Jornal Brasileiro de Patologia e Medicina Laboratorial
ISSN: 1676-2444
jbpml@sbpc.org.br
Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial
Brasil

Viana, Karina Augusta; Martins Filho, Olindo A.; Dusse, Luci Maria S.; Antonelli, Lis R. V.; Avelar, Danielle M. V.; Avelar, Renato S.; das Graças Carvalho, Maria

Evaluation of the interference of platelets in reticulocyte counting by flow cytometry using thiazole orange
Jornal Brasileiro de Patologia e Medicina Laboratorial, vol. 52, núm. 3, junio, 2016, pp. 142-148
Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial
Rio de Janeiro, Brasil

Available in: http://www.redalyc.org/articulo.oa?id=393546394003

J Bras Patol Med Lab, v. 52, n. 3, p. 142-148, June 2016

10.5935/1676-2444.20160025

Evaluation of the interference of platelets in reticulocyte counting by flow cytometry using thiazole orange

Avaliação da interferência de plaquetas na contagem de reticulócitos por citometria de fluxo, utilizando laranja de tiazol

Karina Augusta Viana[1]; Olindo A. Martins Filho[2]; Luci Maria S. Dusse[1]; Lis R. V. Antonelli[2]; Danielle M. V. Avelar[3]; Renato S. Avelar[3]; Maria das Graças Carvalho[1]

1. Universidade Federal de Minas Gerais (UFMG), Minas Gerais, Brazil. 2. Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz (Fiocruz), Minas Gerais, Brazil. 3. Patologia Clínica São Paulo, Minas Gerais, Brazil.

ABSTRACT

Introduction: The reticulocyte count by flow cytometry (FC) – an automated counting method – can present errors due to the presence of interfering factors, contributing to a slight increase in results. However, automated methods have large advantages over the manual method, taken as reference, what justifies efforts to improve their quality. **Objective:** Evaluate platelet interference with the reticulocyte count by FC, using thiazole orange (TO) (FC/TO). **Materials and methods:** The method of reticulocyte count by FC/TO and a modified automated equivalent method, which excluded CD61-positive cells (platelets) from analysis (FC/TO/MOD), were compared to the manual method. **Conclusion:** Results were analyzed according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) to assess interchangeability between the methods, by linear regression analysis and paired *t*-test. The exclusion of interfering fragments from result analysis by the modified method produced results in closer proximity to those of the reference method.

Key words: reticulocyte count; flow cytometry; blood platelets.

INTRODUCTION

The Clinical and Laboratory Standards Institute (CLSI) and the International Committee for Standards in Haematology (ICSH) produced the document H44-A2 – Methods for Reticulocyte Counting (Automated Blood Cell Counters, Flow Cytometry, and Supravital Dyes); approved guideline, second edition[1] –, that comprises a set of technical norms for reticulocyte counting and the quality control for its implementation. This protocol, as well as other authors[2-4], points to the interferences that may occur with automated enumeration, including those caused by the presence of leukocytes and platelets. The same principle that permits reticulocyte counting by flow cytometry (FC) is that which causes interference: the affinity by the fluorochrome, used as a dye in the automated method, for nucleic material[1]. This fluorescent dye, besides binding to the ribonucleic acid (RNA) remaining in the reticulocyte, has affinity for the nuclear material of other nucleated cells, such as the leukocytes, or the remnants of the nuclear materials present in reticulated platelets. As revised by Hoffmann (2014)[5], reticulated platelets are immature platelets in circulation that still contain a small amount of RNA. Robinson *et al*. (1998)[6] suggest that thiazole orange (TO), a fluorescent dye used in automated methods of reticulocyte and reticulated platelets count, would also bind, in a non-specific form, to the granules present in platelets.

Contrasted with the manual method, the automated method presents better precision due to the larger number of cells counted in the sample in a shorter time. In addition, it allows the evaluation of other reticulocyte parameters not previously monitored in internal medicine, for example, the mean reticulocyte volume (MRV) and the reticulocyte immature fraction (RIF)[7-10]. Thus, despite possible errors due to the presence of interferents, which contribute to a slight increase in the automated reticulocyte count, the automated methods have great advantages in relation to the

First submission on 15/12/15; last submission on 08/03/16; accepted for publication on 14/03/16; published on 20/06/16

manual, regarded as the reference, what justifies efforts for the improvement of their quality.

