ORIGINAL ARTICLE

WILEY | ISLH International Journal of Laboratory Hematology

Reticulocyte count and extended reticulocyte parameters by Mindray BC-6800: Reference intervals and comparison with Sysmex XE-5000

M. Buttarello[1] | A. Rauli[2] | G. Mezzapelle[2]

[1]Department of Laboratory Medicine, Azienda Ospedaliera-Università di Padova, Padova, Italy

[2]Clinical Pathology Laboratory, Hospital of Adria, Adria, Italy

Correspondence
Mauro Buttarello, Department of Laboratory Medicine, Azienda Ospedaliera-Università di Padova, Padova, Italy.
Email: mbuttarello@yahoo.it

Abstract

Introduction: In this study, analytic performance (imprecision, carryover, time stability) and diagnostic efficiency of Mindray BC-6800 analyzer to quantify reticulocytes and extended reticulocyte parameters was evaluated. Moreover, reference intervals on adult population were determined. Results were compared with those obtained by Sysmex XE-5000 analyzer.

Methods: One hundred and eighty-four healthy adults of both sexes, and 368 subjects affected by various pathologic conditions (nutritional anemias before and after treatment, hemolytic and posthemorragic anemias, acute and chronic inflammations, malignancy under therapy, and beta thalassemia trait) were selected.

Results: Reference intervals were as follows: reticulocytes ($\times 10^9$/L): 23.2-93.2; immature reticulocyte fraction: 0.015-0.14; mean reticulocyte hemoglobin equivalent (RHE) (pg): 30.9-35.7; mean reticulocyte volume (fL): 97.8-118. Imprecision on reticulocyte count at all concentrations was close to analytic goal based on within-subject biological variation. Carryover (2.3%) was negligible, and time-stability was excellent up to 8 hours.

Conclusion: When compared with XE-5000, BC-6800 shows a good overall correlation on counting despite evidence of difference in the upper limit of reference intervals (93.2 vs 101.3). Comparison of diagnostic efficiency of extended parameters shows a good total agreement of RHE (91.6%).

KEYWORDS
immature reticulocyte fraction, Mindray BC-6800, reference intervals, reticulocyte, reticulocyte hemoglobin content

1 | INTRODUCTION

Reticulocyte count is clinically important both for pathophysiological classification of anemias, and for early identification of normalization of erythropoiesis by the marrow after therapeutic intervention (iron, folate, cobalamin, etc.), or as a way of checking early regeneration after marrow or stem cell transplant. Manual microscopic method is relatively inaccurate because of the subjective morphological definition of reticulocyte and is very imprecise with a coefficient of variation (CV) between 16% at high level and 68% at low concentration.[1] These limitations make the method unreliable above all at low values, that is, in situations in which one must define reduced erythropoietic activity of the marrow (values under the lower limit of the reference interval), or in which small but significant variations that appear in the early recovery of postaplasia or postmarrow transplant must be monitored. Automated analyzers represent a revolution for reticulocyte count using dyes to bind reticulocyte RNA and flow cytometers to perform rapid counts. These methods allow for objective counts, and because

they analyze tens of thousands of cells, they reduce sampling error and are therefore more precise mainly at low values (CV between 3% and 25%).[2] A further advantage is the identification of other reticulocyte parameters, such as the immature reticulocyte fraction (IRF) or the reticulocyte indices that is, to say, the mean hemoglobin content (RHE), and the mean reticulocyte volume (MRV), which are useful in several clinical conditions.[3-9] Problems still exist that essentially depend on the differing sensitivity of dyes used to stain RNA, on the technology used to identify positive cells (fluorescence, light scattering, absorbance), and on softwares that are more or less capable of separating reticulocytes from mature erythrocytes as there is a physiologic continuum between these populations. These differences contribute to the incomplete agreement of counts with the consequent necessity of method-specific reference intervals.[2,4]

The aim of our study was to evaluate the analytic performance (imprecision, carry-over, time stability) of the hematology analyzer Mindray BC-6800, and the reference intervals calculation on reticulocyte count and reticulocyte parameters. To evaluate possible interchangeability, the values obtained have been compared with those obtained by Sysmex XE-5000, our standard equipment, for which there is a large amount of literature on clinical usefulness of extended reticulocyte parameters.[10-13]

2 | MATERIALS AND METHODS

2.1 | Hematology analyzers

2.1.1 | Mindray BC-6800

Mindray BC-6800 (Mindray, Shenzhen, China) is an automated hematology analyzer able to perform a complete blood count (CBC) and leukocyte differentiation. It can also count reticulocytes using a dedicated channel where a fluorescent dye (asymmetric cyanine) can bind to cytoplasmic RNA to allow for reticulocytes separation from mature red cells. As fluorescent signals are proportionate to the RNA content, the analyzer subdivides reticulocyte into three fractions according to their maturity level. IRF is defined as the sum of populations at high and medium immaturity. It also provides, with a forward light scatter measure, some reticulocyte indexes similar to those for erythrocytes such as the mean "reticulocyte hemoglobin equivalent" (RHE) expressed in picograms, and the MRV.

