Automated red blood cell analysis compared with routine red blood cell morphology by smear review

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Summary
The RBC histogram is an integral part of automated haematology analysis and is now routinely available on all automated cell counters. This histogram and other associated complete blood count (CBC) parameters have been found abnormal in various haematological conditions and may provide major clues in the diagnosis and management of significant red cell disorders. Performing manual blood smears is important to ensure the quality of blood count results and to make presumptive diagnosis. In this article we have taken 100 samples for comparative study between RBC histograms obtained by automated haematology analyzer with peripheral blood smear. This article discusses some morphological features of dimorphism and the ensuing characteristic changes in their RBC histograms.

Keywords: RBC histogram, Peripheral blood smear, red cell disorders.

Introduction
The RBC histogram, a graphic representation of particle size distribution, is now routinely available on automated cell analyzers as a standard part of automated complete blood count (CBC) analysis. This histogram in association with other CBC parameters, such as RBC distribution width (RDW) and mean corpuscular volume (MCV), has been found abnormal in various haematological conditions and may provide major clues in the diagnosis and management of significant red cell disorders. In addition, it is frequently used, along with the peripheral blood film, as an aid in monitoring and interpreting abnormal morphological changes, particularly dimorphic red cells.

Aims and objectives
To compare RBC morphology findings on peripheral blood smear with RBC histograms obtained by automated cell counter.

Material and method
In this comparative study of RBC morphology, a total of 100 samples received in central laboratory, civil hospital, Ahmedabad, are evaluated by both, histograms obtained through Cell-dyn 3700 automated haematology analyzer and peripheral blood smear stained by Giemsa stain. Out of 100 samples 17 samples are of iron deficiency anaemia, 5 of megaloblastic anaemia, 6 samples of alcoholic liver disease, 10 of reticulocytosis, 16 of post-iron deficiency anaemia therapy, 5 of beta-thalassemia major and 3 of beta-thalassemia minor showing RBC histograms correlated with that of the peripheral smear. Other 28 samples which do not show correlation with the peripheral smear are-13 with Platelet clumps, 5 with giant platelets, 2 with cold agglutination, 1 with very high leukocyte count, 2 with nucleated RBCs, 3 with post-transfusion & 1 with chronic lymphocytic leukaemia (CLL).

In assessing a histogram, the overall pattern is observed, and the histogram is described by its shape, centre, and spread. This pattern by itself as seen in the red cell distribution curve is meaningless unless it is compared with a reference normal curve and/or confirmed microscopically. Some distributions have simple shapes, such as symmetric and skewed, but others may be more challenging, especially when dimorphic (multiple) populations of red cells are present. Although direct inspection of the distribution curve offers a sensitive method for detecting small populations of microcytic or macrocytic red cells, the estimation of the number of cells from the distribution curve should be avoided. Misleading results can occur because the frequency curve shows only the relative and not the actual number of cells in each size range.5

Figure 1-A shows Typical Normal RBC Histogram

In this study, iron deficiency anaemia (IDA) and beta-thalassemia trait, the red cell distribution curves are shifted to the left, and the percentage of microcytosis is increased. Although their histograms are similar, the degree of anisocytosis, as measured by the RDW, differentiates them. Iron deficiency anaemia is characterized by elevated RDW (FIG 1-B) where as in thalassemia trait RDW is within normal range (FIG 1-C). Although this difference is useful to some extent in distinguishing them, cases involving severe anaemia with homogenous microcytosis give misleading results.
We found that patients with macrocytic anaemia showing a single small peak on the left of the macrocytic cells because of fragmented RBCs and very small cells (FIG 1-E), where as alcoholic liver disease presents with only single macrocytic peak (FIG 1-D).

In case of cold agglutination, the abnormal histogram is the result of a high titer cold agglutinin causing red cells to agglutinate and interfere with their sizing and enumeration with high MCV. (FIG 1-F) It is corrected by smear examination. In cases of Beta-thalassemia major, a high frequency of small cells (<50 fL) is seen at the beginning of the histogram (FIG 1-H). This is due to the small particles seen in this disorder, such as red cell fragments, nucleated RBCs, microspherocytes, and microcytic red cells, producing an erroneous mean cell volume for the intact cell population. In reticulocytosis the histogram is bimodal and is skewed to the right (FIG 1-J).

In samples of Patients with post-IDA therapy shows second population of normocytic cells other than microcytic because of IDA. (Figure 2).
Cases of platelet clumps & giant platelets are misinterpreted as microerythocytes so that correction was done by smear review. Histogram shows following pattern of lower discriminator error.

Cases of extreme leucocytosis or Post-transfusion therapy are having misleading results because they show multiple peak as shown in figure. It is corrected with the help of smear examination.

A case of CLL shows following kind of pattern with abnormal height at upper discriminator.

RBC agglutination, fragmented or very microcytic red cells, platelet clumps, platelet satellitosis, Giant platelets and nucleated RBCs are sources of error in cell counts.

