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Optimizing the management of analytical interferences affecting red blood cells on XN-10 (Sysmex®)

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Abstract

Introduction: Interferences on red blood cells (RBCs) measurement and the associated parameters in haematology analyzers are very common. Many sources of interferences are described but their management remains uncertain depending on the measurement system; we aimed at developing an optimized scheme allowing the accurate management of most interferences affecting RBCs, based on the alternative "optical" parameters from SYSMEX XN-10.

Methods: Samples from 12 groups of relevant interferences were analysed and compared with a control group allowing (1) the determination of deviation thresholds beyond which an interference is likely, and (2) the development of two flowcharts for their subsequent management. These flowcharts were then evaluated among a bank of retrospective typical cases of interferences and in the routine flow of the laboratory.

Results: After verifying the excellent agreement between standard and alternative parameters, the comparative study between analytical channels allowed to determine an acceptable deviation and then discriminate technical concerns caused by cold agglutinins, leukocytosis and plasma-related interferences. This led to the development of flowcharts ensuring the accurate management of these interferences, whether MCHC is <320 or >365 g/L. These proposed flowcharts allowed the correction of 63/65 historical confirmed interferences cases (97%). Furthermore, they corrected 18 results among 901 unselected prospective samples.

Conclusion: The resulting flowcharts allow a relevant correction for most common interferences affecting RBCs and are now definitively included in the routine analytical management and will be directly incorporated in the middleware of the laboratory.

KEYWORDS

analytical interference, flowchart, RBCs count, XN-10

1 | INTRODUCTION

Accurate management of analytic errors from haematology analyzers in the determination of the cell blood count is an everyday concern in clinical laboratories. While many situations may impair measurements and accurate cell counts, interferences affecting red blood cells (RBCs) and the associated parameters are among the most common. Several sources of interferences are well-recognized: plasma changes (due to hemolysis, lipemia, icteric plasma, or immunoglobulins), haemoglobin or red blood cells disorders, leukocytosis.[1–7]

With the development of new generation analyzers, novel technologies and parameters have been introduced to improve both analytical performances and detection of presumed interferences.

The XN-10 (Sysmex, Kobe, Japan) combines impedance and photometry methods for the measurement of standard RBC parameters (haemoglobin (photometry) (HGB), hematocrit (HCT), and mean corpuscular volume (MCV)) and calculates MCH (HGB/RBC) and MCHC (HGB/HCT). It may provide in parallel alternative parameters primarily obtained by the optical method derived from the RET channel, after labelling of intracellular nucleic acids by a fluorescent marker: namely optical RBC count (RBC-O), RBC haemoglobin content (RBC-He); optical haemoglobin (HGB-O) derived from RBC-O and RBC-He. Of note, an alternative evaluation of MCV is available, namely R-MFV (defined as RBCs most frequent volume), which is obtained by the impedance channel however.

Regarding interferences with XN-10, the circumstances associated with spurious increased mean corpuscular haemoglobin concentration (MCHC), which stands as one of the best identified and described interference-related situation, have been studied by Berda-Haddad et al who proposed a decision-tree to ensure accurate results[1] and by Roccaforte et al as well who studied cold agglutinins and RET channel especially[3]; in addition, Aruga et al have recently assessed haemoglobin concentration in case of chylous turbidity.[2] Furthermore, the impact of leukocytosis on RBCs parameters and the subsequent management has been recently evaluated by Schillinger.[4]

Nevertheless, while all of the alternative parameters are expected to improve the management of analytical errors, the concrete impact of each relevant type of interferences on the different measurement channels remains to be determined.

In this study, we aimed at developing flowcharts, which should be relevant for routine practice, allowing the management of most interferences affecting RBCs, based on the alternative "optical" parameters of XN-10 device.

For this purpose, as a first step, an as exhaustive list as possible of relevant interferences and associated-impact on RBCs parameters was established according to literature. Then, after assessing within a control group the correlation between impedance and optical methods for the measurement of RBCs parameters, a deviation threshold beyond which an interference is likely was determined and studied in 12 groups of relevant sources of interferences. Thereafter, these thresholds were combined to propose flowcharts, which were evaluated in a bank of 65 historical cases with significant interferences, and in a series of 901 unselected prospective samples.

2 | MATERIALS AND SAMPLES

2.1 | Materials

The Sysmex XN-10 (Sysmex, Kobe, Japan) is an automated blood cells analyser, with six channels combining three measurement principles: impedance, photometry and fluorescence/optical method. All of the three channels are used for the measurement of RBCs parameters. Regarding impedance, after hydrodynamic focusing, cells are sent between two electrodes; the resulting electric impulsion being proportional to the size of the cells, the RBCs count corresponds to the number of impulsions. Haematocrit is defined using the cumulative pulse size and MCV corresponds to HCT/RBC, while R-MFV corresponds to RBCs most frequent volume. In parallel, the measurement of haemoglobin is obtained with photometry after lysis and dilution, allowing MCHC and MCH to be calculated. Additionally, the RET channel allows a second count of RBCs (termed RBC-O), with optical method using fluorescence flow cytometry after stabilization and warming at 41°C. This alternative measurement then allows the calculation of HGB-O derived from the RBC-O count and RBC-He (HGB-O = RBC-O × RBC-He). A corrected MCHCc is also available (MCHCc = HGB-O/(RBC-O × R-MFV) × 100).

