

Chapter 17

BODY FLUID ANALYSIS: MANUAL
HEMACYTOMETER COUNTS AND
DIFFERENTIAL SLIDE PREPARATION

Use of Hemacytometer

Manual methods using hemacytometer for some body fluids

Used when counts are too low for accurate results on automated counters

Highly viscous fluids require pretreatment before counting

Require well-trained and technically proficient laboratorians

Have poor precision (reproducibility)

Subject to numerous errors due to the multiple steps involved

Diluents

Clear fluids do not require diluent

White blood cell (WBC) counts often use acetic acid to lyse red blood cells (RBCs) and dilute WBCs

- *Note:* Synovial fluid cannot use acids; hyaluronic acid and protein will form a mucin clot

RBC counts use isotonic solutions such as normal saline

Use appropriate calibrated pipettes to make dilutions

TABLE 17.1 Body Fluid Dilution Guidelines for Cell Counts Based on Visual Appearance

Fluid Appearance	WBC Count	RBC Count
Clear	Undiluted	Undiluted
Hazy (slightly cloudy)	1:2* dilution	Undiluted
Blood-tinged	1:2* dilution	Undiluted
Cloudy	1:20 dilution	Undiluted
Bloody	1:2* or 1:20 dilution	1:200 dilution

TABLE 17.2 Diluents for Body Fluid Blood Cell Counts*

Diluent	Cell Counts	Comments
Commercial isotonic diluents	WBC count RBC count	Diluent used in hematology analyzers for cell counting
Isotonic saline (0.85%)	WBC count RBC count	Also known as "normal" saline
Hypotonic saline (0.30%)	WBC count	<ul style="list-style-type: none"> • Lyses RBCs
Dilute acetic acid (3.0%) [†]	WBC count	<ul style="list-style-type: none"> • Lyses RBCs • <i>Do not use</i> with synovial fluids; causes mucin clot and cell clumping
Turk's solution	WBC count	<ul style="list-style-type: none"> • Lyses RBCs • <i>Do not use</i> with synovial fluids; causes mucin clot and cell clumping
Hyaluronidase (0.1 g/L) buffer solution	WBC count RBC count	<ul style="list-style-type: none"> • Prevents mucin clot formation in synovial fluids • Stain enhances nucleated cell identification

Semen Counts

Diluent is sodium bicarbonate, formalin, and an optional, stain trypan blue or gentian violet

Specimens that do not liquefy in 60 minutes require special pretreatment

Resource for standardization of sperm counts

- World Health Organization's *WHO Laboratory Manual for the Examination and Processing of Human Semen*

Hemocytometer Cell Counts

Mix specimen well for 1 to 2 minutes before filling hemocytometer chamber

Can use undiluted or diluted specimen, depending on situation

Calculations must account for dilution, if used

Do counts in duplicate

Duplicates should agree within criteria established by laboratory (usually $\leq 20\%$)

Procedure

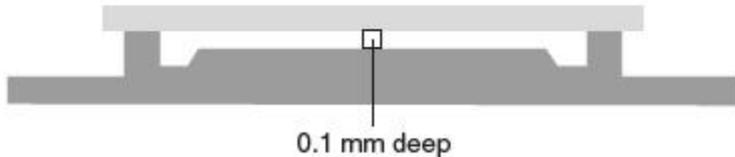
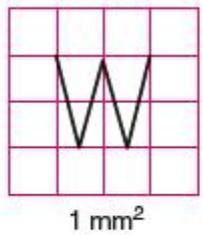
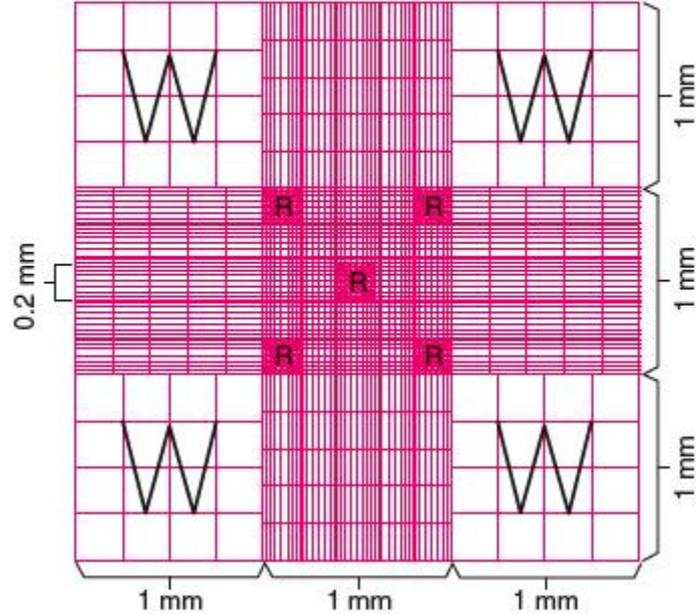
Diluted or undiluted specimen duplicates are loaded into hemacytometer chamber, and cells are allowed to settle in moist chamber to prevent drying out during settling

