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An Electronic Blood-Cell-Counting Machine

P. CROSLAND-TAYLOR, J. W. STEWART and G. HAGGIS

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An Electronic Blood-Cell-Counting Machine

By P. Crosland-Taylor, J. W. Stewart and G. Haggis

THE METHOD of counting red cells has remained virtually unaltered since the latter half of the nineteenth century when Cramer introduced his hemocytometer. It is a tedious and time-consuming method, and many pathologists have abandoned it altogether for routine use.[6,14]

The accuracy of the method when counting all cells in 80 1/400 sq. mm. squares has a coefficient of variation of about 9.5 per cent.[1] This inaccuracy is due mainly to the small number of cells counted in a comparatively small field, but there are also a chamber-filling error and a diluting error when making up the dilute suspension.

A great deal of work has been done in order to produce an instrument capable of counting blood cells, and the literature contains the descriptions and results of several machines designed for this purpose. They vary considerably in principle, mode of operation, complexity and cost. The simplest method is to use the optical properties of a dilute suspension,[10] but it has proved unreliable in the presence of macrocytosis and especially hypochromia.[2] A method of counting which employs the measurement of the electrical conductivity of suspensions of blood cells has been devised by Texter et al.,[15] but apparently has not yet proved satisfactory for routine purposes. An improved machine which measures the variations in conductivity as a suspension of cells passes through an orifice has been described by Brecher et al.[3]

Moldavan[11] in 1934, suggested a photoelectric apparatus for counting erythrocytes, and he enumerated the difficulties of counting cells flowing through a capillary tube. His main difficulty was the insensitivity of the photoelectric cell. The development of an extremely sensitive and powerful photomultiplier tube enabled Lagercrantz,[7] in 1947, to be the first to count erythrocytes with a machine. He used dark-ground illumination. Lagercrantz described an improved apparatus in 1951. Much careful and excellent work has been done in the development of machines using a mechanical scanner and an unruled counting chamber. Machines using this principle have been described by Cooke-Yarborough et al.[4] and Poole.[12] A very complex apparatus using an electronic scanner has been described by Young et al.[17]

The purpose of this communication is to describe an electronic blood cell

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From The Bland-Sutton Institute of Pathology and The Barnato Joel Laboratories, The Middlesex Hospital, London, England.

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We wish to thank Messrs. Evans Electroselenum Ltd., Harlow, Essex, for their willing and patient cooperation and for the use of the production machine we tested We also thank Petrochemicals Ltd., Urmston, Manchester, for the supply of O.P.C. 45 detergent. This is now available only from shell chemicals as "Nonidet P. 40."

counter which has been developed over the past five years, first at the Bland-Sutton Institute, and then in co-operation with Evans Electroseleneum Ltd.

Description of Counter

The general appearance is illustrated in figure 1. The machine consists of four distinct parts.

Firstly, a counting-chamber (fig. 2) in which the cells are aligned into single file. It is mounted on the optical axis of a horizontal dark-ground microscope. The images of the red cells passing through the chamber are projected onto the photomultiplier tube.

Secondly, the water-circulating and filtering apparatus which provides a constant head of water for the counting chamber (fig. 3).

Thirdly, the micropump which supplies a known volume of cell suspension into the counting chamber.

Fourthly, the electronic apparatus which comprises a photomultiplier, amplifiers and scaler unit for recording.

The Chamber

The counting chamber is illustrated diagrammatically in figure 2. It is based on that described by Crosland-Taylor[3] in 1953. The sides are made of glass, the whole chamber being mounted between the condenser and 2/3-in. objective of a dark-ground microscope. Clear distilled water supplied by the circulating system enters the chamber through a wide bore pipe at the base and is sucked out by a thin, movable, hollow needle known as the vortex needle, mounted vertically above.