According to Hove *et al.* (1990)[11], strategies may be used to decrease the trouble of interfering factors in the automated method; some of them may be eliminated simply by an operator's attention to the analysis of results. Other authors[12, 13] employed strategies such as the addition of specific antibodies (anti-CD61, anti-CD45 or anti-glycophorin) to the counting method, so as to eliminate or identify artifacts.

The current study is part of the same research line in which Viana *et al.* (2014)[14] assessed an automated method of reticulocyte count by FC using acridine orange, comparing it to the manual method and the reticulocyte count by FC using TO (FC/TO). However, the objective of the present investigation was to compare reticulocyte counts of blood samples by means of three methods: the manual method; the FC/TO automated method; and the equivalent modified automated method, in which strategies to exclude platelet interference were used.

MATERIALS AND METHODS

This study is part of a larger project named "Methodological innovations in flow cytometry applied to hematology", approved by the research ethics committee of Universidade Federal de Minas Gerais (UFMG) and the research ethics committee of Fundação Centro de Hematologia e Hemoterapia de Minas Gerais (Fundação Hemominas), besides being a continuation of a study already published by Viana *et al.* (2014)[14]. For the conduction of experiments, the following were used: phycoerythrin (PE)-labeled anti-CD61 monoclonal antibody (CD61PE), clone VI-PL2 and TO dye commercially available as BD Retic-COUNT™, both manufactured by Becton, Dickson and Company (BD) Biosciences; brilliant cresyl blue (BCB) stain, produced by Laborclin (Pinhais/Brazil); phosphate buffered saline (PBS) (0.015 M, pH = 7.2), obtained from Centro de Pesquisa René Rachou (Belo Horizonte [MG]), and saline solution (SS) produced by Farmax (Divinópolis/Brazil).

Comparison between methods of reticulocyte count by FC with and without platelet exclusion

Blood samples

For comparison between reticulocyte count by FC with/without platelet exclusion and the manual method (reference) samples to be analyzed were drawn from healthy donors and anemic outpatients of Fundação Hemominas, especially those with sickle-cell disease (SCD) (HbSS).

Samples of 5 ml of whole blood were collected in tripotassium ethylenediaminetetraacetic acid (K_3-EDTA) directly into Vacutainer tubes (Becton, Dickinson and Company) by means of venipuncture from patients and donors at Fundação Hemominas. A small aliquot of these venous blood samples (around 1 ml) was separated for the conduction of the study, being transferred to a 12- × 75-mm polystyrene tube. Thus, 50 samples from healthy donors (group C) were analyzed, with normal reticulocyte count (reference value [RV]: 0.5%-2%). Blood samples from anemic outpatients, especially HbSS patients, with reticulocytosis, were also included in the study. Among these patients, 50 samples with reticulocyte count ranging from 2.1% to 10% (group PI) were analyzed, as well as 50 samples of patients with count above 10% (group PH). Patients were classified within these percentage ranges, in accordance with the results from reticulocyte counts obtained by the manual method.

Each sample underwent two reticulocyte counts by the manual method, using the BCB supravital stain; one count by the automated method using FC/TO, and one by the same automated method modified, in which platelets were removed from analysis (FC/TO/MOD).

The protocols used for reticulocyte enumeration by the manual method and by the automated methods with and without platelet exclusion are described in the item "Strategies for reticulocyte count".

These groups were compared in twos: results from the manual method averages counted twice, and results from the automated methods, each one counted once. As a result, it was possible to assess platelet interference with reticulocyte count by FC/TO.

Strategies for reticulocyte count

Count by the manual method

Reticulocyte enumeration by conventional microscopy was carried out according to the standard protocol routinely employed at the hematology laboratory of Faculdade de Farmácia (FaFar) of UFMG.

To each sample, in a 12- × 75-mm polystyrene tube, aliquots of 50 µl whole blood collected in EDTA and 50 µl BCB dye were added. The mixture was homogenized and incubated for 15 minutes in a water bath at 37ºC, shielded from light. Next, blood smears were prepared in glass slides for microscopy. The count was carried out in a microscope with a 100× immersion objective lens, and the

number of reticulocytes was calculated considering the number of these cells in 1,000 assessed erythrocytes. Results were expressed as reticulocyte percentage values.

