saline* is the diluent of choice, and appropriate dilution factor must be included in the formula for calculating the results. See listed references for additional directions on use of the hemacytometer and performance of cell counts.

2. The number of squares and which squares to be counted generally depends on how many cells are present. If there are very few cells present, counting the cells seen in the entire nine (9) large square grid will produce the more accurate results. If there appears to be a large number of cells present, counting a fewer number of squares or counting the small squares may be acceptable. If initially unsure of the number of squares to count, consult with your lab instructor.

Procedure (This is for a glass hemacytometer)

1. Thoroughly mix the body fluid specimen. Observe and record the body fluid's color and clarity.
2. Clean hemacytometer and coverslip with alcohol. Gently wipe it dry using a soft lint free cloth or lens paper. Be sure that the alcohol is completely dry before attempting to plate the fluid.
3. Using a capillary tube for the older hemacytometer and a 10 uL semi-automatic pipet for the plastic hemacytometer, draw up a small volume of the well mixed fluid.
4. To plate the fluid onto the hemacytometer, touch the end of the filled capillary pipet to the "V Slash" area on the hemacytometer beneath the coverslip. Due to capillary action, fluid will flow from the pipet onto the hemacytometer. Avoid introducing air bubbles and that the hemacytometer is filled properly.
5. Set the hemacytometer in a covered petri dish. Include a damp cotton ball as this will prevent the specimen from drying out. Allow it to set undisturbed for five (5) minutes so that the cells settle into one plane.
6. Remove hemacytometer from the petri dish and wipe any moisture from the bottom of the chamber.
7. Place on the microscope stage and focus on 10X. The background light in the field should be low, as the contrast will improve the cell view.
8. Start by locating the upper left large square on the low power objective. For samples with few cells present, count all 9 of the large hemacytometer squares, which is the entire grid. For samples with numerous cells, count fewer numbers of squares such as the 4 outer large squares or the 25 small squares within the center square. Consult with your lab instructor, if unsure.
9. Once you have located the appropriate starting point on low power, carefully switch the objective of the microscope to 40X to begin your count. If possible, you should count the WBC and RBC simultaneously – but keeping the count results separate.
10. As you move from square to square you must continuously, but gently, focus up and down using the microscope's fine adjustment knob to see the details of each cell.
    a. RBC – smooth, shiny surface, highly refractile, and may have a yellowish or reddish tinge. It may be a round shape or may be crenated (spiky), but its surface will still be smooth and shiny.

b. WBC – have a rough or grainy surface, not very refractile. May have grayish or bluish tinge. Shape is generally round, but may have rougher or more irregular outer edges.

c. Debris/Junk – usually very retractile with indistinct shapes and sizes (not reported).

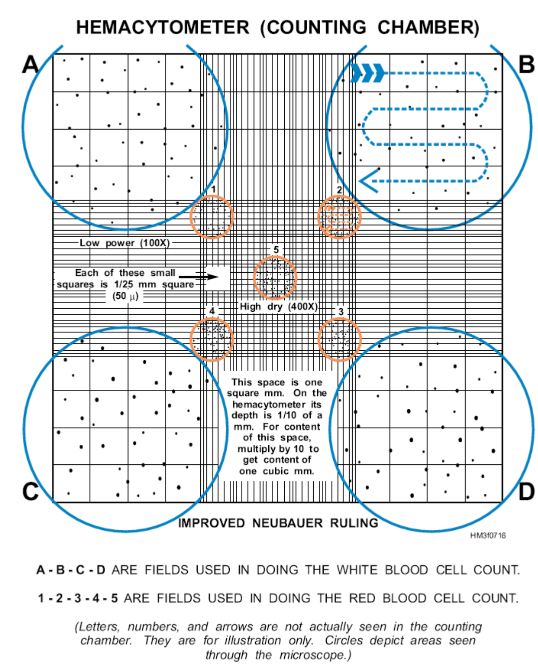
Procedure (This is for a disposable hemacytometer)

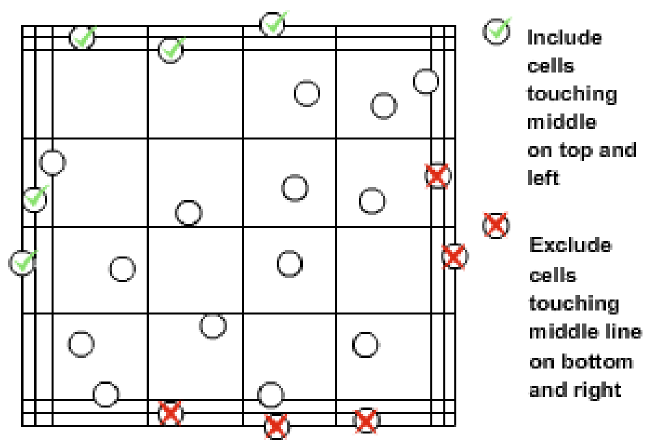
1. Watch the demonstration from your instructor.
2. Obtain a disposable hemacytometer, 10uL automated pipette, and pipette tips from your instructor.
3. Open the hemacytometer package.
4. Mix your sample gently, being sure that all is mixed off the bottom of the tube. The cells settle out fast.
5. Pipetted 10uL of the sample into side A of the hemacytometer.
6. Pipette 10uL of the sample into side B of the hemacytometer.
7. Place the hemacytometer on the stage of the microscope.
8. Count side A, then count side B (depending on what you are counting, see below).
9. Record each side and perform the calculation.
10. Dispose of the hemacytometer in the biohazard container.

