Leaders

The white cell differential: Personal observations

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Introduction

Current technologies for enumerating, sizing, and differentiating blood cells are diverse. Aperture impedance, hydrodynamic focusing, different forms of light scatter, isovolumetric sphering, conductivity, radiofrequency, differential lysis and cytometry are used by different manufacturers in various combinations. Comprehensive statistical software packages such as cluster analyses have also been developed, and are in a continual upgrade process, to analyse the data obtained.

Most of these technologies have been evaluated and compared with each other. Furthermore, the differential white cell count (DWCC) has been the subject of numerous extensive review articles during the past decade. The current technologies have been more recently reviewed. It is interesting that, despite the increasing sophistication of the technologies used to derive the DWCC, the traditional anticoagulant of routine haematology, ethylenediaminetetra-acetic acid (EDTA), has remained unchanged for at least four decades. EDTA and the pre-analytical variables of blood collection are important limitations on the new technology. Moreover, the lack of an acceptable reference method for the DWCC has rendered comparisons of technologies difficult. DWCC quality assurance and assessment methods need to be developed to parallel other laboratory procedures.

The object of the increased technological sophistication is to process blood samples rapidly but with accuracy and precision. Increased workload without a concomitant increase in trained technologist staff has emphasised this in most laboratories. It is essential that the automated DWCC complies with this philosophy in an efficient laboratory. The complete success of the automated DWCC, however, depends on the type and diversity of the local workload. Grossly abnormal samples require identification or "flagging" within the analytical process for further examination and comment. Because of this need for efficiency, single analysts for the simultaneous measurement of the full blood count (FBC) and DWCC have evolved. The evolution process has involved pattern recognition, flow cytometry, flow cytometry, three population DWCC screens, and finally, five population complete DWCC inclusive of comprehensive "flagging" systems. Separate analysers for the DWCC have been operationally unsuccessful despite their potential for increased precision and accuracy by analysing a fixed number of cells during each analysis.

When normal adult samples, and those with white cell distributional abnormalities, were analysed the current DWCC analysers produced precise and comparable results.5-7 Comparability in all studies was excellent for neutrophils and lymphocytes. Eosinophil comparability, derived from a diverse range of methodologies, was also good by all technologies. Comparability, however, was not as good for monocytes and poor for basophils, with significant differences between technologies. Despite the poor comparability and inferred inaccuracy the automated analyses were more precise than their manual counterparts. Interestingly, these conclusions represent a modest improvement on the three population screening approach to the DWCC used by either aperture impedance and differential lysis8 or laser light scatter. Superficially, accurate eosinophil counts on each analysis seem to be the net gain of these technological advances.

In analysing these comparative data it is important to consider the limitations of the independent variable or "reference" method. The standard manual DWCC involves the recognition, enumeration, and classification of 100 white cells. In the hands of a skilled observer it also provides qualitative data for red cells and platelets, and, occasionally, diagnostic information. However, it is subject to considerable distributional, statistical, and observer error. Attempts have been made to overcome the inherent errors of the technique by either preparing monolayer blood films or using recommended counting techniques. These approaches have been successful only to a point whereas it is established that the statistical error can be reduced by counting increasing numbers of cells. Unfortunately, the statistical improvement is not entirely linear and the time necessary to count sufficient cells is unavailable in routine practice. Thus a reference method, HT20-A, has been recommended. This reference method has been considered controversial, but it is based on experienced observers, 400 white cell counts, and should be used when evaluating new DWCC technology.

Between 1000 and 20000 white cells are analysed in the automated DWCC. The number of cells analysed depends on the total white cell count as the analysis is time dependent to ensure throughput. Inevitably, if the white cells are morphologically and cyto-
chemically normal, the automated DWCC is more precise than the standard or reference manual technique. This point is illustrated in the table by a comparison of the intrabatch precision statistics of a range of automated DWCC flow cytometers to the standard and reference manual methods using normal fresh blood anticoagulated with EDTA. In this comparison the superior precision of neutrophil, lymphocyte, and eosinophil counts from all flow cytometers, regardless of methodology, is confirmed. The clinical usefulness of precise and accurate counts of these three cell types is well established. Furthermore intra- and interlaboratory quality assessment of each technology should be feasible with suitable material.

The relative imprecision of monocyte and basophil automated counts is also confirmed with certain methodologies. This may be due in part to the difficulties of statistical analysis of rare cell types. Consequently the clinical usefulness and quality assessment of automated monocyte and basophil counts will be more difficult to establish. It is apparent from this comparison that automated flow cytometry methods, using either alcin or astra blue heparin staining for basophils, or, a-naphyl butyrate esterase staining for monocytes, are more precise. Constraints such as expense, reliability, and efficiency have limited the continued use of this approach. Automated flow cytometry, or the use of fluorescence flow cytometry and monoclonal antibodies however, may represent more precise alternatives than the manual DWCC as reference methodology.

There is some evidence that the new less direct technologies for basophil counting may be associated with operational difficulties. Furthermore, users need to be aware of the "false negative" implications in clinical conditions associated with dysplastic cellular changes when measured by certain analytical technologies. The technologies include those that utilise the normal granularity of neutrophils, eosinophils, or basophils as a discriminant function (personal observations).

