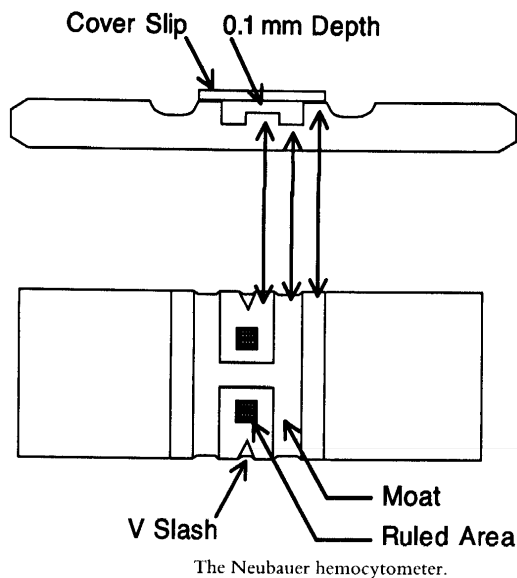
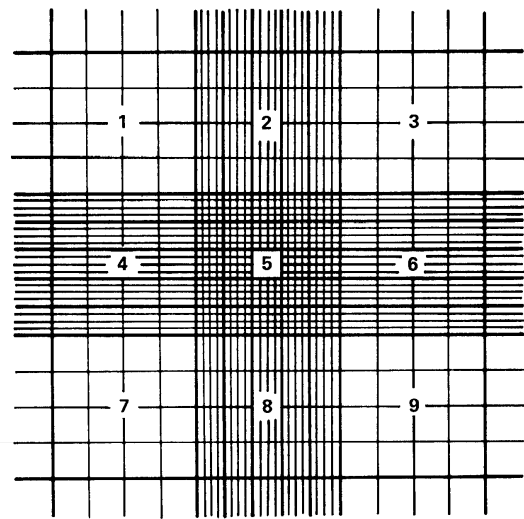


**Body Fluids Lab5 - Cell Counts**

- Points:** Points are awarded for Pre-test (10pt.), Skills (20 pts. total: includes general points awarded for neatness, lab clean-up, teamwork, etc. Student counts are expected to be within  $\pm 20\%$  of instructor values), as well as successful and timely completion of Study Questions. Study Questions are due by the end of the next lab period, or as designated by the course instructor.
- Objectives:** According to standards set by the instructor, the student will be able to:
1. Correctly classify color and transparency
  2. Perform WBC and RBC cell counts on two body fluid specimens within  $\pm 20\%$  accuracy using the hemacytometer.
  3. Use appropriate recording format to report results .
  4. Use quality control results to determine the acceptability of test results.
  5. Answer all pre-test and study questions using related information found in the textbook, lecture guide, and this lab procedure.
- Materials:**
1. Two body fluid specimens
  2. Capillary pipets, and Kimwipes
  3. Hemacytometer with coverslip
  4. Lens cleaner, lens paper, and alcohol prep pads
  5. Microscope
  6. Cell Counter
  7. Petri dish with cover and dampened cottonball
- References:** Current hematology course textbook.  
Current urinalysis / body fluids course textbook.
- Principle:** Body fluid specimens are usually collected in three sterile tubes and 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. In rare cases, four tubes may be drawn in which case Tube 1 may not be used. All body fluid testing should be performed immediately upon specimen receipt as deterioration of specimen components can occur rather 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. CSF is normally colorless and clear in appearance. Yellow, pink or orange are abnormal colors for CSF. The appropriate term for these specimens is 'xanthochromic Refer to the lecture materials for more information on xanthochromia.
- Synovial fluid can be colorless to a pale yellow; its clarity is generally clear, but may have some slight cloudiness due to the presence of synovial cell debris and fibrin. Serous fluids, like synovial fluid is an ultrafiltrate of plasma and may be light yellow in color.
- Serous fluids are normally clear. Serous fluids come from the pleural, pericardial and peritoneal body cavities. Additional information on these fluids is available in your textbook and the lecture materials.
- WBC and RBC cell counts provide extremely important information for the diagnosis and treatment of diseases involving CSF, serous, and synovial cavities. Infections, hemorrhages, and malignancies are of primary concern.
- Most body fluids specimens do not need to be diluted prior to plating on the hemacytometer since they normally do not contain many, if any, cells. When the specimen is NOT diluted, the dilution factor (the number that is put in the calculation formula provided below) is "1". Should a dilution be necessary due to a high cell count, *normal saline* is the diluent of choice. When a dilution is required, the 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.



The Neubauer hemacytometer.



Neubauer counting chamber.

Basic formula  
for calculation of manual cell counts

$$\frac{\text{ave. \# cells counted} \times \text{dilution}}{\text{\# squares counted} \times \text{volume of each square}}$$

**Procedure:**

1. Thoroughly mix body fluid specimen.
2. Record color and clarity.
3. Clean hemacytometer and coverslip and prepare for plating. When finished with your count be sure and clean the hemacytometer with alcohol.
4. Using capillary pipet, carefully draw body fluid specimen up into the pipet until approximately 3/4 full. Hold fingertip on the end of the pipet so fluid doesn't run back out. (Procedure may vary depending on specific equipment available.)
5. 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. (Release fingertip from the end of the pipet.) Due to capillary action, fluid will flow from the pipet onto the hemacytometer. Be sure there are no bubbles and that the hemacytometer is neither under-filled nor over-filled.
6. Place hemacytometer in a covered petri dish along with a dampened cotton ball. This will keep the dish humid and avoid drying of the specimen.
7. Let set for five (5) minutes for all of the cells to settle onto one plane in the fluid.
8. Remove hemacytometer from the petri dish and carefully wipe any moisture from the bottom of the chamber.
9. Place on the microscope stage and focus initially on 10X. The background light in the field should be kept relatively low, as the cells will stand out better. Too bright of light will make the cells difficult to see.
10. For our specimens today we will probably use all nine (9) large squares – the entire grid – for our cell counts. Locate the upper left square.

