INTRODUCTION

Blood cell counts are routinely performed using automated cell counters or hematology analyzers. However, when cell counters are not available or when blood cell counts are extremely low, manual blood cell counts can be performed microscopically using the **hemacytometer**, a special counting chamber.

THE HEMACYTOMETER

The hemacytometer is a slide for performing manual cell counts using the microscope. The hemacytometer is sometimes called a counting chamber.

The traditional hemacytometer is a heavy glass slide with two counting areas. When viewed from the top, two polished raised platforms are surrounded by moats (troughs) on three sides form the shape of an *H*. Each raised platform contains a ruled counting area. The hemacytometer must be used with a **hemacytometer coverglass** of uniform thickness (0.4 mm)

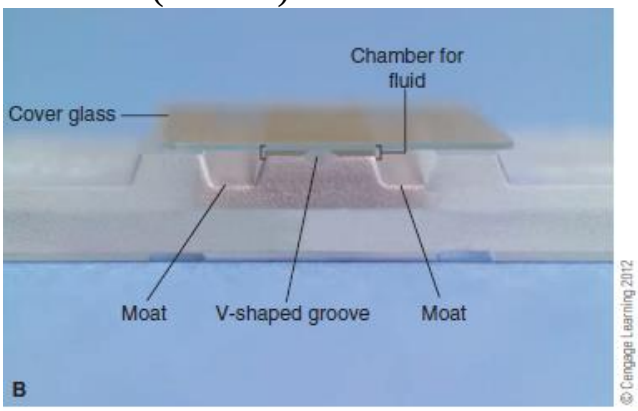


FIGURE 2-20 The hemacytometer: (A) top view of hemacytometer with coverglass in place; (B) side view of hemacytometer with coverglass in place

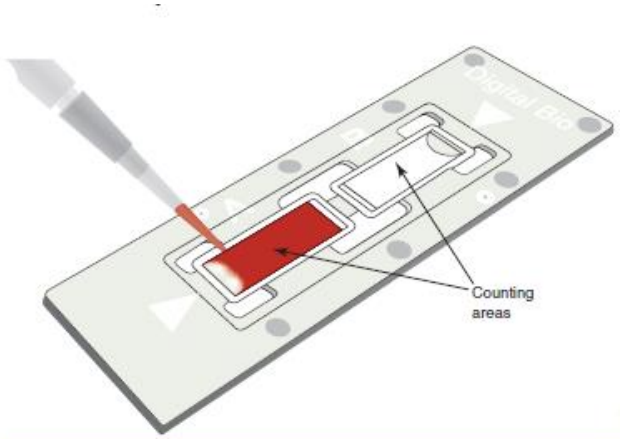


FIGURE 2-21 Diagram of disposable hemacytometer showing counting areas and sample loading points

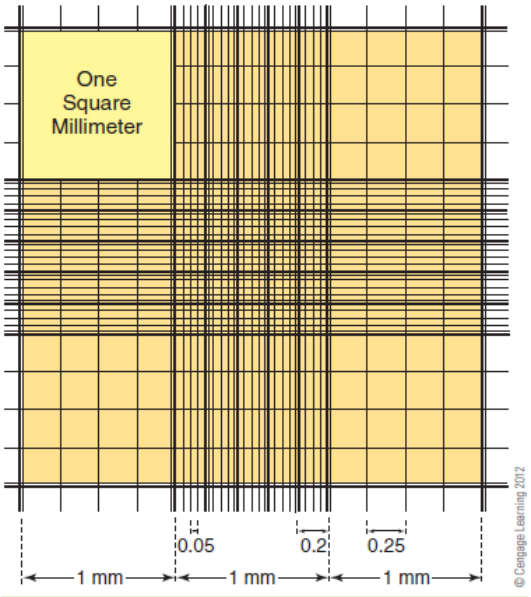


FIGURE 2-22 Ruled area of a hemacytometer chamber showing the dimensions

Ruled Counting Areas

The hemacytometer contains two identical ruled areas composed of etched lines that define squares of specific dimensions. the total ruled area on each side consists of a large square divided into nine equal squares , each 1-mm square (mm2). The total area of the large square is 9 mm2. When the cover glass is in place and fluid is introduced into the chamber.

White Blood Cell Counting Area

The area counted for a WBC count is determined by the degree of blood dilution used for the count. For blood diluted 1:100, all nine large squares of the ruled area are counted. The cells in the four large corner squares are counted when using a 1:20 dilution.

Red Blood Cell and Platelet Counting Areas

The large center square is used for platelet counts and RBC counts. This center square is divided into 25 smaller squares, which are each subdivided into 16 squares.
The entire large center square (circled) is used to count platelets . For RBC counts, only five of the 25 squares in the center square are counted. These can be the four corner squares and the center square (a through e) or five squares in a diagonal line within the large center square.