2.2 | Sysmex XE-5000

The Sysmex XE-5000 (Sysmex, Kobe, Japan) is an automated analyzer which, in a dedicated channel, using a fluorescent dye (polymethine), is able to count reticulocytes. The counter, using a forward light scatter measure and side fluorescence intensity, is capable of differentiating reticulocytes from other red cells, to calculate the IRF, and to obtain the RHE (Ret-He).

Both analyzers were calibrated according to the manufacturer's instructions and checked twice a day using commercial tri-level control provided by the companies.

2.3 | Samples

Healthy subjects (N=184) were selected in accordance with the H44-A2 specifications to calculate reference intervals.[14] Subjects were men (N=81) and women (N=103) aged 19-71 (mean 44). They were excluded if any laboratory results of CBC, markers for liver and renal functions, and iron status, were outside the reference intervals used in our laboratory. Other criteria of exclusion were as follows: pregnancy, clinical evidence of recent medical disorders, and blood donation during the previous 6 months. In addition, samples from 368 subjects affected by various pathologic conditions (nutritional anemias before and after treatment, hemolytic and posthemorragic anemias, acute and chronic inflammations, malignancy under chemotherapy, and beta thalassemia carriers) were analyzed in four different days. For the possible effect due to the presence of red cell inclusions, three samples with basophilic stippling and five with Jolly bodies were identified. All samples were collected in the morning, under fasting conditions with the consent of informed donors by venipuncture into 3 mL K_2EDTA evacuated tubes (Vacutest, Kima, Italy). For some patients, samples were taken from leftover blood collected for CBC. The analysis on both instruments was performed in automated mode within 45 minutes of drawing samples.

The study is in accord with ethical standards established by the institution in which the experiment was performed.

2.4 | Imprecision and carryover

Imprecision on reticulocyte count was estimated with the imprecision profile [2,15] extended to a wide range of values (5 to 230×10^9/L) by performing repeated analysis (8 counts) on 17 samples reinserted in random order. Calculation of carryover was performed by analyzing a pair of samples: A sample with a high reticulocyte concentration (156.7×10^9/L) analyzed three time (i_1, i_2, i_3) immediately followed by 1 sample at a low concentration (35.5×10^9/L) also analyzed 3 times consecutively (j_1, j_2, j_3) using the formula $(j_1-j_3)/(i_3-j_3) \times 100$.[16]

2.5 | Method comparison

Results of the comparison with Sysmex XE-5000 on reticulocyte count, IRF, and RHE were summarized graphically with Bland-Altman plot with 95% confidence intervals which represent the limits of agreement.[16] For RHE, the evaluation was based also on the assessment of clinical agreement on selected groups of patients.

2.6 | Time stability

For the study of preservation time of blood samples, we compared the value of parameters of interest by retesting samples coming from the same group of 368 subjects (between 45 minutes and 8 hours from sampling: <45 minutes, +2, +4, +8 hours maintaining samples at room temperature).

TABLE 1 Reference intervals on reticulocyte and reticulocyte parameters (N=184)

	Mindray BC-6800			Sysmex XE-5000		
	Mean	2.5%	97.5%	Mean	2.5%	97.5%
Reticulocyte (10^9/L)	44.99	23.18	93.2	49.44	23.08	101.3
Immature reticulocyte (fraction)	0.053	0.015	0.14	0.059	0.012	0.15
Reticulocyte hemo-globin equivalent (pg)	32.79	30.90	35.70	32.30	29.40	35.98
Mean reticulocyte volume (fL)	108.87	97.79	117.98	N.A.	N.A.	N.A.

2.7 | Statistical analysis

Reference intervals were calculated with a nonparametric method (middle 95th percentile of the distribution). To evaluate population distribution characteristics, a Shapiro-Wilk test was used. For method comparison, Bland-Altman model was performed. Behavior of parameters of interest as a function of time were compared using analysis of variance (ANOVA). All significance testing were two-tailed, and a P value <.05 was considered significant.

