ARTICLE
https://doi.org/10.22239/2317-269X.01283

Revista Visa em Debate
sociedade, ciência & tecnologia

Fresh frozen plasma: pharmaceutical input for the production of plasma-derived medicines

Plasma fresco congelado: insumo farmacêutico para produção de medicamentos hemoderivados

Marisa Coelho Adati*

Antônio Eugênio Castro Cardoso de Almeida

Álvaro da Silva Ribeiro

Helena Cristina Balthazar Guedes Borges

Marlon Akio da Silva Issobe

Departamento de Imunologia, Instituto Nacional de Controle de Qualidade em Saúde, Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, RJ, Brasil

* **E-mail:** marisa.adati@incqs.fiocruz.br

Received: Mar 14, 2019
Approved: May 15, 2019

ABSTRACT

Introduction: In Brazil, hemotherapy was started as a medical specialty in the 1940s in the Rio de Janeiro and São Paulo axis with the inauguration of the first Blood Bank at the Fernandes Figueira Institute. As a governmental initiative, Law n. 1,075/1950/MS, which provides for the voluntary donation of blood, was promulgated, culminating with the Law n. 10,205/2001, which regulated paragraph 4 of article 199 of the Federal Constitution, regarding collection, processing, storage, distribution and application of blood and its components. Among the components obtained in the Hemotherapy Services, we can highlight the frozen fresh plasma (FFP) that can be transfused and, when becoming a surplus of the therapy, continue to be used to produce industrialized blood products. **Objective:** This study intends to demonstrate the most relevant aspects regarding the recovery of factor VIII content, in the FFP units collected in 72 Hemotherapy Services visited in the country, aiming at its safe and effective use both for therapeutic use and as a pharmaceutical input in the production of blood products. **Method:** The methodology adopted included five steps: Elaboration and validation of the questionnaire applied; Selection of Hemotherapy Services to be visited; Analysis of quality indicators according to the Donabedian Triad; Collection, packaging and transport of FFP units; Analysis of the Factor VIII content in the FFP units collected during the technical visit during the period from 2013 to 2015. **Results:** Among the results obtained, it is important to highlight the concentration of factor VIII (IU/mL), in the FFP units, with the following results: mean 0.68; standard deviation 0.32; coefficient of variation 47.1%, confidence interval 0.64 to 0.71. **Conclusions:** factor VIII content greater than or equal to 0.70 IU/mL was found in 38.5% of FFP units, as specified in the Brazilian Pharmacopoeia, which can and should be used as a pharmaceutical input in the production of blood products. It was also found a disposal of approximately 500 million IU/mL of factor VIII, which represents 83.0% of the annual acquisition by the Ministry of Health of Concentrate of factor VIII for medical uses. Such use could generate significant savings in public coffers.

KEYWORDS: Frozen Fresh Plasma; Hemotherapy Services; Recovery of Factor VIII Content

RESUMO

Introdução: No Brasil, a prática hemoterápica foi iniciada como especialidade médica, na década de 1940, no eixo Rio de Janeiro e São Paulo com a inauguração do primeiro Banco de Sangue no Instituto Fernandes Figueira. Como iniciativa governamental, foi promulgada pelo Ministério da Saúde a Lei n° 1.075, de 27 de março de 1950, que dispõe sobre a doação voluntária de sangue, culminando com a Lei nº 10.205, de 21 de março de 2001, que regulamentou o parágrafo 4º do artigo 199 da Constituição Federal, relativo à coleta, processamento, estocagem, distribuição e aplicação do sangue e seus componentes. Dentre os componentes obtidos nos Serviços de Hemoterapia destaca-se o plasma fresco congelado (PFC) que pode ser transfundido e quando excedente da terapia, seguir para ser beneficiado a medicamentos hemoderivados industrializados. **Objetivo:** Desta forma, este estudo pretende demonstrar os aspectos mais relevantes relativos à recuperação do teor de fator VIII, nas unidades de PFC coletadas em 72 Serviços de Hemoterapia visitados no país, visando seu aproveitamento seguro e eficaz tanto para o uso terapêutico, quanto como insumo farmacêutico na produção de medicamentos hemoderivados. **Método:** A metodologia adotada compreendeu cinco etapas: Elaboração e validação do questionário aplicado; Seleção dos Serviços de Hemoterapia a serem visitados; Análise dos indicadores de qualidade segundo a Tríade de Donabedian; Coleta, acondicionamento e transporte das unidades de PFC; Análise do teor de fator VIII nas unidades de PFC coletadas durante a visita técnica, no período de 2013 a 2015. **Resultados:** Dentre os resultados obtidos, destaca-se a análise do teor de fator VIII (UI/mL) nas unidades de PFC, com os seguintes resultados: média de 0,68; desvio-padrão de 0,32; coeficiente de variação de 47,1%, intervalo de confiança de 0,64 a 0,71. **Conclusões:** O teor de fator VIII superior ou igual a 0,70 UI/mL foi encontrado em 38,5% das unidades de PFC, conforme especificado na Farmacopeia Brasileira e esses podem e devem ser utilizados como insumo farmacêutico na produção de medicamentos hemoderivados. Este estudo também evidenciou o descarte de aproximadamente 500 milhões de UI/mL de fator VIII o que representa 83,0% da aquisição anual, pelo Ministério da Saúde, do medicamento Concentrado de fator VIII. Essa utilização poderia gerar uma expressiva economia aos cofres públicos.

