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Original Article

The impact of automation of extended erythrocyte phenotyping in the routine of a blood transfusion center

O impacto da automação da fenotipagem eritrocitária estendida na rotina de um serviço de hemoterapia

Luciana Corrêa Carneiro[1], Lacy Cardoso de Brito Júnior[2], Carlos Eduardo de Melo Amaral[3]

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ABSTRACT: Objective. Evaluate the impact of automation on expanded erythrocyte phenotyping and the level of agreement between it and the manual methodology in samples from blood donors treated at the blood center coordinating the Fundação HEMOPA from january to december 2019. Material and Methods. 2,700 erythrocyte phenotyping performed by manual and automated methodology using BioRad® IH500 equipment was analyzed. The results were tested for the level of agreement using the Kappa Coefficient test. Results. Of the phenotyped samples, 98,6% (2,662 / 2,700) were in agreement in both methodologies and only 1,4% (38/2700) were in disagreement. Of the 38 discordant samples, 31,6% referred to the Lu(b) phenotype; 15,8% to the Lu(a) phenotype; 13,1% to the Fy phenotype (b); 7,9% to Le(b), E, c phenotypes; 5,3% to N, S, s, Kp (a), P1 phenotypes; and 2,6% for phenotypes M, Jk(a), Jk(b), Fy(a). Conclusions. The level of agreement between data obtained through manual and automated erythrocyte phenotyping techniques was 98.6%. The implementation of this methodology had a positive impact, with an increase of 1,649 more processed samples compared to the same period of the previous year.

Keywords: Automation laboratory; Blood group antigens; Blood group incompatibility; Erythrocyte transfusion.

RESUMO: Objetivos. Avaliar o impacto da automação na fenotipagem eritrocitária expandida e o nível de concordância dessa com a metodologia manual em amostras de doadores de sangue atendidos no hemocentro coordenador da Fundação HEMOPA no período de janeiro a dezembro de 2019. Material e métodos. Foram analisadas 2.700 fenotipagens eritrocitárias realizadas por metodologia manual e automatizada através do equipamento IH500 da BioRad®. Os resultados foram testados quanto ao nível de concordância através do teste de Coeficiente Kappa. Resultados. Das amostras fenotipadas 98,6% (2.662/2.700) foram concordantes em ambas as metodologias e apenas 1,4% (38/2700) foram discordantes. Das 38 amostras discordantes 31,6% referiram-se ao fenótipo Lu(b); 15,8% ao fenótipo Lu(a); 13,1% ao fenótipo Fy(b); 7,9% aos fenótipos Le(b), E, c; 5,3% aos fenótipos N, S, s, Kp(a), P1; e 2,6% aos fenótipos M, Jk(a), Jk(b), Fy(a). Conclusões. O nível de concordância entre os dados obtidos através das técnicas de fenotipagem eritrocitária manual e automatizada foi de 98,6%. Já a implantação dessa metodologia teve um impacto positivo com o aumento em 1.649 amostras processadas a mais em relação ao mesmo período do ano anterior.

Palavras-chave: Automação laboratorial; Antígenos de grupos sanguíneos; Incompatibilidade de grupos sanguíneos; Transfusão de eritrócitos.

Postgraduate Program in Clinical Analysis of the Federal University of Pará in partnership with the HEMOPA Foundation.

1. Dr João de Barros Barreto University Hospital, Federal University of Pará, Biomedical, Master by the Postgraduate Program in Clinical Analysis, Federal University of Pará (UFPA), Belém, Pará, Brazil. https://orcid.org/0000-0003-4087-5605. Email: lucianacarneiro1@hotmail.com.
2. Federal University of Pará, Institute of Biological Sciences, Laboratory of General Pathology - Immunopathology and Cytology, Biomedical, Associate Professor III, Belém, Pará, Brazilhttps://orcid.org/0000-0001-9102-5817. Email: lcdbrito2@gmail.com.
3. Foundation Center for Hemotherapy and Hematology of Pará (HEMOPA), Molecular Biology Management, Responsible for the Molecular Biology Laboratory, Biomedical, Doctor at the Federal University of Pará (UFPA), Belém, Pará, Brazil. https://orcid.org/0000-0003-1245-3851. Email: carlosamaral23@hotmail.com.

Correspondence: Prof. Dr. Lacy Cardoso de Brito Junior. Federal University of Pará. Institute of Biological Sciences. General Pathology Laboratory - Immunopathology and Cytology. Av. Augusto Correa n 01. Guamá District - CEP 66075-900. Belém Pará. e-mail: lcdbrito@ufpa.br, lcdbrito2@gmail.com

INTRODUCTION

The Consolidation Ordinance # 5 dated September 28, 2017[1], recommends performing erythrocyte phenotyping of Rh system antigens (E, e, C, c), Kell (K), Duffy (Fya, Fyb), Kidd (Jka, Jkb), and MNS (S, s) in receptor blood samples, whenever possible, before performing any blood transfusion to identify the presence of any possible irregular anti-erythrocytes antibodies in these patients. Especially when treating alloimmunised patients against erythrocyte antigens or that are or could enter a chronic transfusion therapy scheme.

