



RECOMMENDED METHODS FOR THE VISUAL DETERMINATION
 OF WHITE CELL AND PLATELET COUNTS

Prepared on behalf of the World Health Organization
 by
 The Expert Panel in Cytometry¹ of the
 International Committee for Standardization in Haematology

CONTENTS

	<u>Page</u>
1. INTRODUCTION	2
2. SAMPLING OF BLOOD	2
3. DILUTION OF BLOOD	2
3.1 Pipettes	2
3.2 Diluting fluids	3
3.3 Dilution procedure	3
4. COUNTING CHAMBER	3
5. TECHNIQUE OF HAEMOCYTOOMETRY	6
5.1 Preparation of haemocytometer	6
5.2 Counting procedure	6
5.2.1 White cell count	6
5.2.2 Platelet count	6
6. CALCULATION	6
6.1 Formula	6
6.2 Calculation of white cell count	6
6.3 Calculation of platelet count	6
7. STATISTICAL EVALUATION OF OBTAINED RESULT	7
7.1 White cell count	7
7.2 Platelet count	7
APPENDIX	8



¹ MEMBERS: J.M. England (Chairman), R.M. Rowan (Secretary), M. Bins, B.S. Bull, W.H. Coulter, W. Groner, A.R. Jones, J.A. Koepke, S.M. Lewis, N.K. Shinton, R. Thom, O.W. van Assendelft, R.L. Verwilghen.

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1. INTRODUCTION

Counting of blood elements is an important aspect of diagnostic haematology. In many instances electronic particle counters are used for this purpose, but in smaller laboratories and, especially in developing countries, visual counting may be the only available technique for white cells and platelets. This technique may be used as a back-up and for weekend and night duties, even when an electronic counter is used routinely. Visual counting of red cells is not recommended because the combined errors in dilution and enumeration are too great.

Haemoglobin measurement and haematocrit determination are done easily and sufficiently reliably by the recommended methods.¹ Together they provide sufficient information of the diagnosis of anaemia and polychythaemia. Red cell indices computed from an inaccurate visual red cell count are worthless for the reliable diagnosis of different forms of anaemia.

2. SAMPLING OF BLOOD

Although the blood sample may be taken from a freely bleeding capillary puncture (finger or ear-lobe in adults; heel in young infants) a venous specimen is preferred.

Capillary punctures must be deep enough to allow blood to flow freely. If pressure has to be exerted to obtain a sufficient volume of blood from the puncture, an error will be introduced because of dilution of the blood with tissue fluid. A venous specimen may be collected into any solid anticoagulant; e.g. K_2EDTA , 1.5 mg/ml of blood. Before sampling from a venous specimen the blood should be gently mixed either by tipping the tube end-over at least 20 times or left on a mechanical mixing device for 2-3 minutes.

3. DILUTION OF BLOOD

3.1 Pipettes

Thoma type pipettes in which blood is diluted in the bulb of the pipette are obsolete. They are inaccurate and easily damaged and they require a direct mouth-pipetting procedure.

Sahli type capillary pipettes of 20 μ l capacity (see Fig. 1) are recommended for pipetting the blood, and 0.5 ml bulb pipettes for the diluent. Calibration of the pipettes is needed (see Appendix) unless they are already certified.

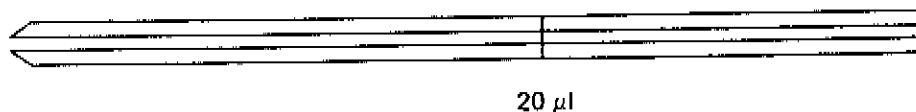


Fig. 1 Sahli type pipettes

WHO 88209

¹ See also the following related WHO publications: Recommended methods for the determination of packed cell volume, LAB/80.4 (under revision); CDC/WHO Laboratory Manual. Anaemia - Fundamental Diagnostic Haematology, 1983. Published jointly by the U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, Georgia 30333, USA and World Health Organization, Geneva, Switzerland.

Careful cleaning and drying of pipettes is essential. They should be cleaned daily in detergent, followed by thorough rinsing with distilled water and drying. It is also advisable to clean the pipettes once a week in a suitable cleaning agent (e.g. HCl 0.1 Molar) followed by thorough rinsing with distilled water and drying.