Count by FC/TO

Reticulocyte count by FC/TO was carried out using BD FACSCalibur™ flow cytometer (BD Biosciences) and the Cell Quest Pro software for data analysis.

For each sample, two 12- × 75-mm polystyrene tubes were used. To the control tube, 1 ml PBS and 5 µl of a whole blood sample collected in EDTA were added. To the other tube, 1 ml solution of the TO stain and 5 µl of the same blood sample were added. Preparations were homogenized and left during 30 minutes at room temperature, shielded from light. Immediately after this waiting time, readings were taken in the flow cytometer. Firstly, reading of the control tube was taken, and afterwards, of the tube containing the sample, to which TO was added.

Blood cells were acquired in a BD FACSCalibur™ cytometer, with a total of 50,000 events. Data were analyzed by the Cell Quest Pro software. So, first the scatter plot was generated in the logarithmic scale, and the acquired events were observed considering the morphometric aspects (forward scatter [FSC] × side scatter [SSC]). Then, the oval region related to the erythrocyte population was selected. Based on this region, other graphs were drawn for analysis of the FL1 positive population, and consequently, the percentage of reticulocytes in the sample.

Reticulocyte percentage was obtained by the difference between results of the tube to which TO was added and the control of each sample.

Count by FC/TO, and platelet exclusion strategy of analysis

For each sample, a 12- × 75-mm polystyrene tube was used. To each tube, 95 µl of SS, 5 µl of whole blood collected in EDTA, and 4 µl of anti-CD61PE antibody were added for platelet staining (anti-CD61PE was used at a 1:25 dilution). The content of each tube was homogenized and left during 30 minutes at room temperature, shielded from light. Later, 1 ml PBS was added, followed by homogenization and centrifugation at 2,200 rpm for 10 minutes in the Rotofix 32® (Hettich Lab Technology) centrifuge. The supernatant was decanted and the excess was carefully taken with tissue paper, without disturbing the cells at the bottom. Next, staining of reticulocytes was carried out with the fluorescent stain. After sample staining with anti-CD61PE antibody, 1 ml TO stain was added. Solutions were homogenized and left at room temperature for 30 minutes. The control tube was prepared similarly, however 1 ml PBS was added to the tube, instead of 1 ml TO stain.

Blood cells were acquired by BD FACSCalibur™ cytometer, collecting a total of 50,000 events. During the process of data acquisition by the flow cytometer, adjustments were made in the equipment so as to obtain the best profile for sample reading.

The acquired data were analyzed by means of scatter plots generated by Cell Quest Pro software, using some strategies for the elimination of platelet interference. Hence, analysis of the data obtained by FC for reticulocyte enumeration using TO (**Figure 1**) was carried out using, initially, FSC (size) versus SSC (granularity), and FL1 versus FL2 (logarithmic scale) dot plots. The homogeneous population distribution in these graphs allowed