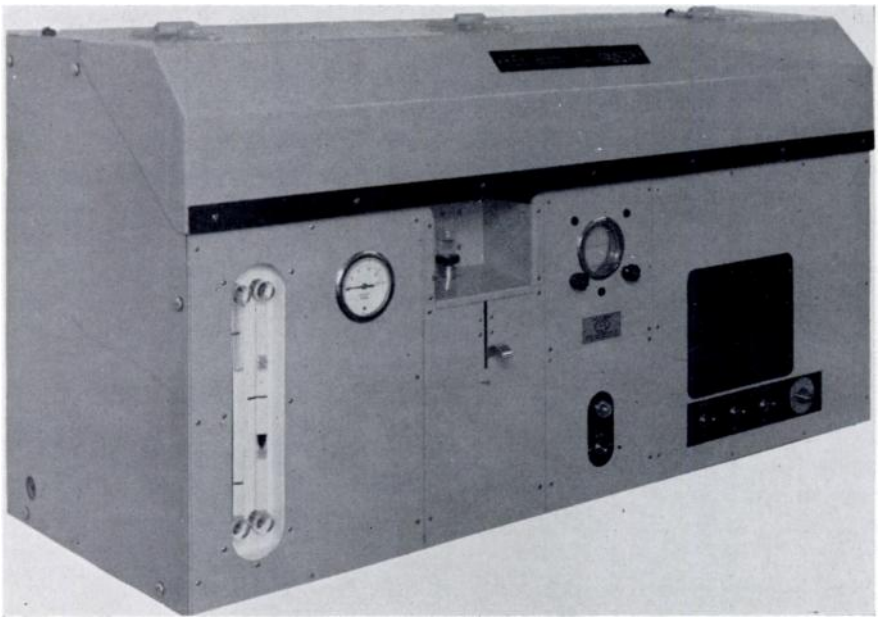

FIG. 1.—Photograph of E E L automatic blood cell counter.

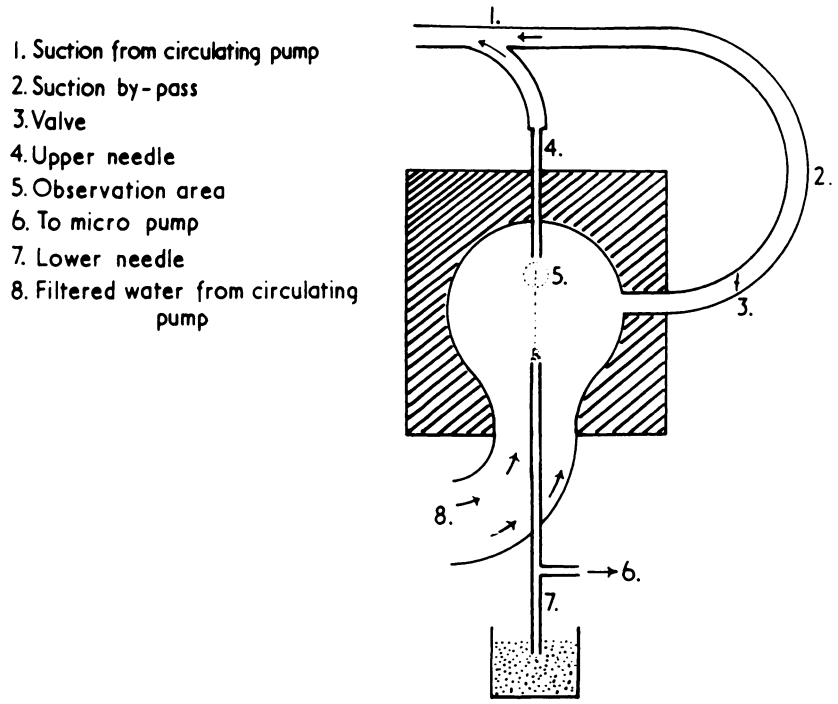

FIG. 2.—Diagramatic representation of counting chamber.

The velocity of the water flowing through the chamber is fastest near the vortex, where it reaches a speed of 100 cm. a second.

