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Estimation of Erythrocytes from a Blood Smear by a "Dry Chamber" Method.

EMIL MARO SCHLEICHER. (Introduced by R. W. Keeton.)

From the Department of Medicine, University of Illinois, College of Medicine, Chicago, Ill.

If one could make an accurate red blood cell count from a smear, it would save time in manipulating the diluting pipette, hemacytometer, and avoid errors arising from sampling, diluting and chance distribution of the cells in the chamber.

The feasibility of estimating the erythrocytes from a blood smear was suggested by a former associate.* The "Dry Chamber" method reported briefly in this communication makes this possible. It has been in use for the past 2 years and is based on 2000 red blood cell counts, checked by the hemacytometer method, using standardized pipettes (Bureau of Standards). For the purpose of discussion the method may be presented under 4 headings.

I. *Making the Standard Smear.* For those not accustomed to making micro-slide preparations it is recommended that oxalated blood be drawn up to the 0.5 mark of a red blood cell dilution pipette, expelling the sample on a slide and then spreading the blood with a sharp cornered counting chamber cover glass, size 20x26,† held at an angle of 45°. The margin-free smear should be about 40 to 50 mm in length, and obviously 20 mm in width. When one has learned to judge the proper size of the drop, successive preparations will vary but little from each other. One may now use finger blood and proceed as is customary in making a red blood cell count. Wipe off the first drop. Permit the second drop to approximate the standard size. Pick up the blood sample with the 20 mm edge of the spreader. Apply to the slide and allow the blood to spread to a uniform layer along the edge of the cover glass. Push the spreader with an even, quick, sure movement towards the other end of the slide. The proper smear should be margin-free, devoid of coagulum, fibrin threads, waves or foamy appearance. This film approximates the volume of whole blood used in the red cell dilution pipette and if the second drop is collected the cellular quantities should be the same by either

* Suggested by Dr. E. A. Sharp, Detroit, Michigan.

† Made to specifications by Thomas and Company, Philadelphia.

method. The length and width of the blood smear assures an even distribution of the cellular elements. The air dried smear is stained, and air dried.

II. *Adjustment of Microscope.* The optical setting given below results in a constant which permits the estimation of the erythrocytes similar to that of the hemacytometer.

Optical Setting.‡

(a) Eye piece 10x.
(b) Tube length 160 mm.
(c) Oil objective 1/12.
(d) Whipple's ocular micrometer disk 7x7 mm square.§

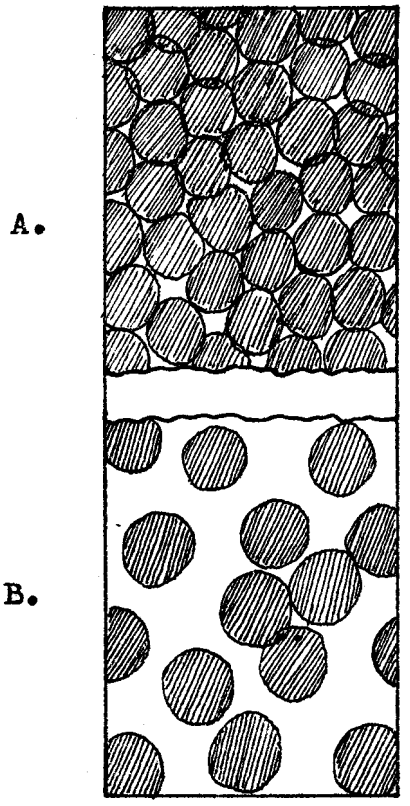

FIG. 1.
Normal Blood.
A. Minimum boundary pattern.
B. Maximum boundary pattern.

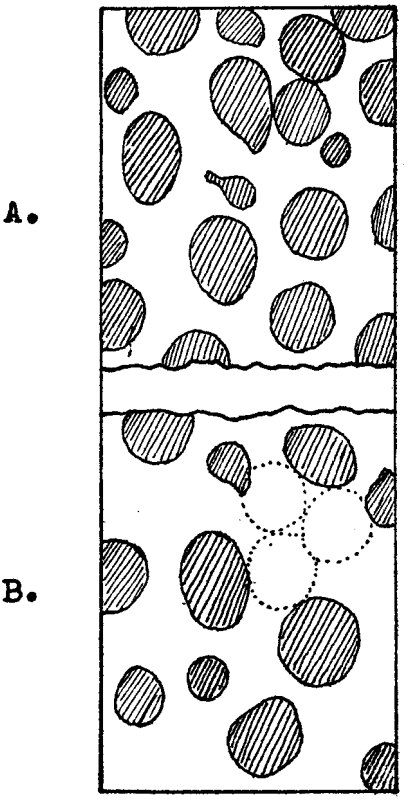

FIG. 2.
Pathologic Blood.
A. Minimum boundary pattern.
B. Maximum boundary pattern.
(The average cell is indicated by the interrupted lines)

‡ Zeiss, Standard Routine Microscope.

