REVISTA ONLINE DE PESQUISA

CUIDADO É FUNDAMENTAL

Universidade Federal do Estado do Rio de Janeiro • Escola de Enfermagem Alfredo Pinto

RESEARCH

DOI: 10.9789/2175-531.2019.v11i2.285-288

Validation Assessment of Irregular Antibodies Investigation at the Regional Blood Center in *Montes Claros* City

Avaliação da Validação da Pesquisa de Anticorpos Irregulares no Hemocentro Regional de Montes Claros

Evaluación de la Validación de la Búsqueda de Anticuerpos Irregulares em la Región Hemocentro de Montes Claros

Karina Marini Aguiar[1]*; *Débora Cristina de Jesus Mota*[2]; *Larúbia Cordeiro Rocha*[3]; , *Caroline Nogueira Maia e Silva*[4]; *Giovani de Souza Loures*[5]; *Rafael Candido Alves Aguiar*[6]

How to quote this article:
Aguiar KM, Mota DCJ, Rocha LC, *et al*. Validation Assessment of Irregular Antibodies Investigation at the Regional Blood Center in *Montes Claros* City. Rev Fund Care Online.2019.11(n. esp):285-288. DOI: http://dx.doi.org/10.9789/2175-5361.2019.v11i2.285-288

ABSTRACT

Objective: The study's purpose has been to assess the validation of irregular antibodies investigation using the Coombs control reagent in blood samples collected over the period from February 2015 to August 2016. **Methods:** It is a observational, retrospective and prospective study, which presents technical procedures bearing a documentary character, and that was performed at the Laboratory of Immunohematology from the Regional Blood Center in *Montes Claros*-MG. **Results:** During the research, it was observed that after the non-validation of some tests and its repetition was then performed alone; the validation was not verified and once again a repetition was necessary until this sample was defined as validated. This fact raises the possibility of other interferences beyond those both known and discussed; bearing in mind that the repetition was carried out in isolation and also all stages of the process were performed under scrutiny. **Conclusion:** The low percentage of non-validated results ratifies that the antiglobulin validation test is a good method to confirm the result of the search for irregular antibodies.

Descriptors: Antibodies, Validation, Blood Donors, Coombs' Test.

[1] Biomedicine Graduate by the *Universidade de Uberaba (UNIUBE)*, MSc in Biotechnology by the *Universidade Estadual de Montes Claros (UNIMONTES)*, PhD student in Health Science at *UNIMONTES*, Biomedical professional at Hemocentro Regional de Montes Claros. *Hemocentro Regional de Montes Claros, Fundação Hemominas*, Brazil.

[2] Pharmacy Graduate by the F*aculdades Integradas do Norte de Minas (FUNORTE),* Trainee at *Hemocentro Regional de Montes Claros. Hemocentro Regional de Montes Claros, Fundação Hemominas*, Brazil.

[3] Pharmacy Graduate by the *FUNORTE,* Trainee at *Hemocentro Regional de Montes Claros. Hemocentro Regional de Montes Claros, Fundação Hemominas,* Brazil.

[4] Pharmacy Graduate by the *Universidade Federal de Minas Gerais (UFMG),* MSc in Biological Science by the UNIMONTES, Pharmacist at *Hemocentro Regional de Montes Claros. Hemocentro Regional de Montes Claros, Fundação Hemominas,* Brazil.

[5] Pharmacy Undergraduate student by the *Faculdade de Saúde Ibituruna (FASI)*, Trainee scholarship holder at *Hemocentro Regional de Montes Claros. Hemocentro Regional de Montes Claros, Fundação Hemominas,* Brazil.

[6] Pharmacist at *Hemocentro Regional de Montes Claros. Hemocentro Regional de Montes Claros, Fundação Hemominas*, Brazil.

Funding: *FAPEMIG.*

Approved by the Research Ethics Committee from the *Faculdades Integradas do Norte de Minas – FUNORTE,* under the No. 7468116.0.3001.5118.

Validation assessment of irregular antibodies investigation at the regional blood center in *Montes Claros* city, 2016. Presented to the *FUNORTE.*

RESUMO

Objetivo: Analisar a validação da pesquisa de anticorpos irregulares (PAI) através da utilização do reagente Controle de *Coombs* em amostras sanguíneas coletadas de Fevereiro de 2015 à Agosto de 2016. **Métodos:** Estudo de natureza observacional, retrospectiva e prospectiva, apresentando procedimentos técnicos de caráter documental, a ser realizado no Laboratório de Imunohematologia do Hemocentro Regional de Montes Claros - MG. **Resultados:** Foi observado durante a pesquisa que após a não validação de alguns testes e realizada a repetição dos mesmos isoladamente, não foi constatado a validação sendo necessário outra repetição até que essa amostra validasse. Esse fato levanta a possibilidade de outras interferências além das conhecidas e discutidas, uma vez que a repetição foi realizada isoladamente analisando criticamente todas as etapas do processo. **Conclusão:** O baixo percentual de resultados não validados ratifica que o teste de validação antiglobulínico é um bom método para confirmar o resultado da pesquisa de anticorpos irregulares.

