J Bras Patol Med Lab. 2020; 56: 1-7.

10.5935/1676-2444.20200036

Validation of a new method for estimating low platelet counts: G&S method

Validação de um novo método para estimação de contagens plaquetárias baixas: método G&S

Gian Carlos Ramirez-Ubillus[1]; Carlos Ricardo Neira-Montoya[2]; Eduardo Eulogio Sedano-Gelvet[2]; Joel Fernando Verona-Cueva[2]

1. San Bartolomé Mother-Child National Teaching Hospital, Lima, Peru. 2. Hospital María Auxiliadora, Lima, Peru.

ABSTRACT

Introduction: Automated hematology analyzers are able to produce low platelet counts with great precision and accuracy. However, these analyzers may produce erroneous counts due to the presence of interferences. Therefore, additional methodologies are required to confirm platelet counts, such as blood smear and a direct count, using the Neubauer chamber. **Objective:** To validate the reliability estimate produced by the G&S method. **Material and method:** One hundred and fifty platelet counts were analyzed in the hematology and emergency laboratories of the Hospital Nacional Docente Madre-Niño San Bartolomé, in Lima, Peru, by four methodologies: one optical platelet count (PLTO), one indirect blood smear count (Indirect), and two direct counts (Direct and G&S). Descriptive and inferential statistics were used to compare the groups. **Results:** A significant difference was observed in the distribution of the four methodologies and, after applying the post-hoc analysis, a similarity between the PLTO and G&S methods was found. Also, the Direct, G&S and Indirect methods showed a positive correlation with the PLTO method. The Bland-Altman test demonstrated that the G&S method presented a high agreement with the PLTO method. **Conclusion:** The G&S method is inexpensive, easy to perform, and has demonstrated statistical reliability concerning the automated methodology, and is useful for confirming low platelet counts after a suspected error by the automated equipment or when this device is not available for use.

Key words: platelet count; platelets; nonparametric statistics; data correlation; methylene blue.

RESUMO

Introdução: *Os analisadores automatizados hematológicos são capazes de realizar baixas contagens de plaquetas com precisão e exatidão. No entanto, esses analisadores podem produzir contagens erradas devido à presença de interferências. Dessa forma, métodos adicionais são necessários para confirmar as contagens de plaquetas, como esfregaço de sangue e uma contagem direta, utilizando a câmara de Neubauer.* ***Objetivo:*** *Validar a confiabilidade gerada pela estimativa do método G&S.* ***Material e método:*** *Cento e cinquenta contagens de plaquetas foram analisadas nos laboratórios de hematologia e emergência do Hospital Nacional Docente Madre-Niño San Bartolomé em Lima, Peru, por quatro metodologias: uma contagem óptica de plaquetas (PLTO), uma contagem indireta por esfregaço de sangue (Indireto) e duas contagens diretas (Direto e G&S). Estatísticas descritivas e inferenciais foram utilizadas para comparar os grupos.* ***Resultados:*** *Observou-se diferença significativa na distribuição das quatro metodologias e, após a aplicação da análise* post-hoc*, obteve-se semelhança entre os métodos PLTO e G&S. Além disso, os métodos Direto, G&S e Indireto mostraram correlação positiva com o método PLTO. O teste de Bland-Altman demonstrou que o método G&S apresentou alta concordância com o método PLTO.* ***Conclusão:*** *O método G&S é barato, fácil de executar e demonstrou confiabilidade estatística em relação à metodologia automatizada, sendo útil para confirmar baixas contagens de plaquetas após uma suspeita de erro pelo equipamento automatizado ou quando não se tem esse aparelho disponível para uso.*

Unitermos: *contagem de plaquetas; plaquetas; estatísticas não paramétricas; correlação de dados; azul de metileno.*

First submission on 08/17/19; last submission on 08/19/19; accepted for publication on 11/05/19; published on 05/14/20

