CORRESPONDENCE

Validation of the reticulocyte channel of Sysmex XN-9000 system for blood cell count in samples with suspected cold agglutination for use in a total laboratory automation setting

Modern haematology platforms provide information regarding blood cell count, differential cellular characteristics, leukopoiesis, erythropoiesis and thrombopoiesis. Among the disorders of red blood cells (RBC), anaemias are a common healthcare problem, for which effective treatment is possible if the cause is correctly recognised. For this purpose, a classification system based on the RBC indices, generated automatically by haematology systems, is used and has taken on clinical importance.[1] Among RBC indices, the mean corpuscular haemoglobin concentration (MCHC) is one of the most indicative parameters. Elevated MCHC can be seen in RBC disease, in cold agglutination (CA) or in haemolysed or icteric samples.[2] Coronary artery disease (CAD) is an antibody and complement-mediated haemolytic anaemia classified as primary, if associated with lymphoproliferative disease, and secondary CAD, when associated with malignant disease and infection, respectively.[3] Diagnosis of CAD occurs by direct antiglobulin test. High mean corpuscular volume (MCV) and false reduction in RBC count are often seen in CAD, together with a false increase of mean corpuscular haemoglobin (MCH) and MCHC. In this condition, preheating at 37°C the blood sample for 2 hours permits to restore the correct values of the perturbed haematological parameters.

Previous studies reported the use of reticulocyte (RET) channel of Sysmex XN series (Sysmex, Kobe, Japan), which, through a short preheating (1 min) at 41°C, may return the correct RBC count and derived indices by spontaneous separation of agglutinated RBC.[4 5] In this study, we aimed to further validate this approach for use in a total laboratory automation framework, with the aim to minimise manual intervention and taking advantage of the information-processing unit (IPU) available on our Sysmex XN-9000 haematology system (IPU V.22.09-00), based on a three module configuration working in parallel on the same track.[6] In the XN analyser, RBC and haematocrit (HCT) are measured at room temperature using an impedance (I) technology, with a hydrodynamic focusing system and the cumulative pulse method, and MCV is calculated from the two previous values. The additional parameter 'RBC most frequent volume' (R-MFV) is also available. Blood haemoglobin (HGB) concentrations are measured by photometry using sodium lauryl sulphate (SLS) reagent. MCH (HGB/RBC) and MCHC (HGB/HCT) are calculated according to Wintrobe formulas.[1]

Using an MCHC value ≥370 g/L, considered in previous studies as the limit for selecting false increases due to CA,[4] we recruited 43 consecutive samples, who underwent a complete blood cell count for diagnostic purposes, excluding repeated samples from the same patient. By default, in our daily practice, these samples are automatically submitted to the 37°C/2 hours treatment to confirm/amend RBC count and related indices. In our validation study, we compared values obtained by I technology after preheating at 37°C/2 hours and after short warming at 41°C by the RET channel using optical technology. In the RET channel, after warming in the incubation chamber, RETs are separated from mature RBCs using a fluorescence signal associated to the presence of intracellular nucleic acids. The HGB in the RET channel (HGB_RET) is derived from the optical RBC (RBC_RET) count and RBC haemoglobin content (RBC-He) using the formula RBC-He x RBC_RET, and the MCHC_RET is obtained as RBC-He/R-MFV ratio. To test the significance of the difference between values, the Wilcoxon test for paired samples was applied since data, checked by Shapiro-Wilk test, were not normally distributed. Methods comparison was carried out by Passing-Bablok regression. Statistical analysis was performed by MedCalc software V.18.11. A p-value<0.05 indicated statistical significance.