3 | RESULTS

3.1 | Reference intervals

Reference intervals (mean and range) are summarized in Table 1. Distribution of absolute reticulocyte count and IRF are log-normal, while for RHE and MRV it is approximately Gaussian. When compared to XE-5000, the interval for reticulocyte count is different in mean and upper threshold (−5.4 and −8×10^9/L, respectively), but lower thresholds are coincident. For IRF, the values are essentially superimposable, while for RHE, there is a positive difference in lower threshold (+1.5 pg), but the mean and upper threshold are similar. MRV is not comparable as it is not available on XE-5000.

3.2 | Imprecision

The imprecision profile of BC-6800 in reticulocyte count is characteristically shaped with a CV% rapidly increasing while the parameter concentration is lowering. For values close to the lower limit of the reference interval, CV is near 7%, while for the upper limit, it is 4.5% (Figure 1). These results are close to the analytical goal based on within-subject biological variation.[17-19] The carryover (2.3%) is negligible.

3.3 | Method comparison

Table 2 shows the comparison between XE-5000 and BC-6800 with data related to Bland-Altman statistics (mean difference with confidence intervals). Figure 2A,B shows the graphical display for reticulocyte count and IRF. The comparison of these parameters was also evaluated with the biparametric matrix: reticulocyte count vs IRF [3,4]
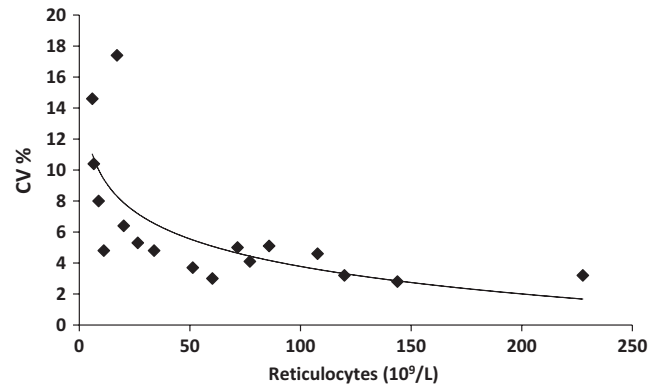
FIGURE 1 BC-6800: imprecision profile of reticulocyte absolute count

TABLE 2 Comparison of methods: analysis of data by Bland-Altman statistics (N=552)

Parameters	Mean difference	95% Confidence intervals	P
Reticulocyte (10^9/L)	−1.8	−2.64, −0.95	<.001
Immature reticulocyte (fraction)	−0.006	−0.008, −0.005	<.001
Reticulocyte hemoglobin equivalent (pg)	0.99	0.87, 1.10	<.001