RESUMEN

Introducción: *Los analizadores automáticos hematológicos son capaces de realizar bajos recuentos de plaquetas con precisión y exactitud. Sin embargo, eses analizadores pueden producir recuentos erróneos debido a la presencia de interferencias. Así, métodos adicionales son necesarios para confirmar los recuentos de plaquetas, como el frotis de sangre y un recuento directo en cámara de Neubauer.* ***Objetivo:*** *Validar la confiabilidad de la estimación del método G&S.* ***Material y método:*** *Se analizaron ciento cincuenta recuentos de plaquetas en los laboratorios de hematología y emergencias del Hospital Nacional Docente Madre-Niño San Bartolomé, en Lima, Perú, por cuatro métodos: un recuento óptico de plaquetas (PLTO), un recuento indirecto por frotis de sangre (Indirecto) y dos recuentos directos (Directo y G&S). Se utilizaron estadísticas descriptivas e inferenciales para comparar los grupos.* ***Resultados:*** *Se observó diferencia significativa en la distribución de los cuatro métodos y, luego de la aplicación del análisis post-hoc, se encontró similitud entre los métodos PLTO y G&S. Además, los métodos Directo, G&S e Indirecto mostraron correlación positiva con el método PLTO. La prueba de Bland-Altman demostró que el método G&S presentó alta concordancia con el método PLTO.* ***Conclusión:*** *El método G&S es económico, fácil de llevar a cabo y demostró confiabilidad estadística en relación con el método automatizado, siendo útil para confirmar bajos recuentos de plaquetas después de una sospecha de error por el equipo automático o cuando el laboratorio no cuente con éste.*

Palabras clave: *recuento de plaquetas; plaquetas; estadísticas no paramétricas; correlación de datos; azul de metileno.*

INTRODUCTION

Hematology laboratories need to confirm low platelet counts in order to support or assist some medical procedures or diagnosis of some disorders that involve these cell fragments, as blood donation, surgeries, genetic diseases related to thrombocytopenia, among others. Throughout history, various platelet count methods have been described[1-3], the immunological platelet counting method (actual reference method) is not routinely used because of the high cost of the monoclonal antibodies and the flow cytometry; thus, most laboratories use hematology analyzers selected in impedance or optical methodology to confirm thrombocytopenic blood samples.

Among the indirect counting methods (peripherally blood), the red blood cells/platelet ratio method[4], has already been proved to be well correlated with the automated methodology. However, in Peru, there is no standardized indirect method to be used, therefore, the reports may present a weak agreement, as was demonstrated by Conde and Rodríguez (2018)[5].

On the other hand, among the direct counting methods (Neubauer chamber), the "Brecher and Cronkite" method, is recommended by the International Council for Standardization in Haematology (ICSH) and the International Society for Laboratory Hematology (ISLH) for thrombocytopenic blood samples[6]. Although, a phase-contrast microscope is required for platelet count, as it is difficult to recognize them under a light field microscope (they have a complete lack of color).

Additionally, a new direct method is proposed, the G&S method, which is aimed to platelet count for thrombocytopenic blood samples to confirm the result of a low platelet counting given by automated equipment or when it is not available and a light field microscope is used, in which platelets can be recognized easily and quickly by their color (blue) and refringence; and also, uses cheap reagents that are easy to acquire for laboratories with a very basic implementation for carrying out important hematological tests, such as a complete blood count (CBC). For instance, Peru has 2296 small health centers, which don't have an automated hematology analyzer, according to the latest decree of the Peruvian Minister of Health (MINSA)[7]. Therefore, this research aimed to validate the reliability generated by the estimation of platelet count in thrombocytopenic blood samples using the G&S method, for that reason, it was compared with other standardized forms of counting in order to demonstrate that it is useful to confirm low platelet counts.

MATERIAL AND METHOD

This scientific research was carried out in the hematology and emergency laboratories of the Mother-Children San Bartolome National Hospital and presented the following features.