Using standard I technology, the median (min–max) RBC, HGB, MCV, HCT and MCHC values of the recruited samples were 4.10 (0.67–6.52) 10^{12}/L, 138 (77–227) g/L, 89.9 (72.6–121.9) fL, 0.372 (0.071–0.607) L/L and 375 (370–1803) g/L, before, and 4.35 (2.15–6.58) 10^{12}/L, 132 (70–225) g/L, 94.6 (76.7–121.0) fL, 0.412 (0.208–0.630) L/L and 343 (292–416) g/L, after the 37°C/2 hours treatment, respectively (figure 1). We excluded from further analysis the sample from a female patient with a diagnosis of Waldenström macroglobulinemia and a monoclonal immunoglobulin (IgMκ) concentration in serum of 46.4 g/L. McMullin *et al* previously showed an inaccurate HGB estimation in this clinical condition due to an unusual reaction of the employed reagent on Sysmex instruments with the pentameric IgM paraprotein.[7] Two samples with serum triglycerides>4.5 mmol/L were also excluded as hypertriglyceridaemia is a known interferent with the measurement of HGB using SLS reagent.[8]

Figure 2 shows the distribution of results related to RBC, HGB and MCHC obtained by the RET channel compared with those of the three parameters routinely measured after preheating at

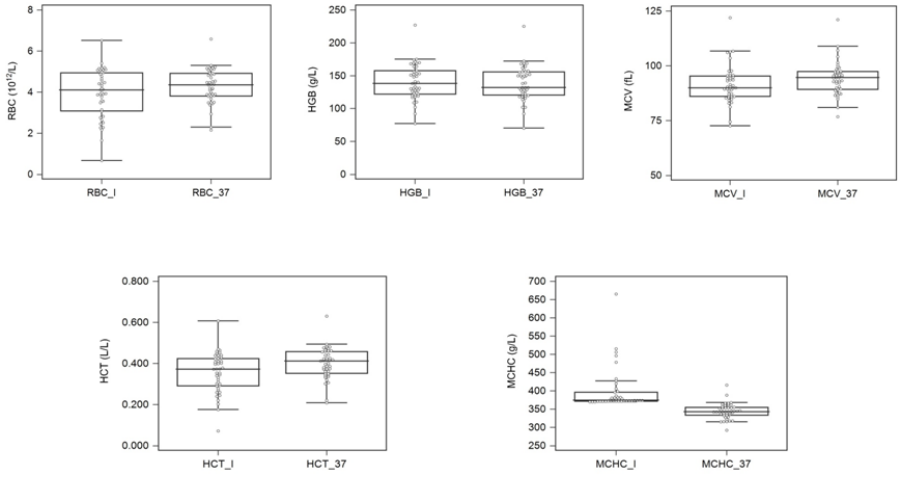

Figure 1 Box and whisker plots showing the baseline values obtained by standard technology of red blood cells (RBC_I), blood haemoglobin concentration (HGB_I), mean corpuscular volume (MCV_I), haematocrit (HCT_I) and mean corpuscular haemoglobin concentration (MCHC_I) compared with the same parameters after preheating at 37°C/2 hours (RBC_37, HGB_37, MCV_37, HCT_37 and MCHC_37). For graphic reasons, the maximum value of MCHC_I (1803 g/L) was omitted.