Descritores: Anticorpos, Validação, Doadores de Sangue, Teste de *Coombs.*

RESUMEN

Objetivo: Analizar lavalidación de la detección de anticuerpos irregulares (PAI) mediante el uso del reactivo de control de *Coombs* en muestras de sangre tomadas de Febrero 2015 a Agosto 2016. **Métodos:** Estudio de naturaleza observacional, retrospectivo y prospectivo, con los procedimientos técnicos de carácter documental, que se sucederá em Laboratorio de Inmunohematología del Hemocentro Regional de Montes Claros - MG. **Resultados:** Se observó durante la encuesta que después de la no validación de algunos testes y sucedida la repetición del mismos individuos, no se ha encontrado la validación, por lo tanto requeiendo otra repetición hasta que la validación de la muestra. Esto plantea la posibilidad de interferencia que no sea el conocido y discutido, ya que la repetición se realiza aisladamente el análisis crítico de todos los pasos del proceso. **Conclusión:** El bajo porcentaje de resultados no validados ratifica la prueba de validación antiglobulínico es un buen método para confirmar el resultado de la búsqueda de anticuerpos irregulares.

Descriptores: Anticuerpos, Validación, Los Donantes de Sangre, Prueba de *Coombs.*

INTRODUCTION

The identification of erythrocyte antigens and the definition of their importance in transfusion practice and hemolytic disease of the newborn occurred in the first half of the twentieth century, characterizing advances in medical research.[1] According to the International Society of Blood Transfusion, there are about 340 antigens, 308 of which are grouped into 36 blood-group systems, and much progress is still being made in the understanding and structural and functional analysis of antigens expressed in Red Blood Cells (RBCs) and in non-erythroid tissues.[1]

There are several types of irregular antibodies in different forms. After the discovery of blood groups, A, B, O in 1900 by Karl Landsteiner, several antigens and antibodies were found in the erythrocyte membranes. These antibodies were classified as significant when they agglutinated the RBCs, reducing their survival; and insignificant when agglutinated, since it did not affect survival in the organism.[2]

These antibodies were named as Rh, KELL, MNSs, Lewis, Duffy and Kidd; they are identified in either serum or plasma from donors and in patients undergoing blood transfusions. This is because each of these antigens has biological functions that are capable of causing reactions to erythrocytes and when in contact with the human antigen causes immediate, late reactions or sudden death of the patient.[3] The Rh, MNS and KELL systems are the most complex, and have 54, 48 and 35 antigens, respectively.[4]

When the individual is exposed to substances foreign to the body, such as erythrocyte antigens, activation of the immune system with a formation of irregular antibodies can occur, resulting in a response, antigens contained in transfused blood components. Irregular antibodies occur in approximately 0.3 to 2.0% of the general population, can be found in the serum/plasma of individuals or linked to the erythrocyte membrane. After the presence of irregular antibodies has been identified, the specificity of these antibodies using a panel of RBCs with known phenotypes in immunogenic systems.[5-6]

The Search for Irregular Antibodies (SIA) should be performed in the blood of donors using methods capable of detecting the presence of clinically relevant antibodies as determined by the Resolution No. 34 of June 11th, 2014, and Ordinance No. 158 of 04 of February 2016, which approve the technical regulation of hemotherapy procedures.[7-8]

Irregular antibodies occur in up to 3% of transfused patients; but in some patients, this risk is more significant about 7 to 10% in poly transfused patients, 6 to 36% in sickle individuals and 3 to 10% in thalassemia.[9] Some authors have reported up to more than 30% alloimmunization in these patients.[9]

The frequency of irregular antibodies in a poly transfused population shows the prevalence in onco-hematologic patients, aplastic anemia, acute myelogenous leukemia of 11-16%, with chronic diseases such as chronic renal failure and upper gastrointestinal bleeding, with results of 11-16%, patients with hemoglobinopathies, such as sickle cell anemia and alloimmunization expressed a percentage of 33.4%.[10-11]

The occurrence of negative results when antibody concentration is below detectable limits represents limitation to the antibody detection test. Usually, this occurs in elderly people or in those not exposed to the corresponding antigen.[12]

The present study aimed to analyze the validation of SIA

through the use of Coombs Control reagent in blood samples from donors who attended the Regional Blood Center of *Montes Claros* city over the period from February 2015 to August 2016. Moreover, study also aims to investigate possible causes for the non-validation of the tests, and to discuss ways of minimizing divergences in the results.

