Pure & Appl.Chem., Vol.54, No.11, pp.2053-2058, 1982.
Printed in Great Britain.

0033-4545/82/112053-06$03.00/0
Pergamon Press Ltd.

AUTOMATION IN HAEMATOLOGY - PRESENT AND FUTURE TRENDS

S.M.Lewis

Royal Postgraduate Medical School, Du Cane Road, London W12 0HS, UK

Abstract - Recent developments in automation of the blood count and differential leucocyte count are reviewed; advantages and limitations of different systems are assessed. These give increased effectiveness of working practices and discrimination of diagnostic data at levels unattainable by manual methods. There are, however, intersystem discrepancies; harmonisation of the systems with appropriate reference materials is needed to achieve comparability of results by different systems.

It was at the time of the first industrial revolution in 1785, that Oliver Evans, a contemporary of James Watt, introduced the concept of automation to perform a repetitive process, when he devised a completely automated self-operating flour mill. The major development of automation, and with it the second industrial revolution, dates from 1924 when, at the Morris motor plant in Coventry, a method was established to provide a complex series of assembly operations which did not require human intervention for any of the intermediate procedures.

This development excited many other industries and led to changes in manufacturing processes, but appeared to make no impact on the clinical laboratory. Thirty years passed before Skeggs (1) described for the first time an automated chemical analytical method. This was an Auto Analyser technique for measurement of blood urea. The subsequent rapid pace of of events in clinical chemistry has been amply demonstrated by the papers presented at this conference. But what of haematology?

The blood count was particularly well suited for adaptation to automated technology. It consists of a series of branching and sequential operations which by manual techniques are labour intensive and time consuming. Moreover, the blood count comprises 90% of the routine work of the haematology laboratory; it is used as a screening procedure on every patient as well as for population health surveys. In 1934 Moldavan had described a photoelectric technique for counting cells in a flow system but its value, as a tool in the clinical laboratory, was not appreciated sufficiently to promote its development and the first electronic blood cell counters were not available commercially until a sudden outburst of productivity saw the development in the 1950s of several types of instrument, notably, Crosland Taylor's flow-through method (2), which has since been incorporated with Moldavan's principle in one group of instruments, and Coulter's electrical impedance method (3).

The initial aim was to achieve a labour saving procedure for particle counting. Other advantages soon became obvious. There was an acceleration in the rate at which reports of results could be produced as well as increased productivity in terms of the number of tests which could be carried out per technician. As a consequence, at least in busy laboratories, there was significant reduction of the labour costs with decreased cost per test. It now became possible to use the red blood count (RBC) as a routine diagnostic investigation where previously it had been considered so time-consuming and laborious that it was performed only for special reasons, for example, the elucidation of obscure anaemias. The speed and ease with which a cell count can be done on an electronic counter has had a major effect on the pattern of clinical laboratory services and the organisation of routine haematology in the district general hospital. An even more important consequence has resulted from the remarkable degree of precision of the blood count measurements which can be achieved. Previously, the best precision attainable for RBC gave a coefficient of variance (CV) of 10%, and that only under ideal conditions so that the significance of the measurement was often discounted. Today, the red cell count can be performed with a CV of 1% and this remarkable degree of precision provides data with a discriminant function which can be used in diagnosis and classification of disease at a level which was inconceivable a few years ago. The mean red cell haemoglobin concentration (MCHC), as obtained from measurement of haemoglobin by colorimeter and packed cell volume by centrifugation was previously considered to be the most sensitive index of early phase iron deficiency and for discriminating thalassaemia minor. But today we consider the MCHC to be the least reliable

of the parameters. Instead, the RBC and the mean red cell volume (MCV) are considered far more reliable measurements, indeed, it is possible to diagnose β thalassaemia and to distinguish it from iron deficiency in 99% of cases by means of the formula (MCV - RBC - 5.Hb - K) where K is a constant factor which is instrument specific (4). Conversely, finding a minor degree of macrocytosis by the MCV has become an important means for detecting the earliest stages of megaloblastosis associated with vitamin B_{12} or folate deficiency and a wide range of diseases associated with dyserythropoiesis. The MCV has also been found to be useful as an indicator of chronic alcoholism. Thus, for example, in a health care screening of an apparently normal adult working population, 17 subjects with increased MCV were identified: they were not anaemic, their serum folate and vitamin B_{12} levels were normal but on subsequent questioning they were all found to have chronic excessive alcohol consumption (5).