In our laboratory XN-10 is used in daily routine, controls are analysed every day using both internal quality control XN Check (Sysmex, Kobe, Japan) and external quality control RANDOX (Riqas) and correlation between the three connected modules is weekly verified with fresh blood samples. Specimens in the study were samples from patients (with both children and adults) from the Nancy University Hospital, requiring a blood count during their care. The samples were collected in K2-EDTA tubes from Becton Dickinson (Franklin Lakes, NJ, USA) and Greiner MiniCollect® Complete, K2-EDTA spray dried for micromethods samples.

2.2 | Samples

According to the French ethical laws, the patients are informed that their biological data obtained in routine care may be anonymously used unless they express an opposition. The local Institutional Review Board deemed the study exempt from review.

2.2.1 | Interferences groups

Many interferences are described in the literature[1–10]; however, some of them were not included in our study, for the following reasons:

1. Presumed (very) low frequency[7] (warm agglutinin disease, carboxyhaemoglobin, sulfhaemoglobin);
2. Considered as analytically insignificant[5,7] (giant platelets, microcytes and schistocytes);
3. Impact on reticulocytes' numeration only (no impact on RBCs)[7,8];

TABLE 1 Categories of interferences selected in the study

Categories	Inclusion criteria	Estimated frequency of the interference in our laboratory	*n*	Expected impact on measurement channels and parameters, according to the literature and laboratory's field experience	Δ Comparison versus control group		Integration for flowchart's development
Leukocytosis	WBC ≥90 × 10^9/L	Monthly	*n* = 52 (104–408 × 10^9/L)	Impedance	ΔRBC%	***	Yes
				RBC, HCT, MCV ↗ MCHC ↘ (<320 g/L) MCH ↘	ΔHGB%	**	
					ΔMCHC%	***	
				Impact visible on RBC histogram	ΔMCH%	***	
Cold agglutinin suspicion	« RBC agglutination? » Positive flat	Daily	*n* = 55	Impedance	ΔRBC%	***	Yes
				RBC, HCT ↘ MCV ↗ MCHC ↗ (>365 g/L)	ΔHGB%	***	
					ΔMCHC%	***	
				Impact Obvious on RBC histogram	ΔMCH%	***	
Lipid histogram	Lipidic abnormalities on WNR and WDF scattergrams	Daily	*n* = 33	Optical	ΔRBC% = ns	ns	No
				MCHC ↗ (>365 g/L)	ΔHGB%	ns	
				Detection by WNR histogram	ΔMCHC%	ns	
					ΔMCH%	ns	
Hereditary spherocytosis	Hereditary spherocytosis	Daily	*n* = 33	Impedance and Optical	ΔRBC%	ns	No
				RBC ↘ MCHC ↗ (>365 g/L)	ΔHGB%	ns	
					ΔMCHC%	ns	
					ΔMCH%	ns	
Sickle cell disease	Sickle cell disease	Daily	*n* = 30	Detection by RBC and RET histograms	ΔRBC%	ns	Yes
					ΔHGB%	***	
					ΔMCHC%	***	
					ΔMCH%	***	
Cryoglobulin	Positive test for cryoglobulinemia on a sample from concomitant blood collection	Annual	*n* = 30	Impedance	ΔRBC%	***	No
				HGB ↗ MCHC ↗ (>365 g/L)	ΔHGB%	ns	
					ΔMCHC%	ns	
					ΔMCH%	ns	
Immunoglobulin	IgG > 18 g/L (concomitant blood collection)	Weekly	*n* = 30 (28.4–87.5 g/L)	Photometry	ΔRBC%	*	No
				HGB ↗, MCHC ↗ (>365 g/L)	ΔHGB%	ns	
					ΔMCHC%	ns	
				MCH ↗	ΔMCH%	ns	
Glycemia	Glycemia ≥22 mmol/L (concomitant blood collection)	Monthly	*n* = 30 (22–53 mmol/L)	MCV, HCT ↗	ΔRBC%	ns	No
					ΔHGB%	ns	
				MCHC ↘	ΔMCHC%	ns	
					ΔMCH%	ns	
Bilirubin	Bilirubinemia >17 µmol/L (concomitant blood collection)	Weekly	*n* = 30 (109–387 µmol/L)	Photometry	ΔRBC%	ns	Yes
					ΔHGB%	***	
				HGB ↗, MCHC ↗ (>365 g/L), MCH ↗	ΔMCHC%	***	
					ΔMCH%	***	
Lipemic sample	Lipemic indice >2 (concomitant blood collection)	Daily	*n* = 30	Photometry	ΔRBC%	ns	Yes
					ΔHGB%	***	
				HGB ↗, MCHC ↗ (>365 g/L), MCH ↗	ΔMCHC%	**	
					ΔMCH%	***	

