TECHNICAL METHODS

A SIMPLIFIED PROCEDURE FOR BLOOD CELL COUNTS AND HAEMOGLOBIN DETERMINATION

BY

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Blood cell counts and haemoglobin determinations are so common in modern medical practice that even minor technical simplifications may result in significant savings of time, effort, and expense. Using standard methods the blood-counting technician to-day carries a multitude of pipettes on his rounds. These pipettes must be carefully filled to a mark with blood, and then with diluting fluid, care being taken to avoid air bubbles. Later, the pipettes must be individually cleansed, using suction apparatus.

The method described in this report requires no pipettes or filling-to-mark procedures at the patient's bedside. It employs the principle of a calibrated capillary tube for blood sampling described by Goldfeder and her co-workers (1948) in animals. The greater simplicity of this method over the orthodox procedure suggested its use in clinical medicine. A comparative study was made in order to evaluate its accuracy as compared with the standard method for determination of the erythrocyte and leucocyte counts and haemoglobin concentration of the peripheral blood.

Procedure

Capillary tubes are prepared and calibrated to contain the amount of blood equal to the 0.5 mark of the Thoma blood cell pipette. This capillary tube is filled with blood obtained by finger puncture, and then diluted with a volume of diluting fluid (Hayem's solution for red cells, 2 per cent acetic acid for white, 0.1N hydrochloric acid for acid haematin formation), and the blood count or haemoglobin determination is made in the usual fashion using a Neubauer Brite-line counting-chamber and Sahli haemometer.*

* The method is applicable to the making of haemoglobin determinations with a photoelectric colorimeter, acid or alkaline diluent. During the war Dr. J. Murray Steele (then commander, U.S.N.) employed a similar procedure with a photoelectric colorimeter.

Mercury is drawn into a standard Thoma white-cell pipette up to the 0.5 mark, and the measured volume of mercury is transferred to a length of capillary tubing through a wide end. Lengths of capillary tubing containing the measured amount of mercury are marked off with a diamond-point pencil, and these calibrated lengths are then separated into individual tubes. Thus, each capillary tube contains exactly the volume of blood ordinarily drawn into the white-cell pipette up to the 0.5 mark. Capillary tubes for the red-cell and haemoglobin determinations are prepared by the same technique using the corresponding pipettes. By this technique, a large supply of calibrated capillary tubes can be prepared at one time.

Red Cell Count.—Diluting fluid (Hayem's solution) is drawn into a standard red cell pipette up to the 101 mark, and then transferred to a small test tube (75 × 10 mm.). The finger-tip is punctured. A calibrated red-cell capillary tube, held in a pair of forceps, is touched to the finger-tip blood, and this results in immediate filling of the tube by capillary action. The blood-filled capillary is then dropped into the test tube containing the diluting fluid,† after which mixing is readily accomplished by shaking. A drop of red-cell suspension is then transferred to a counting chamber by means of a glass rod or applicator stick, and the cells are counted by the usual method.

White Cell Count.—The method is in every way identical with that described for the red cells, except that the corresponding diluting fluid (2 per cent acetic acid), diluting volume,‡ and calibrated white cell capillary tube is used.

† By this method the blood is diluted 1:201, as compared to 1:200 by the standard method. This introduces a negligible error of 0.5 per cent in the final red cell count.

‡ The standard white-cell pipette dilutes blood 1:20. By the method here described the dilution is 1:21, a difference of 5 per cent, which is barely significant. This small error can be corrected by using a recalibrated pipette or by withdrawing from the delivered diluting fluid the equivalent excess volume, or simply by adding 5 per cent to the final white count.

TABLE I

BLOOD COUNTS ON TWENTY-FIVE PATIENTS

Patient	Haemoglobin (g./100 ml.)				Red cells (millions/mm. ³)				White cells (hundreds/mm. ³)			
	Capillary		Standard		Capillary		Standard		Capillary		Standard	
1	12.0	11.5	12.0	12.0	4.4	4.3	4.1	4.7	78	76	78	70
2	12.0	11.0	11.0	11.5	3.2	3.1	4.0	2.5	168	158	132	148
3	12.5	13.0	12.5	13.0	3.2	3.5	4.2	3.9	80	86	74	86
4	12.0	12.0	12.5	12.0	3.4	3.2	4.5	3.7	70	69	70	63
5	6.5	6.5	5.5	6.0	1.3	1.3	1.5	1.2	34	32	34	32
6	12.5	13.0	12.5	12.5	3.4	3.5	4.0	3.5	114	116	100	110
7	12.0	13.0	13.0	12.0	4.5	4.6	4.7	3.5	90	80	96	82
8	10.5	11.0	11.0	10.5	3.0	2.8	3.4	3.4	106	106	98	114
9	12.0	11.5	11.5	11.5	4.6	4.4	4.6	4.7	132	120	80	118
10	14.0	14.0	14.0	13.0	3.7	3.4	3.7	3.6	84	79	142	118
11	13.0	13.0	13.5	12.5	5.2	5.0	4.8	4.3	104	94	100	84
12	14.0	14.0	14.0	14.0	4.3	4.3	4.4	4.4	104	98	90	94
13	12.5	12.0	12.5	12.5	5.2	4.9	4.8	4.5	102	100	86	78
14	13.0	14.0	14.0	13.0	4.9	4.8	5.2	5.8	68	74	70	58
15	10.5	11.0	10.5	10.5	5.4	4.9	5.1	4.7	118	130	130	120
16	14.0	14.0	13.0	13.0	5.7	4.5	4.9	4.3	100	96	104	96
17	13.5	13.0	13.5	14.0	3.8	3.8	4.0	4.1	78	92	98	76
18	13.0	13.0	13.0	12.5	4.2	4.3	3.4	3.8	88	106	92	106
19	13.5	14.0	14.0	14.0	3.3	3.4	3.5	3.7	68	76	78	72
20	12.0	12.0	12.0	12.0	3.7	3.5	4.0	4.0	82	80	82	78
21	14.0	14.0	14.5	14.0	4.2	4.3	4.2	4.2	68	64	70	68
22	12.0	12.5	12.5	13.0	3.2	3.2	4.0	3.4	78	78	76	64
23	12.0	12.0	12.0	12.5	3.0	3.2	3.1	3.0	84	84	74	74
24	13.5	14.0	13.0	13.0	3.1	3.4	3.3	3.6	178	190	188	194
25	12.5	12.0	12.0	12.0	3.7	3.7	3.6	3.7	62	66	66	70

