An evaluation of the Vickers Instruments J12 cell counter

D. E. PEGG AND A. C. ANTCLIFF¹

From the Westminster Medical School and the Westminster Hospital, London

SYNOPSIS The Vickers J12 cell counter uses an optical hydraulic counting system to perform rapid automatic counts of leucocytes and erythrocytes in liquid suspension. The effect of sample storage, the use of different diluents, variation in cell dimensions and other relevant factors have been studied. A counting accuracy of $\pm 1.19\%$ for replicate erythrocyte counts and $\pm 2.94\%$ for leucocyte counts was obtained.

The introduction of automatic blood cell counting methods has been brought about by the large errors involved in visual haemocytometer techniques (Berkson, Magath, and Hurn, 1940) and also by the progressively increasing clinical demands on haematology laboratories, particularly those dealing with the control of patients receiving cytotoxic drugs. A number of instruments have been developed: the earliest techniques were based on light transmission or light scattering by fairly concentrated cell suspensions (Ponder, 1935; Blum, 1946; Brackett, Mattern, and Olson, 1953), but these were generally insufficiently accurate, particularly when counting abnormal cells. The more modern devices have all employed direct enumeration of individual cells, either by automatic scanning of a modified haemocytometer (Lagercrantz, 1952) or by counting cells suspended in a fluid flowing through a detector zone (Crosland-Taylor, 1953; Coulter, 1956). The latter technique is probably the most convenient and certainly possesses the greatest potential accuracy. The Coulter counter, which is now well known in clinical laboratories, uses an electrical impedance detection system, whereas the Vickers Instruments J12 cell counter, the subject of this study, uses an optical hydraulic counting cell modified from the original design of Crosland-Taylor (1953). A somewhat similar cell was used in the EEL blood cell counter (Crosland-Taylor, Stewart, and Haggis, 1958).

PRINCIPLE OF THE J12 COUNTER

The counting cell is shown in Fig. 1: fluid enters via the tube A, passes up through cavity B past two

FIG. 1. Counting cell.

parallel opposing glass windows C, and leaves by orifice D. The orifice D is positioned just above the horizontal axis of the windows, and immediately below the windows, in the same vertical axis as orifice D, is a fine inlet tube E which passes down into the sample to be counted, F. Fluid is sucked through the chamber by a pump attached to the exit orifice D, and so long as the inflow tube A is unconstricted the pressure difference across the inlet tube E is so low that very little fluid is aspirated from the sample F. However, a solenoid valve is provided to constrict the inflow line intermittently, and when this is done the pressure in the cavity B drops, the pressure across tube E rises, and fluid from the sample F is aspirated into the chamber. Flow conditions in the chamber are substantially laminar, so that fluid aspirated

¹Present address: Department of Chemical Pathology, Postgraduate Medical School, Hammersmith, London.

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FIG. 2. Functional diagram of instrument.

through tube E passes upwards surrounded by a fluid sheath, accelerates rapidly in the region immediately below the orifice D, and thereby becomes greatly reduced in diameter. A narrow horizontal section of this zone is illuminated in dark ground via one window, and the light flashes produced by the passage of cells are detected by a photomultiplier tube positioned opposite the other window.

The complete counter is illustrated diagrammatically in Figure 2. The sample is contained in pot F, and the counting chamber inlet tube E and a pair of metering electrodes, G and H, dip into it. The separation of the electrode points is such that the

volume contained between them is exactly 0.5 ml. When the instrument 'start' button is depressed the solenoid valve I operates, aspiration of the sample starts, and the fluid level in the sample pot falls. When it reaches the point of the electrode G, the scaler unit starts to count, and when the fluid level breaks contact with the lower electrode H, both suction and counting cease. The fluid circuit is completed by pump J, 'Millipore' filter K which removes cells and debris, fluid reservoir L, and pulse-damping chamber M, which also incorporates a pressure relief valve N from the pump outlet.

The pulses from the photomultiplier tube are amplified and fed to the digital readout scaler through a variable pulse height discriminator. The discriminator control, arbitrarily calibrated 0-10, causes the scaler to respond only to pulses above a variable minimum voltage, and since the voltage of each pulse is related to the linear dimensions of the particle producing it, this control enables the instrument to discriminate between blood cells and the very much smaller background particles and debris. The correct discriminator setting for red cell counts is determined by plotting a curve of registered counts against discriminator setting using a single red cell suspension on which an accurate chamber count has been performed (Fig. 3); when a normal blood sample is used the spread of cell sizes is so small that a plateau is produced which corresponds to the correct cell count. A similar calibration is carried out for white cells (Fig. 4), but in this case the variation in cell size and the presence of red cell debris prevent a plateau from being formed; the optimum setting is usually two or three units lower than that used for red cell counts.