A second hollow steel needle enters at the base of the chamber. This lower needle has another joining it about its middle at a T junction. Blood cells enter the chamber through this needle from the diluted blood sample which is placed at its lower end, as shown in the diagram in figure 2. The side arm is connected to the micropump by means of a polythene tube. The upper end of this needle lies within the chamber in the stream of circulating water, so that all red cells leaving it are swept upwards towards the vortex, gaining in speed as they travel. As the cells enter the vortex they cross the optical axis of the microscope as a very fine column of cells, not more than 20 μ wide.

The large bore pipe, leading from the side of the chamber and known as the suction by-pass (See figure 2), is connected to the low pressure side of the water circulating system. This pipe is normally closed by a spring-loaded valve. On opening the valve, water rapidly flows through the counting chamber; this results also in a rapid replacement of test-cell suspension in the lower needle. This "flushing" action is utilized when changing the sample to be counted.

The Micropump

This is connected to the lower needle at the side arm of the T junction shown in figure 2. It consists of a steel pin of known diameter, spring-loaded to maintain contact with a rotating cam geared to an electric motor. Rotation

of the cam forces the pin into a small chamber filled with water, and this (via the polythene tube connected to the lower needle at the T junction) displaces an equal volume of sample from the lower needle into the chamber. The vloume of this lower needle is much greater than that of the displacement pump (some 5 to 25 times greater).

The Water Circulating System

This is illustrated diagrammatically in figure 3. An electric constant-speed centrifuged pump forces water through a filter and into an open reservoir. The purpose of this reservoir is to supply a constant head of filtered water to the counting chamber. The negative pressure required for the vortex needle and suction by-pass is provided by a Sprengle pump working off the main flow from the centrifugal pump as shown in figure 3. The system is so arranged that there is always an excess of filtered water which overflows from the open reservoir and is recirculated.

The Electronic Circuit

The cells flowing through the chamber under dark-ground illumination cause flashes of light which are focussed by the microscope on the photomultiplier, converting them into electrical impulses of proportional intensity. These pulses are then passed through the amplifier, thence to the scaler unit where they are recorded.

A control, known as the discriminator, is fitted to prevent all electrical

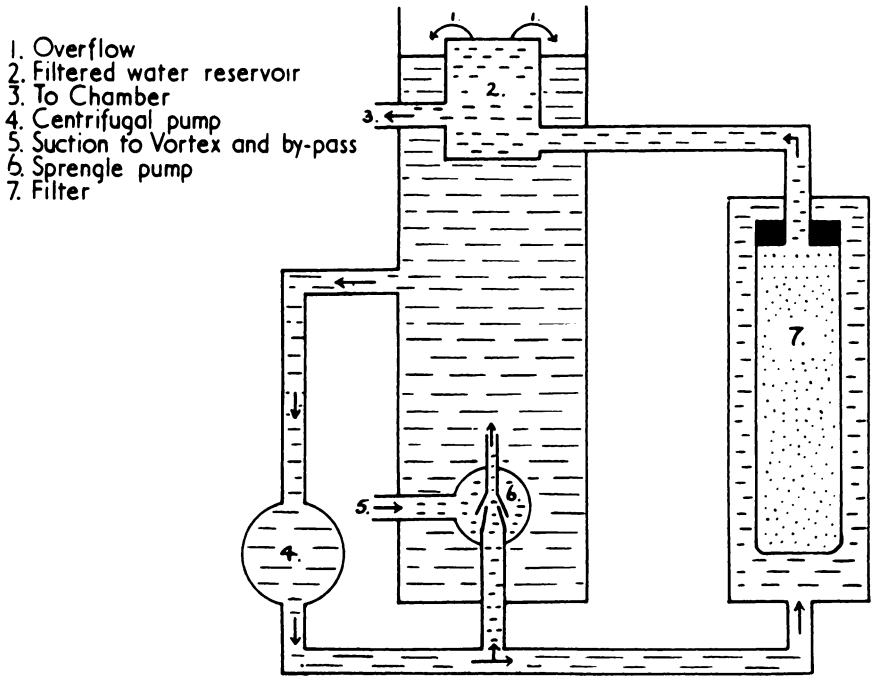

FIG. 3.—Diagramatic representation of water circulating system.

pulses of less than the selected voltage from being counted. It is necessary because minor fluctuations in the electrical circuits and particles such as platelets in the sample cause very small voltage pulses which must be excluded from the recorded count if accurate erythrocyte and leukocyte counts are to be made.

