Evaluation of the new red cell research parameters on the Sysmex XE-5000

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Abstract
Background: The Sysmex XE-5000 analyser includes four new red cell research parameters, namely the percentages of microcytic (%Micro R), macrocytic (%Macro R), hypochromic (%LScRBC) and hyperchromic (%HScRBC) red cells. The aim of this study was to establish reference intervals for these four parameters and look at their values in different anaemic populations. They were also compared with estimates of microcytic, macrocytic and hypochromic red cells obtained from the Cellavision DM96.

Methods: 405 samples were analysed in reticulocyte mode for the full study. Also 112 macrocytic sample results were obtained retrospectively to compare with the DM96, along with 82 stained slides from the full study. Results were compared statistically using the Kolmogorov-Smirnoff test, independent t-test, Pearson correlation and receiver-operating characteristic (ROC).

Results: The values for the four studied parameters were not normally distributed so percentile analysis was used to determine reference intervals. The values were statistically different in each anaemic population except for the iron deficient and thalassaemic group. These parameters proved to be reproducible and stable for at least 16 hours at room temperature.

Conclusions: These new parameters correlate better with MCV and MCH results from the analyser than CellaVision estimates. The %LScRBC and %Micro R are useful for predicting restricted erythropoiesis in iron deficiency and thalassaemia. The %LScRBC may provide extra information for clinicians in determining renal patients with functional iron deficiency.

Key words: Hypochromia, microcytosis, macrocytosis, restricted erythropoiesis.


Introduction
All modern haematology analysers produce values for mean cell volume (MCV) and mean cell haemoglobin (MCH). These represent the average size and haemoglobin content of red cells produced in the bone marrow over the last 120 days. Until recently, ADVIA instruments (Siemens, Tarrytown, USA) were the only analysers to use flow cytometry to quantify red cell sub-populations. The Sysmex XE-5000 analyser (Sysmex Corporation, Kobe, Japan) is now capable of estimating percentages of microcytic, macrocytic, hypochromic and hyperchromic red cells.

The proportions of hypochromic and microcytic red cells reflect the body's iron status over the previous couple of months. These parameters have been shown to be a useful indicator of functional iron deficiency (1) and disrupted haemoglobin synthesis in thalassaemias (2). In our laboratory we routinely use the Cellavision DM96 automated microscopy analyser (Cellavision AB, Lund, Sweden). This instrument performs a white cell differential and presents a composite picture of the red cells taken from the stained blood film. It also displays an estimate of percentages of microcytic, macrocytic and hypochromic red cells. These estimates were also compared with those obtained from XE-5000.

The aim of this study was to evaluate these new parameters, establish reference intervals for a healthy population and investigate their diagnostic performance in different types of anaemia.

Derivation of the new parameters
Red blood cells (RBCs) and platelets are counted by a Sheath Flow DC detection method. As the cells pass through an aperture, the change in electrical resistance between two electrodes allows precise size distribution of the RBC population. The percentage of microcytic red cells is calculated from the number of cells between 60 fL and the low discriminator for the red cell population. The %LScRBC and %Micro R are useful for predicting restricted erythropoiesis in iron deficiency and thalassaemia. The %LScRBC may provide extra information for clinicians in determining renal patients with functional iron deficiency.

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Materials and methods

During a 4-month period, samples were selected from the routine workload at Middlemore Hospital Laboratory (Auckland, New Zealand). The “healthy” population (N=148) were selected from patients in the Emergency Department, with no clinical disease history and a normal full blood count and biochemical profile. The “healthy” population (N=148) were selected from routine monthly bloods sent from the Dialysis Unit.

Three “non-healthy” patients groups were selected for the full study. The chronic kidney disease group (CKD; N=99) were selected from samples where haemoglobinopathy screens had been previously performed and serum ferritin levels were <20 μg/L. The Thalassaemic group (THAL; N=58) were selected from samples where haemoglobinopathy screens had been previously performed. There were 31 alpha and 27 beta thalassaemia traits in this group. The iron deficiency anaemia group (IDA; N=100) were selected from samples where iron studies had previously been performed.

Each sample was analysed in the reticulocyte mode of the Sysmex XE-5000, within 12 hours of collection. The new parameter results were obtained from the XE-5000 Research screen.

At the end of the study, an additional “non-healthy” macrocytic population was also included. Only retrospective % Macro R results were obtained from the XE-5000 data base (MACRO N=112) from patients with an MCV >103 fL.

For the comparison between the CellaVision DM96 and XE-5000, a selection of 82 stained blood films from the four “non-healthy” populations were put back on the DM96 analyser for red cell characterization. These values are not stored in the database if red cell characterization is not being routinely reported.

For the reproducibility study, an iron deficient and a normal sample were sampled 10 times in the reticulocyte mode. For the stability study, a thalassaemic sample, kept at room temperature, was sampled every two hours. This was used as it could potentially have to most unstable red cell population.

Statistical analysis was by the Kolmogorov-Smirnoff test, T-test, variance, pearson correlation and receiver operation curves using the statistics package SPSS v13.0 (SPSS, Chicago, USA).

Results

Kolmogorov- Smirnoff test
In the “healthy” population the distribution of values for the four parameters were found not to be normal with a right hand skew to the data. P values for %Micro R, %LScRBC and %HScRBC were <0.001 and 0.021 for %Macro R.