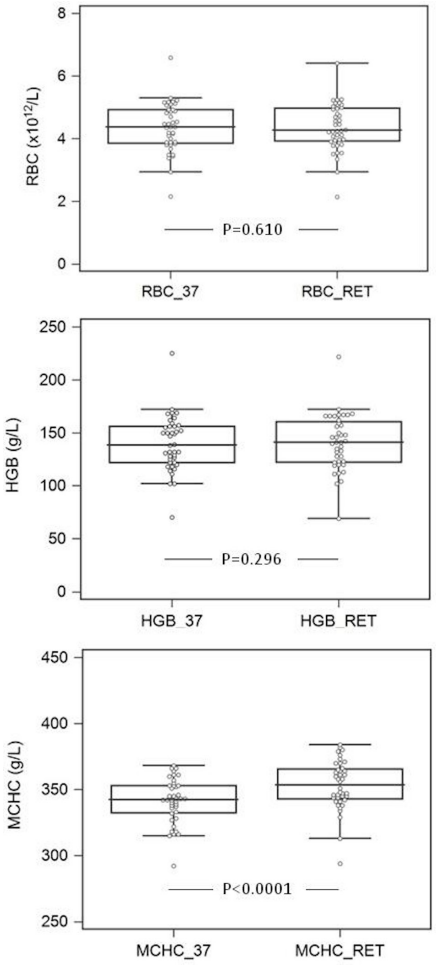

Figure 2 Box and whisker plots showing the distribution of results related to red blood cells (RBC), blood haemoglobin concentration (HGB) and mean corpuscular haemoglobin concentration (MCHC) obtained by the reticulocyte (RET channel (RBC_RET, HGB_RET, and MCHC_RET) compared with those of the three parameters routinely measured in the selected samples (n=40) after preheating at 37°C/2 hours (RBC_37, HGB_37 and MCHC_37).

37°C/2 hours. The statistical comparison demonstrated no significant differences for RBC and HGB, with regression equations (95% CIs in parentheses) showing no significant deviation from the identity equation: RBC_RET=0.975 (0.922 to 1.027) RBC_37+0.12 (−0.10 to 0.37) 10^{12}/L and HGB_RET=1.040 (0.929 to 1.129) HGB_37–3.7 (−14.4 to 10.4) g/L, respectively. On the contrary, a significant difference was observed between MCHC_RET and MCHC_37 (median (IQR), 353.5 (343–365.5) g/L vs 342.5 (332.5–353) g/L, respectively). Different approaches in estimating RBC volume used to calculate MCHC (HCT/RBC by the standard I technology and R-MFV in the RET channel) may explain this difference.

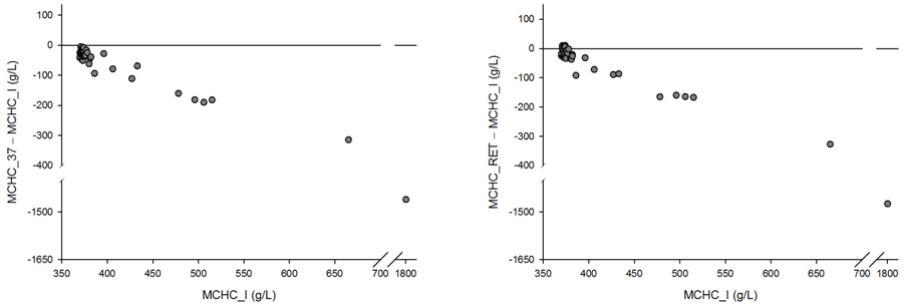

Figure 3 Plots showing the differences between the mean corpuscular haemoglobin concentration (MCHC) values obtained after preheating at 37°C/2 hours of blood samples (MCHC_37, left) or by the RET channel (MCHC_RET, right) and those obtained using the standard impedance technology (MCHC_I).

However, as shown by difference plots depicted in figure 3, both MCHC_37 and MCHC_RET equally resolved the falsely altered MCHC values>385 g/L obtained using the standard I technology. Indeed, the stratification of samples based on the initial MCHC values showed that for the group with MCHC>385 g/L (n=11), MCHC_37 and MCHC_RET values fully overlapped (median (min–max), 327 (292–368) g/L and 338 (294–364) g/L, respectively; p=0.465).

Our study results support the introduction of an automatic reflex test when the critical MCHC value of 385 g/L is exceeded in the standard blood cell count by the Sysmex XN-9000 and a CA condition is suspected. In this case, repeating the test using the RET channel, followed by calculation of optical parameters by Extended IPU, can guarantee the CA reversibility for these samples. The implementation of this reflex approach in a total laboratory automation setting may solve the problem of spuriously high MCHC, by both minimising manual operations and offering accurate information to clinicians in a few tens of minutes instead of waiting 2 hours for sample preheating.

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