Methodology

Quantitative, descriptive and cross-sectional research.

Sampling

The sampling consisted of all blood samples referred to the laboratories described, and the chosen quantity was 150 samples; all of them were thrombocytopenic blood samples only, selected by a non-probability convenience sampling. The inclusion criteria were venous blood samples extracted with dipotassium or tripotassium ethylenediaminetetraacetic acid (EDTA) with less than 4 hours of storage at room temperature and venous blood samples taken from patients at the Mother-Children San Bartolome National Hospital during July 2018. Also, the exclusion criteria were presence of clots, lipemic, icteric or hemolyzed venous blood samples, and inadequate volume.

Data collection

Firstly, a participation request was delivered to two medical technologists (MT) who performed the counts. Then, the data collection sheets, and the standardized operating procedure sheet created for the G&S method were provided as well. Then, blood smears (Indirect) were performed for an indirect platelet count, the automated analyzer, the Brecher and Cronkite (Direct) and the G&S methods were used, too. The results were tabulated in Microsoft Excel 2010 software.

Finally, to obtain the coefficient of interobserver variability only, the two evaluators performed counts using the G&S method, in parallel, for the last 20 samples.

Procedures

Optical platelet count: PLTO

The automated hematology equipment used was the Abbott CELL-DYN Ruby and its methodology is based on the scattering of light in two dimensions (two-dimensional optical counting).

Direct count: Brecher and Cronkite method

This method uses a diluent liquid composed of ammonium oxalate and distilled water; their effects cause red blood cell plasmolysis, nuclear degeneration of leukocytes, prevent platelet aggregation and their adhesion to other elements[8, 9]. In this research, it is named just Direct to differentiate from other alternative methods created from it[10-11].

This count was performed following the suggestions described in the articles: The reproducibility and constancy of the platelet count[12], and Morphology and enumeration of human blood platelets[13].

G&S method: application of the HAMA methylene blue

This new direct counting method proposed by the main author of this research was standardized only for thrombocytopenic samples and to confirm a report of automated equipment. It uses ammonium oxalate, which lysis red blood cells, and methylene blue, which is oxidized by sodium bicarbonate by boiling. This last action produces azures (derivatives with a smaller number of methyl groups) that create a better electrostatic attraction with the acid glycosaminoglycan molecules (sialic acid) found in the platelet membrane[14]. Lastly, platelets can be recognized in the Neubauer chamber not only for their refringence and morphology but also now, for their color.

This new way to prepare this dye, also proposed by the main author of this research, called HAMA methylene blue, was prepared as follows: dilute 1 g of methylene blue in 100 ml of distilled water, add 1 g of sodium bicarbonate and mix them. Then, boil the solution (100°C); once achieved, time 20 minutes. Finally, conserve the solution at room temperature, after filter with Whatman 2° paper. Once frozen, this solution is ready to use and its stability is undefined, as the majority of dyes (the preparation for this study was performed in 2016, with no variation in quality or contamination by microorganisms).

Procedure

• For every 20 µl of blood, dilute with 425 µl of 1% ammonium oxalate in 12 × 75 mm plastic tubes (glass should not be used because platelets can adhere to the tube walls). After that, continue mixing for 20 seconds (slowly), then wait 18 minutes for incubation period at room temperature; clean briefly the excess blood on the surface of the tip with a piece of paper towel before the mix.

• Then, add 15 µl of the HAMA methylene blue and mix five times. Immediately, dispense 20 µl of the total solution in only one side of the Neubauer chamber. Let stand on a Petri dish with a piece of wet cotton for 2 minutes to allow platelet sedimentation.

• Finally, the platelet counting is carried out by a light field microscope, with the 40× objective, the condenser totally down, almost completely closed diaphragm, and in the red blood cell counting area (central secondary quadrant), except for the tertiary quadrant central (only four quadrants, upper left and right; lower right and left). Platelets can be differentiated from other elements due to their intense blue color, refringence, and round to oval shape. The total number of platelets counted was multiplied by 1437, resulting in the number of platelets/µl of blood.