11. Carefully switch the objective of the microscope to 40X and begin your count. You may or may not be able to count WBC and RBC simultaneously. Remember, there is no set squares to use for body fluid cell counts. You must decide based on the number of cells that appear to be present. For instance, if there are very few cells, count the entire hemacytometer. If the fluid is loaded with cells, you may only want to count the one center square. Then calculate your result accordingly.
12. As you move from square to square you must *continuously* but gently focus up and down using the fine adjustment knob on the microscope. This will allow you to see the details of each cell to judge whether it is an RBC, WBC, or junk.
  - a. **RBC** – will have a smooth, shiny surface, will be highly refractile, and may have a yellowish or reddish tinge to it. It may be a round shape or may be crenated (spiky) but its surface will still be smooth and shiny.
  - b. **WBC** – will have a rough or grainy surface, will not be very refractile, and may have more of a grayish or bluish tinge to it. Its shape is generally round, but may have rougher or more irregular outer edges.
  - c. **Junk** – is usually *very* refractile and has indistinct shapes and sizes. Junk annoys us, but it is NOT reported in our lab.
13. 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, re-plate it. Otherwise count the cells on the other side of the hemacytometer.
14. QC precision check. Precision is the ability to get the same result repeatedly. In this application, the 'count' in the two sides of the hemacytometer must agree with each other within a specified range.  
To determine if the two counts are in close enough agreement, you must perform the following simple statistical mathematic steps:
  - a. Add together the results obtained from both of the hemacytometer chambers. Now, take the square root of this number AND multiply that number by 2. (Set this information aside for the moment.)
  - b. Find the difference between what you obtained on side 1 and what you got on side 2. IF this number is less than the number you obtained in step a, your precision is acceptable and you can continue with the calculations.
15. If your counts are acceptable, use the basic hemacytometer formula for calculating your WBC and RBC counts.
16. Example results

Side 1 count 20 WBC

Side 2 count 15 WBC

(Difference = 5) (AVE = 17.5)

$$20 + 15 = 35 \quad \text{square root of } 35 = 5.916 \times 2 = 11.8$$

Since my difference (5) is less than the 11.8, the precision is acceptable, and I can continue with calculating the count.

$$\frac{\text{ave. \# cells counted} \times \text{dilution}}{\text{\# squares counted} \times \text{volume of each square}}$$

17.5 X 1 (because I did not make any dilution)

9 large squares X 0.1

= 19.4

Since results are reported out in whole numbers per  $\mu\text{L}$ : 19 WBC/ $\mu\text{L}$

/ 20 points

Name \_\_\_\_\_

Date \_\_\_\_\_

### Body Fluid Cell Counts

	Body Fluid #1	Body Fluid #2
Patient Name		
ID Number		
Type of Fluid		
Color		
Clarity		
WBC*		
RBC*		

\* Write the formula used to calculate the RBC & WBC counts. Show your calculations used to determine the WBC & RBC counts.

**EXERCISE 8: Body Fluid Cell Counts**

**Body Fluids**

/ 20 points

Name \_\_\_\_\_

Date \_\_\_\_\_

**Body Fluid Cell Counts Study Questions**

Unless otherwise noted, each question is worth one point. Using lecture notes, reading assignments and information presented in this lab, answer the following questions.

1. Complete the following table.

Tube number	Lab Department to which it should go for analysis.
Tube 1	
Tube 2	
Tube 3	

2. True or False? 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. Give the basic hemacytometer formula for calculating manual cell counts.

4. Using the following information, calculate the body fluid cell count. Report your result in the space provided using correct units. No dilution was used. (2 pts)

WBC: All 9 large squares were used for the count

Side 1 = 14 WBC

Side 2 = 20 WBC

RBC: 5 of the 25 small squares were used for the count

Side 1 = 33

Side 2 = 41

WBC = \_\_\_\_\_

RBC = \_\_\_\_\_

5. Complete the following table. (3pts)

Type of fluid	Normal color	Normal clarity
CSF		
serous		
synovial		

6. List five (5) reasons for performing a CSF analysis. (5 pts)

1.
2.
3.
4.
5.

7. Define xanthochromia.

8. State four (4) ways a traumatic tap can be distinguished from a cerebral hemorrhage in CSF analysis by stating the characteristic and whether it is seen in a traumatic tap or CNS hemorrhage. (2 pts)

Characteristic	Indicate which: Traumatic tap ? / seen in CNS hemorrhage?
1.	
2.	
3.	
4.	

9. List the CSF normal values for the following. Be sure to use correct units. (2 pts total)

- a. protein - \_\_\_\_\_
- b. glucose - \_\_\_\_\_
- c. WBC - \_\_\_\_\_
- d. RBC - \_\_\_\_\_

10. List the most common types of CSF infections seen in: (2 pts total)

- a. newborns - \_\_\_\_\_
- b. children - \_\_\_\_\_
- c. adults - \_\_\_\_\_
- d. immunocompromised patients - \_\_\_\_\_