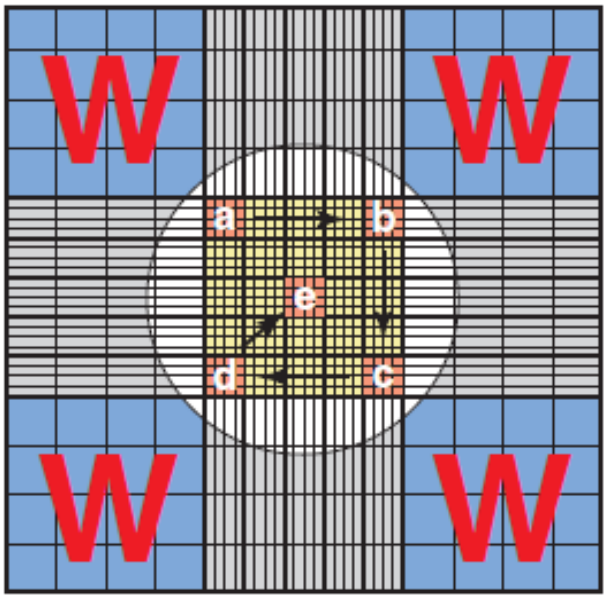


FIGURE 2-23 Cell counting areas: Cells in the four large corner squares (shown in blue) are counted for the white blood cell count; all cells in the entire center square (yellow, circled) are counted for the platelet count; the four red corner squares and center red square within the large center square (labeled a-e) are used for the red blood cell count

Dilutions and Cell Diluting Fluids

The first step is to dilute the blood using the **cell diluting fluid**.

TABLE 2-4. Comparison of manual red blood cell, white blood cell, and platelet counting procedures

CELL TYPE	CHARACTERISTIC OF DILUTING FLUID	BLOOD DILUTION MADE	DILUTION FACTOR	RULED SQUARES COUNTED	AREA COUNTED (mm^2)
Red blood cells	Isotonic for RBCs	1:200	200	5 squares within large center square	0.2
White blood cells (Leuko-TIC method)	RBC lysing	1:20	20	4 large corner squares	4.0
White blood cells (LeukoChek method)	RBC lysing	1:100	100	Entire ruled area (9 large 1 × 1 mm squares)	9.0
Platelets	RBC lysing	1:100	100	Entire large center square	1.0

Loading the Hemacytometer

Once the blood dilution is made, the hemacytometer is loaded. A clean hemacytometer coverglass should be positioned so that it covers both ruled areas of a clean hemacytometer. The hemacytometer is loaded by touching a filled capillary tube or **micropipet** to the point where the coverglass and the raised platform meet .

Viewing the Ruled Areas

The hemacytometer is placed on the microscope stage with the low power (10) objective in place,

Depending on the blood dilution system used, the four large corner squares or the entire nine large squares are used for WBC counts.

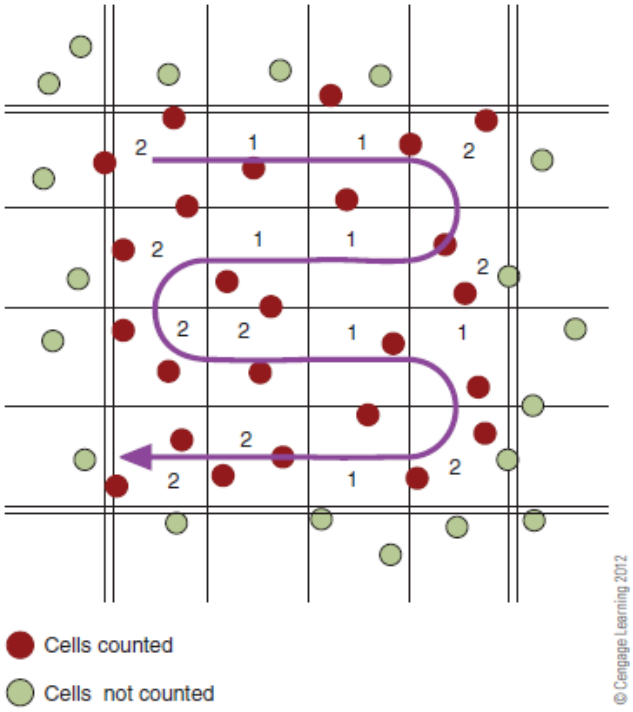
After the WBC area has been observed, the central square used for RBC counts should be located. The high power (40) objective should be carefully rotated into place.