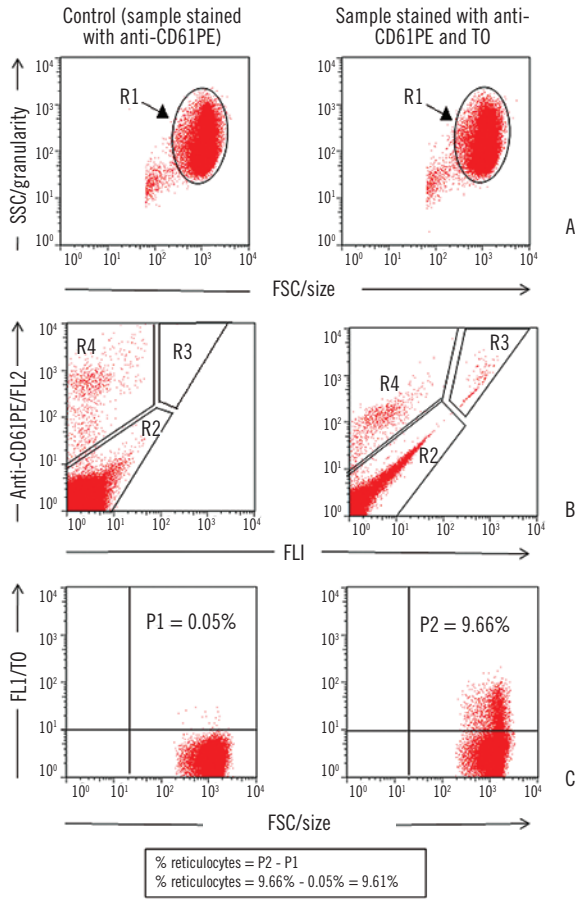

FIGURE 1 – *Strategy of analysis for reticulocyte count by FC/TO and CD61PE*

A) selection of the population of interest, R1, based on morphometric aspects for the construction of graph B (FL1 × anti-CD61PE/FL2); B) identification of the homogeneous population, R2, and possible interferents – R3 and R4; C) quantification of the percentage of positive cells in the upper right quadrant (stained with TO) present in the R2 region – graph drawn with the combination of gates in R1 and R2 regions.

FC/TO: method of reticulocyte count by flow cytometry, using thiazole orange; SSC: side scatter; FSC: forward scatter.

for the placement of windows over the regions corresponding to erythrocytic lineage cells (R1 and R2). With the combination of R1 and R2 regions, the gate used for the determination of reticulocyte percentages in the sample was established, producing FSC (size) versus FL1 dot plots. So, quadrants were placed to determine the percentage of TO-stained cells. Reticulocyte percentage was estimated by the difference between the results of the tube to which TO stain was added and the control tube of each sample.

Statistical analysis

The obtained data were tabulated in Excel program and analyzed by the SPSS v.13.0 statistical software package.

For comparisons involving the manual method and CF methods, excluding or not platelets, linear regression models were adjusted and analyses of the correlation coefficient were conducted. Besides, tests evaluated differences between the results of manual methods and methods using FC, by means of the *t*-paired test, as recommended by CLSI (2004).

Because data were not normally distributed, Wilcoxon test and Spearman's correlation coefficient were also used, as recommended by an experienced Statistics professional.

RESULTS

Comparison between methods of reticulocyte count by FC with and without exclusion of CD61-positive cells and the manual method

In all the preparations to which CD61PE was added, some cells were stained with this antibody (Figure 1 – item B). Therefore, these cells stained with the monoclonal antibody (R4) that presented the same morphometric characteristics of erythrocytes (R1 region – FSC × SSC) were excluded in the production of the FL1 × FL2 scatter plot; just cells of the R2 region were selected for the analysis of reticulocyte percentage. With selection of the R2 region, it was also possible to exclude another type of interferent by excluding R3 cells, which, according to some studies and researches, are leukocytes that also present morphometric aspects similar to those of erythrocytes, staying in the R1 region. Consequently, by the FC/TO/MOD method, reticulocyte counting permitted to remove possible platelet interference (R4) by the addition of CD61PE. The exclusion of leukocytes (R3), in its turn, in both the FC/TO method and the FC/TO/MOD, is only possible by the analysis of the scatter plot, which exhibits leukocytes with greater fluorescence emission, due to the addition of TO stain alone.

The comparison between results of FC methods and the manual method was made by graphs produced by the Excel program, presenting confidence intervals (CI) of 95% for binomial variables, which represent the standard error of the reference method. Linear regression analyses and paired *t*-test were also conducted by SPSS v.13.0 statistical software package. The graph comparing the tested FC methods and the manual method (reference), as recommended by CLSI[1], as well as the linear regression lines, are represented in **Figures 2** and **3**, in which the tested method was FC/TO without platelet exclusion and with exclusion of these cells, respectively.

The *t*-test was carried out with the results obtained from all the analyzed samples ($n = 150$), and a significant difference was observed between results of the manual × FC/TO groups ($p < 0$).