as shown in Figure 3. To verify the agreement between methods, it is useful to see where BC-6800 placed patients previously defined as "aplastic or with early marrow recovery" by XE-5000. This is shown by black dots in Figure 3. From the position of these dots, it is evident that all patients were placed in the same area by both analyzers. This indicates that, in spite of numerical differences in reference intervals, in clinical use, results obtained with the matrix by the two analyzers are superimposable. The samples with red cell inclusions did not demonstrate systematic differences on the counts of both analyzers (reticulocytes from 37 to 55×10^9/L at BC-6800 and from 35 to 60×10^9/L at XE-5000. In Figure 2A, there are four samples with markedly higher results with BC-6800 than with XE-5000. These belonged to two patients with beta thalassemia major, 1 with beta thalassemic trait, and 1 with chronic hemolytic anemia. In the first
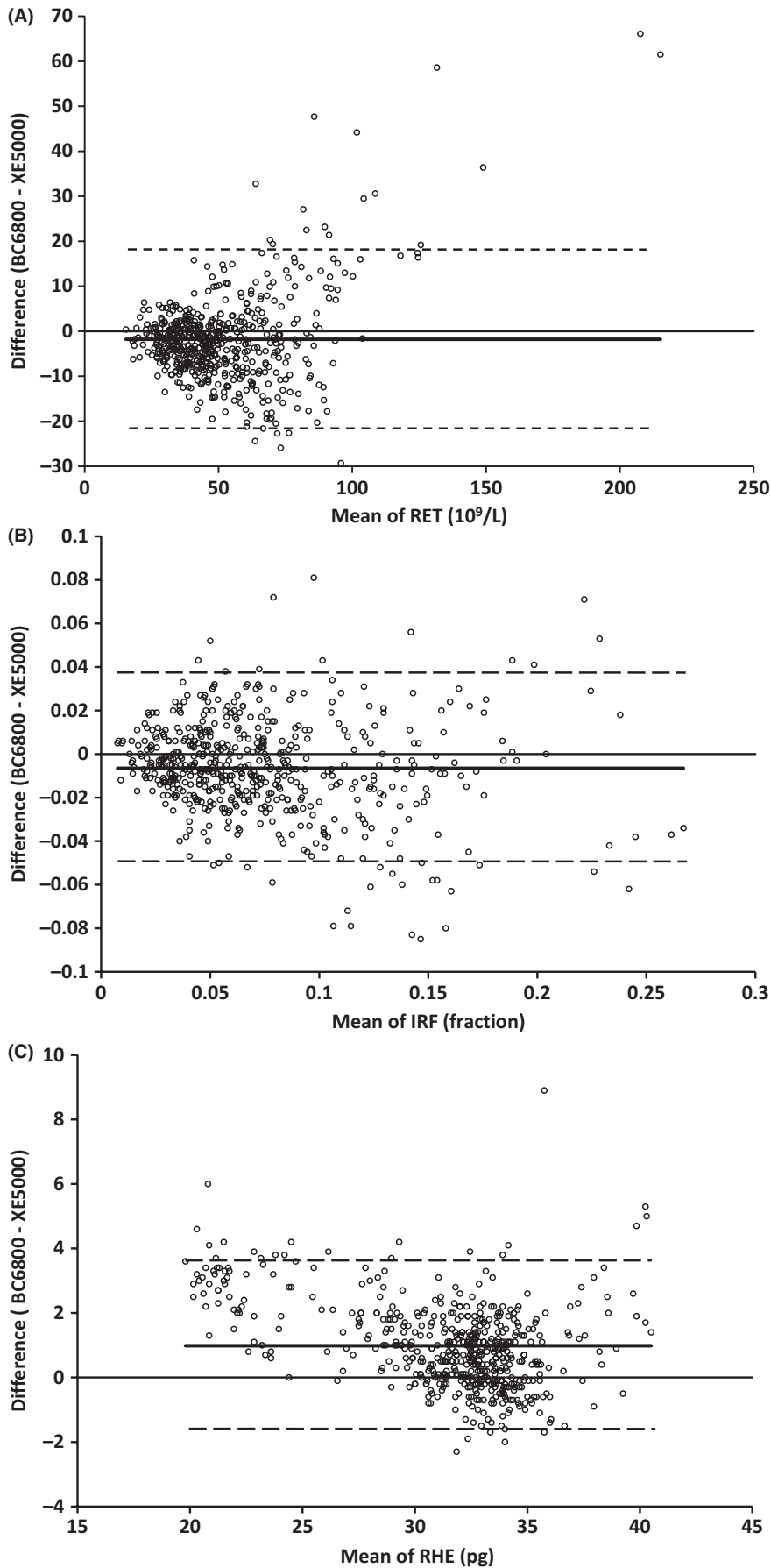
FIGURE 2 Bland-Altman plots comparing the reticulocyte count (A), the immature reticulocyte fraction (B), and the reticulocyte hemoglobin equivalent (C) of BC-6800 and XE-5000.The dotted lines represent 95% limits of agreement
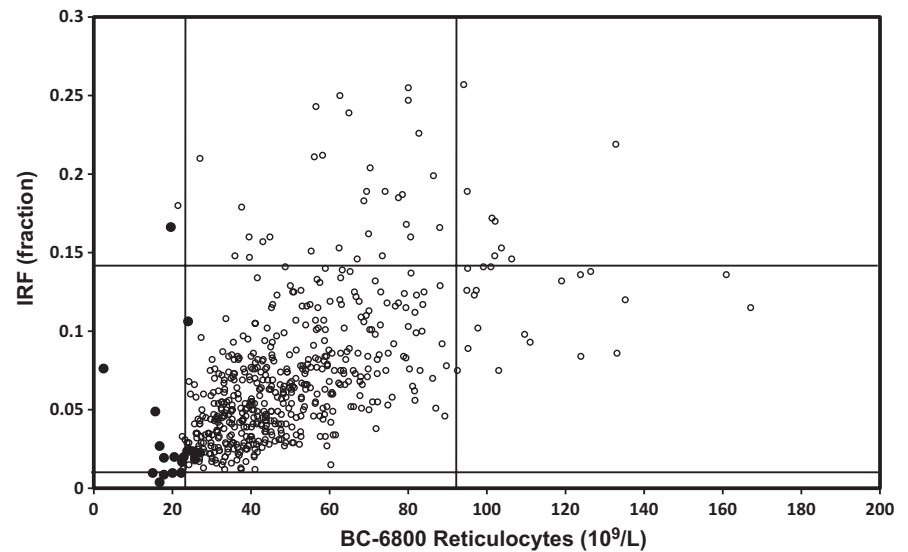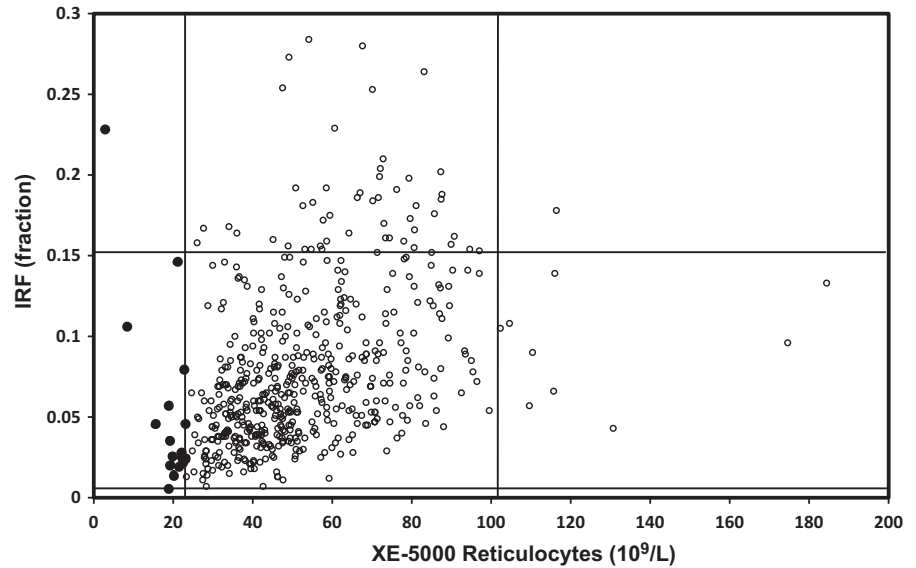
FIGURE 3 Two parameters matrix comparing the relationship of immature reticulocyte fraction vs absolute reticulocyte count of BC-6800 and XE-5000. The black dots show the samples that XE-5000 placed in the "aplastic" category. The vertical and horizontal lines represent the reference intervals