TABLE 1 (Continued)

Categories	Inclusion criteria	Estimated frequency of the interference in our laboratory	*n*	Expected impact on measurement channels and parameters, according to the literature and laboratory's field experience	Δ Comparison versus control group		Integration for flowchart's development
Icteric sample	Icteric indice >4 (concomitant blood collection)	Monthly	$n = 30$	Photometry	ΔRBC%	ns	Yes
					ΔHGB%	*	
				HGB ↗, MCHC ↗ (>365 g/L), MCH ↗	ΔMCHC%	***	
					ΔMCH%	ns	
Haemolysed sample	Haemolysis indice >3 (concomitant blood collection)	Daily	$n = 30$	Impedance and Optical	ΔRBC%	ns	Yes
					ΔHGB%	ns	
				RBC HCT ↘ HGB ↗, MCH ↗, MCHC ↗ (>365 g/L)	ΔMCHC%	ns	
					ΔMCH%	ns	

Note: Hemolysis Lipemia and Icterus (HIL) indices are obtained by STA-R Max (Stago®) on a concomitant blood sample. ns: not significant.
Abbreviations: HCT, haematocrit; HGB, haemoglobin (photometry); HGB-O, optical haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCHCc, corrected mean corpuscular haemoglobin concentration; ΔMCHC, deviation for MCHC; ΔMCH, deviation for MCH; ΔHGB, deviation for haemoglobin; MCV, mean corpuscular volume; R-MFV, RBCs most frequent volume; ΔRBC, deviation for RBC count; RBC-0, optical RBC count; RBC, RBC count-impedance; RBC-He, RBC haemoglobin content; WBC, white blood cells; WDF, WBC differential channel; WNR, white cell nucleated channel.
*$p < .05$, **$p < .01$, ***$p < .001$.

4. Sample withdrawal, in accordance with the local preanalytical and analytical procedures (coagulated samples, excess of EDTA due to insufficient sample volume or insufficient blood aspiration).

Accordingly, 12 selected interferences were engaged for evaluation (Table 1). For each selected interference, raw data from 30 samples at least were collected (without age or sex criteria) by extraction in anonymous. CSV files from the analyser. All samples displayed data with the three measurement channels (impedance, photometry, and fluorescence/optical).

2.2.2 | Control group

To evaluate the correlation between optical and impedance methods and their ability to provide similar results for RBC parameters, a "control group" (i.e., a priori devoid of interferences) was established including all blood counts analysed during 1 month, excepted those presenting the following exclusion criteria:

- Presence of any Sysmex alarm flag,
- Leukocytes count >90 $\times$ 10^9/L
- Abnormal MCHC (inferior to 320 g/L or superior to 365 g/L), since the vast majority of relevant interferences systematically impacts MCHC, as evidenced in Table 1.

2.3 | Methods

The design of study is summarized in Figure 1.

2.3.1 | Agreements and acceptable deviation between impedance and optical methods

Pearson's correlation was used to assess the agreement between impedance and optical parameters in the control group: RBC versus RBC-O; HGB versus HGB-O; MCH versus RBC-He; MCHC versus MCHCc. In addition, the agreement for RBCs volume (MCV vs. R-MFV) was evaluated, although both derive from the same measurement channel (impedance).

Then, regarding the following red blood cells parameters, RBC, HGB, MCH, and MCHC, a deviation percentage (termed Δ) between impedance and optical method was determined as follow within each group of interference and then compared with the control group:

$$\Delta\text{RBC}\,(\%) = ((\text{RBC-O} - \text{RBC})/\text{RBC}) \times 100.$$

$$\Delta\text{HGB}\,(\%) = ((\text{HGB-O} - \text{HGB})/\text{HGB}) \times 100.$$

$$\Delta\text{MCH}\,(\%) = (\text{RBC-He} - \text{MCH})/\text{MCH} \times 100.$$

$$\Delta\text{MCHC}\,(\%) = (\text{MCHCc} - \text{MCHC})/\text{MCHC} \times 100.$$

2.3.2 | Criteria for the development of flowcharts

After careful literature review and/or according to the assessment of a significant deviation between impedance and optical method (Table 1), MCHC was chosen as input for the flowcharts; interferences groups were the included in the study according to the (1) existence of significant difference(s) of Δ as compared with the control group, and (2) the critical thresholds defined as the 3rd and/or 97th percentiles of the distribution within the control group.