PALAVRAS-CHAVE: Plasma Fresco Congelado; Serviços de Hemoterapia; Recuperação do Teor de Fator VIII

INTRODUCTION

Since the dawn of mankind, human blood has fascinated men because it is associated with the concept of the right to life and linked to ethical and moral values, which make blood a men's patrimony[1]. Whole blood and blood products are vital in medical procedures and are therefore considered essential technologies[2].

Blood transfusion for different therapeutic proposals has a relatively recent origin. This practice became more widespread during World War II, but the concept of transfusion dates back to a long time ago. There are reports on the subject by Hippocrates 430 years before Christ. The earliest report of the modern era is from 1492 and refers to a transfusion to Pope Innocent VIII, who probably suffered from chronic kidney disease[3].

In 1937, Bernard Fantus, from Chicago's Cook County Hospital, established the first Blood Bank in which blood was collected in glass vials and stored in refrigerators for more than 10 days. In 1941, Phillip Levine discovered the Rhesus (Rh) system, associated with the fatal condition of hemolytic disease in newborns. In 1943, Loutit and Mollison developed the adenine citrate dextrose (ACD) anticoagulant solution, extending the life of red blood cells. In 1944, Edwin Cohn and his collaborators developed plasma fractionation into different protein fractions[4].

In Brazil, the practice of hemotherapy became a medical specialty in the 1940s, in Rio de Janeiro and São Paulo. On December 7, 1942, the first Blood Bank was open at the Fernandes Figueira Institute (Rio de Janeiro) to obtain blood for this hospital, as well as to support the war effort by sending human plasma to battlefront hospitals[5].

After the beginning of this practice, the Brazilian Ministry of Health enacted Law n. 1.075, of March 27, 1950, which provides for voluntary blood donation[6]. However, over the years, Blood Banks opted for paid donations, which encouraged and increased the occurrence of donors like beggars, alcoholics and patients with infectious diseases[7]. Furthermore, there was an increase in the number of small blood collection banks that operated unethically, without quality standards and without inspection[7,8].

To regulate this practice and control the blood collected and transfused in Brazil, the National Commission of Hemotherapy (CNH) was created in 1964. In 1976, it was renamed as the National Council of Hemotherapy (CTH) of the National Health Council (CNS), with normative and advisory functions[8,9]. This Commission prepared the first normative instrument in this area in Brazil. Its scope ranged from the registration of institutions that performed hemotherapy activities to the export of human plasma[9]. Law n. 4.701 was enacted on June 28, 1965. It regulated hemotherapy activities in Brazil and determined the principles of the National Blood Policy, like: unpaid voluntary donation, donor and recipient protection measures, organization of the collection, storage and distribution of blood and blood components[10].

In the 1970s, two important laws for health surveillance activities were enacted: Law n. 6.360, of September 23, 1976[11], which provides for the health surveillance of medicines, drugs, pharmaceutical and related ingredients, cosmetics, sanitizers and other products; and Law n. 6.437, of August 20, 1977[12], which specifies the violations of federal health legislation, establishes sanctions and other measures that are in force up to today. It is noteworthy that quality deviations are regulated by these laws up to today[11,12].

In 1980, CTH was replaced by the National Blood and Blood Products Program (Pró-Sangue), created by Interministerial Ordinance n. 7, of April 30, 1980, of the Ministry of Health and Social Security, as a special program that instituted the Blood Centers - Public Blood Banks, proposing a total reorganization of blood therapy activities. This ordinance can be seen as an official response to the growing dissatisfaction of the society with the system's poor control[13].