Principle of Operation

A sample pot containing a 1:200 dilution of blood in red cell fluid is placed on a platform below the lower needle. By moving a handle the platform is raised so that the end of the lower needle is in the suspension. By raising the handle to its limit against a spring, the suction by-pass valve is opened, which forcibly sucks the cell suspension through the lower needle. As the handle is released the valve closes and the lever comes to rest on a stop. In this position, the lower needle is still immersed below the surface of the cell suspension, and the stream of cells flowing upwards into the vortex may be examined through a spy-hole near the photomultiplier in order to check that alignment is correct. By lowering the handle further the sample pot containing the cell suspension is taken clear of the lower needle. The water circulating system is so arranged that the slight negative pressure in the chamber is not sufficient to overcome the resistance of the capillary attraction of the fluid suspension in the lower needle. Under these conditions, fluid with cells in suspension remains in the lower needle, but any fluid forced in by the micropump via the T junction on this needle immediately releases an equal volume of sample from the upper half of the tube, which is then carried by the water flow through the counting area. The action of lowering the handle also resets the scaling unit to zero, and the micropump is started. After 10 seconds the micropump delivery is complete and the number of cells in the delivery volume is shown on the scaler.

Methods Employed in Testing the Machine

1. *Repeatability.* One hundred counts were made from a single sample of a 1:200 suspension of normal blood. The recommended procedure is to take the coefficient of variation of the 100 counts and the 25 averages of four.

2. *Calibration.* To check the accuracy of the calibration, 23 different red cell suspensions were counted visually by the standard hemocytometer technic and, subsequently, by the machine.

Lost Counts Due to Abnormal Cell Suspensions

The extent to which this occurs may be examined by two methods:

1. By examining the fall in the counts as the discriminator voltage is increased. This effect is due to the removal of smaller pulses before they reach the counting circuits. The slope of the curve when machine count is plotted against the discriminator voltage indicates the likelihood of undercounting. This has been done on (a) a normal cell suspension, (b) a case of microcytic anaemia, and (c) a case of pernicious anaemia. The abnormal cases were chosen because the cells showed considerable anisocytosis.

2. By examination of the ratios of visual: machine count for any significant

correlation between an increased ratio with any particular type of cell suspension.

Lost Counts Due to Diminished Lamp Intensity

The microscope lamp is a low voltage coiled filament lamp and is underrun by operating the transformer at 200 instead of 230 volts. Using a single suspension of normal blood, we made a series of four counts at discriminator voltage settings of 20, 30, 40 and 50 volts, and repeated the process of altering the lamp transformer voltage to 230 and 160 volts.

Lost Counts Due to Coincident Pulses

A suspension of normal blood was accurately measured and diluted to give final dilution values of 1:100, 1:200, 1:400 and 1:800 parts of diluting fluid. Ten counts were made on each of these four samples, and the average of these is recorded in table 4. Any rise of count with dilution, when all are multiplied to give results as if they had been diluted 1:100, is assumed to be due to changes in coincidence losses.

Results

1. *Repeatability.* The repeatability of the Counting Machine is shown in table 1. The coefficient of variation for single counts is 2.1 per cent, and for groups of four, it is 1 per cent. The time taken for this experiment was under one hour.

2. *Calibration.* The results of visual counts and their comparison with machine counts is shown in table 2. The mean ratio of the visual:machine count is 1.007; a mode of distribution is 0.98.