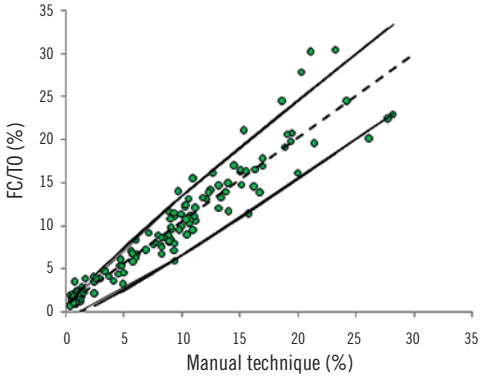

FIGURE 2 – *Reticulocyte count (%) by the manual method and by FC/TO* (n = *150)*

Continuous lines: 95% binomial confidence interval; traced line: linear regression between the evaluated methods; Y = 0.827 + 0.97X; $r^2 = 0.92$; r = *0.959.*
FC/TO: method of reticulocyte count by flow cytometry, using thiazole orange.

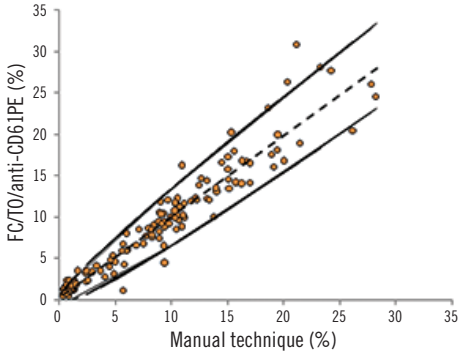

FIGURE 3 – *Reticulocyte count (%) by the manual method and by FC/TO, excluding CD61-positive cells* (n = *150)*

Continuous lines: 95% binomial confidence interval; traced line: linear regression between the evaluated methods; Y = 0.297 + 0.983X; $r^2 = 0.93$; r = *0.964.*
FC/TO: method of reticulocyte count by flow cytometry, using thiazole orange.

Between the manual and the FC/TO/MOD methods, no significant difference was observed between results ($p = 0.28$).

The data obtained through the employment of all assessed reticulocyte count methods did not present normal distribution. This is why Wilcoxon test was performed to compare groups, as well as Spearman's (r) correlation coefficient (**Table**). Results revealed good correlation between methods. Wilcoxon test also did not indicate significant difference between manual × FC/TO/MOD groups ($p > 0.05$).

TABLE – Comparison between the results yielded by different methods of automated reticulocyte count and the manual method

	Manual method	FC/TO	FC/TO/MOD
Median	7.885	8.474	8.049
Minimum	0.35	0.58	0.4
Maximum	28.25	30.41	30.8
IQR	10.8	11.053	10.398
p*	-----	$0_{(1)}$	$0.073_{(1)}$
SCC (r)	-----	$0.966_{(1)}$	$0.97_{(1)}$

*IQR: interquartile range; SCC: Spearman's correlation coefficient; FC/TO: method of reticulocyte count by flow cytometry, using thiazole orange; FC/TO/MOD: method of reticulocyte count by flow cytometry, using thiazole orange, which excluded CD61-positive cells (platelets) from analysis; *: Wilcoxon test; $_{(1)}$ value compared with the manual method.*

DISCUSSION

The automated methods evaluated in this study involved TO, a fluorescent reagent used to determine the reticulocyte percentage of peripheral blood in flow cytometers such as FACSCalibur™, FACSort™, FACSCan™ or FACStrak™. As stated in the manufacturer's manual, TO is a stain that binds deoxyribonucleic acid (DNA) with a ratio of 1:2 dye molecules per base pair, has positive charge and membrane permeability. TO binds RNA and DNA, forming a fluorescent nucleotide-reagent complex that absorbs light at 475 nm (blue light) and emits fluorescence at 530 nm (green light), being detected at the fluorescence 1 (FL1) channel. Considering the above, the evaluated reagent, besides exhibiting affinity for reticulocytes, would also bind cells presenting nucleic material, as leukocytes, nucleated erythrocytes and reticulated platelets, what would cause interference with the count.

The results yielded in this study confirmed the presence of interferents in reticulocyte count by FC, using the fluorescent stain, and some strategies were used to improve result precision. In their study, Hove *et al.* (1990)[11] stated that leukocytes and nucleated blood cells can be identified as distinct population groups in scatter plots, and they can be excluded from result analysis by an attentive operator, so as to decrease interference with reticulocyte count. The analysis of cells acquired in this study followed this recommendation, once these interfering populations were excluded (Figure 1 – item B: population R3) from both method FC/TO and FC/TO/MOD in result assessment. Furthermore, other procedures performed by the researchers in this work proved that the R3 region is in fact a leukocyte population (results not published yet). Thus, the study allowed assessing just platelet interference with reticulocyte count by FC.