two samples, there was a similar percentage of erythroblasts among the two analyzers (23.5 and 18.7 vs 24.7 and 20.3) while in the other two the erythroblasts were absent. Also the IRF values were similar among the two methods (from 0.08 to 0.13 vs 0.05 to 0.13) and no other possible interference was highlighted in the blood smear. In 2 of these samples (beta thalassemia major and hemolytic anemia), BC-6800 reported the flag "cellular debris." Figure 2C shows the plot of RHE with a positive mean difference (0.99). This complies with a tendency to produce higher results mainly at low value of the reference interval. Figure 4 shows the clinical usefulness (ability to distinguish between normal and abnormal conditions) of RHE when compared to Ret-He of XE-5000, keeping in mind that clinical use of results is particularly useful at subnormal values (diagnosis of iron deficiency erythropoiesis).[10-13] Table 3 shows the disagreement in samples classification (31 from a total of 368ones). These differences are identified by black dots in Figure 4.

FIGURE 4 Plot of reticulocyte hemoglobin equivalent: BC-6800 vs XE-5000. The vertical and horizontal lines represent the reference intervals. The black dots indicate the disagreement in samples classification

TABLE 3 Instrument agreement of reticulocyte hemoglobin equivalent: distributional classification (N=368)

Sysmex XE-5000	Mindray BC-6800			
	Low	Normal	High	Total
Low	105	4	0	109
Normal	19	210	2	231
High	0	6	22	28
Total	124	220	24	368

3.4 | Time stability after venipuncture

Regarding the time laps in measuring samples stored at room temperature, Table 4 shows results obtained. The evaluation was stopped after 8 hours from venipuncture as it is a reasonable limit that should not be exceeded. In fact, for a longer time, not all the other parameters of the CBC are stable regardless of storage temperature.[20-22] For reticulocyte count, there are no significant differences between 45 minutes and 8 hours. For other parameters (IRF, RHE, MRV), differences are statistically significant already after 4 hours, but always close to the "optimal" bias based on biological variability (0.125 $[CVi^2+CVg^2]^{1/2}$), where CVi and CVg represent the within subject and between subject variability) [17,19].