Lost Counts Due to Abnormal Cell Suspensions

Examination of the effect of discriminator voltage on the machine count: The result of plotting the machine count against the discriminator voltage

Table 1.—*Coefficient of Variation on Repeat Counts E E L Machine*

Red Count in Millions/cu.mm.							
4.26	4.35	4.24	4.43	4.41	4.34	4.47	4.49
4.46	4.25	4.32	4.44	4.33	4.48	4.48	4.33
4.25	4.34	4.44	4.29	4.35	4.38	4.42	4.30
4.37	4.40	4.33	4.46	4.28	4.44	4.39	4.62
4.25	4.20	4.28	4.43	4.32	4.43	4.36	4.45
4.38	4.38	4.47	4.48	4.17	4.48	4.44	4.43
4.36	4.45	4.45	4.39	4.64	4.35	4.26	4.25
4.57	4.39	4.41	4.25	4.26	4.39	4.39	4.16
4.44	4.25	4.42	4.36	4.46	4.51	4.35	4.31
4.41	4.51	4.41	4.31	4.31	4.30	4.55	4.29
4.37	4.29	4.28	4.31	4.44	4.30	4.28	4.30
4.45	4.49	4.55	4.41	4.43	4.48	4.38	4.41
4.38	4.35	4.41	4.40				

Coefficient of Variation Single Counts = 2.1 Per cent.
Coefficient of Variation in Groups of 4 = 1 Per cent.

TABLE 2

Hb. Gm.	PCV	VISUAL COUNT	MACHINE COUNT	MCHC	MCV	VISUAL MACHINE
11.6	32	2.72	2.57	36	124	1.05
10.9	32	3.67	3.74	34	85	.98
6.1	19	1.88	1.83	32	104	1.03
12.2	37	4.22	4.30	33	86	.98
9.9	33	3.82	3.80	30	87	1.00
3.3	14	1.90	1.56	24	90	1.15
3.3	14	1.67	1.57			
11.9	36	3.60	3.69	33	97	.98
9.1	33	3.60	3.73	28	88	.96
10.9	34	3.65	3.65	32	93	1.00
3.3	14	1.80	1.75	24	85	1.09
9.9	30	3.41	3.54	33	85	.96
15.2	46	4.74	4.90	33	94	.96
5.4	23	2.10	2.14	24	107	.98
11.0	33	4.05	4.13	33	80	.98
9.6	29	2.88	2.96	33	99	.98
3.2	9.5	0.93	0.84	34	115	1.11
10.6	32	3.40	3.40	33	94	1.00
10.0	36.5	5.36	5.62	27	65	.95
10.3	32	3.80	4.04	32	79	.94
14.4	42	4.90	4.54	34	93	1.09
15.0	45	5.40	5.24	33	86	1.03
13.3	38	4.0	4.08	35	93	.98

is shown in figure 4 for normal, hypochromic anemia and macrocytic anemia. The recommended discriminator voltage is indicated by the arrow.

The slope of the graph for the hypochromic anemia in figure 4 implies that with microcytic and hypochromic cells the working margin is less than that for other bloods. This was anticipated, but at the discriminator voltage recommended we could not detect any error with this type of blood.

Lost Counts Due to Diminished Light Intensity

In the same way that variations in the machine sensitivity or discriminator voltage alter the count by the machine, so do variations in lamp intensity. The degree to which this occurs in normal blood is shown in table 3, the normal discriminator voltage in this machine being 20 volts with 200 volts on the lamp transformer.

The change in count is small for normal blood. The effect on abnormal blood might be greater, hence the need to under-run the bulb and change it every three months before it blackens and reduces the light intensity.

