REVIEW ARTICLE

Automated Techniques in Haematology

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ABSTRACT

***Background**: Manual techniques have been mostly in use in developing countries until recently because of its low cost even though it is laborious. Many laboratories are however changing to automated techniques since many samples can be analysed within a short time. It is likely that more laboratories will use these instruments either as semi automated or fully automated multichannel instruments. There is therefore a need for laboratories to become more responsive to the needs of physicians making request by providing help in the presentation and interpretation of results.*

***Method**: Available literature on automation in Haematology was sourced for using both manual library search and Medline search.*

***Results**: Quality control in automated blood cell counts and related topics are extensively researched but there is still dearth of knowledge on automation in other areas of Haematology and on the clinical implications of automation.*

***Conclusion:** It is therefore necessary to make provision for automated facilities in laboratories in view of the high precision and cost effectiveness of these machines. This review is geared towards a critical look at this technology and its application to disease states.*

***KEYWORDS**: Automated techniques; Haematology.*

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INTRODUCTION

Automated techniques have replaced manual techniques in determining the various elements of a complete blood count, this is so because they are not as laborious as the manual techniques and many samples can be analyzed within a short time. Manual techniques are however still in use in many developing countries because of its low cost. Some laboratories however now use cell counters because of their precision and it is likely that many more will use these instruments either as semi automated or fully automated multichannel instruments.

The maintenance of such instruments may be a draw back since blood cell counters vary considerably in the ease of maintenance both from the mechanical and electronic standpoints. Another drawback is the difficulty in recognizing the existence of erroneous results when measurements are carried out on a single machine, as the error may be constant in the particular instrument. Counts also may vary from instrument to instrument and even with different models of the same instrument [1,2].

To check this it is necessary to calibrate the instrument from time to time [3] and to employ quality assurance procedures to check on precision and accuracy [4]. It is also important to prepare a blood film for every sample analysed by the electronic counter since examination of such films will assist in identifying certain errors.

The information provided by electronic blood cell counters is abundant, and the clinician may be confused by so much data, interpreting so many variables and correlating these with clinical data may be challenging for the physician receiving the report. It is therefore necessary to review the importance of this technology and its application to disease states.

Red Cell Indices

The red blood cell (RBC) values provided by blood cell counters are haemoglobin (Hb), haematocrit or packed cell volume (PCV), number of red blood cells (nRBC), mean cell volume (MCV), mean cell haemoglobin (MCV), mean cell haemoglobin concentration (MCHC) and red cell distribution width (RDW). The MCV, MCH and MCHC are referred to as red cell indices or “absolute” values, they are calculated from the results of the red cell count, Hb concentration and PCV. These indices have been widely used in the classification of anaemia.

The measurements that are directly obtained by most blood cell counters are nRBC, Hb, MCV and RDW. The PCV, MCH and MCHC are calculated from combinations of these primary measurements, their replicate errors are therefore higher [5]. MCV is calculated either from the mean height or a selected span of the pulses generated during the red cell count or from the sum of the pulse height divided by the number of pulses which are generated during the count. Hb is measured as HiCN in a standard procedure; PCV is deduced from the red cell count and MCV; MCH is deduced from the Hb and red cell count; whilst MCHC is calculated from the measured Hb and the deduced PCV. The range of normal 'absolute' values varies slightly depending on whether they are based on automated measurements or on PCV determined by centrifugation, these differences are small and without clinical significance except in iron deficiency, thalassaemia and polycythaemia [6].

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Age, sex, race, body build, ethnicity, altitude and other variables affect the reference values

Until the advent of electronic cell counters the only measurement, which could be relied upon was the MCHC calculated from the relatively accurate Hb and PCV measurements. MCVs and MCHs based on visual red cell counts were relatively inaccurate and of less clinical value. Electronic cell counters can give highly reproducibly values for MCV and MCH, but their accuracy is limited by variable shape factors and red cell flexibility as well as by differences in values assigned to the materials used to calibrate them. They are nonetheless, reliable aids to recognizing minor degrees of macrocytosis or to diagnosing iron deficiency at an early stage [7-9].

Anaemia can be classified based on red cell indices as microcytic or macrocytic anaemia and the following measurements are useful in the classification.

MCV: This describes the size of the RBC, the reference range is 86 $\pm$ 10 fl. Values above 100fl indicates macrocytosis, microcytosis is described by values below 70fl. The MCV is rarely higher than 150 fl or lower than 50 fl. Values are often raised in megaloblastic anaemia (commonly due to folate or Vitamin B12 deficiency).

MCH : This defines the haemoglobin content of the red cell. The reference range is 29.5 $\pm$ 2.5 pg, hypochromia results from values which are usually below 27 pg. The MCH is rarely higher than 50pg nor lower than 15 pg. Low MCH occur in iron deficiency and the thalassaemias.

MCHC: This is often not used in the classification of anaemia because it may be normal or diminished in macrocytic anaemias and often diminished in microcytic hypochromic anaemias. Much attention should not be paid to the MCHC and the nRBC since they are not useful in the classification of anaemia.