4 | DISCUSSION

The large diffusion of automated methods depends on the possibility of replacing manual visual counts, which are subjective, highly imprecise and tedious, and on the advantages deriving from a precise and objective count mainly at low values, necessary for the diagnosis of hypoplastic anemias or for the early monitoring of bone marrow regeneration. A further advantage is the identification of other reticulocyte parameters, such as the IRF which is useful in classification of anemias based on marrow response, mainly using two-dimensional matrices of IRF vs absolute reticulocyte count, and in differential diagnosis of reticulocytopenia in distinguishing bone marrow aplasia from early erythropoietic response. Other uses include monitoring therapy efficacy in nutritional anemia because the increase in IRF precedes the increase in total reticulocyte count by several days.[3,4,23] Other useful parameters available only with automated analyzers are reticulocyte indexes such as RHE and MRV. These indexes are important because low values indicate iron-deficient erythropoiesis, even in conditions in which traditional biochemical markers such as ferritin and transferring saturation are inadequate as in inflammations or anemia from a chronic disease. Moreover, they are useful in monitoring an early response to therapy because they change significantly after only 48-72 hours from administration of iron, cobalamin or folate in nutritional anemias.[9,24] Increase of different analyzers on the market, among which Mindray BC-6800 represents one of the last introduced, brings in the pressing need for results comparison and harmonization. The reference interval in absolute value between the two analyzers is different,

TABLE 4 Time stability evaluation (N=368)

Parameters	T0	T2			T4			T8			Optimal bias
	Mean	Mean	Mean difference	P	Mean	Mean difference	P	Mean	Mean difference	P	
Reticulocytes (10^9/L)	50.88	50.66	0.22	.413	50.33	0.55	.052	50.42	0.46	.124	3.9% (1.98)*
Immature reticulocyte fraction (fraction)	0.070	0.068	0.002	< .051	0.067	0.003	<.001	0.067	0.003	<.001	2.4% (0.002)*
Reticulocyte hemoglobin equivalent (pg)	31.97	31.90	0.07	.081	31.81	0.16	<.001	31.80	0.18	<.001	0.49% (0.15)*
Mean reticulocyte volume (fL)	106.22	105.99	0.23	.078	105.72	0.50	.002	105.65	0.57	<.001	0.41% (0.43)*

*Parenthesis: absolute values.

mainly on the upper limit, and the disagreement needs for specific reference intervals. Results disagree also with previous published results obtained with Mindray BC-6800 on adult population, especially on the upper limit: 93.2 vs $115 \times 10^9/L$.[25] This may depend on the selected population, but the difference is so large to requires further study before reaching a final conclusion. Also comparison between methods on all analyzed samples shows a negative mean difference ($-1.8 \times 10^9/L$). In this study, the number of samples with erythrocyte inclusions was modest (three with basophilic stippling, five with Jolly bodies and no one with inclusions from malarial parasite), and therefore, a more extensive evaluation would be necessary to assess the effect of all possible interferences. For the four samples with marked differences in counting, no interference from erythroblasts or a higher proportion of young reticulocytes can be hypothesized considering the similar values between the two analyzers. However, these differences may depend on the different ways in which the software, in certain samples, determines the separation between reticulocytes and mature erythrocytes. It should be noted, however, that, for diagnostic purposes, both analyzers showed an increase in reticulocytes although more pronounced for BC-6800 than for XE-5000.