Hitherto, as described in the previous section, the red cell parameters used for clinical purposes have been mean values of cell volume and cell haemoglobin concentration. In 1933, Price-Jones developed a diagnostic procedure for distinguishing various types of anaemia by measuring the diameters of 100-200 erythrocytes in the blood film using a microscope with a micrometer eyepiece. He expressed the mean and distribution of cell size as the Price-Jones curve. For a time this test was considered to be a valuable diagnostic aid but its laboriousness resulted in it becoming outmoded and obsolete, especially with the advent of the electronic counters which measured MCV so precisely. It is, however, now beginning to be appreciated that MCV alone does not provide adequate information on the actual distribution of cell sizes in a blood specimen. Thus, for example, in nutritional anaemias, where there may be combined iron deficiency and folate deficiency the MCV may well be normal. The introduction of the Channelyzer provided a means for measuring cell size distribution. Several studies have demonstrated the value of volume distribution curves to identify double populations and in studying kinetics of erythrocyte subpopulations (6). This principle has now been incorporated into the Coulter Model S Plus and the Technicon H 6000 system. The use of a multivariant analysing computer is a further refinement which allows an even greater range of measurements of individual cells including quantitation of erythrocyte morphological characteristics of size, shape, circumference, as well as staining intensity as an index of haemoglobin concentration (7). These features can be defined objectively and presented in the form of histograms, and in measurable units.

The platelet count has become an important haematological test, helpful as a screening procedure in the diagnosis of various blood dyscrasias, essential in the diagnosis and management of thrombocytopenias and crucial in patients receiving cytotoxic drugs or irradiation. The introduction of electronic instrument systems has greatly improved counting precision and, in at least some of the systems, accuracy as well. The new generation of instruments includes the platelet count as a routine measurement, and also provides measurements of platelet size distribution. This has introduced an entirely new diagnostic approach as platelet size and platelet volume are more important criteria of platelet function in health and disease than the traditional platelet count alone. It has been shown, for example, that (a) platelet aggregation velocity is related to the number of large platelets, (b) the presence of increased microthrombocytes is indicative of increased platelet destruction and (c) megathrombocytes are preferentially sequestered by the spleen. There is also a suggestion that markedly heterogeneous platelet production is a reflection of disordered megakaryocyte function (8-10). Almost certainly these new measurements will have significant influence on clinical investigation and management of patients with platelet disorders.

Whilst recognising the usefulness of automation in the laboratory, it is essential to put into perspective the extent to which an automated system is required in any particular laboratory. The world market is being flooded with laboratory equipment of various types for use at all levels of health care; an indiscriminate acceptance of the total range of available instruments is not possible even in the most affluent countries, even if it were desirable. It is thus necessary to identify and select techniques and instruments appropriate to the individual laboratory and to the health service at a national level. In each laboratory account must be taken of the overall daily work load and the rate at which specimens are received by the laboratory, whether the laboratory serves a specific clinical unit or provides a more general service to the district. Thus, in one laboratory all the needs may be satisfied by a simple haemoglobin/WBC counter and the information provided gratuitously by the major instruments may perhaps be more embarrassing than useful. In other circumstances, however, this added information, even though not of immediate use for patient care, may provide the basis for establishing reference ranges and thus improve the predictive value of data obtained in screening programmes as well as on individual patients. In analysing cost benefit, the time taken to process each specimen has become an especially important factor, because as systems become increasingly complex, they have become slower in operation. Thus, the Coulter S is capable of handling 120-150 samples per hour but the new model S Plus can handle only 60 specimens per hour.

Another problem concerns the performance reliability of the instruments. As discussed above, present-day instruments have a remarkable degree of precision; but this has highlighted the

problem of the extent to which instruments which function with different principles give different analytical answers. The cell counters in use today are, in effect, comparators which can be adjusted arbitrarily, to that to obtain a true measurement of the count, the instruments must be calibrated by means of reference preparations with authenticated assigned values. Unfortunately, blood itself is not stable for a sufficiently long period to be a realistic reference material and, despite extensive work on this subject, no completely satisfactory artificial material has yet been developed. Monosized polystyrene latex particles have recently become available which have shown promise in a preliminary trial by the EEC Bureau of Reference (BCR) (11). It is important to recognise the limitations of each type of instrument, especially in relation to its performance with abnormal blood. Thus, the Coulter counter shows discrepancies in sizing red cells which have reduced deformability such as those of iron deficiency, thalassaemia, sickle cell disease, spherocytosis (12). With laser beam optics, cells are sized by light scattering, but this method, too, is not necessarily accurate as abnormal cells are likely to absorb and reflect a variable amount of light (13). There is poor correlation between platelet sizing by Coulter ZBI/Channelyzer and by Coulter S Plus (14). It may be argued whether the important requirement is greater accuracy (i.e. "truth") or comparability with greater sensitivity for clinically relevant information (12).