RDW : The extent of the red cell size variation distribution is measured by the RDW i.e. it is a measure of the degree of anisocytosis. It is useful in distinguishing between iron deficiency and β - thalassaemia trait. A low MCV with a normal RDW suggest thalassaemia trait ; a low MCV with an increased RDW indicates iron deficiency [10,11]. It is also useful in differentiating high MCV due to aplastic anaemia from that due to megaloblastic anaemia, the RDW is normal in aplastic anaemia but high in megaloblastic anaemia [5]. Its unit of measurement is the coefficient of variation (CV) or SD. The normal range of RDW as coefficient of variation (CV) is 12.8 $\pm$ 1.2%. Values above 15% are regarded as increased.

White Blood Cell (wbc) Count

The WBC parameters provided by cell counters are the total number of leucocytes and the differential count. People differ considerably in their leucocyte counts. Some tend to maintain a relatively constant level over a Long period of time [12], others have counts which may vary by as much as 100% at different times. Variation in women may be related to the menstrual cycle [13]. Leucocytosis and leucopaenia are defined when the WBC count is above or below the reference respectively. The reference value for this environment is 3-12 $\times$ 10^9 /L [14]. The normal range for the WBC also depends on age, sex and other variables.

Differential count provided by some blood cell counters is calculated based on both size and the granularity of the WBC. Counts are performed on diluted blood in which red cells are either lysed or are rendered transparent. Differential counting could provide either a three-part or a five to seven-part differential count. A three part differential count assign cells to categories usually designated:

- Granulocytes or large cells, this will include eosinophils and basophils.
- Lymphocytes or small cells.
- Monocytes (mnonuclear cells) or middle cells.

A five to seven part differential count on the other hand classifies cells as neutrophils, eosinophils, basophils, lymphocytes and monocytes [15-17]. Some counters will designate some cells as “large unstained cells”, these cells are larger than the normal lymphocytes and lack peroxidase activity and are often atypical lymphocyte [17], and other counters could identify large immature cells. Automated instruments performing three part or five to seven part differential counts are able to 'flag' or reject counts from samples with nucleated red cells, myelocytes, promyelocytes, blasts and atypical lymphocytes [15,18]. In general, automated counts have compared reasonably favourably with routine manual counts if the instruments are assigned only two functions - performing differential counts on normal samples, and 'flagging' abnormal samples.

In the presence of a significant number of nucleated red cells the total count is neither a true white cell count nor a true total nucleated cell count and the absolute white cell counts calculated from the total will be erroneous. Some instruments have the potential to produce information on enzyme activity index (MPXI). An increased MPXI has been observed in infections, some myelodysplasias, leukaemias, AIDS and megaloblastic anaemia, whereas reduced MPXI occurs in inherited and acquired neutrophil peroxidase deficiency [17,19].

Platelet Measurements

The platelet measurements provided by blood cell counters are the total number of platelet, the mean platelet volume (MPV), platelet distribution width and the plateletcrit.

Platelet Count

The reference value for the total number of platelet among Nigerians is. Lower platelet counts have been observed in apparent healthy Africans and West Indians than in Caucasians [20,21]. Platelet counts are also higher in women than in men [21].

Mean Platelet Volume (MPV)

The reference values for the MPV range between 8 and 12fl, this depend on many variables, one of which is the platelet count. The higher the platelet count, the lower the normal value of the MPV. Low platelet counts with normal MPV occur in immune thrombocytopaenic purpura (ITP) while thrombocytopaenia with high MPV values occur in Bernard-Soulier syndrome. Myeloproliferative disorders are associated with both high platelet count and MPV.

Platelet Distribution Width (PDW)

This is a measure of platelet anisocytosis. The PDW has been found to be useful in distinguishing between essential thrombocythaemia in which the PDW is increased from reactive thrombocytosis in which the PDW is normal.

Plateletcrit

This is the product of the MPV and the platelet count. It is indicative of the volume of circulating platelet in a unit volume of blood. The plateletcrit does not provide any useful clinical information.

Other Automated Techniques

Automated techniques have been introduced in other areas of haematology, these include its use in coagulation and serology. The use of this technology in coagulation and serology is far from being commonplace in developing countries. This may be due to the fact that the technology in this field is more recent. The main problem with its use in coagulation is the difficulty in using homemade reagents with such instruments. Apart from its ease of use and the high precision system, automated haemostasis analyzers have the advantage of reduced volume and so could be of advantage in paediatric patients.

The microtyping system is a sensitive method both for ABO blood grouping and crossmatching, which has changed the traditional method of the use of glass slides. An accuracy of 99.9% was found at first reading with ABO blood grouping [22]. In automated serology techniques the three key aspects of reagent preparation, the test process and interpretation of results must be standardized. In addition, all the steps of the test process: pipetting, incubation, centrifugation, suspension must be carried out according to standard operative procedures (SOPs). The use of SOPs should therefore be put in place in our laboratories in readiness for this technology.

CONCLUSION

The need for laboratory physicians to provide strong support to clinical services and to community health by means of tests that are technically reliable, clinically relevant and diagnostically informative has long been noted, this is particularly necessary in developing countries. It is therefore necessary to make provision for automated facilities in Haematology laboratories in view of the high precision and cost effectiveness of these machines.

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