The greater the number of correctly phenotyped donors, the lower the risk for transfusion reaction occurrences, immediately or afterward, in receptors[2-6]. Various authors have converged on this principle and have dedicated studies on many phenotyping tests on blood donors of the most important blood types for transfusions[7-9].

Sometimes this work is performed manually, a fact that requires an experienced operator and dedication to the task due to the large number of samples collected daily, making this job arduous, repetitive, and many times associated with failures in the sample processing and analysis; delaying the final delivery of the results; and even absenteeism for health treatment of the operator due to repetitive strain injury (RSI)[10-15].

Hence various manufacturers of laboratory supplies have offered automated erythrocyte phenotyping equipment to the market, capable of making the process quicker and safer. Thus, beginning in 2017**,** recommended by the "*Associação Americana de Bancos de Sangue*" (American Blood Bank Association) (AABB), the "*Gerência de Imunohematologia Eritrocitária*" (Erythrocyte Immunohemotology Management) (GEMER) from the "*Fundação Hemopa*" (Hemopa Foundation) purchased the IH500 equipment from (BioRad®) to use in the erythrocyte phenotyping process for donors.

Then that study sought to evaluate the impact of automation on the expanded erythrocyte phenotyping method on samples from blood donors, based on the quantitative results released annually after implementing that methodology and the level of concordance between the data sets collected by comparing the expanded manual and automated erythrocyte phenotyping techniques.

METHOD

Casuistry

Transversal and analytical studies were performed in the period from January to December 2019, including data from 2,700 samples of routine blood donors abiding by the "Gerência de Imunohematologia Eritrocitária" (Erythrocyte Immunohemotology Management) routine (GEMER) from the "Hemocentro Coordenador da Fundação" (Blood Bank Coordinating Foundation) HEMOPA.

Inclusion and exclusion criteria

Erythrocyte phenotyping results were included from blood donors ages 16 years old and older of both sexes, who: (1) permanently reside in the Bélem metropolitan region (Ananindeua, Belém, Benevides, Castanhal, Marituba, Santa Bárbara do Pará, and Santa Izabel do Pará); (2) who preferably are repeated donors, and who have performed at least two blood donations at the "Hemocentro coordenador da Fundação" (Blood Bank Coordinating Foundation) HEMOPA; and (3) presented negative results in all serologic screening tests and other hematological diseases. Unacceptable donors were excluded who submitted incomplete data in the data records or from previous erythrocyte phenotyping.

Ethical aspects

As this study was performed without any direct contact with the researchers and their research subjects or the use of their personal data, the researchers have committed to maintaining data security obtained by signing the Data Protection, Secrecy, and Use Term from the institution responsible for supplying the data. The Status as defined by the CNS Resolution # 466/2012 identified the same just by using the respective numbering from the HEMOPA Foundation.

Erythrocyte phenotyping by the manual methodology

All the samples from the selected blood donors, compliant with the inclusion criteria, were submitted to erythrocyte phenotyping for the following antigens Rh (E, e, C, c), Kell (K), k, Kp (Kpa, Kpb), Duffy (Fya, Fyb), Kidd (Jka, Jkb), MNS (S, s), Lewis (Lea, Leb), P1, and Lutheran (Lua, Lub) by a manual methodology and, afterward by an automated methodology using the IH500 (BioRad®) equipment.

The manual erythrocyte phenotyping technique was performed using a specific antiserum for each antigen present on the BioRad® microtiter plates (manufacturer: DiaMed) suspended in Sephadex gel.

For the Rh (C, c, E, e) and Kell phenotyping systems, red blood cell suspensions were prepared in a proportion of 1:20, that is, 0.5 ml dilutant-2 (Liss) and 25 µl of red blood cell concentrate with the posterior dispensation of 12.5 µl of this solution in each microtiter plate that contains anti-C, anti-c, anti-E, anti-e, and anti-K antibodies from human sources, suspended in gel.

For each of the MNS (S and s) and Duffy (Fya,

Fyb) phenotype systems, they were prepared as red blood cell suspensions in a 1:100 proportion, that is, 1,000 µl of dilutant -2 (Liss) and 10 or 12.5 µl of red blood concentrate, with the posterior dispensation of 50 µl of that solution in each microtiter plate that contains two microtiter tubes with polyspecific anti-human globulin (anti-IgG from a rabbit and anti-C3d monoclonal) suspended in gel. Afterward, the respective anti-M, anti-N, anti-S, anti-s, anti-Fya, and anti-Fyb antiserums were added to each microtube and incubated for 10 minutes at room temperature (18-25ºC).