3.2 Diluting fluids

3.2.1 For white cell count: a diluent of 2% (20 ml/l distilled water) glacial acetic acid lightly coloured with gentian violet is used to lyse the red cells and stain the white cells.

3.2.2 For platelet count: a diluent is required to lyse the red cells. This is 1% ammonium oxalate (10 g/l distilled water) prepared in small volumes (e.g. 0.5 l) using clean glassware. The diluent solution should be filtered through a micropore filter (pore diameter 0.22 μm) and kept at 4°C. If the solution has been stored for more than a week it should be re-filtered before use.

3.3 Dilution procedure

A dilution of 1 in 26 is convenient in most cases for white cell and platelet counts. For samples with markedly increased counts, further dilution will increase accuracy. The 1 in 26 dilution is made by adding 20 μl of blood to 0.5 ml diluting fluid in a 75 x 10 mm glass or plastic tube. After tightly sealing the tube with a plastic stopper, mix by rotation for at least 1 minute for the white cell count and 10 to 15 minutes for the platelet count.

4. COUNTING CHAMBER

This consists of a depressed area of a glass slide which is converted to a volumetric chamber when overlaid by a coverglass (see Fig. 2, page 4). Incomplete cleaning, bad positioning of the coverglass or bowing of a thin (<0.45 mm) coverglass will result in marked errors in the depth of the chamber. The coverglass should be of such a size that when placed correctly on the counting chamber, the central ruled areas lie in the centre of the rectangle to be filled with the cell suspension.

A large number of counting chambers have been described, e.g. the improved Neubauer, Thoma, Burkner, Türk and Schilling chambers. Only the improved Neubauer chamber is recommended by ICSH and will be described here (Fig. 3). The ruled area of the improved Neubauer chamber is 3 x 3 mm giving 9 large squares each of 1 x 1 mm. The depth of the chamber with the coverslip in position is 0.1 mm. Therefore individually each of the large squares has a volume of 0.1 μl . The four 1 x 1 mm squares in the corners (marked by 'WBC' in Fig. 3, page 5) are available for white cell counting. Sufficient 1 x 1 mm squares should be examined to count at least 100 white cells. Each 1 x 1 mm square is subdivided into 16 squares. Cells which touch the edges of these squares are counted according to the rules illustrated in Fig. 4, page 5.

The central 1 x 1 mm area consists of 25 groups (0.2 x 0.2 mm) of 16 squares separated by closely ruled triple lines as shown in the enlargement in Fig. 3. For the platelet count it is normally sufficient to use the five 0.2 x 0.2 mm groups marked by 'P' in Fig. 3 which are together equivalent to a volume of 0.02 μl with the coverslip in position. Each of the five groups is subdivided into 16 squares. Platelets which touch the edges of these squares are counted according to the rules illustrated in Fig. 4. If <200 platelets are counted in the five groups shown then the entire central area should be counted (equivalent to a volume of 0.1 μl).