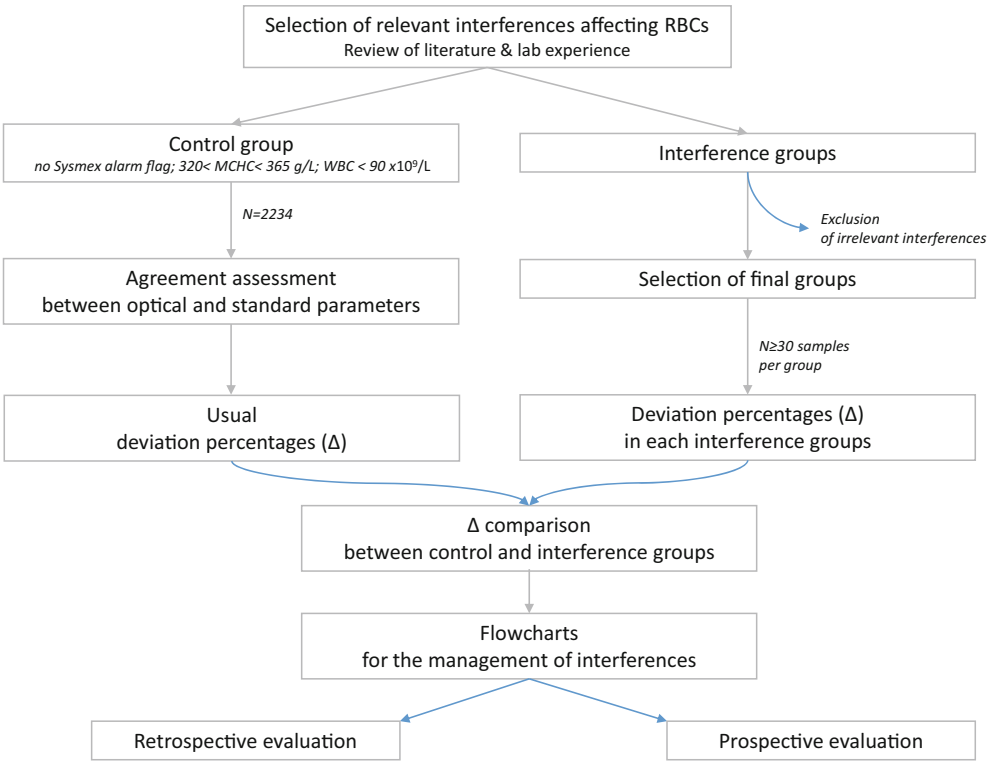

FIGURE 1 Study design. MCHC, mean corpuscular haemoglobin concentration; WBC, white blood cells

2.3.3 | Evaluation of flowcharts

The resulting flowcharts were first retrospectively evaluated by testing on a bank of 65 historical cases from our laboratory showing significant interferences, to assess whether they may provide relevant corrected values. The flowcharts were then tested within 901 unselected prospective routine samples from our laboratory, among which 225 samples presenting MCHC <320 and 43 with MCHC >365 g/L.

2.3.4 | Statistical analysis

The agreement between impedance and optical methods in the control group and in the 12 interferences groups was assessed by Pearson's correlation. Gaussian distribution of the control group was verified by Shapiro–Wilk test. Descriptive statistical analyses are performed with Kruskal Wallis test; percentages of deviation (Δ) were compared using Dunn's test and then modelized with Tukey box.

Analyses were carried out using PRISM® v 5.0 statistical software and XLSTAT® 2012 from Microsoft Corporation®, a *p*-value <0.05 was considered as statistically significant.

3 | RESULTS

3.1 | Control group and correlation between impedance and optical methods

The control group included 2234 samples; the agreement between impedance and optical methods was excellent regarding RBC versus RBC-O ($r^2 = 0.99$); HGB versus HGB-O ($r^2 = 0.98$); MCH versus RBC-He ($r^2 = 0.91$); agreement between MCHC and MCHCc was correct ($r^2 = 0.50$) (data not shown). This allowed the determination of an acceptable range comprised between the 3rd and 97th percentiles for Δ RBC (%) Δ HGB (%) Δ MCH (%) and Δ MCHC (%) (Table 2).

3.2 | Interferences and acceptable deviation between impedance and optical methods

Twelve groups of interferences were selected for the study; comparisons of Δ RBC (%) Δ HGB (%) Δ MCH (%) and Δ MCHC (%) between control group and each group of interferences are detailed in Table 1 and Figure 2; among the 12 groups, 3 (presence of lipids on scattergrams; hereditary spherocytosis; hyperglycemia) did not show any statistical difference as compared with the control group and were thus excluded.

Distribution characteristics of the control group are detailed in Table 2, 3rd and 97th percentiles especially, which will be used to propose 2 flowcharts, in a case of abnormal MCHC, <320 g/L and >365 g/L (Figure 3A and B, respectively). Only interferences both with MCHC <320 and MCHC >365 g/L and easily correctable impact were considered. Therefore, 2 additional interferences were excluded from evaluation, namely cryoglobulinemia and high Ig titre, because of normal MCHC.