Since the P1, Lewis (Lea, Leb), and Lutheran (Lua, Lub) phenotype systems were prepared from red blood cell suspensions with 5% dilutant-1 (Bromelina) in a 1:20 proportion, this is, 0.5 ml of dilutant-1 and 25 µl of red blood cell concentrate, incubated at room temperature for 10 minutes, and posterior dispensation of 10 µl of that solution from each microtiter plate that contains monoclonal antibodies anti-P1, anti-Lea, anti-Leb, and polyclonal anti-Lua, anti-Lub from suspended human sources in a gel.

And finally, the k, Kp (Kpa, Kpb), and Kidd (JKa, JKb) phenotyping systems were performed by preparing red blood cell suspensions that were also diluted with a 5% dilutant-1 (Bromalin) and in a 1:20 proportion, that is, 0.5 ml of dilutant-1 and 25 µl of red blood cell concentrate incubated at room temperature for 10 minutes, and posterior dispensation of 10 µl in each microtiter plate that contains human anti-k, human anti-Kpa, human anti-Kpb, anti-Jka monoclonal, and anti-Jkb polyclonal antibodies, on each microtiter plate that contains anti-k human polyclonal, human anti-Kpa, human anti-Kpb, anti-Jka monoclonal and anti-Jkb monoclonal antibodies suspended in gel.

The plates were centrifuged at 910 rpm for 10 minutes, and afterward, the reactions were read based on gel agglutination standards.

Erythrocyte phenotyping by the automated methodology

Following that, the blood donor samples from the "Hemocentro coordenador da Fundação" (Blood Bank Coordinating Foundation) Hemopa were phenotyped in the IH500 (BioRad®) equipment for the Rh (E, e, C, c), Kell (K), k, Kp (Kpa, Kpb), Duffy (Fya, Fyb), Kidd (Jka, Jkb), MNS (S, s), Lewis (Lea, Leb), P1, and Lutheran (Lua, Lub) phenotypes.

The IH500 (BioRad®) equipment utilizes the Gel-centrifugal technique as its analysis methodology. It is a microtechnique based on using plates containing microtubes with Sephadex or Polyacrylamide Gel to analyze the agglutination reactions between the antigen-antibody complex (positive for the studied antigen) or red blood cells without reactivity (negative for the searched antigen) using the IH-Com (BioRad®) data management software and interpreting the results.

Statistical analysis

The researchers obtained data based on erythrocyte phenotyping from blood donors by these two methodologies by accessing the record books from the erythrocyte phenotyping manual or the blood bank management system (BBMS), obtained from the automated phenotyping method. Afterward, they performed comparative and statistical analyses.

The statistical methods employed descriptive statistics using qualitative variables for these evaluations to determine the absolute and relative frequencies and estimate the expected margin of error that did not occur in the discordant results in over 5% of the total analyzed samples.

The discordant data between these two techniques were evaluated by comparative analysis by applying the Kappa Coefficient Test to describe the intensity of concordance between the two methods utilizing the Bioestat 5.0 software, considering the significant values as $p \leq 0.05$.

RESULTS

The automation method for erythrocyte phenotyping on blood donors from the "Hemocentro Coordenador da Fundação" (Blood Bank Coordinating Foundation) HEMOPA was only implemented in 2018. However, only the following year (2019), all the blood donor samples from that foundation were phenotyped and confirmed by the automated methodology. Hence, in that year, a total of 7,542 phenotyping blood donor samples were analyzed by this methodology.

From that total, only 2,700/7,542 (35.8%) phenotyped samples were included in this study after manual and automated erythrocyte phenotyping was performed, according to the established exclusion criteria, to perform a comparative analysis of the results effectively. 2,662/2,700 (98.6%) of the samples from that total were concordant for all the studied phenotyped by both methodologies, and 38/2,700 (1.4%) samples were discordant after performing both methodologies.

Of the 38 discordant samples studied by both methodologies, 35/38 of the discordant samples, there was only 01 discordant phenotype from each sample (Table 1). For the other 3/38 samples, some discordances occurred for 02 phenotypes in the same sample. And in 1/38 of the discordant samples, five discordant phenotypes were observed in the same sample. Thus, a total of 46 discordant phenotypes were considered for statistical analysis in one summation of 38 samples.