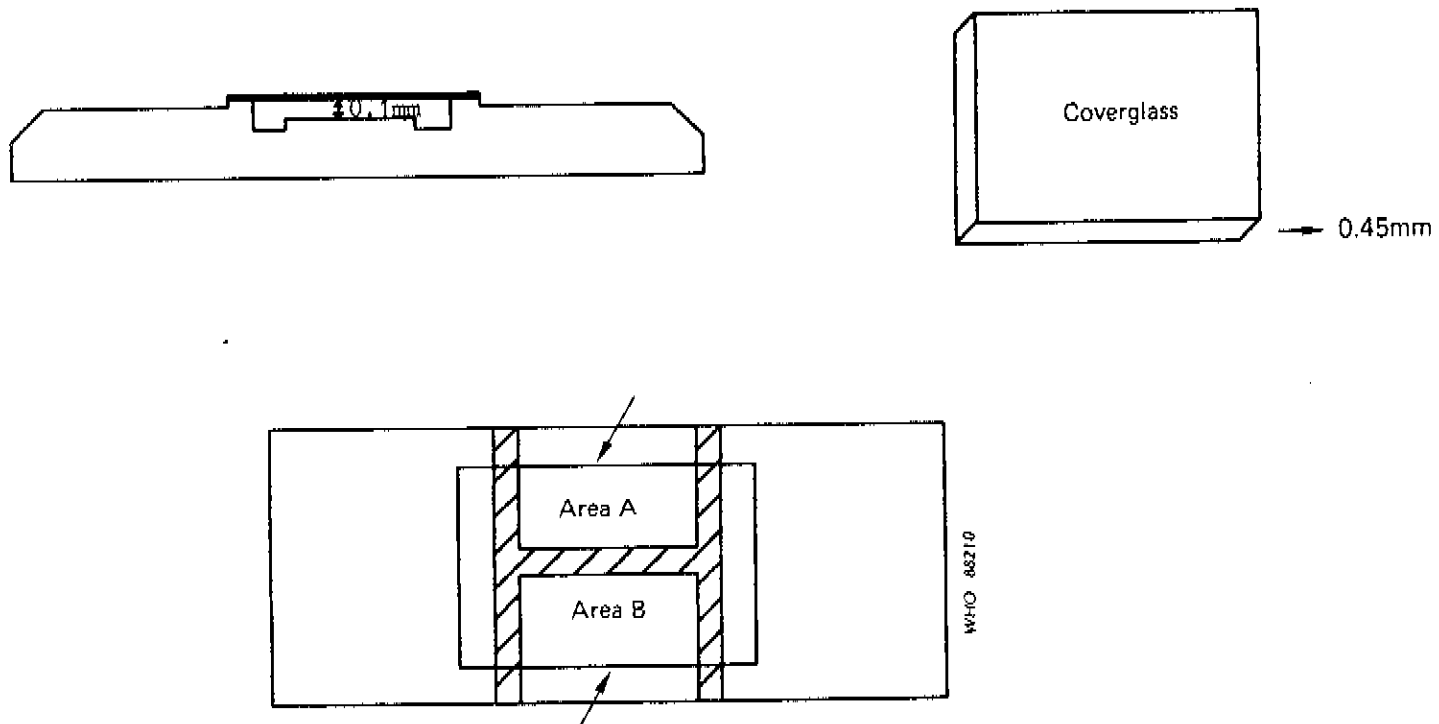


Fig. 2 Design of counting chamber

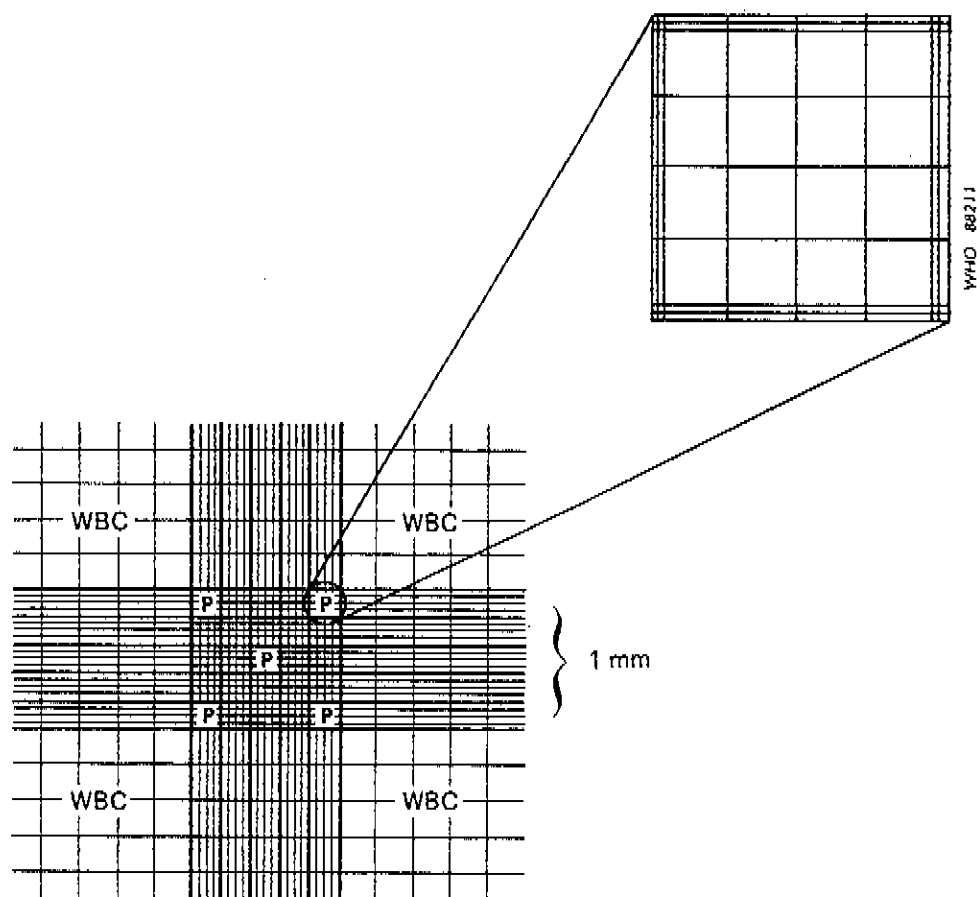


Fig. 3 Improved Neubauer Counting Chamber

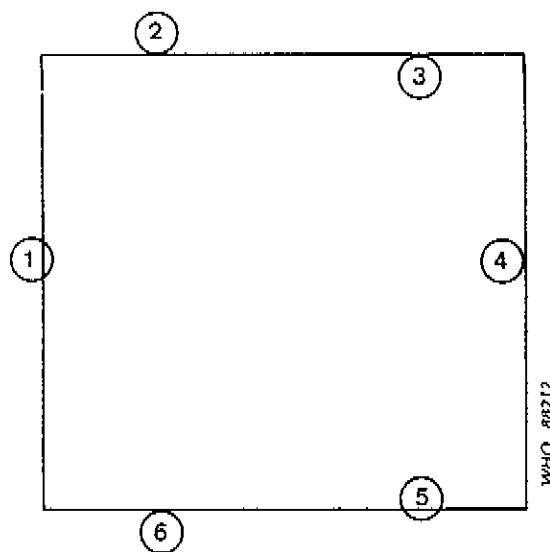


Fig. 4 Counting procedure: cells 1, 2 and 3 are not counted in the square shown while 4, 5 and 6 are included in the count for the square shown.

5. TECHNIQUE OF HAEMOCYTOMETRY

5.1 Preparation of the haemocytometer

It is essential to clean the haemocytometer with bleach, wash in distilled water and then allow to dry. To ensure that the correct volume is attained the coverglass is firmly placed on top of the chamber so as to produce 3-5 Newton's rings.

As soon as the dilution has been prepared, fill the counting chamber using a Pasteur pipette or a length of capillary glass tubing (which has been filled with the mixed suspensions by capillarity). Care should be taken that the counting chamber is completely filled in one action and that no fluid flows into the surrounding moat. If this happens the filling procedure should be repeated using another clean dry chamber. The charged haemocytometer is placed in a Petri dish containing a small wad of moist filter paper or absorbent cotton wool. For white cell counting it is left for 2-3 minutes to allow the cells to settle. For platelet counting a longer period is required for settling and the covered dish is left for 30 minutes. In rare instances platelets may take longer to settle.

5.2 Counting procedure

The haemocytometer is carefully transferred to the microscope stage after it has been confirmed, by checking by means of a spirit-level, that the stage is horizontal. The preparation is examined with a 4 mm dry objective and a X6 or X10 eyepiece. If bubbles or debris are seen anywhere in the chamber, the filling procedure should be repeated with another clean dry chamber. All cells lying on or touching two of the four sides (e.g. right and lower) should be counted, while those on the two other sides (e.g. left and upper) should be left uncounted (Fig. 4).

5.2.1 White cell count: examine sufficient areas to count at least 100 white cells (Figs. 3 and 4). For the purpose of the calculation note the number of white cells counted and the volume in which they were counted.

5.2.2 Platelet count: platelet counting is improved when a phase contrast microscope is used. Examine sufficient areas to count at least 200 platelets (Figs. 3 and 4). For the purposes of the calculation note the number of platelets and the volume in which they were counted.