FIG. 3. Instrument counts of red cells at various discriminator settings. The true count, determined by a chamber count of about 2,000 cells, was $4.00 \times 10^6/\text{c.mm.}$

FIG. 4. Instrument counts of white cells at various discriminator settings. The true count, determined by a chamber count of about 2,000 cells, was 6,300 /c.mm.

MATERIALS AND METHODS

The initial evaluation of the counter was performed using normal venous blood collected into E.D.T.A. (disodium ethylenediaminetetra-acetate) anticoagulant and a variety of suspending fluids. When satisfactory results had been achieved, comparisons were made with counts performed by other methods, using fresh E.D.T.A.- anticoagulated samples arriving in the laboratory for routine investigations.

All suspending fluids were prepared from distilled water and Analar reagents, with the exception of Dulbecco's solution, which was prepared from the Oxoid tablets and ampoules. Merthiolate (0.4 mg./litre) was added to all solutions not including formalin, and the solutions were filtered under positive pressure through Oxoid cellulose acetate membranes. Diluted samples were generally prepared in 10 ml. quantities in 3 in. $\times$ $\frac{3}{4}$ in. polystyrene tubes with polythene caps; these were cheap enough to be disposable and arrived from the suppliers sufficiently clean to be used without washing (Luckham Ltd.). All blood samples and dilutions were thoroughly mixed on a rotating mixer (Matburn Ltd.).

For white cell counts a dilution of 1 : 500 was used; this gives a direct readout in hundreds of cells per c.mm. of blood, and for red cell counts a dilution of 1 in 50,000 was made giving a direct readout in ten thousands of cells per c.mm.

Haemocytometer counts were made with Manners chambers for accurate calibrating counts, and with the improved Neubauer chamber for comparative purposes; in each case Dacie's recommendations, employing bulk dilution, were followed (Dacie and Lewis, 1963). Coulter counts were performed on a Coulter model A counter in the manner described by Brecher, Schneiderman, and Williams (1956) and Richar and Breakell (1959).

RESULTS OF RED CELL COUNTS

SUSPENDING FLUIDS Various possible suspending

fluids were tested; these included 0.15 M sodium chloride with and without the addition of phosphate buffer at pH 7.4, phosphate buffer saline with calcium and magnesium (Dulbecco's solution, Dulbecco and Vogt, 1954), 3% sodium citrate, and Dacie's formol-citrate (Dacie and Lewis, 1963). It was found that cells suspended in 3% sodium citrate, in 0.15 M sodium chloride, or in buffered sodium chloride produced irregular low instrument counts; this was attributed to mechanical lysis while passing through the instrument. Cells suspended in Dulbecco's solution usually gave satisfactory results, but occasional unexplained low counts made this solution unreliable as a final red cell diluent. However, cells suspended in formol-citrate solution always gave reliable results, and it was found that a secondary dilution in formol-citrate after a primary dilution in Dulbecco's solution was also completely reliable.

SAMPLE MIXING In early experiments using hand mixing of the dilute cell suspensions, serious errors were encountered, and these were eventually traced to inadequate mixing of the primary dilution, and to a lesser extent of the second dilution too. In a typical series of six counts made on separate dilutions of the same blood sample without special attention to mixing, a spread of 4.3 to 6.0×10^6 cells/c.mm was obtained. When mechanical mixing was investigated this error was greatly reduced; a range of 4.90 to 5.02×10^6 cells/c.mm was obtained. However, a further pitfall was discovered: it was found that if samples were rotated in a bottle having a narrow neck, the oscillation of the large air bubble from the neck introduced many small bubbles into the sample, and these were registered as particles by the counter: moreover, once the bubbles had been introduced into the suspension they were extremely stable, and did not clear over a period of many hours. This trouble was not encountered with the cylindrical containers since these do not produce a neck bubble, and it was possible to leave suspensions in these containers on the mixer for two hours without loss or gain of counts. When mixing was performed soon after preparing the dilution, three minutes was sufficient to reduce distribution errors to a minimum, but if the primary dilution had been stored for any length of time more prolonged mixing was needed, but 30 minutes was always adequate.

STORAGE OF SAMPLES Primary dilutions of six blood samples were prepared in Dulbecco's solution and in formol-citrate and stored at 4°C. Secondary dilutions and counts were performed at intervals up to

four days. There was no significant alteration in count in either group.