It should also be remembered that despite the sophistication of present-day instruments, we still depend on manual techniques for primary calibration. This highlights the importance of the need to maintain the manual technical skills, and to ensure this by appropriate technician training programmes, despite the temptation to leave it all to the machines.

Differential leucocyte counting (DLC)

Perhaps the greatest impact of automation in haematology is in the differential leucocyte count. It has been traditional to perform the DLC by counting 100 or 200 cells in a Romanowsky stained film. But the 95% confidence limit of such counts is so wide that the results are only meaningful when there is gross elevation in the number of cells and it is of little value for estimating cells seen in low proportions. Automated DLCs are based on four principles: (a) flow microfluorimetry, (b) continuous flow cytochemistry, (c) computerised pattern recognition and (d) leucocyte volume analysis (15, 16).

Flow microfluorimetry has the potential to provide an effective DLC system. Cells in liquid suspension are stained with a fluorescent dye or combination of dyes and made to flow in single file past a high intensity exciter light source. Fluorescent emissions are detected in photomultiplier tubes which are filtered in order to select specific wavelengths of fluorescence. Pulses are generated in proportion to the fluorescence of the cells. By staining with acridine orange which emits a green fluorescence in combination with DNA and a red fluorescence in combination with RNA, it is possible to construct a scatter plot of cellular DNA content against cellular RNA content. A number of authors have demonstrated that by this means several classes of leucocytes can be distinguished (17). However, although this principle is now used for cell sorting in experimental haematology, no data is yet available on the performance of such a system for routine DLC.

Continous flow cytochemistry is the system chosen by Technicon in their H 6000 system. It is based on certain cytochemical reactions by which different cells can be identified. The blood, after suitable dilution, streams into three channels in which the red cells are lysed and leucocytes are stained supravitally. In one channel basophils are detected by staining with alcian blue, in the second channel cytochemical detection of esterase activity distinguishes monocytes and in the third channel the remaining cells are classified on the basis of their size and their peroxidase activity. The stained cells pass through a flow unit in which their size is measured by light scatter and their stain uptake is measured by light absorption. The major advantage of this system is that 10,000 cells are counted in one minute, thus ensuring a high level of precision. However, it is difficult to judge the accuracy of the instrument, as the point of reference is complex and the apparent accuracy at the clinical level varies between different evaluation reports (18). The false positive rate seems to be in the order of 12-15% and the false negative rate 0.8-10%. Some authors have noted discrepancies between manual and Technicon neutrophil counts resulting from apparently low levels of neutrophil peroxidase activity. The clinical significance of this phenomenon, which has been demonstrated in apparently healthy individuals, remains to be elucidated. Nonetheless, the instrument is a valuable screening tool, especially, if the results are considered, not in the light of traditional differential cell counting, but in terms of the overall pattern in healthy subjects and the identifiably different patterns in various diseases. Moreover, the procedure of quantitative cytochemistry has potential value for subclassifying leukaemias, for identifying the effects of chemotherapy and for detecting early remission of leukaemia on treatment.

Computerised pattern recognition provides a contrasting philosophy. By contrast to the flow systems which introduce new principles, this is based on a traditional procedure, which is well established and familiar in all laboratories. The concept of computerised pattern recognition was introduced 15 years ago by Prewitt and Mendelsohn (19) and in recent years it has undergone considerable further developments (20, 21). It is used in several commer-

cial systems, e.g. Abbott (ADC 500), Perkin Elmer (Diff 3), Leitz (Hematrak). Although it uses sophisticated computer technology to achieve high resolution, its basis is the traditional classification of cells by their morphological features in Romanowsky stained thin films. The films are scanned under a microscope equipped with a computer-controlled mechanical stage. When a nucleus (i.e. a suitably-sized optically dense spot) is detected by a crude sensing device, the stage is halted and the field is scanned by a high resolution photosenser such as a television camera or a flying spot scanner which generates an electronic analog signal corresponding to a densimetric scan of the image. The signals are reduced through an array of numbers (termed pixels) which are readily processed by computer. The data which are extracted describe various morphological features of the cell under view. The final stage of the process is pattern classification; by this means, a heterogeneous population may be subdivided into its component subgroups on the basis of a defined set of descriptive numerical parameters. These are derived by means of an initial training experiment in which known cells were presented to the instrument and appropriate features recorded. Thus, the instrument will be no more reliable than the original trainer. Moreover, reliable performance depends on the same consistency of staining as in the original training slides, and to achieve this without carefully standardised stains is not easy. There is considerable hesitation in acceptance of this instrument in routine haematology laboratories. But the resistance, based on scpeticism as much as on financial considerations, is gradually breaking down and the value of the automated differential cell counter both as a labour saving device and as a diagnostic tool in its own right is becoming accepted. The potential applications are considerable. Thus, for example, it may provide a facility to identify subtle but quantifiable morphological features which may have diagnostic significance. Moreover, although the present generation of instruments function relatively slowly, as they speed up with further refinements of their computer processing, it should be possible to identify minor distortions of the cell populations as clinically significant. Also by defining reference values with statistical significance within a narrow range, relatively small shifts within the differential count may be seen to have clinical discriminatory function.