6. CALCULATION

6.1 Formula

The same calculation is used for the white cell and platelet counts using the formula:

$$\text{Count}/1) - \frac{\text{No. of cells counted}}{\text{Volume counted } (\mu\text{l})} \times \text{dilution factor} \times 10^6$$

6.2 Calculation of white cell count

e.g. 150 cells in 3 squares each 1 x 1 mm,
i.e. 0.3 μl at 26X dilution

$$\frac{150}{0.3} \times 26 \times 10^6 = 13 \times 10^9/1$$

6.3 Calculation of platelet count

e.g. 300 cells in 5 squares each 0.2 x 0.2 mm
i.e. 0.2 μ l at 26X dilution

$$\frac{300}{0.02} \times 26 \times 10^6 = 390 \times 10^9/l$$

7. STATISTICAL EVALUATION OF OBTAINED RESULT

The standard deviation (SD) of the count is approximately the square root of the count and coefficient of variation is obtained by $\frac{SD}{MEAN} \times 100\%$.

Ninety-five per cent of the results will lie within ± 2 CV of the true value.

7.1 White cell counts

150 cells counted as in example 6.2 white cell count = $13 \times 10^9/l$.

$$SD = \sqrt{150} = 12.25 \qquad CV = \frac{12.25 \times 100}{150} = 8.2\%$$

$$WBC \times 2 \times CV = 2 \times \frac{8.2}{100} \times (13 \times 10^9/l) = 2.1 \times 10^9/l.$$

$$WBC \pm (2 \times CV) = 13.0 \pm 2.1 \times 10^9/l = 10.9 - 15.1 \times 10^9/l.$$

7.2 Platelet count

300 cells counted as in example 6.3, platelet count = $390 \times 10^9/l$

$$SD = \sqrt{300} = 17.3 \qquad CV = \frac{17.3 \times 100}{300} = 5.8\%$$

$$PLT \times 2 \times CV = 2 \times \frac{5.8}{100} \times (390 \times 10^9/l) = 45 \times 10^9/l.$$

$$PLT \pm (2 \times CV) = 390 \pm 45 = 345 - 435 \times 10^9/l.$$

APPENDIX

1. CALIBRATION OF VOLUMETRIC PIPETTES

The pipette is filled to the calibration mark with distilled water, which is then transferred to a pre-weighed beaker in accordance with the normal usage of the pipette. The beaker is reweighed. The ambient temperature is noted. The volume of the pipette (in ml) is calculated by dividing the weight of the water (in g) by one of the following specific gravities depending on temperatures:

<u>Temperature (°C)</u>	<u>Specific Gravity</u>
18	0.9986
19	0.9984
20	0.9982
21	0.9980
22	0.9978
23	0.9976
24	0.9973
25	0.9971
26	0.9968
27	0.9965
28	0.9963
29	0.9960
30	0.9957

The calibration must be performed in duplicate for each pipette

2. CALIBRATION OF MICROPIPETTES

The tip of a tuberculin syringe, the barrel of which has been lubricated with soft grease, is attached by means of a piece of thick rubber or plastic tubing to the base of the pipette to be calibrated. The unit is clamped vertically on a retort stand. The plunger of the syringe is withdrawn slightly, and the pipette is submerged in a beaker containing mercury. The pipette is then filled with mercury by further aspiration with the syringe. When the mercury has reached the calibration mark of the pipette, the beaker is removed. A weighing bottle, the weight of which has been determined, is held beneath the pipette, and the measured amount of mercury delivered into this by manipulation of the syringe. The weight of the bottle plus the mercury is determined and the weight of the mercury alone is then obtained by subtraction. The ambient temperature is noted.

The volume of the pipette (in μ l) is calculated by dividing the weight of the mercury (in mg) by one of the following specific gravities depending on temperature:

<u>Temperature (°C)</u>	<u>Specific Gravity</u>
18	13.556
19	13.549
20	13.546
21	12.544
22	13.541
23	13.539
24	13.536
25	13.534
26	13.532
27	13.529
28	13.527
29	13.524
30	13.522

The calibration must be performed for each pipette.

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