Lost Counts Due to Coincident Pulses

When the sample equivalent to 10 million/cu. mm. was subsampled and diluted to give counts equivalent to approximately 5 million, 2.5 million and 1.25 million, there was an increase in the value of the machine count over the expected count. This increase is shown in table 4, and is due to coin-

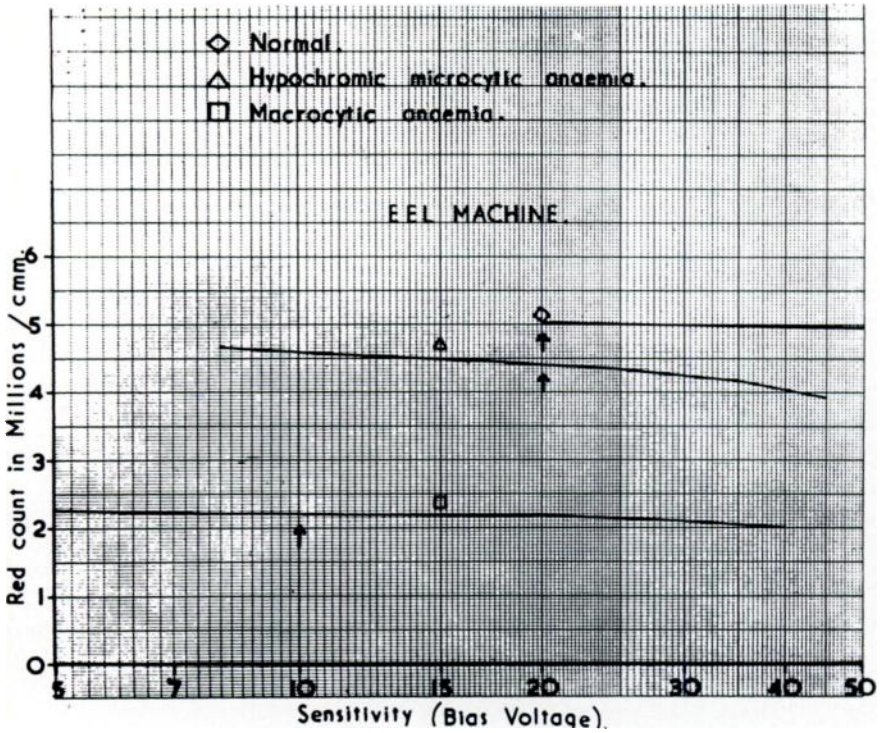

FIG. 4.—Graph showing bias voltage curves obtained using normal blood and blood from hypochromic microcytic and macrocytic anemias. The arrows indicate recommended operating voltages on the machines tested.

cidence loss. The change in coincidence loss is small over the range tested, indicating that the loss itself is small.

DISCUSSION

This machine, developed for counting blood cells, has the advantage of giving more reproduceable results than visual counts. We tried the machine on various abnormal specimens, and within the limits of our tests the machine counted all samples accurately. No difference was detected between macrocytic or microcytic samples or with any other abnormality of the erythrocytes which we tried; the sole exception was in cases of leukemia with high leuko-

TABLE 3.—*Sensitivity Test on E E L*

Red count in M/cu.mm. at various discriminator voltages and lamp brightnesses				
Lamp Voltages	Discriminator Voltages			
Lamp Volts	20	30	40	50
160	4.93	4.80	4.71	4.35
200	5.06	4.97	4.93	4.95
230	5.36	5.11	5.01	4.95

TABLE 4

DILUTION OF ORIGINAL SAMPLE	AVERAGE MACHINE RED CELL COUNT	MULTIPLED TO EQUAL FINAL DILUTION 1/100
1/100	10.27 M/cm.	10.27
1/200	5.23 M/cm.	10.46
1/400	2.65 M/cm.	10.60
1/800	1.318 M/cm.	10.54

cyte counts. Here the leukocytes are counted with the erythrocytes, and to obtain the latter count, the leukocyte count has to be deducted.

The exact calibration of this machine depends on the calibration of the micropump. The loss of counts due to coincidence varies with the cell concentration and may be calculated theoretically if the pulse duration, dead time and the counting rate are known. In the machine tested, the pulse duration was 40 μ seconds which gives a theoretical loss of 2 per cent for a 5M/cu. mm. count, 4 per cent for a 10 M/cu. mm. count, and 0.5 per cent for a 1.25 M/cu. mm. count. If table 4 is consulted and the values obtained there corrected by these factors, then the counts when compensated read as follows:

Dilution of Original Sample	Multiplied to Equal Final Dilution 1/100	Count Corrected For Coincidence Loss
1/100	10.27 M/cm.	10.67 M/cm.
1/200	10.46 M/cm.	10.66 M/cm.
1/400	10.60 M/cm.	10.70 M/cm.
1/800	10.54 M/cm.	10.59 M/cm.