The 1990s witnessed the promulgation of Ordinance n. 1.376, of September 19, 1993[14], which provides for the technical standards for the collection, processing and transfusion of blood, components and derivatives, and Ordinance n. 121, of November 24, 1995[15], which provides for the rules for implementation, oversight and inspection of Hemotherapy Units. Their objective is to guarantee the safety of the products, as well as the organization of hemotherapy activities. When any irregularity or quality deviation is found, the service is fined or shut down, based on Law n. 6.437/77[11,12].

In 2001, Law n. 10.205, of March 21, was enacted. It regulated paragraph 4 of article 199 of the Federal Constitution, concerning the collection, processing, storage, distribution and application of blood and its components, establishing the institutional order indispensable for the execution of these activities[16]. Article 23 of Law n. 10.205/2001 allows non-therapeutic apheresis only for the purpose of obtaining blood products. This can be done exclusively by the public sector and the activity is regulated by a specific standard, which defines fresh frozen plasma (FFP) as a pharmaceutical ingredient for the production of blood products. However, this is not in practice yet[16].

Currently, hemotherapy activities are regulated by the following laws: Resolution of the Collegiate Board (RDC) n. 34, of June 11, 2014[17], which provides for Blood Cycle Good Practices, and Consolidation Ordinance n. 5, of September 28, 2017[18], which deals with the consolidation of the rules on health initiatives and services of the Unified Health System (SUS)[18].

Therefore, from 1995 until the current regulation, the country's Hemotherapy Services (HS) are regularly inspected at least once a year by state Health Surveillance agencies (VISA), together with the National Health Surveillance Agency (Anvisa) of the Ministry of Health, with the objective of reducing the health risk of products obtained from donated and collected whole blood. These are called blood components and include packed red blood cells,

fresh frozen plasma, platelet concentrate, i.e. the main blood components authorized for consumption[15,17,18].

The object of this study is the human plasma that preserves the factor VIII content within the specification, the so-called FFP[19]. When the plasma is not properly preserved or when the fraction called cryoprecipitate (which contains factor VIII) has been removed, this plasma is called common plasma (CP)[19]. For preservation and later recovery of the most important fraction of plasma, factor VIII, among other fractions involved in blood coagulation, satisfactory conditions in the handling and processing of plasma are required, such as proper collection, fractionation, freezing and storage[17,18]. Therefore, FFP is the plasma obtained by apheresis or from a whole blood unit by centrifugation, completely frozen within 24 h after collection, in order to recover factor VIII[17,18]. Coagulation factor VIII, also called antihemophilic factor, is a labile protein, essential for the treatment of Hemophilia A, an X-linked hemorrhagic disease[17,18,19].

The whole process of fractionation of FFP units, including careful donor selection, clinical, hematological, serological screening, storage, distribution and use, requires clear rules for constant monitoring and an instrument for monitoring, evaluation and control of products under health surveillance[11,17,18].

Careful donor selection and serological control are intended to minimize the risk of blood component contamination with infectious diseases[17,18]. It should be noted that each unit of whole blood collected with approximately 500 mL and, concomitantly, different fractionated blood components is individually assessed by serological tests for the presence of: HIV-1/2 including group O; hepatitis B and hepatitis C antigen and antibody; anti-HTLV-I/II; Chagas disease and syphilis, as well as molecular testing for HIV, HBV and HCV, according to current legislation[17,18].

After freezing, FFP can follow three distinct pathways: be transfused as such; be fractionated to cryoprecipitate, which contains coagulation factor VIII and also be transfused; or go to the industry to be processed into blood products. FFP-derived drugs are: human albumin, normal or specific human immunoglobulin, factor VIII concentrate, factor IX concentrate, prothrombin complex, biological glue, among others[17,18].

Thus, FFP is the active pharmaceutical ingredient (API) for the production of blood products. Like any drug, it is fundamental to intervene in the evolution of diseases, aiming at curing or minimizing their effects on the human body[17,18].

According to Said, drugs are some of the most powerful ways modern medicine has to treat diseases. However, just as they can cure or alleviate disease, they can also cause health problems [20].