METHODS

It is a observational, retrospective and prospective study, which presents technical procedures bearing a documentary character, and that was performed at the Laboratory of Immunohematology from the Regional Blood Center in *Montes Claros*-MG. To collect data, it was used information about the results of the validation of the irregular antibody test of blood donors, cataloged in the worksheets "Immunohematology Service" and "Validation of the search for irregular antibodies" over the period from 2015 to 2016. A descriptive analysis was performed using a Microsoft Office Excel spreadsheet (version 2010). The research was carried out according to the Resolution No. 466/2012 from the National Health Council, after consent of the person responsible for the Blood Center and approval of the Research Ethics Committee from the *Faculdades Integradas do Norte de Minas (FUNORTE)*, under number No. 57468116.0.0000.5141.

RESULTS AND DISCUSSION

The Regional Blood Center in *Montes Claros*-MG is responsible for all the demand for transfusion procedures, public and private, of the macro-region of Northern region of the *Minas Gerais* State. It attends on average about 2000 candidates for a donation per month, where about 1,500 blood bags/month are collected. According to the Ordinance No. 158 of February 4th, 2016, and the Resolution No. 34 of June 11th, 2014, the search for irregular antibodies must be performed in the blood of donors at each donation using methods capable of detecting the presence of clinically relevant antibodies. This technique is developed in the Laboratory of Immunohematology that participates in the external quality control of the Brazilian Association of Hematology and Hemotherapy with managed and satisfactory results, in addition to having a daily internal quality control protocol.

The SIA of the blood donors is made according to standard operating procedure, in calibrated and validated centrifuges for washing and reading the results, being performed by tube methodology using polyethylene glycol as a potentiating agent. Agglutination readings are performed by the addition of Human Antiglobulin (HAG) serum and, after a negative result; they are validated using the Coombs control reagent. If any sample is not validated, repeat the test.

Data were collected from February 2015 to August 2016 over a period of 19 months between the two years. During this period, 24,136 tests for irregular antibodies were performed, where 853 tests did not validate after the addition of the Coombs Control RBCs, which corresponded to 3.53% of the tests performed, observed in **Figure 1**.

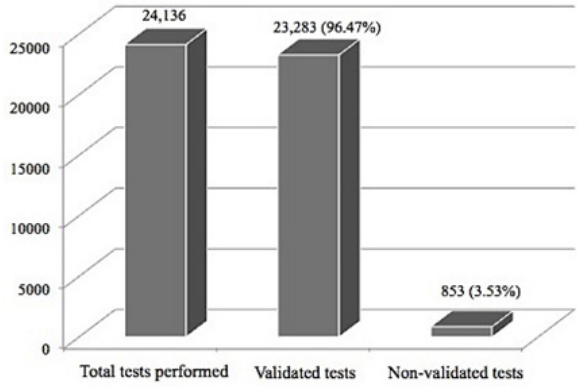

Figure 1 – : The research quantitative and the results validation for irregular antibodies investigation. Regional Blood Center in *Montes Claros-MG*, Brazil, February/2015 to August/2016.

Data regarding the agglutination intensity of the validated tests were also collected. 535 tests agglutinated with traces, which corresponds to 2.2% of tests performed; 1,853 tests agglutinated with intensity of 1+, equivalent to 7.7% of the total of tests performed in the period evaluated; 18,812 tests agglutinated with intensity of 2+, corresponding to 77.9% of the total of tests; 2,897 tests agglutinated with 3+, which is equivalent to 12% of the tests; and 39 tests agglutinated with 4+ corresponding to 0.2% of the tests, as shown in **Figure 2**.

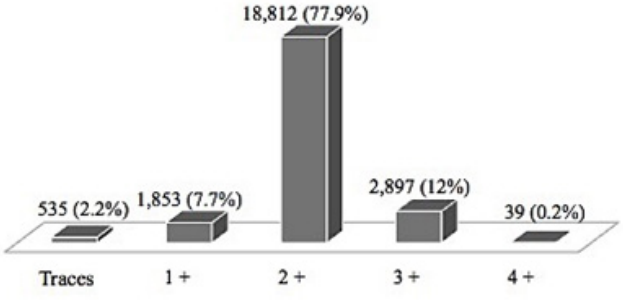

Figure 2 – : The research quantitative and the results validation for irregular antibodies investigation. Regional Blood Center in *Montes Claros*-MG, Brazil, February/2015 to August/2016.