Indirect platelet count

In this research, it appears as Indirect and was carried out based on the suggestions described in the article: Estimation of the platelet count basis on red blood cells/platelet ratio[4].

Statistic analysis

After the collection period, the data was processed and analyzed using the Microsoft Excel 2010, SPSS 15.0, GraphPad Prism® programs, and descriptive and inferential statistical analyses were carried out as well. They were: the establishment of counting ranges, arithmetic mean, standard deviation and calculation of the coefficient of interobserver variability; normality tests and the Friedman test, followed by Dunn's post-hoc test to compare the groups were calculated as well. Finally, Spearman's correlation coefficient, residues analysis, and Bland-Altman test were applied. Values of $p < 0.05$ were considered significant.

Ethical aspects

This research was approved by the chief of the clinical pathology service and by the ethics committee of the Mother-Children San Bartolome National Hospital.

RESULTS

Morphology, refringence and platelet staining according to the G&S method

In all observations, the platelets were round to oval, blue (**Figure 1**) refractory (**Figure 2**) and the numerical value was compared with the other counting methods. During the visualization, some leukocytes and red blood cells were also observed; these were differentiated by their morphology, little or no both refringence, and coloration.

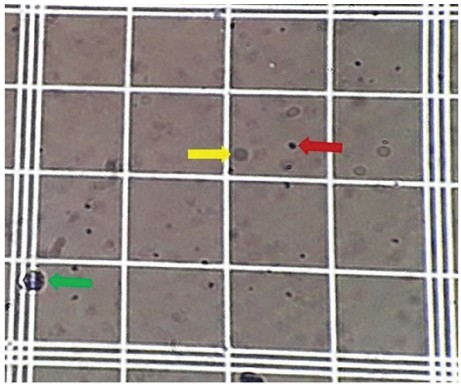

FIGURE 1 – *Platelet staining displayed at 40× according to the G&S method*
Green arrow: leukocyte; yellow arrow: erythrocyte; red arrow: platelet.

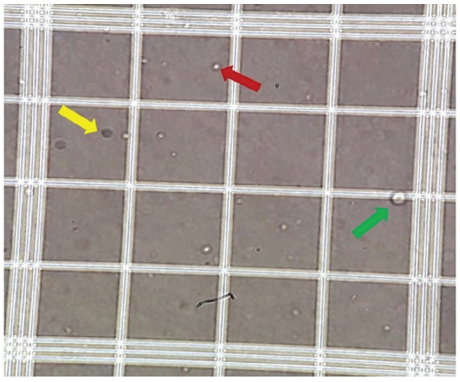

FIGURE 2 – *Platelet refringence displayed at 40× according to the G&S method*
Green arrow: leukocyte; yellow arrow: erythrocyte; red arrow: platelet.

Establishment of counting ranges and calculation of arithmetic mean ± standard deviation (SD)

The counting ranges were ($\times 10^3$/µl): 41-130; 37-118; 43-135; and 55-145 platelets for the PLTO, Direct, G&S, and Indirect methods, respectively. The arithmetic mean ± SD were ($\times 10^3$/µl): 83.47 ± 6.86; 74.81 ± 5.42; 85.45 ± 7.2; and 97.3 ± 8.75 platelets for the PLTO, Direct, G&S, and Indirect methods, respectively.

Determination of the coefficient of interobserver variability for the G&S method

The final range was between 7.5%-8.4%.

Application of normality test or normality contrast

It was used to verify whether the normality hypothesis is correct so that certain analyzes present statistical reliability, such as for the analysis of variances (Anova) or Dunn's post-hoc test. The Kolmogorov-Smirnov test was used and it rejected the normality hypothesis ($p < 0.05$) for the PLTO, Direct, and G&S counting methods.