§ Standard Methods f. the Exam. of Water and Sewage, Am. Health Assn., 8th edition, pp. 178-79, 1936.

III. *Determination of the "Usable Area".* Cover the blood smear with a thin film of immersion oil and examine microscopically under low power for the determination of the usable area and its boundaries. A usable area is defined as that portion of a standard smear which shows the cellular elements evenly distributed according to the following specifications:

Minimum and Maximum Boundaries of the Usable Area for Normal and Pathological Blood.

A. *Normal Blood* (Fig. 1). (a) Minimum boundary: The erythrocytes should touch or slightly overlap each other..

(b) Maximum boundary: The erythrocytes should not be more than one average cell diameter apart.

B. *Pathologic Blood* (Fig. 2). (a) Minimum boundary: The erythrocytes should not be more than one average cell diameter apart.

(b) Maximum boundary: The erythrocytes should not be more than 3 average cell diameters apart.

The above specifications for the boundaries should be observed in at least 14 out of 15 low power micrometer squares. When low power is used with the micrometer square *in situ,* the square will resolve about 15 times into the 20 mm width of the smear. The boundary limits are read off the vernier scale of the mechanical stage and the midpoint determined by adding the boundary figures and averaging them. For example: Minimum boundary at 40 mm; maximum boundary at 60 mm; midpoint is at 50 mm.

Bring the 50 mm mark opposite the "0" mark on the fixed small vernier scale. Change to the oil immersion objective and move it directly vertical to the edge of the smear. The upper side of the ocular micrometer square should be parallel with the edge of the smear. Move the objective vertically a distance of 10 mm toward the center of the blood film; thus, the *"optimal micrometer square"* of the usable area is established (Fig. 3).

The proper fixing of these boundaries is essential since the

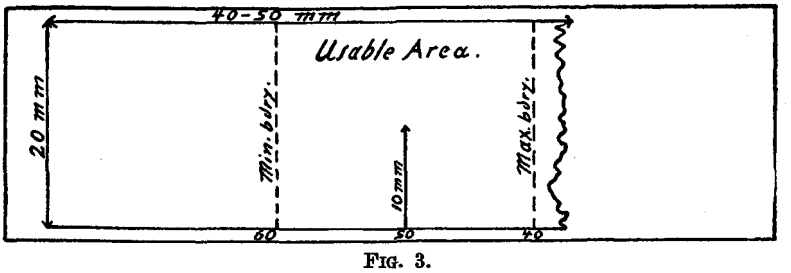

FIG. 3.

Margin free blood smear showing Usable Area with arrow point at optimal micrometer square.

magnitude of the numerical deviation from the counting chamber quantity depends on the proper placing of these limits.

Counting of the Erythrocytes. Count the erythrocytes within the boundary of the optimal micrometer square observing the same rule as used for the hemocytometer method. Mark the figure down. Count one large micrometer square adjacent to each side of the optimal micrometer square, thus counting 5 large squares consisting of 500 small squares in a cross-like fashion (Fig. 4-A). Average the total number of erythrocytes to obtain the number per ocular micrometer square (Fig. 4-B).

IV. *Comparison Between the Hemacytometer Unit and the Dry Chamber Unit.*

1. Hemacytometer Unit: In the counting chamber it should be recalled that the unit is 1 sq mm, subdivided in 1/25 sq mm. The later unit is made up of 16 small squares 1/20 mm each. Only the cells on 5/25 sq mm or 80 small squares are counted. The number of erythrocytes of undiluted blood is found by multiplying the RBC per 5/25 sq mm by 50x200, or 10,000, which is equivalent to adding 4 zeros.