Table 1. The frequency of erythrocyte phenotyping in blood donor samples that obtained discordant results when tested by the manual and automated methodologies in the HEMOPA blood bank coordinating foundation unit in the period from January to December 2019

Phenotype	Number of repetitions of the Discordant phenotype	Frequency (%)	Phenotype	Number of repetitions of the Discordant phenotype	Frequency (%)
Lu(b)	12	28,9	s	02	5,3
Lu(a)	06	15,8	Kp(a)	02	5,3
Fy(b)	05	13,1	P1	02	5,3
Le(b)	03	7,9	M	01	2,6
E	03	7,9	Jk(a)	01	2,6
c	03	7,9	Jk(b)	01	2,6
N	02	5,3	Fy(a)	01	2,6
S	02	5,3			

Statistical analysis was performed on those discordant erythrocyte phenotype samples by the Kappa coefficient test to evaluate if there is discordancy beyond what is expected not only by chance but also the level of concordance among the samples. Hence, our results have proven excellent replicabilities for all the involved and tested phenotypes in both the tested manual and automated methodologies (Table 2).

Table 2 – The discordant erythrocyte phenotype results from the Kappa coefficient test from blood donor samples that occurred simultaneously when tested by the manual and automated methodologies at the HEMOPA blood bank coordinating Foundation unit in the period from January to December 2019

Discordant phenotypes in both methodologies	Observed concordance	Kappa	p (unilateral)	Replicability conclusion
Lu(b)	0.9960	0.8716	<0,0001	Excelente
Lu(a)	0.9978	0.9257	<0,0001	Excelente
Fy(b)	0.9982	0.9374	<0,0001	Excelente
Le(b),E,c	0.9989	0.9615	<0,0001	Excelente
N,S,s,Kp(a),P1	0.9993	0.9740	<0,0001	Excelente
M,Jk(a),Jk(b),Fy(a)	0.9996	0.9868	<0,0001	Excelente

Legend: p – statistical significance level; Lu(a) - Lutheran-A; Lu(b) – system antigen Lutheran-B; Fy(a) - system antigen Duffy-A; Fy(b) – system antigen Duffy-B; Le(b) - system antigen Lewis-B; P1 - system antigen P; M,N,S,s – system antigen MNSs; Jk(a) – system antigen Kidd-A; Jk(b) - system antigen Kidd-B; Kp(a) – system antigen Kell-A; E – system antigen Rh ; c - system antigen Rh.

DISCUSSION

The recent scientific and technological advances have favored the implementation of automation in various fields of laboratory medicine, specifically as they generate essential benefits to the patient as decreased processing and analysis time of samples, decreased the delivery time of results, and improved diagnostic precision and safety[13-18].

Generally, employing automation in laboratory practice has also added advantages to institutions that host and to their staff as increased productivity, reduced exposure to staff member from biological and ergonomic hazards, and fosters a greater security level to all sample processing phases thereby obtaining more precise results[14-18].

Blood banks have observed explicitly that the implementation of automated methodologies has increased transfusion safety by minimizing any possible errors associated with manual processing of samples and enhancing the optimization of inputs and reducing the delivery time of the results based on the interfacing between the equipment and the result system[10,11,14,15,17-20].

This study on the implementation of the automated erythrocyte phenotype methodology for analyzing blood donors displayed similar results as those reported by other authors in the literature[13-19,21] who also compared the impact on immunohematology laboratories in blood banks and their result concordance between manual and automated techniques.

Bhagwat et al.[13] for example, in their study in India, observed that 95.1% concordance between ABO/Rh phenotyping results in 1,000 tested samples by manual and automated techniques. Schoenfeld et al.[11] in their case, analyzed the erythrocyte phenotype in 304 samples from the ABO/Rh and Rh+Kell blood groups and obtained 100% concordance from both the manual and automated techniques.

Park et al.[14] used automated equipment for erythrocyte phenotyping like what we used in our study (IH500 - BioRad®) observed 100% concordance in the manual and automated techniques for erythrocyte phenotyping 200 samples from the ABO/Rh blood groups. However, we could not find any case report in the literature like ours, whereas we performed a comparative study on expanded erythrocyte phenotyping using both manual and automated techniques.

Furthermore, regarding this, in our study, we verified the automated methodology for expanded erythrocyte phenotyping in blood donor samples, utilizing the IH500 (BioRad®) equipment that generated an increase of 1,649 samples compared with the same period in the previous year, when the manual methodology was used exclusively. That fact increased the possibility of detecting a great deal of donors with rare phenotypes in the population and even in transfusions of phenotype-compatible red blood cell concentrates.

CONCLUSION

The concordance level was 98.6% between the manual and automated data set obtained by the expanded erythrocyte phenotyping techniques. Thereby, the implemented automated methodology increased the number of 1,649 blood donor samples processed compared to the same period in the previous year, when the manual methodology was exclusively used.

Authors participation: *Carneiro LC* - In the conception of the work; acquisition, analysis and interpretation of research data; in writing and critical review. *Brito Junior LC* - In the conception and design of the work; analysis and interpretation of research data; in writing and critical review with intellectual contribution; and final approval of the version to be published. *Amaral CEM* - In the acquisition and analysis of research data.

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