The analytic goal for imprecision which is currently widely shared is based on biological variability. Among various clinical conditions, monitoring has the most restrictive criteria (CVa<0.5 CVi). Studies published on biological variability of reticulocyte count provide essentially concordant results: CVi between 9.5 and 11%,[26-28] with analytical goals from 4.75% to 5.5%, respectively. With imprecision profile we can identify imprecision in connection with critical concentrations. This method when extended to a wide range of concentrations and with a sufficient number of samples (to limit the effect of possible outliers) represents a valid alternative to ANOVA performed on duplicates of samples of different concentration (ie, low, normal, and high),[16] or of many consecutive measurements on the same sample. Figure 1 shows that the goal for BC-6800 is reached at normal and high concentrations. At low concentration, imprecision (from 5% to 10%) is greater than the goal, but it is negligible by a clinical point of view, and it is better than that of many previous generation of analyzers.[2] Stability of all parameters over time after 8 hours from sampling is satisfactory with variations lower or approximate to the more restrictive criteria (so-called optimal bias) based on biological variation.[17] On evaluation of clinical usefulness of RHE, total agreement with XE-5000 is good (91.6%). At low values, where the results are particularly useful in diagnosis of iron deficiency erythropoiesis, are present 23 discrepancies (4 low with XE-5000 but normal with BC-6800 and 19 normal with XE-5000, but low with BC-6800). In this case, all values were included in a little interval between 29.5 and 30.9 pg. Less interesting from a diagnostic point of view (increased in macrocytic nutritional anemia), are the high values of RHE, but their rapid decline, likewise to that of MRV, is useful in monitoring early response to therapy as reported in Figure 5. This figure shows also the overlap between iron-deficiency and beta thalassemia trait because in this last condition RHE is always reduced independently from iron stores.[13,29] The main limitation of this study is that methods for measuring reticulocyte extended parameters are not standardized, and reference material for these determinations
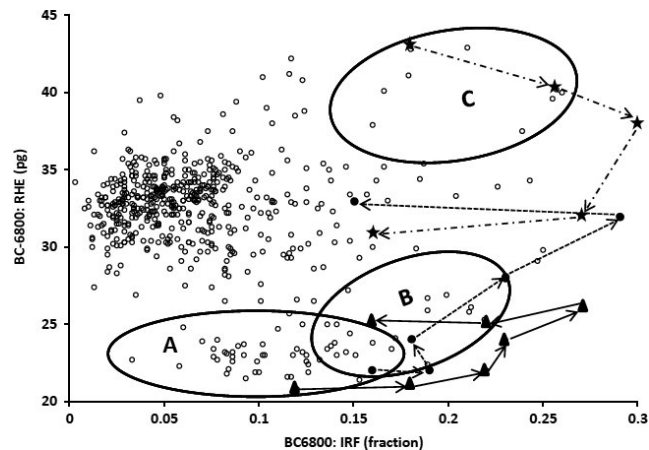
FIGURE 5 Plot of reticulocyte hemoglobin equivalent vs immature reticulocyte fraction. The insets represent some well-differentiate behavior in certain areas of the plot: (A): beta thalassemia trait with or without iron deficiency; (B): iron deficiency at diagnosis; (C): macrocytic nutritional anemia at diagnosis. Superimposed are the trends for three patients after therapy: •) iron deficiency;▲) beta thalassemia trait with iron deficiency; *) cobalamin deficiency

is lacking. This makes it impossible to explore specific causes for the observed discrepancies between the two analyzers.

In conclusion, BC-6800 provides a precise reticulocyte count at all concentrations with a negligible carry-over. Stability of all parameters is excellent up to 8 hours. Clinical agreement with XE-5000 is good mainly using bivariate matrices on classification of anemia, and RHE in diagnosing iron-deficiency conditions. The incomplete numerical agreement on count and in some parameters such as RHE makes it necessary the use of specific reference intervals and clinical decision values.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Buttarello M, Bulian P, Venudo A, Rizzotti P. Laboratory evaluation of the Miles H.3 automated reticulocyte counter. A comparative study with manual reference method and Sysmex R-1000. *Arch Pathol Lab Med*. 1995;119:1141-1148.
2. Buttarello M, Bulian P, Farina G, et al. Flow cytometry reticulocyte counting. Parallel evaluation of five fully automated analyzers: an NCCLS-ICSH approach. *Am J Clin Pathol*. 2001;115:100-111.
3. Davis BH. Immature reticulocyte fraction (IRF): by any name, a useful clinical parameter of erythropoietic activity. *Lab Hematol*. 1996;2:2-8.
4. Buttarello M, Bulian P, Farina G, Petris MG, Temporin V, Toffolo L. Five fully automated methods for performing immature reticulocyte fraction. Comparison in diagnosis of bone marrow aplasia. *Am J Clin Pathol*. 2002;117:871-879.
5. Brugnara C, Laufer MR, Friedman AR, Bridges K, Platt O. Reticulocyte hemoglobin content (CHr): early indicator of iron deficiency and response to therapy. *Blood*. 1994;83:3100-3101.

6. d'Onofrio G, Chirillo R, Zini G, Caenaro G, Tommasi M, Micciulli G. Simultaneous measurement of reticulocyte and red cell indices in healthy subjects and patients with microcytic and macrocytic anemia. *Blood*. 1995;85:818-823.

7. Brugnara C. Reticulocyte cellular indices: a new approach in the diagnosis of anemias and monitoring of erythropoietic function. *Crit Rev Clin Lab Sci*. 2000;37:93-130.

8. Piva E, Brugnara C, Chiandetti L, Plebani M. Automated reticulocyte counting: state of the art and clinical applications in the evaluation of erythropoiesis. *Clin Chem Lab Med*. 2010;48:1369-1380.