The actual difference between these counts is not great, and for ordinary purposes the coincidence losses were assumed to be 2 per cent, whatever the count. The correction was achieved by deliberately setting the micropump to deliver 2 per cent too much suspension, and thus the answer could be read directly off the scaler.

The method of checking the calibration of the machine by examination of the ratios of visual:machine counts has the advantage of giving an immediate answer to the question of whether the machine count is seriously wrong.

It has already been shown in table 1 that the machine count is reproducible, and, therefore, any error on its parts will also be reproducible. Thus, any error of the machine will be seen in the average machine as compared with average visual count. In this series the mean of the visual:machine ratios is 1.007 and the mode of the distributions of this value is 0.98. This means that the average visual and average machine counts agree within 2 per cent, and, therefore, there is no consistent error in the machine count.

The operation of the machine is quick and simple, a single count taking just over 10 seconds. It has been our practice to count each sample four times and to take the average count. By this means, sub-sampling errors are reduced and the coefficient of variation is only 1 per cent, whereas it is 2.1 per cent for a single count.

TABLE 5

Dilution 1:10 Visual count: 6,000
561
508
547
569
593
542
516
584
616
511
Average = 548.9

TABLE 6

Dilution 1 in 200	Visual count: 200,000
1093	1048
1008	1099
1085	1083
1026	1056
1079	1086
1043	1086
1058	986
1044	1085
1042	1088
1082	1021

Maintenance

The regular maintenance which is required is the replacement of the bulb every three months before signs of blackening are evident, and the change of filter and circulating water when the glass walls of the chamber are being cleaned. This must be done every two weeks because a deposit forms on the glass chamber walls. This deposit, even in minute amounts, greatly impairs the efficiency of the optical system and might cause the machine to undercount hypochromic or microcytic cells.

Leukocyte Counts

We have tested a model using a micropump capable of delivering 1 cu.mm., and this model was able to count white cells using a 1:10 suspension of blood. The suspension fluid consisted of 1 per cent acetic acid containing 1 per cent of a nonionic detergent (O.P.C. 45, Petrochemicals Ltd.). The machine count agreed with the visual count, but we have not yet had the opportunity to fully test the ability of the machine to count leukocytes under all conditions. We have done a small number of counts on an experimental basis, and the results of two short experiments are shown in tables 5 and 6.

It is important that the blood for leukocyte counts should not be oxalated, as oxalate crystals are also counted. Sodium citrate, 3 mg./ml. of blood, or disodium versenate, 1 mg./ml. of blood, are satisfactory anticoagulants.

Summary

An electronic blood cell counting machine operating on a flow principle is described. It is quick and easy to use; it can perform a red cell count in ten seconds with an accuracy of ± 2.1 per cent (that is, 5000 cells counted in 10 seconds for a 5 M count). Leukocyte counts can also be performed in ten seconds with an accuracy of ± 9 per cent for a single count (500 cells counted in a 5000 cu.cm. count).

Summario in Interlingua

Es describite un machina electronic pro le numeration de cellulas de sanguine. Su functionamento es basate super le principio del fluxo de cellulas in "fila indie." Le uso del machina es rapide e facile. Illo pote completar un numeration erythrocytic intra dece secundas con un accuratessa de ±2, 1 pro cento (i.e. 5.000 cellulas numerate in 10 secundas pro un numeration de 5M). Numerationes leucocytic es etiam effectuabile in dece secundas. Hic le accuratessa es ±9 pro cento in un numeration unic (50 cellulas numerate in 10 secundas in un numeration de 5.000 cm^3).

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