3.3 | Evaluation of flowcharts' performances

Regarding the retrospective evaluation, the algorithms were tested on a historical bank of 65 cases of significant interferences and allowed

TABLE 2 Distribution's characteristics of ΔRBC (%), ΔHGB (%), ΔMCH (%), ΔMCHC (%) in the control group ($N = 2234$)

	ΔRBC (%)	ΔHGB (%)	ΔMCH (%)	ΔMCHC (%)
Minimum	−8.59	−12.50	−13.29	−13.52
3rd percentile	**−4.44**	**−6.20**	**−5.35**	**−6.48**
25th percentile	−1.91	−2.68	−1.89	−2.17
Median	−0.53	−0.85	0.00	0.11
75th percentile	0.68	1.10	1.63	2.07
97th percentile	**3.09**	**4.65**	**4.29**	**5.46**
Maximum	13.27	9.48	8.22	10.16

Note: Bold values corresponds to the 3rd and 97th percentiles which were further used for the development of flowcharts.
Abbreviations: ΔHGB, deviation for haemoglobin; ΔMCHC, deviation for MCHC; ΔMCH, deviation for MCH; ΔRBC, deviation for RBC count.

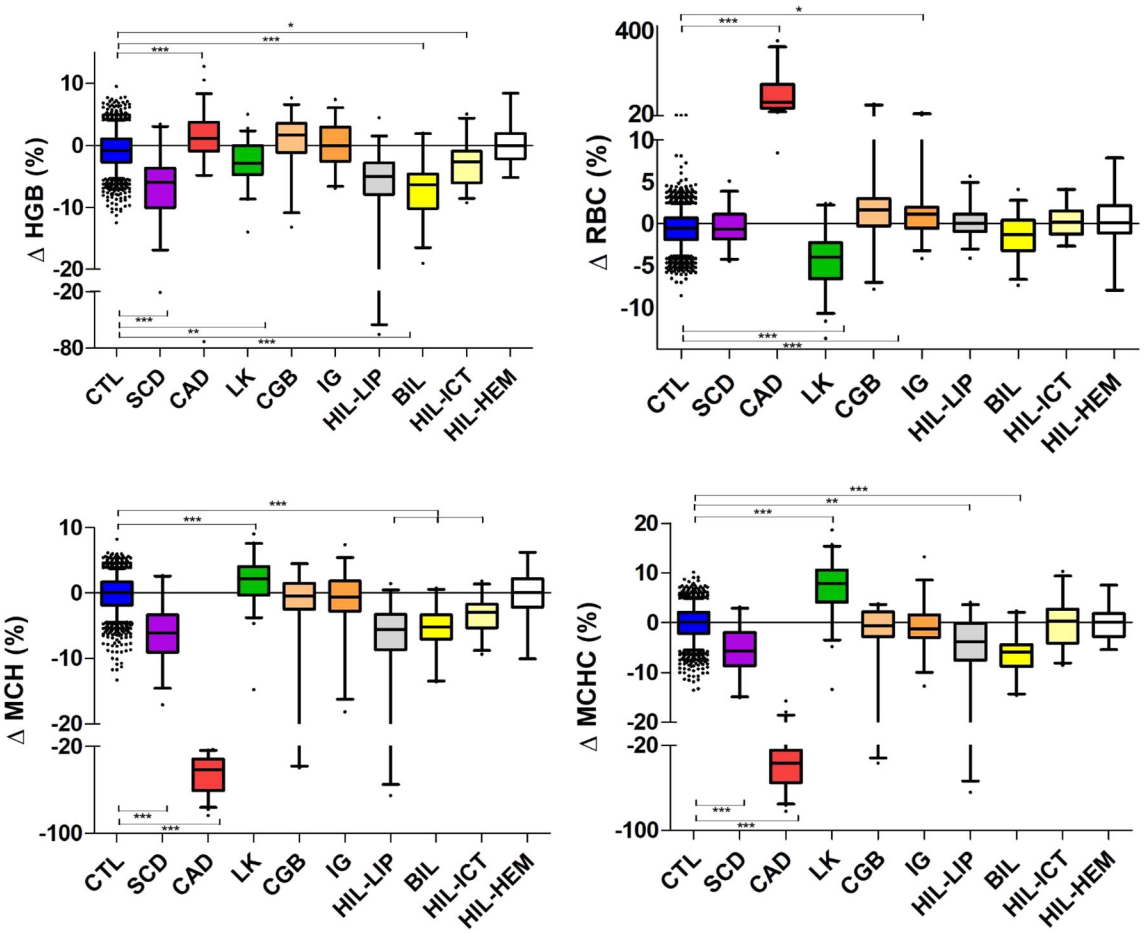

FIGURE 2 Variation of the deviation percentage (Δ) in the selected groups of interference as compared with controls; Only groups included retained for flowchart's design or with at least one significant Δ are shown, (Tukey boxplot; * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$). BIL, bilirubin; CAD, cold agglutinin disease suspicion; CTL, control groups; CGB, cryoglobulin; IG, immunoglobulin; HIL-LIP, lipemic sample; HIL-ICT, icteric sample; HIL-HEM, haemolyzed sample; LK, leukocytosis; SCD, sickle cell disease

the direct subsequent correction of 63 counts (97%). The description of these samples is given in Appendix (Table S1). The first case was related to a highly lipemic plasma, unexpectedly associated with a decreased MCHC (a spuriously increased MCHC is expected instead). After questioning, it appeared that the blood sample was withdrawn concomitantly with parenteral nutrition, leading a critical dilution and decreased MCHC; therefore, in accordance with the algorithm A, measurement of RBC with optical channel was not performed. The subsequent correction was impossible, a new sample was mandatory. The second case involved a sample with a very high level of cold agglutinins: the irrelevant MCHC and ΔHGB < −6.2% suggest a plasma interference, whereas plasma aspect was strictly normal. The