It is important to consider that the DWCC produced by most automation is derived from a nucleated cell count and therefore depends on complete lysis of red blood cells for its accuracy. Abnormalities of the red blood cell membrane, either hereditary, such as certain haemoglobinopathies, or acquired, such as renal and liver disease, may render red blood cells resistant to lysis in some automated systems. Furthermore, myeloperoxidase deficiency, either hereditary or acquired, will produce anomalous results on automated systems that employ this characteristic of white cells in their analytical process. Nucleated red blood cells, dependent on the size of the nuclei, will also interfere with the production of a DWCC on most analytical systems. The frequency of these anomalous DWCC results depends on the type of workload under investigation. Thus users with a high frequency of work from haemoglobinopathy, renal, liver, haematology, oncology and paediatric clinics must ensure the validity of their data from the technology used to produce DWCC.

The detection of clinically important abnormal cells on DWCC automated analysis is a function of the technology specific "flagging" systems that are currently based on self adjusting thresholds and cluster analysis algorithms. Scatterplots and printouts are used to represent this analysis. New categories of cells may be created in this process as well as additional information regarding their nuclear configuration, cytoplasmic contents, and maturity. This information may have additional clinical utility, and is important in the interpretation of the DWCC analysis. It is also important to regard it in context with other aspects of the analytical data produced as part of the FBC, and any cumulative data for the patient, when considering false negative or positive results in terms of the DWCC analysis.

Technological and analytical advance is inevitable in this field. The outcome is likely to be only managerially advantageous when the anticoagulant EDTA is modified or superseded. The most deleterious cellular effect of EDTA is on platelet volume measurements, but important white cell EDTA related changes have been reported with a range of DWCC analysers. Preliminary encouraging data have been reported with an alternative anticoagulant but its usefulness remains to be established with all technologies and in routine practice. Quality control of the automated DWCC is feasible with a high degree of sensitivity.

### Table: Coefficients of variation and methodology for a range of flow cytometers compared with manual methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Code</th>
<th>Expansion</th>
<th>Manufacturer</th>
<th>DWCC-analytical method summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD Hemalog D</td>
<td>HD</td>
<td>H6</td>
<td>Tecnicen</td>
<td>Light scatter; myeloperoxidase (acid); butyrate esterase; alcian blue stain</td>
</tr>
<tr>
<td>H6 Hemalog D</td>
<td>H6</td>
<td>H1</td>
<td>Tecnicen</td>
<td>Light scatter; myeloperoxidase (alkali); myeloperoxidase (acid); butyrate esterase; alcian blue stain</td>
</tr>
<tr>
<td>VCS</td>
<td>VCS</td>
<td>H1</td>
<td>Tecnicen</td>
<td>Light scatter; myeloperoxidase (alkali); myeloperoxidase (acid); butyrate esterase; alcian blue stain</td>
</tr>
<tr>
<td>STKS</td>
<td>STKS</td>
<td></td>
<td>Couter</td>
<td>Light scatter; myeloperoxidase (alkali); myeloperoxidase (acid); butyrate esterase; alcian blue stain</td>
</tr>
<tr>
<td>NE8</td>
<td>NE8</td>
<td></td>
<td>Symsys</td>
<td>Light scatter; myeloperoxidase (alkali); myeloperoxidase (acid); butyrate esterase; alcian blue stain</td>
</tr>
<tr>
<td>CD3</td>
<td>CD3</td>
<td>Cell Dyn 3000</td>
<td>Sequoia Turner</td>
<td>Light scatter; myeloperoxidase (alkali); myeloperoxidase (acid); butyrate esterase; alcian blue stain</td>
</tr>
<tr>
<td>ARG</td>
<td>ARG</td>
<td>Cobs Bio</td>
<td></td>
<td>Light scatter; myeloperoxidase (alkali); myeloperoxidase (acid); butyrate esterase; alcian blue stain</td>
</tr>
<tr>
<td>Man</td>
<td>Man</td>
<td>Manual</td>
<td></td>
<td>Manual; 100 cell DWCC; 1 experienced technologist; wedge film, n = 20</td>
</tr>
<tr>
<td>Man2</td>
<td>Man2</td>
<td>Manual2</td>
<td></td>
<td>Manual; 400 cell DWCC; 2 experienced technologists; wedge film, n = 20</td>
</tr>
</tbody>
</table>
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moving average approach. Unfortunately, quality assessment schemes may have to be derived which are analyser specific due to the inherent problems of the analysis of fixed blood with a diversity of technologies. Cluster analysis and operational software will be upgraded to refine the accuracy of the analytical process. The successful linkage of DWCC analysers to laboratory computer systems, particularly “expert” systems, will have important operational advantages for the routine haematology laboratory in the future.

In conclusion, the automation of the DWCC has significant advantages when compared to the manual alternative. A diversity of sophisticated technology has evolved to produce DWCC data simultaneously with the FBC and other technology dependent cellular variables. These data require careful interpretation in routine practice. The usefulness of each technology depends on the control of preanalytical variables of blood collection, the anticoagulant used, and the nature of local workload. Quality assessment of the automated DWCC technologies has not evolved in a similar manner.