Application of non-parametric statistics

As the reported values rejected the hypothesis of normality, non-parametric tests were carried out to determine if there are significant differences among all platelet counting methods. The Friedman test showed that there is enough evidence to reject the null hypothesis ($p < 0.05$) and conclude that there are

significant differences (> 7.81 for four groups) among the four methods; its value in this study was 152.6.

Subsequently, we proceeded to identify which group or groups specifically are significant concerning the others, then, the Dunn multiple comparison test was performed and it showed that there are no significant differences ($p < 0.05$) only between the G&S and the PLTO methods. To improve this interpretation, box-and-whisker plots were performed (**Figure 3**).

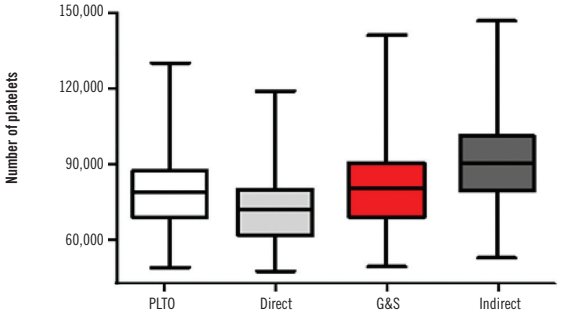

FIGURE 3 – *Box-and-Whisker plots of all the methods performed*

PLTO and G&S methods showed similar distribution.

Spearman correlation coefficient

This statistic showed a positive correlation between the methods Direct, G&S, and Indirect (0.987, 0.998 and 0.981, respectively) compared to the automated method (PLTO).

Analysis of residuals

Residuals values were plotted, and the best result was provided by the G&S method (**Figure 4**) which values presented less distance from the average (0).

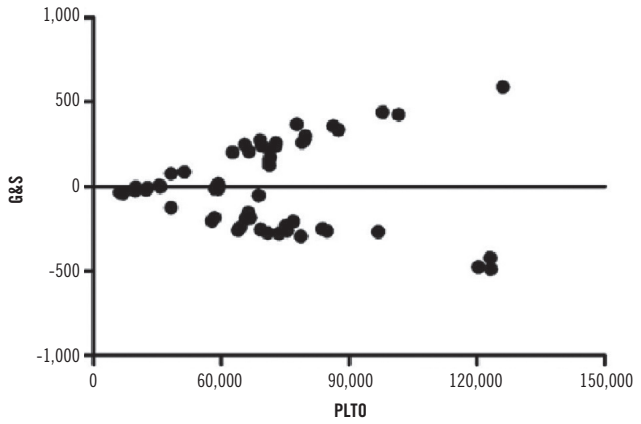

FIGURE 4 – *Residues of the G&S method in relation to PLTO*

The values were around the 0-accuracy line.

Bland-Altman test

The methods Direct, G&S, and Indirect (**Figures 5**, **6** and **7**, respectively) showed that most differences are located around the bias (4245.7 platelets/µl, 27.8421 platelets/µl, and -28984.4 platelets/µl, respectively).

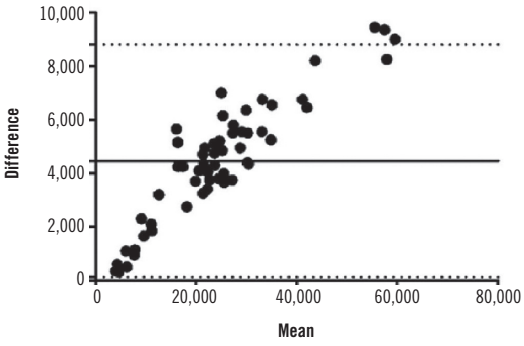

FIGURE 5 – *Bland-Altman test for the Direct method*

The results were above the 0-agreement line.