COINCIDENCE CORRECTION Red cell suspensions with concentrations corresponding to initial blood counts of 0.5 to 12×10^6 red cells per c.mm. were accurately prepared; the true red cell concentrations were determined from the counts made on the higher dilutions, and a graph of true count against observed count obtained (Fig. 5). It will be seen that a coincidence correction is required only when red cell counts exceed 7×10^6 /c.mm. (The corresponding white cell count is $70,000$ /c.mm.) With such blood samples it would be preferable to make a further dilution and avoid altogether the need for coincidence correction tables.

FIG. 5. The effect of coincidence error on instrument red cell counts.

REPLICATE RED CELL COUNTS Large volumes of a 1 in 50,000 dilution of normal blood in formol-citrate were prepared and serial red cell counts made. In 14 series of counts comprising a total of 276 counts a mean standard deviation of 1.19% was obtained.

COMPARISON WITH COULTER MODEL A COUNTER Primary dilutions in Dulbecco's solution were prepared from 51 clinical blood samples and secondary dilutions made in formol-citrate. Each suspension was counted on both the Coulter counter and the J12 counter. The results are shown in Fig. 6, from which it can be seen that all but one of the duplicate cell counts agreed within 5%.

EFFECT OF VARIOUS RED CELL SIZES In order to assess the influence of cell size on the performance of the J12 instrument, counts were made on red cell suspensions prepared from the blood of four

FIG. 6. Comparison of red cell counts made on the Coulter and J12 instruments. The central line indicates perfect agreement and is flanked by lines indicating a 5% spread.

different species—goat, sheep, rabbit, and man. The red cells of these species have mean cell diameters measured in the formol-citrate suspending fluid of 3.0μ , 4.2μ , 6.0μ , and 7.0μ respectively. The curves of recorded counts against discriminator setting are shown in Fig. 7, where it will be seen

FIG. 7. Curves showing instrument counts at various discriminator settings with four species of red cells: A—goat, B—sheep, C—rabbit, D—human.

that although the curves are progressively displaced to the right with decreasing cell size, the plateaux do overlap and a correct estimate of cell concentrations of all species is obtained at discriminator settings of 4 to 5.

RESULTS OF WHITE CELL COUNTS

SUSPENDING FLUIDS A satisfactory white cell fluid must achieve complete red cell lysis without loss of white cells, and must reduce the red cell stroma debris to particles which are fine enough to avoid being counted by the machine, and do not block the Millipore filter too quickly. The manufacturers recommend the use of an acetic acid-formalin-Cetavlon (cetrimide) mixture, but this has the disadvantage that a separate primary dilution must be made if a red cell count is also required. We therefore investigated methods of lysing a primary dilution which could also be used for red cell counts. When the primary diluent was formol-citrate the addition of acetic acid and/or Cetavlon achieved slow lysis with large stroma fragments. When the primary diluent was Dulbecco's solution, red cell lysis was immediate and debris was much finer; in fact, debris was less than with the recommended diluent. These observations were confirmed by making a series of counts on 14 blood samples diluted in the three solutions; the large debris particles contained in samples prepared in the two less satisfactory diluents were shown to produce an increase in recorded count of 18% in the case of the manufacturers' diluent, and of 68% when formol-citrate suspensions were lysed with acetic acid and Cetavlon. Since Dulbecco's solution had been found to be so satisfactory as a primary red cell diluent, the following technique was adopted for routine use.

- 1 Dilute the blood 1 : 500 in Dulbecco's solution (20 c.mm. in 10 ml.) and mix.
- 2 Withdraw 0.1 ml. if a red cell count is required, and dilute this in 9.9 ml. of formol-citrate.
- 3 Add 0.1 ml. of a mixture of equal volumes of glacial acetic acid and Cetavlon to the primary dilution, mix, and count.

EFFECT OF MIXING The need for adequate mixing, particularly after prolonged storage, was only slightly less than it was for red cell counts. All samples were mixed on the Matburn rotating mixer.

EFFECTS OF STORAGE White cell suspensions, in which the red cells were lysed before storage, were kept at 4°C. for periods up to seven days. There was no significant alteration in counts over this period.

REPLICATE WHITE CELL COUNTS Large volumes of white cell suspension in acetic acid-formalin-Cetavlon diluent

FIG. 8.

FIG. 9.

FIGS. 8 AND 9. Comparison of white cell counts made on the Coulter and J12 instruments and by a visual haemocytometer technique. The central line indicates perfect agreement and is flanked by lines indicating a 20% spread.

were prepared and serial white cell counts performed on samples dispensed from a burette. In 21 series of counts comprising a total of 428 counts a mean S.D. of 2.94% was obtained.