9. Buttarello M. Laboratory diagnosis of anemia: are the old and new red cell parameters useful in classification and treatment, how? *Int J Lab Hematol*. 2016;38(suppl1):123-132.

10. Garzia M, Di Mario A, Ferraro E, et al. Reticulocyte haemoglobin equivalent; an indicator of reduced iron availability in chronic kidney diseases during erythropoietin therapy. *Lab Hematol*. 2007;13:6-11.

11. Urrechaga E, Borque L, Escanero JF. Potential utility of the new Sysmex XE 5000 red blood cell extended parameters in the study of disorders of iron metabolism. *Clin Chem Lab Med*. 2009;47:1411-1416.

12. Urrechaga E, Borque L, Escanero JF. Erythrocyte and reticulocyte indices in the assessment of erythropoiesis activity and iron availability. *Int J Lab Hematol*. 2013;35:144-149.

13. Buttarello M, Pajola R, Novello E, Mezzapelle G, Plebani M. Evaluation of the hypochromic erythrocyte and reticulocyte hemoglobin content provided by the Sysmex XE-5000 analyzer in diagnosis of iron deficiency erythropoiesis. *Clin Chem Lab Med*. 2016;54:1939-1945.

14. Clinical and Laboratory Standard Institute (CLSI). *Method for Reticulocyte Counting. Approved Guideline H44-A2*. (2nd ed.). Wayne, P.A.: Clinical and Laboratory Standard Institute; 2004.

15. Sadler WA, Smith MH. Use and abuse of precision profile: some pitfalls illustrated by computing and plotting confidence intervals. *Clin Chem*. 1990;36:1346-1350.

16. Briggs C, Culp N, Davis B, et al. ICSH guidelines for the evaluation of blood cell analysers including those used for differential leucocyte and reticulocyte counting. *Int J Lab Hematol*. 2014;36:613-627.

17. Fraser CG, Hyltoft Petersen P, Libeer JC, Ricos C. Proposal for setting generally applicable quality goals solely based on biology. *Ann Clin Biochem*. 1997;34:8-12.

18. Fraser CG, Hyltoft Petersen P. Analytical performance characteristic should be judged against objective quality specifications. *Clin Chem*. 1999;45:321-323.

19. Sandberg S, Fraser CG, Horvath AR, et al. Defining analytical performance specifications: consensus statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine. *Clin Chem Lab Med*. 2015;53:833-835.

20. Zini G. Stability of complete blood count parameters with storage: toward defined specifications for different diagnostic applications. (Editorial). *Int J Lab Hematol*. 2014;36:111-113.

21. Buoro S, Mecca T, Seghezzi M, et al. Assessment of blood sample stability for complete blood cell count using the Sysmex XN-9000 and Mindray BC-6800 analyzers. *Rev Bras Hematol Hemoter*. 2016;38:225-239.

22. Daves M, Zagler EM, Cemin R, et al. Sample stability for complete blood cell count using the Sysmex XN haematological analyser. *Blood Transfus*. 2015;13:576-582.

23. Buttarello M, Plebani M. Automated blood cell counts. State of the art. *Am J Clin Pathol*. 2008;130:104-116.

24. Buttarello M, Temporin V, Ceravolo R, Farina G, Bulian P. The new reticulocyte parameter (Ret-y) of the Sysmex XE 2100. Its use in the diagnosis and monitoring of posttreatment sideropenic anemia. *Am J Clin Pathol*. 2004;121:489-495.

25. Grillone R, Grimaldi E, Scopacasa F, Conticelli M, Dente B. Evaluation of the reticulocyte counting by the Mindray BC 6800 automated hematology analyzer: comparison with ABX Pentra 120, Coulter LH 750, and microscopy. (Letter to the editor). *Int J Lab Hematol*. 2015;37:e3-e6.

26. Ricos C, Alvarez V, Cava F, et al. Current databases on biological variation: pros, cons, and progress. *Scand J Clin Lab Invest*. 1999;59:491-500.

27. Sandberg S, Rustad P, Johannesen B, Stolsnes B. Within-subject biological variation of reticulocytes and reticulocyte-derived parameters. *Eur J Haematol*. 1998;61:42-48.

28. Zhang P, Tang H, Chen K, Chen Y, Xu D. Biological variations of hematologic parameters determined by UniCel DxH 800 hematology analyzer. *Arch Pathol Lab Med*. 2013;137:1106-1110.

29. Noronha JFA, Grotto HZW. Measurement of reticulocyte and red blood cell indices in patients with iron deficiency anemia and β-thalassemia. *Clin Chem Lab Med*. 2005;43:195-197.