Staining of platelets and leukocytes with monoclonal antibody is a strategy employed to study the removal of these cells interference with reticulocyte count. In their study, to ensure exclusion of other types of cells – leukocytes and reticulated platelets – from reticulocyte count, Ivory *et al.* (2007)[12] used monoclonal antibodies – anti-CD16/CD45 and anti-CD61 –, respectively, in the identification of these cells. The CD45 antigen is expressed in lower levels in granulocytes and in higher levels in lymphocytes[15], while CD16 is expressed in higher levels in granulocytes[12]. Platelets present several proteins (antigens), among which glycoprotein IIb/IIIa (GpIIb-IIIa) stands out. This is the most abundant on the platelet surface and acts as a fibrinogen receptor, establishing interaction among platelets, what is important in the process of platelet aggregation[16]. For this reason platelets can be evaluated by staining with specific antibodies that bind these antigens. The anti-CD61 antibody, in its turn, has affinity for GpIIIa; the anti-CD41a antibody, for GpIIb[17]. Ivory *et al.* (2007)[12] observed that the number of counted reticulocytes increased around 25% (14%-41% interval) when counting considered the presence of platelets (CD61- and TO-positive cells) in relation to cases when they were not considered. Interference from platelets would be higher than that from leukocytes, since platelets are present in larger numbers in peripheral blood.

The present study, when carrying out experiments modifying an automated method by FC/TO, involved the strategy of using anti-CD61PE antibody for platelet identification, so as they could be excluded from result analyses.

The analyses of data from this study showed that the correlation of results from FC/TO/MOD × manual experiments ($r = 0.964$) (Figure 3) increased in relation to results involving FC/TO × manual ($r = 0.959$) (Figure 2). The last result is close to that reported by Peng *et al*. (2001)[18], using Retic-COUNT reagent and Elite ESP flow cytometer (Beckman Coulter), which obtained a value of $r = 0.962$.

As the gathered data did not prove normal, Wilcoxon test was performed, to assess difference between groups, because it is more adequate for data of this nature (Table). This test showed statistical difference in results between the manual method

and the automated method with no exclusion of platelets (FC/TO) ($p < 0$). However, between the manual method and FC/TO/MOD there was no difference between groups ($p = 0.073$). Spearman's correlation coefficient was also calculated, and it was the highest coefficient observed between the manual and the modified methods, confirming the results encountered by linear regression. At the same time, the previous data also underwent analyses employing the *t*-test, as recommended by CLSI, with coincident results.

Based on the cited studies, the non-removal of interfering cells like platelets from results of FC reticulocyte counting would make the method useless in cases of reticulocytopenia. Consequently, this issue must be better evaluated. In a study by d'Onofrio *et al*. (1997)[19], the authors questioned the fact that samples with reticulocytopenia are not analyzed by the FACScan method, since the reticulocyte values in them would be overestimated. Thus, the author suggests that an explanation for the overestimate of samples with low reticulocyte values is the presence of possible interferents.

The analyzed blood samples presented reticulocyte counts ranging between 0.5% and 10% or over, considering the results of the manual method. The CLSI recommends that method comparability studies are conducted with representative samples, comprising normal samples, with reticulocytosis and reticulocytopenia. However, the reference value in reticulocyte enumeration has its lower limit very close to zero. And it was not possible to obtain samples from patients that presented reticulocytopenia, as, for instance, patients with aplastic anemia or undergoing chemotherapy. The current study did not assess blood samples with values lower than the normal value for reticulocyte count. Therefore, one can infer the sensitivity of the modified method in the case of intense reticulocytopenia just from evaluating the linear regression line obtained with the evaluated samples.