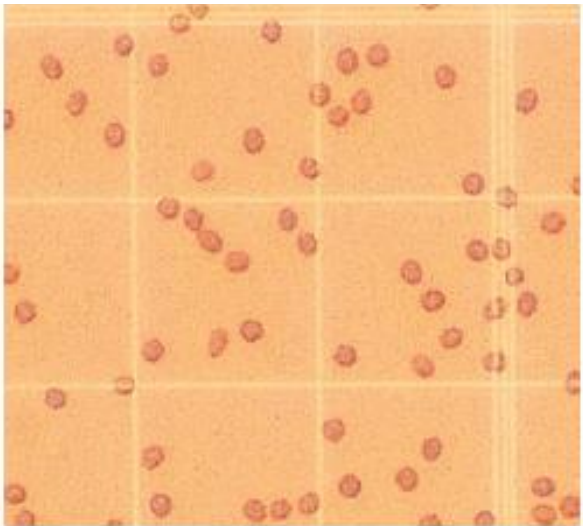
The large center square used for platelet counts and the five small squares used for an RBC count should be located.

The Cell Counting Pattern and Boundary Rule

The count should begin in the upper left corner of a square and proceed in a serpentine manner. All cells that lie completely within a square, and those that touch the left boundary of the square, or touch the top boundary of the square, are considered to be in that square and are counted. Cells that touch the right boundary or lower boundary of a square should not be counted in that square even if they lie mostly within the square .

The cells in the appropriate squares should be counted on both sides of the chamber. The results for the two sides are totaled and an average calculated. The average is used in the formula to calculate the cell counts.





1. Count the cells:

Side 1		Side 2	
Square	**Cells counted**	**Square**	**Cells counted**
a	110	a	105
b	100	b	115
c	90	c	100
d	95	d	106
e	105	e	94
Total	500	Total	520

2. Compute the average:
   A. 500 + 520 = 1020 cells
   B. 1020 ÷ 2 = 510 average

3. Calculate the count:

$$\textbf{RBC} = \frac{\textbf{Average \# cells counted} \times \textbf{Dilution factor}}{\textbf{Area counted (mm}^2\textbf{)} \times \textbf{D(0.1 mm)}}$$

$$\text{RBC} = \frac{510 \times 200}{0.2\ \text{mm}^2 \times 0.1\ \text{mm}}$$

$$\text{RBC} = 510 \times 10{,}000/\text{mm}^3\ (\text{or } \mu\text{L})$$

$$\text{RBC} = 5{,}100{,}000/\mu\text{L}\ (\text{reported as } 5.1 \times 10^6/\mu\text{L or } 5.1 \times 10^{12}/\text{L})$$

FIGURE 2-26 Sample calculation of a red blood cell count using a 1:200 dilution

A. Perform white blood cell count using 1:20 dilution:

1. Count the cells:

Side 1		Side 2	
Square	**Cells counted**	**Square**	**Cells counted**
1	30	1	36
2	29	2	32
3	33	3	33
4	32	4	35
Total	124	Total	136

2. Compute the average:

$$\frac{124 + 136}{2} = 130$$

3. Calculate the count:

$$\textbf{WBC} = \frac{\textbf{Average cells counted} \times \textbf{Dilution factor}}{\textbf{Area counted (mm}^2\textbf{)} \times \textbf{Depth (0.1mm)}}$$

$$\text{WBC} = \frac{130 \times 20}{4\ \text{mm}^2 \times 0.1\text{mm}}$$

$$\text{WBC} = 130 \times 50 / \text{mm}^3\ (\text{or } \mu\text{L})$$

$$\text{WBC} = 6{,}500/\ \mu\text{L}\ (\text{reported as } 6.5 \times 10^3/\ \mu\text{L or } 6.5 \times 10^9/\ \text{L})$$

TABLE 2-7. Reference ranges for white blood cell counts

AGE	AVERAGE		RANGE	
	CONVENTIONAL UNITS (CELLS/μL)	SI UNITS (CELLS/L)	CONVENTIONAL UNITS (CELLS/μL)	SI UNITS (CELLS/L)
Newborn	18,000	1.8×10^{10}	9,000–30,000	$9.0–30.0 \times 10^{9}$
One year	11,000	1.1×10^{10}	6,000–14,000	$6.0–14.0 \times 10^{9}$
Six years	8,000	8.0×10^{9}	4,500–12,000	$4.5–12.0 \times 10^{9}$
Adult	7,400	7.4×10^{9}	4,500–11,000	$4.5–11.0 \times 10^{9}$

TABLE 2-5. Reference ranges for red blood cell counts

AGE/GENDER	REFERENCE RANGES	
	CONVENTIONAL UNITS CELLS/μL	SI UNITS CELLS/L
Adult male	$4.5–6.0 \times 10^{6}$/μL	$4.5–6.0 \times 10^{12}$/L
Adult female	$4.0–5.5 \times 10^{6}$/μL	$4.0–5.5 \times 10^{12}$/L
Newborn	$5.0–6.3 \times 10^{6}$/μL	$5.0–6.3 \times 10^{12}$/L