Number of squares counted in each chamber depends on total number of cells present and laboratory standard operating procedures

Calculate number of cells according to standard formula

BOX 17.2 Manual Cell Count Using a Hemacytometer

- Using a disposable pipette, fill both sides of a standard or disposable "improved" Neubauer hemacytometer (Fig. 17.1) with well-mixed undiluted or appropriately diluted body fluid.
- Allow the chamber to remain undisturbed for 3 to 5 minutes for the cells to settle (and RBCs to lyse, depending on the diluent used).
- Examine the hemacytometer chambers for an even distribution of cells without overlap or clumping. If overlapping or clumping is present, the specimen needs to be recharged. Mix the specimen well or possibly prepare a dilution of the fluid. Clean the hemacytometer or use a new disposable hemacytometer and fill the chambers; examine for even distribution.
- CSF, synovial, pleural, pericardial, and peritoneal fluids
 - If *less than* an estimated 200 cells are present in all nine squares¹:
 - Count cells in all nine large squares in both chambers of the hemacytometer.
 - Area counted: $9 \times 1 \text{ mm}^2 = 9 \text{ mm}^2$ on each side = 18 mm^2 .
 - If *more than* an estimated 200 cells are present in all nine squares¹:
 - Count cells in the four large corner squares (the "W" squares) in both chambers of the hemacytometer. See the "W" squares in Fig. 17.1.
 - Area counted: $4 \times 1 \text{ mm}^2 = 4 \text{ mm}^2$ on each side = 8 mm^2 .
 - If *more than* an estimated 200 cells are present in *one large square*¹:
 - Count cells in five red blood cell squares (i.e., the four corner squares and the center square within the central large square on both sides of the hemacytometer). See the "R" squares in Fig. 17.1.
 - Area counted: $5 \times 0.04 \text{ mm}^2 = 0.20 \text{ mm}^2$ on each side = 0.40 mm^2 .
- Semen
 - Spermatozoa concentration
 - Count sperm present in five red blood cell squares (i.e., the four corner squares and the center square within the central large square on both sides of the hemacytometer). See the "R" squares in Fig. 17.1. (An alternate approach is to count two large "W" squares.)
 - Area counted: $5 \times 0.04 \text{ mm}^2 = 0.20 \text{ mm}^2$ on each side = 0.40 mm^2 .
 - Round cell count
 - For the "round cell" (germ cells and WBCs) count, count the round cells in the four large corner squares and the center large square (the "W" squares) in both chambers of the hemacytometer (see Fig. 17.1).
 - Area counted: $5 \times 1 \text{ mm}^2 = 5 \text{ mm}^2$ on each side = 10 mm^2 .
- The number of cells counted in each chamber of the hemacytometer must agree within a percentage or absolute cell number. If counts from both sides do not agree, the cell count procedure *must be repeated*. Note that each laboratory establishes the acceptable precision criteria required between counts on each side of the hemacytometer (e.g., the number of cells counted in each chamber must agree within 20% or ± 8 cells, whichever is greater).