Buttarello *et al.* (2001)[20] comparatively assessed five different automated methods, verifying a tendency to overestimate the lowest values and underestimate, to a lesser extent, the values for samples with high reticulocyte counts. This observation was confirmed by the linear equation $Y = a + bX$, in which the intercept (a) was greater than zero and the slope (b) was less than 1. As regards the methods assessed in this study (Figures 2 and 3), by means of analysis of the linear regression line [value of intercept (a)], both the FC/TO method and its modified technique, a tendency was also verified to overestimate the lower values found by the manual method (intercept greater than zero). This tendency is, however, higher in FC/TO ($Y = 0.827 + 0.97X$) than in FC/TO/MOD ($Y = 0.297 + 0.983X$). Savage *et al.* (1985)[21], in a review, cited the recommendations by the College of American Pathologists (CAP) concerning new manual or automated methods of reticulocyte count. Thus the CAP advises that the *r* value must be greater than 0.95; and the intercept value of linear regression line, less than or equal to 0.25 when the new method is correlated with the manual (reference) method. The FC/TO/MOD method comes closest to these recommendations.

Hove *et al*. (1990)[11] conducted a linear regression analysis using data obtained by the manual method and by FC using FACScan and the TO reagent, including or excluding outliers. When these were included in the analysis, an *r* value of 0.89 ($Y = 0.36 + 0.9X$) was obtained. When they were excluded, the coefficient of correlation was higher, with $r = 0.97$ ($Y = 0.261 + 0.87X$). The author attributes the presence of outliers to possible interferents that could not be removed in the employment of the technique.

We can affirm that both the literature and the experiments conducted in the present study indicate the need to use multiparametric analyses for distinction between reticulocytes and interferents, among them, platelets in reticulocyte count by FC. Minimizing platelet interference by using a specific antibody (CD61PE) serves as an irrefutable proof that the methods available in the market must be improved. We must emphasize that in highly specific situations, as the monitoring of bone marrow transplantation and cases of reticulocytopenia in general, result accuracy in reticulocyte counting is enormously important. On the other hand, in laboratory routine, the elimination of interferents using a specific antibody renders this method unfeasible, considering its high costs. It is different with the strategy of leukocyte elimination, which does not need a specific antibody, and can be recommended and conducted in laboratories.

Considering the limitations of the existing methods – both the manual and the automated –, and the clinical importance of an accurate reticulocyte count in critical situations, further studies must be designed to yield results without interference, using simple, rapid and low-cost procedures.

CONCLUSION

The conducted studies indicated the presence of platelet interference with the method of reticulocyte enumeration by flow cytometry using thiazole orange for cell staining. This interference can be excluded by a combination of specific antibody and the establishment of a gate in the scatter plot analysis, improving the accuracy of the obtained results.

RESUMO

Introdução: *A contagem de reticulócitos por citometria de fluxo (CF) – um método de contagem automatizada – pode apresentar erros devido à presença de interferentes, contribuindo para uma ligeira elevação dos resultados. No entanto, os métodos automatizados possuem grandes vantagens em relação ao manual, tido como referência, o que justifica esforços para a melhoria de sua qualidade.* ***Objetivo:*** *Avaliar a interferência de plaquetas na contagem de reticulócitos por CF, utilizando laranja de tiazol (*thiazole orange *[TO]) (CF/TO).* ***Materiais e métodos:*** *O método de contagem de reticulócitos por CF/TO e um método equivalente automatizado modificado, no qual se excluíram células CD61-positivas (plaquetas) da análise (CF/TO/MOD), foram comparados com o método manual.* ***Conclusão:*** *Os resultados foram analisados de acordo com as recomendações do Clinical and Laboratory Standards Institute (CLSI) para avaliar a intercambialidade entre os métodos, por meio da análise de regressão linear e do teste* t *pareado. A exclusão de interferentes da análise dos resultados pelo método modificado demonstrou maior proximidade dos resultados com aqueles do método de referência.*

Unitermos: *contagem de reticulócitos; citometria de fluxo; plaquetas.*

REFERENCES

1. NCCLS. Methods for reticulocyte counting (Automated blood cell counters, flow cytometry, and supravital dyes); Approved guideline – second edition. NCCLS document H44-A2. Vol. 24, n. 8, 2004. 35p.

2. 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(10): 1369-80.

3. Riley RS, Ben-Ezra JM, Tidwell A, Romagnoli G. Reticulocyte analysis by flow cytometry and other techniques. Hematol Oncol Clin North Am. 2002; 16(2): 373-420.