Haemoglobin Determinations.—Dilute (0.1N) hydrochloric acid is added to the comparison tube of the Sahli haemometer in the usual manner. Using a calibrated haemoglobin capillary tube, finger-tip blood is obtained and the blood-filled capillary dropped into the comparison tube, which is then shaken to accomplish mixing. Distilled water is added until the colour matches the standard.

Data

Blood counts were made on twenty-five patients employing both the standard method as described by Todd and Sanford (1943) and the modified capillary method. Each determination was made twice by each method, totalling four complete blood counts on each patient. The results are shown in Table I. The data in Table II demonstrate that the figures obtained by paired determinations using capillary tubes for cell counts differ from each other less than the results with standard pipettes.

The statistical significance of these differences of the means of columns 3 from 4 and of column 5 from 6 have been calculated,* and yield a probability of about 2 in 100 that these differences are due to chance alone. On the other hand, the mean differences between paired determinations of haemoglobin showed the same value by both methods.

Discussion

The advantages of the capillary tube method described are especially apparent in hospitals and clinics where many blood counts are done daily. Only one set of pipettes is required for any number of complete blood counts. The capillary tubes can be calibrated readily in the laboratory,

* Comparison of the mean difference of paired red counts by the standard method to the mean difference of paired red counts by the capillary method yielded a *t* value of 2.15. The *t* value for the same comparison for white counts was 2.6. *t* is calculated from the formula:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sigma_{\bar{X}_1 - \bar{X}_2}} \text{ where } \sigma_{\bar{X}_1 - \bar{X}_2} = \sqrt{\frac{s^2}{n_1} + \frac{s^2}{n_2}} \quad s^2 = \frac{\sum (x - \bar{x})^2}{N(N-1)}$$

TABLE II
TABLE OF DIFFERENCES BETWEEN PAIRED DETERMINATIONS

	Haemoglobin (g./100 mm. ³)		Red blood count (million/mm. ³)		White blood count (hundred/mm. ³)	
	1 Stan- dard	2 Capill- ary	3 Stan- dard	4 Capill- ary	5 Stan- dard	6 Capill- ary
1	—	0.5	0.6	0.1	8	2
2	0.5	1.0	1.5	0.1	16	10
3	0.5	0.5	0.3	0.3	12	6
4	0.5	—	0.8	0.2	7	1
5	0.5	—	0.3	—	2	2
6	—	0.5	0.5	0.1	10	2
7	1.0	1.0	1.2	0.1	14	10
8	0.5	0.5	—	0.2	16	—
9	—	0.5	0.1	0.2	38	12
10	1.0	—	0.1	0.3	24	5
11	1.0	—	0.5	0.2	16	10
12	—	—	—	—	4	6
13	—	0.5	0.3	0.3	8	2
14	1.0	1.0	0.6	0.1	12	6
15	—	0.5	0.4	0.5	10	12
16	—	—	0.6	1.2	8	4
17	0.5	0.5	0.1	—	22	14
18	0.5	—	0.4	0.1	14	18
19	—	0.5	0.2	0.1	6	8
20	—	—	—	0.2	4	2
21	0.5	—	—	0.1	2	4
22	0.5	0.5	0.6	—	12	—
23	0.5	—	0.1	0.2	—	—
24	—	0.5	0.3	0.3	6	12
25	—	0.5	0.1	—	4	4
Total	9.0	9.0	9.6	4.9	275.0	152.0
Average	0.36	0.36	0.384	0.196	11.0	6.08

and a supply of each kind can be kept available. The tubes may be re-used indefinitely after they have been placed in a detergent or in a cleaning solution, rinsed, and dried. The capillary tube method permits all the measuring of diluting fluids to be done in the laboratory at one time, away from the patient and the bedside, and simplifies the blood sampling. There is no problem of obtaining too large or too small a quantity of blood by missing the mark. The technique of capillary filling works as well on oxalated blood as on finger-tip blood. The procedure is highly economical because it eliminates the need for large numbers of blood-cell-counting pipettes. The cost of capillary tubes is negligible.

Summary

A modified method of blood cell counting and haemoglobin determination by the use of calibrated capillary tubes is described. When it was compared with the standard procedure it was found to be accurate and to give results which fluctuate less than those of the orthodox method. The new technique is economical in that it is time saving and eliminates the need for large quantities of cell-count and haemoglobin pipettes.

REFERENCES

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