However, these instruments are expensive and only justified if fully utilised. It is, therefore, necessary to ask whether the large numbers of differential counts which are performed in hospital laboratories are really necessary. In a recent analysis (22) it was concluded that, except for an initial screening of each new patient, repeat differential counts were hardly ever necessary and by using the total WBC as an indicator of developing neutropenia or neutrophilia abandoning the differential count would have resulted in loss of information in only about 1% of instances. From this it may be concluded that differential counts are an expensive and almost worthless indulgence.

Leucocyte volume analysis provides a compromise between this view and the traditonal demand for DLC. England et al (23) using a Coulter counter linked to a pulse height analyser resolved lymphocyte and neutrophil peaks in blood samples. By computer analysis of the form of the distribution curve, it is possible to identify several types and to distinguish abnormalities of minor cell classes, such as B CLL, from other forms of lymphocytic leukaemia (24). This principle has now been incorporated in the newest model of the Coulter S Plus II, in which percentage and absolute number of lymphocytes are included in the routine blood count.

Blood transfusion has two different requirements from automation - the tests themselves and the organisation of a busy department with the reponsibility of maintaining a balance between available stock and the demand for this stock with its limited shelf-life. To meet the organisational needs almost any computer facility can provide the means for record keeping and operating an effective blood distribution system. This was recently discussed at length by the working party on Automation of the International Society of Blood Transfusion and reported in an issue of Vox Sanguinis (25). As far as blood grouping and antibody detection are concerned, Technicon introduced a blood grouping analyser in 1963 (26); it had a relatively slow and checkered career because of a number of technical and procedural weaknesses which have only recently been overcome. The recently introduced end product is the Auto Grouper 16-C; this has automatic sample identification, automatic interpretation and recording of results, and it reacts with sufficient sensitivity to identify most antibodies at least as competently as by manual techniques (27). Its challenger is the Groupomatic 360-C (28) which carries out haemagglutin reactions by discrete analysis at about the same rate of 300-350 samples per hour and with similar reliability.

What will be the future trend of automation in haematology? First of all, an important trend is closer collaboration between the engineers and physicists from industry, and the practising haematologists. This should help us to avoid the present-day problem of instruments being launched before they are ready. I have already referred to this problem in the blood-grouping situation and also to the problem which exists with instrument variation whereby an instrument may perform well within the specifications of its manufacturer but will produce results which differ from those obtained by the machine of another manufacturer. The problem exists with even simple levels of automation, as illustrated with coagulometers.

There are several makes of coagulometer on the market, all of which give measurements which are precise and linear, but they differ in their end point reading (29) and this results in prothrombin time measurement which may be clinically misleading if the differences are not appreciated. The importance of this is perhaps not fully realised by the manufacturers. The need for interlaboratory comparability of measurement irrespective of methodology remains our goal. As far as haematology is concerned it is a function of the International Committee for Standardization in Haematology to bring together the experts with this objective.

Collaboration between manufacturer and user should have another dividend. Today there are few haematology laboratories in western Europe without some level of automation, and instrumentation plays a major role in laboratory function. The question arises how best we, as users, can benefit from future developments. Clearly, the most widely used tests are the most likely to be automated but, in the past, the advances have come essentially from the manufacturers' concepts of what they consider to be good for the haematologist, whilst from the haematologist's viewpoint, the availability of the facility has largely determined what tests should be done. Perhaps, clinical judgement is not necessarily the most convincing argument to the manufacturer who is concerned with both custom and economics. It is interesting to note some of the areas which have not yet been considered seriously by the manufacturers albeit that the need has been identified by the user. One example is the reticulocyte count - unarguably a measurement with clinical and scientific importance which is not performed as often as it should be because it is time-consuming and relatively unreliable as performed routinely but which is an ideal test for an automated procedure. Unfortunately, no manufacturer has yet taken up this challenge, but I am sure that an automated reticulocyte counter will come in due course, as will other advances, especially in the precise measurement of a chemical and physical profile of individual blood cells.