Counting the cells

To obtain valid reproducible results, it is important to count the fields in a prescribed order. "B" in the picture drawing below left shows the direction to follow for counting cells within the square. (Do not be concerned with other information on this drawing at this time.)

It is critically important that you count only those cells within the grid's square as well as any that touch the *left* or *upper lines*, as indicated in the picture drawing on the right. Strict adherence to this pattern of counting is necessary to <u>avoid double counting of cells</u> or <u>not counting those cells that should be counted.</u>





After finishing the count on one side of the hemacytometer, check to make sure your specimen is not drying up under the coverslip. If it is, replate it. Otherwise, count the cells on the other side of the hemacytometer. When finished with your count be sure and clean the hemacytometer with alcohol. Allow it to dry and carefully return it to its box/drawer.

Precision check. The counts on each side of the hemacytometer must agree within 4 cells of each other if the cell count is less than 50. If the cell count is greater than 50, the two sides must be within ten percent (10%) of each other.

How do you do the math for the 10% value?
Example: Side A = 300
Side B = 350
The larger number, 350, is multiplied by 10%, which is 35. Subtract 35 from 350 and the answer is 315 and side A is 300. Therefore, side A and side B are not within 10% of each other. This count would have to be performed again.

15. The Cell Count:

Basic Formula		
$\frac{\text{Average number of cells counted X the Dilution Factor}}{\text{Number of squares counted X Volume of each square}}$	= =	# Cells/uL

Continuing with above example:

Average number of WBC cells counted (20 + 21 = 41 ÷ 2 = 20.5)
Dilution Factor = 1 (no dilution was made; therefore, this number is 1)
Number of squares counted = all 9 large squares
Volume of each (large) square = 0.1 mm

Calculation: $\frac{20.5 \times 1}{9 \times 0.1} = 22.7$ *Body Fluid results are reported in whole numbers; therefore, the tech would report 23 WBCs/uL

Body Fluid Analysis _____/20 points
XYZ Research Facility
Austin, Texas 78701

Perform two body fluid cell counts on the patients provided and record the results using the proper units.

Patient name		
Patient ID		
Specimen type		
Color		
Clarity		
WBC count results	* **Show WBC calculation here**	
RBC count results	* **Show RBC calculation here**	
Testing performed by		**Date/Time**

Patient name		
Patient ID		
Specimen type		
Color		
Clarity		
WBC count results	* **Show WBC calculation here**	
RBC count results	* **Show RBC calculation here**	
Testing performed by		**Date/Time**

Laboratory Exercise #8: Study Questions

Student Name ___________________________ Date ________________________ ____ / 24 points

Instructions: Answer the questions as appropriate; unless otherwise stated, each question is worth one point. Lab Study Questions are due by the end of the following lab period. Using lecture notes, reading assignments and information presented in this lab, answer the following questions.

1. Complete the following table.

Cerebral Spinal Fluid

Tube number	Laboratory Department
Tube # 1	
Tube # 2	
Tube # 3	

2. True or False? (Circle one)

CSF and other body fluids can be allowed to sit for up to four (4) hours before laboratory testing takes place. **Briefly explain your answer.**

(3 pts total)

3. When performing an RBC count on a CSF sample the technician gets the counts below. Determine whether the counting procedure was precise enough to proceed with the cell calculation. Show your work and explain your answer and any action to be taken.
   Side 1 = 290 RBCs
   Side 2 = 270 RBCs

4. Give the basic hemacytometer formula for calculating manual cell counts.

5. Using the following information, calculate the body fluid cell count. Report your result in the space provided using correct units. No dilution was used. (3 pts)

WBC: All 9 large squares were used for the count
Side 1 = 18 WBC
Side 2 = 20 WBC

RBC: 5 of the 25 small squares were used for the count
Side 1 = 39
Side 2 = 41

WBC =

RBC =

6. Complete the following table. (3pts)

Type of fluid	Normal color	Normal clarity

CSF		
serous		
synovial		

7. List at least five (5) reasons for performing a spinal tap procedure and CSF analysis. (5 pts)

1.
2.
3.
4.
5.

8. Define xanthochromia.

(2 pts)
9. State four (4) ways a traumatic spinal tap can be distinguished from a cerebral hemorrhage in CSF analysis, according to the lecture guide and textbook authors.

Characteristic	Indicate which Traumatic tap OR CNS hemorrhage?
1.	
2.	
3.	
4.	

(2 pts total)
10. List the CSF normal values for the following. Be sure to use correct units, according to the lecture guide and textbook authors.

CSF protein	
CSF glucose	
WBC count	
RBC count	

(1 pt total)
11. According to the textbook's authors (Mundt & Shanahan) what microorganisms most commonly cause bacterial meningitis?

EXTRA CREDIT
What meningitis causing microorganism is most commonly associated with a patient having a cerebral shunt?

What meningitis-causing microorganism is commonly found in an immunocompromised patient?