Fresh frozen plasma as an active pharmaceutical ingredient

Human plasma is the aqueous component of the blood, consisting of approximately 85% - 90% water. It accounts for 6% to 8% of total body water, between 40-50 mL/kg of body weight, and has various constituents, like proteins, nutrients, crystalloids, hormones and vitamins[21]. FFP is plasma obtained in two ways: fractionated from the whole blood unit, centrifuged and separated and rapidly frozen to a volume of 150 mL or more, with waiting time between collection and freezing recommended in national and international literature between 6 and 24 h[17,18].

As for how to obtain FFP, the international concept considers two ways: Source Plasma and Recovered Plasma. Source Plasma is the FFP obtained by apheresis, a fully automated procedure that allows the plasma to be collected directly from the donor and at the same time red blood cells return to circulation, while the plasma is immediately frozen. The frequency of donation can be twice a week in the USA and up to 24 times a year in Europe, with a volume between 600 and 800 mL[22]. These donations are restricted to the production of blood products and these services are regularly controlled by the blood product industry. A quarantine period of at least 60 days is also adopted. If in this period the donor presents positive serology, this plasma bag is discarded. In the USA, virtually all of the plasma collected for the production of blood products is obtained from Source Plasma (Plasma for Fractionation), as specified by the Food and Drug Administration (FDA), and is usually from paid donors[21,22].

A second way to obtain FFP is the Recovered Plasma, obtained by centrifugation and separation of whole blood, with a minimum volume of 150 mL. This donation is of limited frequency, two to four times a year, usually from unpaid donors. These services are regularly controlled by the country's health authority and have no quarantine period, since blood donors are insufficient and this form of procurement is inspected by the country's health authorities[17,18].

In Brazil, according to current legislation, FFP is obtained from Recovered Plasma, collected from rigorously selected altruistic donors[17,18].

FFP has different characteristics and specifications according to the method of production, time between whole blood collection and human plasma fractionation, freezing time and recovery of factor VIII content, as shown in the Table.

As shown in the Table, different FFP specifications are intended to recover the coagulant fraction, cryoprecipitate or factor VIII. This protein is precipitated under extreme heat shock conditions, so it is also called cryoprecipitate. According to some authors, if the cryoprecipitate is fractionated or stored outside the relevant specification, there can be substantial loss of factor VIII[21,22,23,24,25,26,27]. Therefore, the blood component that has both therapeutic indication and interest in the production of blood products is the FFP that complies with the Brazilian Pharmacopoeia specification. It is also called plasma for fractionation, regardless of how it is obtained[22,22,23,24,25,26,27,28].

The coagulant fraction of the FFP is essential for the production of factor VIII concentrates and for patients with coagulopathies,

Table. Differences in freezing time and temperature definitions and parameters for fresh frozen plasma.

Guideline	Specification of fresh frozen plasma unit	Freezing temperature (T) and factor VIII content (FVIII)
Blood Products Advisory Committee/FDA[22]	FFP collected by apheresis or fractionated from whole blood up to 8 h before freezing. *A-FFP24RT24 - defined as apheresis-collected plasma, stored for up to 24 h after collection at room temperature and before freezing;* *WB-FFP24RT24 - defined as plasma obtained from collected whole blood up to 24 h after collection and before freezing.*	No mention to the freezing temperature or the FVIII content.
American Association of Blood Banks (AABB)[23]	FFP collected and separated from whole blood or collected by apheresis; FFP24 is the plasma separated and frozen within 24 h.	Temperature for PFC and FFP24 equal to or below -18ºC; No mention to the FVIII content.
United Kingdom[24]	FFP separated from whole blood and frozen within 8 h.	No mention to the temperature; FVIII content of 0.70 IU/mL in at least 75% of the collected units.
Council of Europe[25]	FFP collected and separated from whole and frozen blood within 8 h.	Temperature of -70°C; FVIII content equal to or greater than 0.70 IU/mL.
World Health Organization[26]	FFP is plasma separated from whole blood or apheresis collected within 24 hours for the production of labile coagulation factors and stable plasma proteins.	Temperature of -20°C to -30°C; FVIII content equal to or greater than 0.70 IU/mL.
RDC n. 46 of May 18, 2000[27]	FFP whose freezing process has been completed within 8 hours of collection.	Temperature of -20°C or below; No mention to the FVIII content.
European Pharmacopoeia[28]	FFP as plasma for fractionation separated from whole blood or by apheresis, collected within 24 h and rapidly frozen.	Temperature of -20°C or below; FVIII content equal to or greater than 0.70 IU/mL.
RDC n. 34/2014[17]	FFP separated from whole blood by centrifugation or obtained by apheresis, completely frozen within 8 h or between 8 and 24 h.	Temperature of -20°C or below; No mention to the FVIII content.
Consolidation Ordinance n. 5/2017[18]	FFP separated from whole blood by centrifugation and frozen within 6 h. FFP24 - plasma separated from a whole blood unit by centrifugation and completely frozen between 8 and 24 h, maximum time for separation is 18 h after collection if the whole blood unit is kept refrigerated at 4+/-2°C. Cryoprecipitate-Free Plasma (CFP) - plasma from which cryoprecipitate has been removed. Common Plasma (CP) - plasma whose freezing was not within the stated technical specifications or resulting from the transformation of expired FFP or FFP24 or CFP. It cannot be used for transfusion and is exclusively intended for the production of blood products.	Temperature of -30°C or below; No mention to the FVIII content.