Independent T-Test
In each of the 4 groups sampled in reticulocyte mode, the means for %Micro R, %Macro R, %LScRBC and %HScRBC were significantly different from each other (p<0.001). The only exception was the THAL and IDA group, where the means for %Micro R (p=0.891), %Macro R (p=0.116) and %HScRBC (p=0.393) were not significantly different. However, the %LScRBC was significantly different (p<0.001) between these two groups. The mean %Macro R was also significantly different in the MACRO group compared with the other 4 groups (p<0.001)

Variance

The means and standard deviations (in brackets) of each population group are displayed in the table below. As the values were not normally distributed, standard deviations were not used to calculate the reference intervals from the “healthy” population. Instead, these were derived by calculating the 2.5% and 97.5% distribution quantiles using the statistical programme R (Bell Laboratories, New Jersey, USA).

Pearson correlation coefficients

The table below shows the correlation coefficients for the comparison of %Micro R and MCV, %Macro R and MCV and %LScRBC and MCH for each population group.

The table below shows the correlation coefficients as above for the 82 slides analysed on the CellaVision DM96 compared with the XE-5000.

%Micro R vs MCV
%Macro R vs MCV
%LScRBC vs MCH
Cellavision % MICRO vs MCV
Cellavision % MICRO vs %MICRO R
Cellavision % HYPO vs %LScRBC

Figure 2. Reticulocyte scatter showing derivation of %LScRBC and %HScRBC.
Receiver operating characteristics (ROC)
The reticulocyte haemoglobin concentration (Ret-He) has been shown to be a useful parameter to indicate restricted erythropoiesis in various studies (3). A Ret-He level of 28.5 pg was chosen as a cutoff in the ROC analysis. The area under the curve (AUC) for %LScRBC was 0.978 and for %Micro R was 0.983.

Reproducibility and stability
Reproducibility testing showed coefficients of variation (CV) between 0.01 and 0.05 for the four new red cell parameters. All parameters remained stable for 16 hours at room temperature before the %LScRBC value started to decrease.

Discussion
The means for each new parameter were shown to be significantly different in each of the four population groups studied, except for the IDA and THAL group where the percentages of microcytic, macrocytic and hyperchromic cells were very similar. These findings agree fairly well with a Spanish study (4). However, those authors found no significant difference between the %LScRBC in these two populations with restricted erythropoiesis. This was probably due to the fact that the criteria for their iron deficient population included many borderline and mild iron deficiencies. They also found the values for their “healthy” population were normally distributed and used two standard deviations for their reference intervals. The reference intervals they established (%Micro R 0.2-1.9, %Macro R 5.0-12.0, %LScRBC 0.6-0.0 and %HScRBC 0.5-1.1) were similar to those found in the present study using quantile analysis.

There was a strong overall correlation between the MCV and the %Micro R and %Macro R and between MCH and %LScRBC in the total samples tested. This was also so in each population group with the exception of the “healthy” group where the correlation coefficient between MCH and %LScRBC was only -0.49. The MCV correlation would be expected to be good as the values are determined from the red cell distribution plot. The cut off values (60 fL and 120 fL) are fixed by the manufacturer but it would be useful if these could be changed by the operator.

The Cellavision DM96 estimates percentages of microcytic, macrocytic and hypochromic red cells from the stained blood film. During our initial evaluation of the DM96 we decided these estimates did not always correlate with the film and indices and so we do not report them. The correlations between the new XE-5000 parameters and the MCV and MCH were certainly much better than those for the DM96. In our laboratory we use IT3000 Middleware (Roche, Basel, Switzerland). This software runs our film making rules and we also use it for entering blood film comments and manual differentials. IT3000 has the ability to display these new parameters on our enquiry screen and so we can see the percentages of microcytes and macrocytes on every sample. This has become a very useful tool in helping to decide about red cells in the blood film, especially where there is an increased red cell distribution width or dimorphic population.

The AUCs were both close to 1.0 for %LScRBC and %Micro R indicating low false positive predictions for determining restricted erythropoiesis in both thalassaemias and iron deficiencies. The %LScRBC may also prove to be a useful parameter in helping to decide if a patient has functional iron deficiency. This is a common occurrence in CKD where dialysis patients may have adequate iron stores yet cannot utilise this iron to support erythropoiesis when given erythropoietin. These patients typically have normal or high serum ferritin levels but low transferrin saturation and require parenteral iron infusions. The % hypochromic red cells from ADVIA instruments have been demonstrated to be a good predictor of iron deficiency; however this parameter has been shown to be unstable as the sample ages. An Italian study found the %LScRBC to be a better predictor of iron responsiveness than baseline serum ferritin or transferrin saturation in dialysis patients (5). The AUC in that study was 0.72 with a best cut-off value of 2.7%

Our protocol at Middlemore is to maintain a saturation of 0.2 – 0.4 and ferritin >200 μg/L in dialysis patients. In the CKD population that was sampled, 12% had ferritins <200, 16% RET-He <28.5, 14% saturations <0.2 and 32% with %LScRBC <2.7. A %LScRBC cut-off of 5% may be a better indicator, equating to 16% of these patients.

Conflicts of interest
The author declares no conflicts of interest.

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References