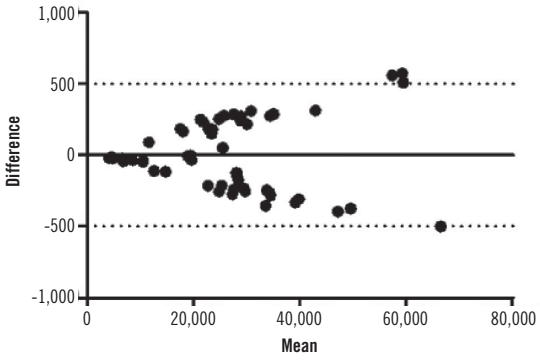

FIGURE 6 – *Bland-Altman test for the G&S method*

The results were around the 0-agreement line.

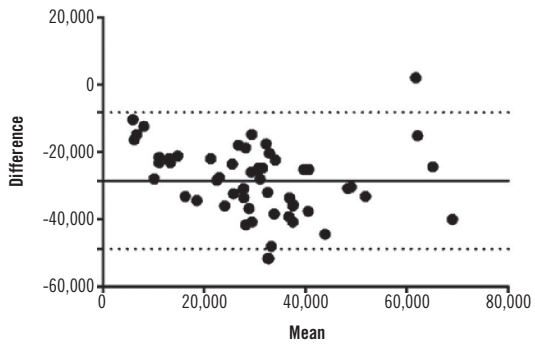

FIGURE 7 – *Bland-Altman test for the Indirect method*

The results were below the 0-agreement line.

DISCUSSION

The automated method (optical platelet count) was taken as a reference in this study due to the reliability generated by this methodology, which has also been taken as a reference by other studies[15, 16]. However, these principles have limitations, the correlation decreases in presence of interferents, such as fragmentation of erythrocytes or leukocytes, lipids, cryogglutinins, among others; this was demonstrated by Congona (2011)[17]. Therefore, the hematology laboratory is always requiring other methodologies to confirm these low counts.

The method that showed a greater dispersion of data concerning its average was the Indirect, it is, therefore, the one that presented less precision in relation to the others. A decrease in the coefficient of interobserver variability was observed regarding to results reported in other studies[18, 19], in which the amount of 20 samples for its calculation has also been used in other investigation researches[20, 21]. This demonstrated that the method G&S improves platelet recognition by observers because, now, color can also be taken into account.

In addition to this, the residues generated by this method (G&S) also showed less distance from the mean (0), which means it that has the highest degree of accuracy. The fact that the G&S method presented the lowest bias (Bland-Altman test), did not generate admiration, due to the ease of recognition of platelets and because of the correct quantity of sampling chosen (similar sizes have already been considered in other studies)[15, 16].

Additionally, in the case of Figure 5, the differences between the measurements are above 0, means that the platelet counts by the Direct method are smaller than in the PLTO (reference in this study) method. In Figure 7, the differences between the measurements are below 0, means that platelet counts using the Indirect method tend to overestimate the counts. On the other hand, Figure 6 shows that the difference is a value close to 0 and was within the limits of agreement, showing that the G&S and the PLTO methods produced very similar results.

Due to this overestimation generated by optical or impedance methodology, indirect count methods are not recommended for low platelets. This advice has also been reported in other studies[22, 23]. For example, the guide provided by the Institute of Public Health of Chile: Recomendaciones para la interpretación del hemograma: serie roja, blanca y plaquetaria[24]. A limitation produced by the G&S method is that it generates results in 20 minutes, however, considering that only one side of the Neubauer chamber is used for the procedure, two results (two patients) can be obtained in that time. Moreover, the preparation time of the dye HAMA methylene blue (20 minutes) is considered negligible once when 100 ml is prepared, more than 6600 tests (15 µl per test) can be performed.