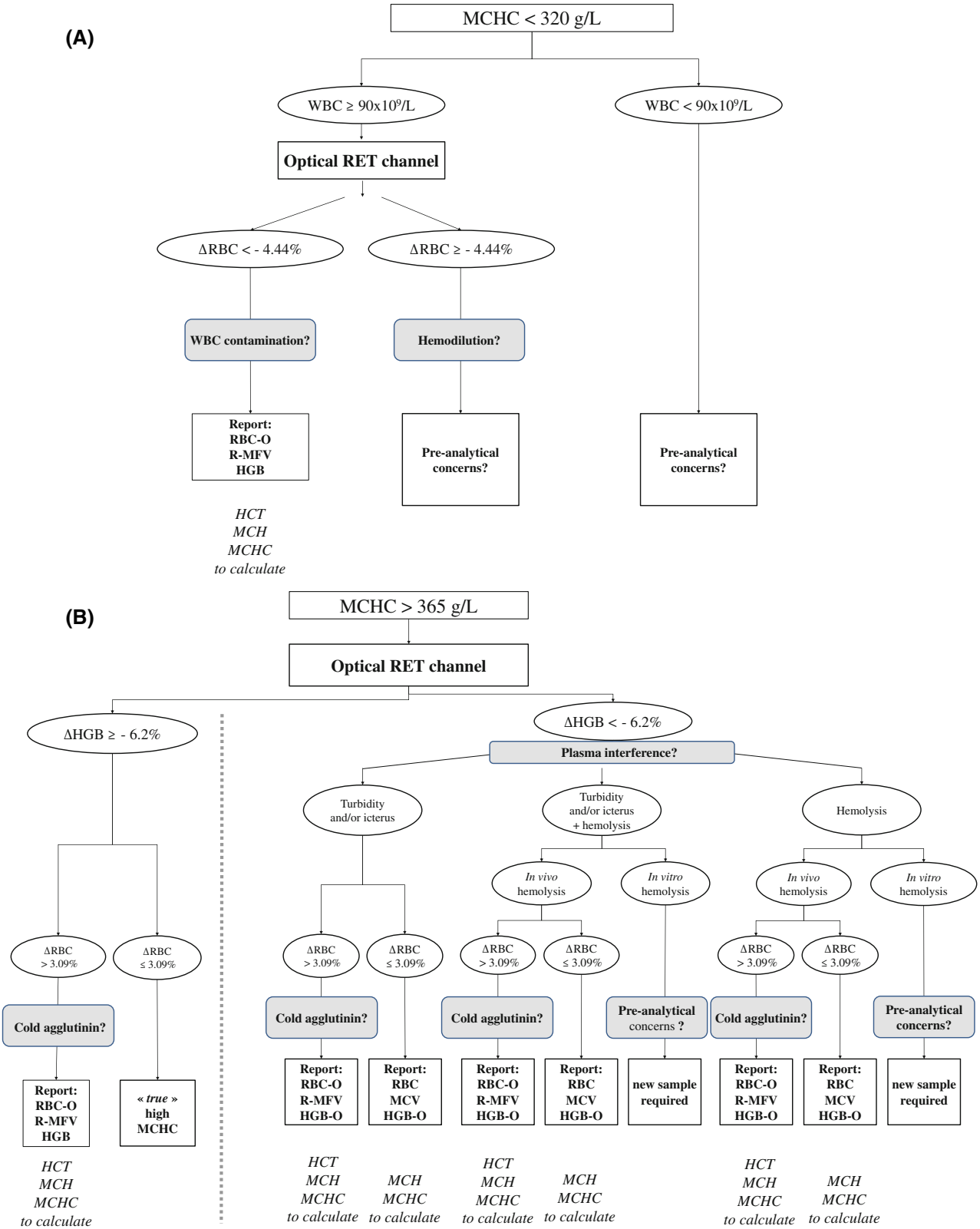

FIGURE 3 Flowcharts for the management of interferences whether Mean Corpuscular Haemoglobin Concentration (MCHC) is (A) < 320 g/L or (B) > 365 g/L. HGB, haemoglobin (photometry); R-ΔHGB, deviation for haemoglobin; HGB-O, optical haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCHCc, corrected mean corpuscular haemoglobin concentration; MFV, RBCs most frequent volume; RBC, RBC count—impedance; RBC-He, RBC haemoglobin content; RBC-O, optical RBC count; ΔRBC, deviation for RBC count; WBC, white blood cells count

presence of cold agglutinins was then suspected after review on RBCS impedance plots. Despite the measurement at +41°C in the optical RET channel, the interference remained significant, and no corrected value was purchased. A new sample with warm transport was required.