4. Riley RS, Ben-Ezra JM, Tidwell A. Reticulocyte enumeration: past & present. Lab Med. 2001; 10(32): 599-608.

5. Hoffmann JJML. Reticulated platelets: analytical aspects and clinical utility. Clin Chem Lab Med. 2014; 52(8): 1107-17.

6. Robinson MSC, Mackie IJ, Khair K, et al. Flow cytometric analysis of reticulated platelets: evidence for a large proportion of non-specific labelling of dense granules by fluorescent dyes. Br J Haematol. 1998; 100(2): 351-7.

7. Gonçalo AP, Barbosa IL, Campilho F, Campos A, Mendes C. Predictive value of immature reticulocyte and platelet fractions in hematopoietic recovery of allograft patients. Transplant Proc. 2011; 43: 241-3.

8. Failace R. Hemograma: manual de interpretação. 5 ed. Porto Alegre: Artmed; 2009. 424p.

9. Rodak BF. Hematology: clinical principles and applications. 2 ed. Indiana: W.B. Saunders Company; 2002. 835p.

10. Briggs C, Grant D, Machin SJ. Comparison of the automated reticulocyte counts and immature reticulocyte fraction measurements obtained with the ABX Pentra 120 Retic Blood Analyzer and the Sysmex XE-120 Automated Hematology Analyzer. Lab Hematol. 2001; 7: 75-80.

11. Hove LV, Goossens W, Duppen VV, Verwilghen RL. Reticulocyte count thiazole orange. A flow cytometry method. Clin Lab Haemat. 1990; 12: 287-99.

12. Ivory K, Sarria B, Fairweather-Tait SJ, Hughes DA. Reticulated platelets interfere with flow cytometric reticulocyte counts. Int J Lab Hematol. 2007; 29: 352-60.

13. Serke S, Huhn D. Improved specificity of determination of immature erythrocytes (reticulocytes) by multiparameter flow-cytometry and thiazole orange using combined staining with monoclonal antibody (anti-glycophorin-A). Clin Lab Haemat. 1993; 15: 33-4.

14. Viana K, Martins Filho AO, Dusse LMSA. Reticulocyte count: comparison among methods. J Bras Patol Med Lab, 50(5): 339-45.

15. Villamor N, Kirsch A, Huhn D, Vives-Corrons JL, Serke S. Interference of blood leucocytes in the measurements of immature red cells (reticulocytes) by two different (semi-) automated flow-cytometry technologies. Clin Lab Haematol. 1996; 18(2): 89-94.

16. Landi EP, Marques Júnior JFC. Caracterização da ativação plaquetária nos concentrados de plaquetas por citometria de fluxo. Rev Bras Hematol Hemoter. 2003; 25(1): 39-46.

17. Beltrame MP. Avaliação das glicoproteínas plaquetárias na síndrome de Bernard-Souliere e trombastenia de Glanzmann. 2004. [dissertation]. Programa de Pós-Graduação em Medicina Interna, Universidade Federal do Paraná; 2004.

18. Peng L, Yang H, Jiang H, Su J, Peng Z. Automated reticulocyte counting using the Sysmex RAM-1. Clin Lab Haemat. 2001; 23: 97-102.

19. D'onofrio G, Kim YR, Shulze S, et al. Evaluation of the Abbott Cell Dyn 4000 automated fluorescent reticulocyte measurements: comparison with manual, FACS and Sysmex R1000 methods. Clin Lab Haemat. 1997; 19: 253-60.

20. Buttarello M, Bulian P, Farina G, et al. Flow cytometric reticulocyte counting. Parallel evaluation of five fully automated analyzers: an NCCLS-ICSH approach. Am J Clin Pathol. 2001; 115(1): 100-11.

21. Savage RA, Skoog DP, Rabinovitch A. Analytic inaccuracy and imprecision in reticulocyte counting: a preliminary report from the College of American Pathologists Reticulocyte Project. Blood Cells. 1985; 11: 97-112.

CORRESPONDING AUTHOR

Karina Augusta Viana
Rua Presidente Roosevelt, 118; Glória; CEP: 30830-320; Belo Horizonte-MG, Brasil; e-mail: karina.av@hotmail.com.