For this reason, there are studies that highlight the value of low platelets direct counting (Neubauer chamber) for hematology laboratories, such as those performed by Anilema (2016)[25] and Zabala (2013)[26]; and even in procedures carried out by Leena (2014)[15] for blood bank laboratories (platelet-poor plasma) as well. Also, there are guides that recommend direct counting methods, such as the one carried out by Kitchen S *et al.* (2010)[27] whose title is Diagnóstico de hemofilia y otros trastornos de la coagulación, and the current manual of laboratory procedures in basic hematology techniques of the National Institute of Health of Peru (2005)[28].

CONCLUSION

The G&S method is cheap, easy to perform and demonstrated statistical reliability compared to the automated methodology, and is useful to confirm low platelet counts after a suspected error by the automated equipment or when it is not present.

Further studies in which only pathological samples are considered, or each interferent is analyzed independently, are recommended.

ACKNOWLEDGMENTS

To Augusto Adán Marimón Pacheco and Fredy Barboza León for the support in platelet counting.

To Carlos Orlando Velasquez Figueroa and Diego Arturo Paniagua Napán for the advice provided on automation in hematology. To the company BIOSIX IMPORT S.A.C. for the donation of 1% ammonium oxalate.

REFERENCES

1. Fink N. Automatización en hematología. Hematología. Enero-Abril. 2005; 9(1): 2-11.

2. Campuzano G. Del hemograma manual al hemograma de cuarta generación. Medicina & Laboratorio. 2007; 13: 11-12.

3. Hernández L. Avances y aplicación clínica de la citometría hemática automatizada. Rev Cubana Hematol Inmunol Hemoter. 2013; 29(1): 24-39.

4. Brahimi M, Osmani S, Arabi A, et al. Estimation of platelet count on the basis of red cell: platelet ratio. Iraqi Journal of medical sciences. 2009; 26(1): 21-4.

5. Conde S, Rodríguez R. Concordancia en el recuento e identificación morfológica de plaquetas en frotís sanguíneo entre tecnólogos médicos de hospitales e institutos especializados de Lima metropolitana y Callao, Octubre 2017-Marzo 2018. [thesis]. Perú: Universidad Norbert Wiener, Facultad de Ciencias de la Salud; 2018.

6. Harrison P, Kenneth A, Chapman S, et al. An interlaboratory study of a candidate reference method for platelet counting. Am J Clin Pathol. 2001; 115: 448-59.

7. Superintendencia Nacional de Salud. Intendencia de Investigación y Desarrollo. Infraestructura del sector salud por tipo de establecimiento, según departamento, 2016 (número de establecimientos). Lima: Compendio Estadístico Peru; 2017.

8. Lynch M, Raphael S, Mellor L, Spare P, Inwood M. Obtención de muestras de sangre y citometría hemática. In: Folch R, editor. Métodos de laboratorio. México: Nueva Editorial Interamericana; 1972. p. 713.

9. Lewis S, Bain B, Bates I. Hematología en laboratorios de recursos escasos. In: Elsevier España, editor. Dacie y Lewis hematología práctica. España: Elsevier; 2008. p. 580-1.

10. Rodak B. Pruebas de rutina en hematología. En: Giovaniello O, Oxemberg J, Rondinone S, Taveira J, editors. Hematología fundamentos y aplicaciones clínicas. Argentina: Médica Panamericana; 2004. p. 159-60.

11. Crocker J, Burnett D. Análisis de plaquetas. In: Tovar M, editor. La ciencia del diagnóstico de laboratorio. México: McGraw-Hill-Interamericana; 2007. p. 290.

12. Brecher G, Cronkite E. Morphology and enumeration of human blood platelets. J Appl Physiol. 1950; 3: 365-77.

13. Brecher G, Cronkite E, Schneiderman M. The reproducibility and constancy of the platelet count. Am J Clin Pathol. 1953; 23: 15-26.

14. Lillie R. Quinone-iminedyes. In: Lillie R, Stotz E, Emmel V, Darrow M, editors. H. J. conn's biological stains. United States: The Williams & Wilkins Company; 1969. p. 430.