Both flowcharts were then tested in 901 prospective unselected routine samples from our laboratory, including 225 samples with MCHC <320 and 43 with MCHC >365 g/L, in which the algorithms were therefore applied (Figure S1). The algorithms allowed the correction of 18 samples: 5 samples with 246 < MCHC <293 g/L associated with 213 < WBC < 465 × 10^9/L; 13 samples with 372 < MCHC <1516 g/L without any plasma interference. The direct correction by the algorithm was not possible in one sample only: this case involved a sample with a very high titer of cold agglutinins, in which the algorithm was first applied and corrected values were obtained. However, after review of blood smear where RBCS clumps were particularly abundant, a further warming of the sample was achieved before performing an extra count (30 min à 37°C), where values of RBCs parameters significantly differed from the initial corrected values given by the algorithm (RBC 5.03 then 4.7 × 10^{12}/L; HGB 138 then 143 g/L; MCV 90.3 then 94.1 fL; MCH 27.4 then 30.2 pg; MCHC 304 then 321 g/L).

4 | DISCUSSION

Our study aimed at optimizing the management of analytical interferences on RBCs parameters using the alternative parameters given by SYSMEX XN-10. These parameters, obtained by optical method on RET channel, have been reported as potentially useful in the case of interferences, such as increased MCHC, very high leukocytosis, cold agglutinins, or chylous turbidity.[1–4,11,12] Twelve categories of interferences were initially included in our evaluation: leukocytosis >90 × 10^9/L, cold agglutinins' suspicion, presence of lipids on scattergrams, hereditary spherocytosis, sickle cell disease, cryoglobulin, high immunoglobulins titer, hyperglycemia, bilirubinemia, lipemic sample, icteric sample, hemolysis.

Before considering the alternative parameters for the management of interferences, we first confirmed, as previously reported,[1,4] their excellent agreement with "standard" parameters obtained with impedance and/or photometry, within a control group including 2234 blood samples, without any age or sex criteria; of note, the agreement between MCHC and MCHCc was weaker ($r^2 = 0.50$). Indeed, they are not obtained by a direct measurement since both derive from a calculation, thus enhancing the variability of the final result. To establish this control group, the presence of a Sysmex alarm flag, a MCHC <320 g/L or >365 g/L and leukocytes count >90 × 10^9/L, were the only exclusion's criteria. To ensure the most consistent control group as possible, the cut-offs for MCHC were defined as follow: 365 g/L, being the flag defined by the manufacturer and verified by the literature,[1] 320 g/L, as the low normal value, in the very true pathological sense, since no further data is available both from the manufacturer or literature. This allowed therefore the recruitment of blood counts devoid of spurious data (as presenting a presumed accurate MCHC no additional alarm flag) as well as a larger representation of "normal" blood counts from different kinds of populations, as compared with the study of Berda-Haddad, in which control group only included blood counts from adult subjects, with MCHC <365 g/L.[1]

We then determined an acceptable deviation (Δ) for RBC-O, RBC-He, HGB-O, and MCHCc, ranging from the 3rd to the 97th percentile of the distribution within the control group. The deviation percentage for RBCs volume was not evaluated since both MCV and R-MFV derive from the impedance channel. Noteworthy, their correlation was excellent as well (not shown), allowing the R-MFV to be used in case of spurious MCV (see below). In fact, in case of interference, there is no further alternative to get an information on RBCs volume.

Interestingly, as reported in the literature,[1,5,7] the vast majority of relevant or frequent interferences on RBCs impacts MCHC, which may be increased or decreased as well, resulting in the development of two flowcharts whether MCHC is <320 or >365 g/L.

Regarding MCHC <320 g/L, the key points are leukocytosis and RBCs: in case of leukocytosis >90 × 10^9/L associated with ΔRBC < −4.44%, leukocytes probably interfere with red blood cell count by impedance, leading to spurious RBCs and MCV (which is calculated using RBC impedance count) and have to be replaced by RBC-O and R-MFV, respectively. Interestingly, Schillinger has recently proposed a flowchart of leukocytosis-related interferences on XN-10, with a method close to ours, based on RET-channel parameters (ΔHGB) especially.[4] The threshold for leukocytosis however differs (100 vs. 90 × 10^9/L in the present study). The determination of a "unique" relevant cut-off for leukocytosis stands as a very difficult issue: in fact, the degree and the severity of the interference are not only related to the sole WBC count, but it is also dependent on the type of cells, the homogeneity of the population and/or underlying disease.[4,5] We decided to lessen the cut-off to 90 × 10^9/L and to choose ΔRBCs as input, because of a previous case of chronic lymphocytic leukaemia managed in our laboratory, presenting a leukocytes count of 96 × 10^9/L, with a significant interference with RBC counts. A data-driven study should have been preferable for the determination of a precise cut-off, but it will probably require a great number of samples to be relevant, and consequently a very long duration of inclusion or a multicentric study, which did not fit with the design of our study. Except the specific situation of extreme leukocytosis, a MCHC <320 g/L is in all probability related to haemodilution and another sample shall be tested.