Another trend is an increasing awareness of the need for adequate quality assurance, by both external quality assessment and continuous instrument monitoring. Blood counters are beginning to have built-in computer interphases to analyse continuously either modal or mean values of the blood count indices (MCV, MCH and MCHC). Once established the indices should, under constant conditions of measurement, be unchanging for a total patient population (30); this provides a method for self-appraisal by the counter which can thus rapidly diagnose a fault in its system, identify its aetiology and even indicate the cure.

But I would like to emphasize that the haematology laboratory cannot function as a private arrangment between the computer and the automated instruments. There will always be unexpected diagnostic situations which will require human skill for their interpretation. Thus, whilst instrument systems may become increasingly independent of human supervision, it is well to remember that haematology has always been and essentially remains a clinical art. It is vital that we distinguish between having dependable instruments and becoming instrument dependent.

REFERENCES

1. L.T. Skeggs, Amer. J. Clin. Path. 28, 311-322 (1957).
2. P.J. Crosland Taylor, Nature, 171, 37-38 (1953).
3. W.H. Coulter, Proc. Nat. Electronics Conf. 12, 1034-1042 (1956).
4. J.M. England and P.M. Fraser, Lancet, 1, 449-452, (1973).
5. K.W. Unger and D. Johnson, Amer. J. Med. Sci. 267, 281-289 (1974).
6. J.M. England, S.M. Ward and M.C. Down, Brit. J. Haemat. 34, 589-597 (1976).
7. S.A. Bentley and S.M. Lewis, Brit. J. Haemat. 32, 205-214 (1976).
8. S. Karpatkin, Blood, 51, 307-316 (1978).
9. R.M. Rowan, C. Fraser, J.H. Gray and G.A. McDonald, Clin. Lab. Haemat. 1, 29-40 (1979).
10. M. Kraytman, Blood, 41, 587-598 (1973).
11. S.M. Lewis. Advances in Haematological Methods: The Blood Count, Ch 19, CRC Press, Boca Raten (1981).
12. N. Mohandas, M.R. Clark, S. Kissinger, C. Bayer and S.B. Sohet, Blood, 56, 125-128 (1980).
13. S.M. Lewis and S.A. Bentley, J. Clin. Path. 30, 54-64 (1977).
14. R.M. Rowan, C. Fraser and J.H. Gray, Clin. Lab. Haemat. 3, 165-168 (1981).
15. S.A. Bentley and S.M. Lewis, Topical Rev. Haemat. 1, 50-64 (1980).
16. S.M. Lewis, Blut, 43, 1-6 (1981).
17. L.R. Adams and L.A. Kamentsky, Acta Cytol. 15, 289-291 (1971).
18. J.A. Koepke (ed), Differential Leukocyte Counting, CAP Conference, Aspen (1978).
19. J.M.S. Prewitt and M.L. Mendelsohn, Ann. NY Acad. Sci. 128, 1035-1053 (1966).
20. A. Rosenfeld and A.C. Kak, Digital Picture Processing, Academic Press, New York (1976).
21. J.W. Bacus and E.E. Gose, IEEE Trans. Syst. Man. Cybern SMC, 2, 513-526 (1972).
22. G. Brecher, R.E. Anderson and P.D. McMullen, Blood Cells, 6, 431-454 (1980).
23. J.M. England, C.C. Bashford, M.G. Hewer, N.C. Hughes Jones and M.C. Down, Lancet, 1, 492-493 (1975).
24. C. Costello, J.M. Wardle, D. Catovsky and S.M. Lewis, Brit. J. Haemat. 45, 209-214 (1980).

25. R.L. Hirsch and P. Cazal, Vox Sang. 40, 214-221 (1981).
26. C. McNeil, W.M. Helmick and A. Ferrari, Vox Sang. 8, 235-241 (1963).
27. J. Rechsteiner, W.J. Lockyer and L.W. Friedman, Vox Sang. 40, 192-200 (1981).
28. A. Muller, M. Garetta and M. Hebert, Vox Sang. 40, 201-213 (1981).
29. B.L. Evatt, D. Brigan, D. Triplett, J. Koepke and G. Waters, Clin. Lab. Haemat. 3, (1981).
30. B.S. Bull, R.M. Elashoff, D.C. Heilbron and J. Couperus, Amer. J. Clin. Path. 61, 473-481 (1974).