15. Leena J, Devaraju S, Saldhana C. A comparative study of Platelet counts by manual and automathed methods in platelet poor plasma. Int J Rec Rec Trends Sci Technol. 2014; 12(2): 262-5.

16. Pan L, Chen Ch, Huang W, Sun Ch. Enhanced accuracy of optical platelet counts in microcytic anemia. Lab Medicine. 2014; 45(1): 32-6.

17. Congona R. Influencia de interferentes en el recuento plaquetario en pacientes hemato-oncológicos mediante el principio de impedancia y recuento óptico/fluorescente en el analizador SYSMEX XE-2100 FULL. [thesis]. Peru: Universidad Nacional Mayor de San Marcos, Facultad de Medicina; 2011.

18. Banfi G, Salvagno GL, Lippi G. The role of ethylenediamine tetraacetic acid (EDTA) as in vitro anticoagulant for diagnostic purposes. Clin Chem Lab Med. 2007; 45(5): 565-76.

19. Brambila E, Castillo R, Lozano P. Comparación entre tres métodos manuales empleados en la cuenta diferencial de leucocitos respecto a un equipo automatizado. Bioquimica. 2003; 28(3): 4-12.

20. García F. Evaluación de la calidad de vida y remodelado cardíaco en pacientes sometidos a ablación de flúter auricular dependiente del istmo cavo-tricuspídeo. [thesis]. España: Universidad de Santiago de Compostela Facultad de Medicina; 2009.

21. Hernández S, Fernández C, Baptista L. Selección de muestra. In: Rocha M, editor. Metodología de la investigación. México: McGraw-Hill/ Interamericana; 2014. p. 177.

22. Rachid M, Al-Farsi R, Al-Hashmi S, Al-Riyami H, Khan H, Al-kindi S. Comparative analysis of four methods for enumeration of platelet counts in thrombocytopenic patients. J Appl Hematol. 2015; 6(3): 119-24.

23. Gómez S. Comparación del método de fluorescencia, impedancia y microscopía óptica en el recuento de plaquetas en distintos grupos de pacientes. Conferencia Sociedad Española de Hematología y Hemoterapia. In: Sesión laboratorio básico y automatización en hematología. España; 2017.

24. Retamales E. Recomendaciones del informe del frotis sanguíneo serie plaquetaria. In: Moscoso H, Ramírez V, editores. Recomendaciones para la interpretación del hemograma: serie roja, blanca y plaquetaria. Chile: Comité Editor Instituto de Salud Pública; 2015. p. 20-2.

25. Anilema M, Baños G. Importancia de las determinaciones de proteinuria de 24 horas y contaje de plaquetas como ayuda diagnostica de preeclampsia en mujeres embarazadas que acuden al hospital Gineco Obstétrico Isidro Ayora en la ciudad de Quito durante el período octubre 2015-marzo 2016. [thesis]. Quito: Universidad Nacional de Chimborazo, Facultad de Ciencias de la Salud; 2016.

26. Zabala N, Hannaqui E. Comparación del contaje plaquetario empleando diferentes metodologías, en pacientes con purpura trombocitopénica y síndromes mielodisplásicos. Saber. 2013; 25(3): 259-64.

27. Kitchen S, McCraw A, Echenagucia M. Platelet count. In: Ahmed M, Lam C, de Bosch N, et al., editors. Diagnosis of hemophilia and other bleeding disorders. Canada: World Federation of Hemophilia; 2010. p. 30-4.

28. Muñoz M, Morón C. Recuento de reticulocitos y plaquetas. In: Cabezas C, Cabrera R, editors. Manual de procedimientos de laboratorio en técnicas básicas de hematología. Peru: Comité Editor Instituto Nacional de Salud; 2005. p. 60-2.

CORRESPONDING AUTHOR

Gian Carlos Ramirez-Ubillus ID 0000-0002-9236-8667
e-mail: scarlosc1@hotmail.com