In case of MCHC >365 g/L, ΔHGB value appears critical to seek an interference: If ΔHGB ≥ −6.2% the measurement of haemoglobin is not impacted by the interference and the value is thus correct. It is worth to notice that recently Schillinger also concludes that a deviation of 6% between HGB and HGB-O should question on a possible interference on the photometric measurement of haemoglobin.[4] This study seats however in a different point of view from ours, by evaluating RBC parameters in a setting of well-defined diagnosis of haematological malignancies with extreme hyperleukocytosis; on the contrary, we focused on a priori technical management of routine samples, with minimal information on context. Considering the

relative low occurrence of such diseases, their inclusion would not have fit with our study design. Nevertheless, this point obviously stands as a further improvement for the management of interferences and could be subject to additional studies, since the conclusions of Schillinger need to be confirmed in larger cohorts.

Additionally, ΔRBC value should be carefully checked, since if >3.09%, a cold agglutinin related-interference should be suspected. As a consequence, the optical parameter, RBC-O is preferred, since obtained via the RET-channel at higher temperature (41°C); HCT, MCH and MCHC must be then recalculated, by using R-MFV as discussed above.

If ΔHGB < − 6.2%, a plasma-related interference is possible. The visual aspect of plasma is therefore crucial to determine whether the interference may be due to hemolysis, icterus or lipid-induced turbidity. Similarly, ΔRBC value should be carefully checked to detect a possible additional cold agglutinin. In any case, if ΔRBC value ≤3.09% (suggesting that RBC is correct), only Hb value has to be checked and replaced by HGB-O. In the same manner as before, optical parameters will be preferred when ΔRBC value>3.09%. One can question on the central position of hemolysis in our flowchart when results did not evidence significant impacts on the deviation percentages. As a matter of fact, the management of hemolysis is a very complex issue.[13,14] In fact, even if mild-to-moderate hemolysis is considered as non-significant on blood count, its management completely differs depending on the associated aetiology (in vivo or in vitro). Indeed, in case of suspected in vitro hemolysis (i.e. after blood collection), the sample should be withdrawn and another blood collection is required.[5,9,13]

However, if a true in vivo hemolysis could not be excluded, it must be systematically considered (for instance by checking biochemical data such as haptoglobin if available…), since the related consequences on clinical management are critical, a reliable blood count being thus mandatory to provide for physicians. Therefore, checking for hemolysis was included in our flowchart and this is reinforced by its very frequent occurrence (almost daily). Finally, in the particular case of in vivo hemolysis, the resulting flowchart gives reliable results allowing to provide crucial data on RBCs and our results stand as a significant improvement compared to the previously published decision-tree, which did not allow to validate any results.[1]

In the light of the above, the question may raise why optical method is not definitely recommended as the "standard" for the determination of RBCs parameters especially, as such parameters are expected to be almost completely free of interferences. However, several points limit their widespread implementation in routine practice: first, according to the manufacturer, the cost of the technology may be substantially higher than impedance in terms of routine use: in fact, with SYSMEX technology for instance, RET-channel implies the labelling of intracellular nucleic acids by an additional reagent containing a fluorescent marker. As a consequence, to get optical RBC parameters, it is mandatory to involve RET-channel, and consequently, consume further reagent; second, the duration of analysis is quite longer as well: as an example, with XN-10, the analysis duration with optical RET channel is around double the duration with impedance; finally, the availability of the technology depends on the analyser which in turn is dependent on the expected activity in the laboratory. Indeed, according to the size of the laboratory, the expected population of patients and diseases, the level of expertise and the expected production rate, the most appropriate device will differ, and consequently will not offer an identical level of performances.

Therefore, considering those limitations and the relatively low occurrence of analytical interferences (confirmed by our prospective study, 19 within 901 unselected routine blood counts—2%), we decided to focus on the development of an upgraded scheme for the detection of presumed interferences on SYSMEX XN-10, rather than a systematic use of the optical RET-channel.

To summarize, this study provides further improvement in the management of relevant and common interferences impacting RBCs by supplying 2 flowcharts whether MCHC is <320 or >365 g/L, building on optical parameters from XN-10. Furthermore, these flowcharts do not require the calculation of "RBC score" as compared with previously proposed decision-trees[1]; indeed such "RBC score" includes « FRC » parameter (Fragmented Red cell), whose analytical performances are currently reconsidered.[15] In conclusion, both flowcharts are now fully included in our routine management for haematology analyzers, the next step being direct incorporation in the middleware of the laboratory.

AUTHOR CONTRIBUTIONS

Sylvain Henry designed the study, collected data, performed analyses and wrote the manuscript; Delphine Gérard designed the study and wrote the manuscript; Sylvain Salignac performed analyses and reviewed the manuscript; Julien Perrin designed the study and wrote the manuscript. All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data generated and analyzed in this study are included in this article and supplementary materials. Further enquiries can be directed to the corresponding author upon reasonable request.

PATIENT CONSENT STATEMENT

According to the French ethical laws, the patients are informed that their biological data obtained in routine care may be anonymously used unless they express an opposition.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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