COMPARISON WITH COULTER MODEL A AND HAEMOCYTO-METER COUNTS Series of 50 white cell counts on un-

selected clinical blood samples were performed on each counter and by the conventional haemocytometer techniques. The results are shown in Figures 8 and 9. The agreement of most duplicate cell counts was within 20%.

DISCUSSION

The standard deviation of the serial red cell counts was 1.19%, part of which will have been contributed by errors in keeping the large bulk of cell suspension used in these tests evenly mixed; the instrument error is probably less than 1%. This may be compared with a standard error of 2% quoted for the Coulter counter (Brecher *et al.*, 1956) and 8% for 'normal, careful' haemocytometer counts (Magath, Berkson, and Hurn, 1936; Berkson *et al.*, 1940). The importance of adequate sample mixing, particularly of the first dilution when a two-stage dilution technique is used, was very clearly demonstrated by our tests: this is, of course, just as important for other types of counter. The agreement between the J12 and the Coulter counter of 5% is perhaps a little worse than might have been expected of counts made on the same cell suspension: we attribute this increased figure to two factors: uneven distribution of cells in the suspension from which the two samples were drawn, and the different sensitivity of the two detection systems to populations of particles with varying sizes. Our measurements revealed remarkably slight variations in counting performance when red cells varying between $3.0\ \mu$ and $7.0\ \mu$ in diameter were used, and since all the plateaux coincided at discriminator settings of 4 to 5, one would not expect anisocytosis or microcytosis to introduce significant counting errors: counts on blood samples from patients with pernicious anaemia and severe iron-deficiency anaemias have fully confirmed this expectation. The Coulter counter, on the other hand, produces grossly different threshold curves for different species of red cell (Mattern, Brackett, and Olson, 1957).

The standard deviation for serial white cell counts, 2.94%, is considerably greater than for red cell counts; this is probably due to the greater anisocytosis of white cells, and also to the influence of background debris from the lysed red cells. This figure may be compared with 2.63% for a much shorter series on the Coulter counter (Richar and Breakell, 1959), 12% on careful haemocytometer counts (Berkson *et al.*, 1940) and 20% for routine clinical haemocytometer counts (Richar and Breakell, 1959). In view of these figures for visual counts, the 20% spread in the correlation between haemocytometer and J12 counts is not surprising, and in fact an almost identical spread was obtained in a comparison of Coulter and haemocytometer counts.

The similar spread of 20% between the Coulter and the J12 counter is attributed to the same factors as influenced the comparative red cell counts.

The preparation of solutions presented no problems. Filtration under positive pressure through Oxoid membranes is recommended as a cheap, rapid and effective process, giving particle counts on the readout of anything from zero to 005, that is, the equivalent of up to 500 white cells or 50,000 red cells per c.mm. Particle counts tended to fall when the dispensed solutions were stored. Such levels of background count are insignificant for red cell counts, but could amount to 10% of a normal white cell count and even more in leucopenic states; consequently it is advisable to subtract the background count from white cell counts. Suction filtration tended to give higher particle counts owing to the presence of small bubbles in the filtrate. The stability of both red and white cells in the solutions finally selected is of great practical value and is vastly superior to the stability of cells in the solutions commonly used for the Coulter counter.

The instrument required a moderate amount of attention during use; bubbles occasionally entered the counting chamber and produced erratic counts, but this was usually obvious both from the nature of the count recorded and from the pattern displayed on the 'magic-eye' indicator. Nevertheless, we found it most useful to have a cathode ray oscilloscope permanently attached to the counter (a socket is provided for this purpose), since the oscilloscope provides a much more precise picture of counting conditions and immediately reveals the presence of dirt in the circulating fluid or on the windows, bubbles or dirt in the chamber, or optical misalignment. Bubbles can be rapidly and simply cleared from the chamber. The windows of the chamber eventually become dirty and have to be removed periodically and cleaned; about twice a month in our experience. Very occasionally small fragments of dirt become lodged on the outlet orifice of the counting chamber. All these faults, once suspected from the oscilloscope pattern, can be readily identified by using the small swing-over viewing microscope.

The sample counting time with this instrument is 19 seconds, but to this must be added the time taken to aspirate the fluid above the first electrode, usually 12 to 25 seconds. When performing a prolonged series of counts the mean time per sample was one minute. Incipient blockage of the filter is denoted by a progressively increasing sample counting time, and complete blockage is indicated by operation of the pressure release valve and cessation of sample aspiration. In our experience the filter needs changing

after about 400 white cell counts, or 1,000 red cell counts.

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