<table>
<thead>
<tr>
<th>Variations observed</th>
<th>Possible causes</th>
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<td>Left of curve does not touch baseline</td>
<td>Schistocytes and extremely small red cells</td>
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<tr>
<td>Bimodal Peak</td>
<td>Transfused cells, Therapeutic response</td>
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<td>Right portion of curve extended</td>
<td>Red cell autoagglutination</td>
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<tr>
<td>Left shift of curve</td>
<td>Microcytes</td>
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<td>Macrocytes</td>
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Results
Out of 100 samples analysed, RBC histogram interpretation of 72 cases shows correlation with Peripheral blood smear & other 28 cases shows discrepancies in results. This means 72% histograms correlated with the findings of the peripheral smear and 28% had discrepancies.

Discussion
The well-known Coulter principle of counting and sizing red cells provides the basis for generating the histogram. This method relies on the change in conductance as each cell passes through an aperture. Normal RBC histogram has a normal Gaussian or bell-shape curve without left or right shift & two sides of the curve coincide when folded in half.

A histogram is a graphic representation of a collection of data based on cell size and/or cell number depicting variations in the process. Because graphics can show data in ways that are meaningful and quickly understood, the histogram is a very powerful tool in red cell morphological analysis. At times, a histogram can provide invaluable information that may not even be apparent in the automated numerical data.

The results of RBC histograms obtained in this study are compared with that of RBC histograms given in the book “The ABC of CBC by DP Lokwani.” Same results of IDA are obtained showing leftward shift of curve showing microcytic population & in post-IDA therapy bimodal peak with two different
populations of RBCs are seen. Errorneous results may be possible with Post-transfusion therapy or infection or tumor anaemia or extreme leukocytosis showing multiple peaks with RBC anisocytosis. Increased RDW combined with low MCV distinguishes IDA from thalassemia minor. Same results are seen in this study too. In megaloblastic anaemia single peak with high MCV or a second peak because of RBC fragments are obtained, similar to this study.

Cold agglutination is a time, temperature, and agglutinin titre dependent reaction, the frequency curves may vary in shapes. It disappears when these samples are incubated at room temperature. Histograms obtained in this study shows similarity with the results given in the book mentioned before. Although the size ranges for RBC histograms are between 24 fL and 360 fL, the instrument counts only those cells with volume sizes between 36 fL and 360 fL as red cells. Those cells counted in the 24 fL to 36 fL range are rejected and not included in the RBC count. They are enumerated and displayed in the histogram area between the 24 fL and 36 fL range, however, allowing the lower end of the histogram to be monitored. Normally, the space below 36 fL remains clear, but in certain conditions the histogram may begin above the baseline or show a high takeoff on the far left of the curve (FIG 1-G to FIG 1-I), which generally indicates the presence of small particles. These particles include red cell fragments, microspherocytes, nucleated RBCs, nonlyzed RBCs, elliptocytosis, macrothrombocytes, platelet clumps, bacteria, parasitic organisms, and other interfering substances such as cryoglobulin, cold agglutinin, and macroglobulinemia.

Potentially, a number of factors may affect the histogram of the aperture impedance cell distribution analysis. These include the coincident doublet of red cells, red cell agglutination, inclusion of reticulocytes with mature red cells, alteration in red cell shape, and inclusion of leukocytes in certain diseases. These factors, in 1 way or another, influence the histogram’s appearances and accordingly will have a variable effect on any measurements made from the histogram. To reduce the effect of these problems, manufacturers design their instruments and reagent systems to specifically prevent and correct for interferences. They develop mathematical algorithms for particle counting and produce numeric data, graphic data, scatter plots, and interpretative comments that will assist or alert the users to potential incorrect results. In addition, to avoid interference in the calculations of RDW, the information below 20% of scale on the red cell histogram are excluded (Figure 3). These misleading data include cell coincidence, aperture artifacts, doublets and triplets, and agglutinates on the right side and platelet clumps, and megathrombocytes on the left side of the histogram.

![Figure 3: Calculation of RDW.](image)

The RDW is calculated from the width of the histogram at 1 SD from the mean divided by MCV. The normal RDW-CV is 11.5% to 14.5%. The RDW-SD is the arithmetic width of the distribution curve measured at the 20% frequency curve. The normal RDW-SD is 39 to 47 fL.

From this study we that RBC histograms assists in identification of smear picture & its correct interpretation gives better idea about diagnosis & management of haematological diseases. But sensitivity of histogram is only 72%, it should be reviewed once through smear for confirmation.

From reviewing histograms, one can get a good idea of what to expect when actually evaluating the peripheral blood film. The speed and reliability of the modern analyzers allow time to evaluate abnormal blood films, consider diagnostic clues, and correlate clinical findings to histograms and other hematologic parameters with greater confidence and efficiency, all of which produce high returns in terms of patient health care.

**Conclusion**

On basis of this study, we come to the conclusion that though automated analyzers reduces overall workload by its advances of graphical representation, it should be confirmed by microscopry.

**References**