Calculations

Total number of cells counted is multiplied by dilution factor and divided by volume to obtain cells per microliter

$$\begin{aligned} & \text{Cells counted (A)} \times \text{Dilution factor (B)} \div \text{Volume (C)} \\ & = \# \text{ cells}/\mu\text{L (mm}^3\text{)} \end{aligned}$$

If cells per liter is desired, then cells/ μL is multiplied by 10^6

$$\# \text{ cells}/\mu\text{L (mm}^3\text{)} \times 10^6 \mu\text{L/L} = \# \times 10^6 \text{ cells/L}$$

Volume

Actual volume of fluid counted in cubic millimeters (microliters) is calculated using the area of squares counted times depth of chamber (0.1 mm)

Example 1

$$\begin{aligned} &\text{Cells counted (A)} \times \text{Dilution factor (B)} \div \text{Volume (C)} \\ &= \# \text{ cells/mL (mm}^3\text{)} \end{aligned}$$

Example 2

$$\# \text{ cells/mL (mm}^3) \times 10^6 \text{ mL/L} = \# \times 10^6 \text{ cells/L}$$

Slide for White Blood Cell (WBC) Differential on Fluids

Prepare immediately after collection to preserve cellular morphology

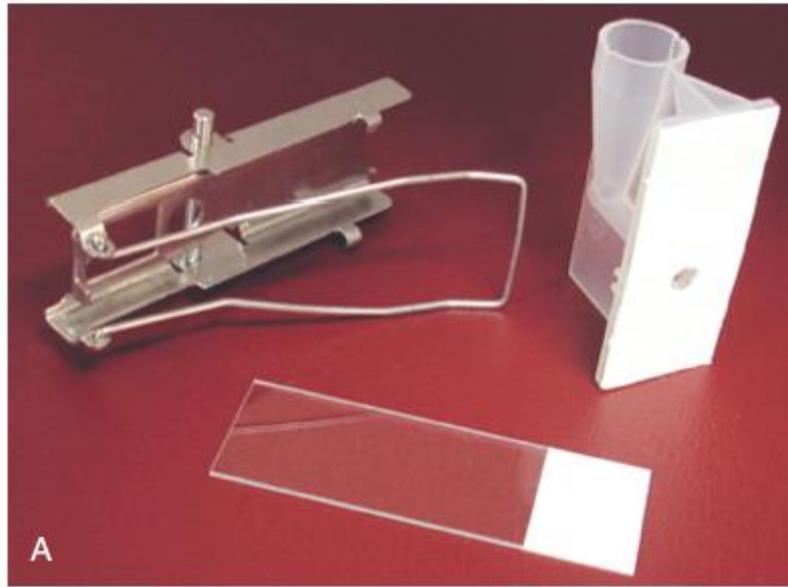
Cytocentrifugation is preferred technique

Cytocentrifuges are commercially available and use specially designed assemblies for each sample

Centrifugal force pulls body fluid from sample aliquot in chamber to slide; cells adhere to slide in a monolayer “button,” and liquid is absorbed



Thermo Fisher Scientific Inc, Waltham, MA.



Notes on Cytocentrifugation

Some cells are lost to filter paper during centrifugation, but this affects all cell types equally; distribution for differential remains accurate

Each laboratory should establish optimal time and speed of centrifugation for its cytocentrifuge

For specimens with low protein content such as cerebrospinal fluid (CSF), a drop of 22% albumin to sample chamber before adding fluid will enhance adherence of cells to slide and reduce distortion

Slides are stained with Wright or Wright-Giemsa

BOX 17.4 Distortions Associated With Cytocentrifugation

- Cells at center of cell button often smaller and have denser nuclear chromatin.
- Nuclear distortions, such as clefting or lobulation or holes in nuclei
- Nuclear lobes of PMNs localized at cell periphery
- Cytoplasmic vacuoles and/or granules localized at cell periphery
- Formation of irregular cytoplasmic processes

From Kjeldsberg CR, Knight JA: Laboratory methods. In Body fluids, ed 3, Chicago, 1993, American Society of Clinical Pathology Press.