2. Dry Chamber Unit: The ratio between the hemacytometer unit 1/25 sq mm and that of the ocular micrometer square is 1 : 0.72 and is referred to in this paper as "Dry Chamber Unit" or D.C.U. This unit has been found to be constant in a series of 2000 red blood cell counts done by both methods. In order to find the approximate equivalent erythrocyte number per 1/25 mm the number per micrometer square is multiplied by the D.C.U. 0.72, the resulting number is again multiplied by 5 to obtain the sum of

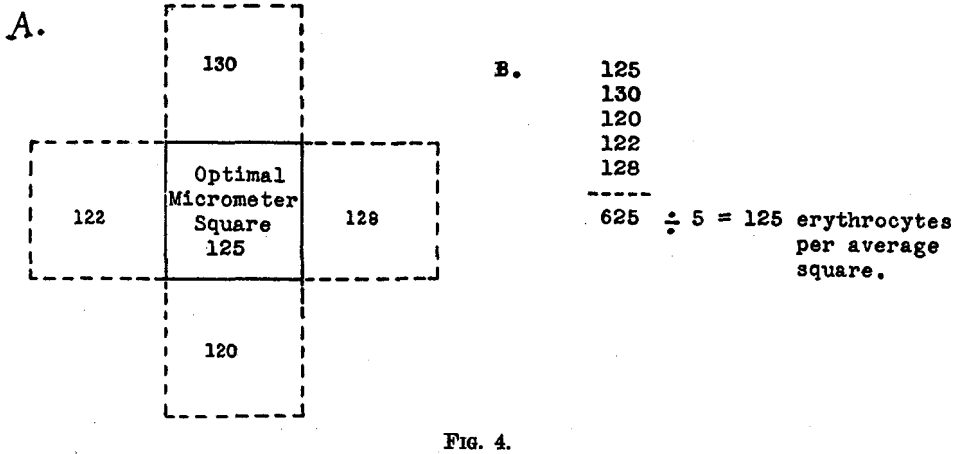

FIG. 4.

A. Arrangement of the additional fields to be counted. The number within the squares representing erythrocytes.

B. Method of arriving at the erythrocytes per average square.

TABLE I.
Comparison of Erythrocyte Counts Made by Hemacytometer and Dry Chamber Methods.

Diagnosis	Hemacytometer R.B.C. in mill.	Dry chamber R.B.C. in mill.	Experimental error of dry chamber
Pernicious anemia	1.360	1.368	+ 8
Sickle cell anemia	1.950	1.944	— 6
Acute hemorr. anemia	2.610	2.628	+18
Cooley's anemia	3.342	3.348	+ 6
Cong. elliptocytosis	3.730	3.708	—22
Cong. hemol. icterus	4.325	4.325	0
Chron. myel. leukemia	4.740	4.752	+12
Normal	5.040	5.040	0
Pern. anemia after liver therapy	6.130	6.105	—25
Polycythemia vera	8.750	8.712	—38

erythrocytes per 5/25 sq mm. To the product add 4 ciphers, as is done when the counting chamber sum of 5/25 sq mm has been determined. For example: RBC per average micrometer square is 125 erythrocytes; then $125 \times 0.72 \times 5 = 450$ cells, equivalent to 5/25 sq mm on the counting chamber. Add 4 ciphers to obtain the equivalent number of erythrocytes per cubic millimeter, or X number of erythrocytes $\times$ $0.72 \times 5 \times 10{,}000 =$ RBC equivalent to mm^3.

A total of 2000 comparative counts disclosed that the number of cellular elements found within the optimal square does not depend upon the size, shape, adhesiveness, nor specific gravity, but that their distribution in the standard smear is proportional to their mass.

The quantitative error of the Dry Chamber method is within $\pm 200{,}000$ permitted for the hemacytometer counts (Table I).

Summary. A method is described that permits estimation of erythrocytes from a blood smear. A specific optical setting is used yielding a ratio of the "Dry Chamber Unit" to the hemacytometer unit of 1: 0.72. On the blood smear a usable urea is determined. Its boundaries are described. The midpoint fixes the position of the Optimal Micrometer Square. Within this area the erythrocytes are counted in the same manner as done for the hemacytometer method. The average number of erythrocytes of 5 ocular micrometer squares is used for the estimation of the equivalent RBC number found with the hemacytometer method. A total of 2,000 comparative counts have been made on bloods ranging from 720,000 to 8,750,000 cells per mm^3. The Dry Chamber Method yields totals that are comparable with that of the hemacytometer. The quantitative error of the Dry Chamber count is within $\pm 200{,}000$, the standard error allowed for the counting chamber.