The agglutination intensity results in which most of the tests agglutinated with 2+ or 3+ are in accordance with the thecnical leaflet from the reagent used, which establishes those levels as the specified grade.

In view of the 853 tests not validated after the addition of Coombs Control RBCs, corresponding to 3.53% of the tests performed, may be justified by several factors that may influence the non-validation of the indirect Coombs' test, and those factors added to an extensive routine might lead to false results during the irregular antibodies investigation.

Each step of the test should be performed carefully to ensure reliable results. Incubation of the RBCs with antisera allows the antibody molecules to bind to the antigen present on the RBCs membrane, and it is important to monitor the optimal incubation temperature of the test that is optimal at 37°C for most of the IgG antibodies.[12]

Another factor that may also contribute to non-validation of the tests is saline lavage to remove all residual free globulins that can neutralize the HAG reagent.[12]

Given the aforementioned, by facing a very extensive routine, the technician may not realize that the three required washes were carried out and proceed to the next step without completing the proper washing of the RBCs. It is critical to discard the entire supernatant after the final wash and proceed with the drying of the tubes, as the remainder of saline dilutes the HAG reagent and decreases the sensitivity of the test.

During the research, it was observed that after the non-validation of some tests and its repetition was then performed alone; the validation was not verified and once again a repetition was necessary until this sample was defined as validated. This fact raises the possibility of other interferences beyond those known and discussed, bearing in mind that the repetition was carried out in isolation and also all stages of the process were performed under scrutiny.

CONCLUSIONS

The low percentage of non-validated results endorses that the antiglobulin validation test is a good method to confirm the result of the irregular antibodies investigation. Furthermore, the negative result, when present, may occur due to process-related interferences that influence the technique.

ACKNOWLEDGEMENTS

We acknowledge the Regional Blood Center of Montes Claros-MG and the personnel from the Laboratory of Immunohematology: Adenilson Teixeira de Melo, Maria Eunice Gonçalves Lima and Antônio Carlos Dias Souto; and also from the Pharmacy: Anne Carolline Amaral Batista.

REFERENCES

1. ISBT. Blood Group Allele Terminology [monograph on the internet]. [Acesso em 11 maio 2016]. Disponível em: http://www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/blood-group-terminology/blood-group-allele-terminology/.
2. Lima PR, Destefani AC. Irregular Antibodies Identified Previously In Blood Transfusions: A Review Of The 21st Century. Open Science Journal 2(1); Jan 2017.
3. Castilho L. O futuro da aloimunização eritrocitária. Ver Bras Hematol Hemoter. 2008;30(4).
4. Castilho L, PELLEGRINO JÚNIOR J, REID ME. Fundamentos de Imunohematologia. São Paulo: Atheneu; 2015.
5. Brecher ME. Technical Manual of American Association of Blood Banks. 15º ed. Copyright 2005 Bethesda, Maryland (USA).
6. Girello AL, Kuhn TIB. Fundamentos de imuno-hematologia eritrocitária. São Paulo: Editora SENAC. São Paulo; 2002.
7. BRASIL. Ministério da Saúde. Portaria nº 158 de 04 de fevereiro de 2016. Redefine o regulamento técnico de procedimentos hemoterápicos. Brasília, 2016 [Acesso em 11 nov 2016]. Disponível em http://portal.anvisa.gov.br.
8. BRASIL, Ministério da Saúde. Resolução nº 34 de 11 de junho de 2014. Dispõe sobre as boas práticas no Ciclo da Sangue. Brasília, 2014 [Acesso em 03 fev 2016]. Disponível em: http://portal.anvisa.gov.br>.
9. Aguiar KM, Maia CN, Teles LF, Oliveira TRGM, Ruas MO. Identificação de anticorpos irregulares no Hemocentro Regional de Montes Claros, MG. EFDeportes.com, Revista Digital. Buenos Aires; Jan 2013.
10. Blumberg N, Peck K, Ross K, AvilaE. Immune response to chronic red blood cell transfusion. Vox Sang; 1983; 44: 212-17.
11. Coles SM, Klein HG, Holland PV. Alloimmunization in two multitransfused patient Populations. Transfusion; 1981; 21: 462-66.
12. Harmening DM. Técnicas Modernas em Banco de Sangue e Transfusão. 4º ed. Rio de Janeiro: Revinter; 2006.

Received on: 04/25/2017
Required Reviews: None
Approved on: 05/16/2017
Published on: 01/15/2019

***Corresponding Author:**
Karina Marini Aguiar
Rua São Vicente de Paula, 186
Roxo Verde, Minas Gerais, Brazil
E-mail address: k_marini@hotmail.com
Telephone number: +55 38 3221-9994
Zip Code: 39.400-370

The authors claim to have no conflict of interest.