including Hemophilia A, an X-linked hereditary pathology, with a frequency of 1:10,000 births, caused by factor VIII deficiency, which makes blood coagulation difficult and favors the occurrence of bleeding and hemorrhages[29,30,31].

FFP produced in the various Brazilian HS is intended for therapeutic use. Since its use is restricted, there is often overstocking in these HS. This affects the maintenance of the cold network, since the storage is done at temperatures below -20°C, at a cost of approximately BRL 1 million per year. Additionally, it is a pharmaceutical input for the production of blood products[32].

Given this fact, by strategy and initiative of the Ministry of Health, in 2004, through Law n. 10.972, of December 2, 2004, the Brazilian Company of Hemoderivatives and Biotechnology (Hemobrás) was created with the objective of optimizing the use of surplus FFP of therapeutic use, in order to achieve self-sufficiency in the production of blood products[33]. Today, Hemobrás, considered the largest producer of blood products in Latin America, is not yet fully operational, but, according to the company's website, this is expected to happen by 2020.

According to the National Registry of Hemophiliacs of the Ministry of Health, Brazil has about 6,900 patients with Hemophilia A, and the factor VIII concentrate is essential for the survival of these patients. This supply is fully funded by the SUS, and Brazil is one of the few countries to offer it to patients free of charge[32]. To this end, the federal government, through international bidding, acquires 100% of factor VIII concentrates imported from Europe and the USA. According to Ministry of Health information, about 600,000,000 IU of factor VIII are imported annually at the average cost of USD 0.20/IU, totaling a financial impact of approximately USD 120,000,000 for the procurement of these drugs and for the care of hemophiliac patients[32]. According to Soares, the liter of FFP in international quotations costs about USD 70.00 to USD 120.00, so Brazil is in a conundrum[33]:

> [...] at the same time that it encourages voluntary blood donation, that is, when the donor donates motivated by the feeling of human solidarity and the exercise of citizenship, it is disposing of thousands of liters of human plasma every year[32].

The discrepancy between the annual procurement of factor VIII by the Ministry of Health and the annual disposal of thousands of liters of FFP motivated the study on the quality and efficacy of this input obtained in Brazilian HS, aiming at its safe and effective use for both therapeutic use and as a pharmaceutical ingredient for the production of blood products.

Therefore, this study aims to demonstrate the most relevant aspects related to obtaining and recovering factor VIII content in FFP units collected in 72 HS visited across the country. The monitoring is intended to be an instrument of assessment for health surveillance, understood as a set of measures adopted by societies over time to reduce and prevent health problems in the population[34].

METHOD

Design and validation of the questionnaire

For the design and validation of the questionnaire applied at the time of the technical visit, we used the Marconi and Lakatos model containing the following characteristics: a) reliability: the results will be the same, regardless of who applied it; b) validity: the data collected are necessary for the research, c) effective: accessible vocabulary with clear meaning[35].

In the validation of the questionnaire some concepts were revised, especially those regarding the quality indicators treated as a unit of qualitative or quantitative analysis. These types of analysis are used to represent or measure a problem, condition, topic or event that has to be observed in a real situation[35]. The revised questionnaire after the pre-test had information about the quality indicators of the work processes.

Selection of hemotherapy services to be visited

The country's HS are hierarchically organized at different levels of complexity, as set forth in RDC n. 151, of August 21, 2001. The Coordinating Blood Centers, located in the state capitals, are considered of high complexity and perform the activities of Donor Cycle and Blood Cycle[36]:

1. Donor Cycle: donor registration, clinical and hematologic screening, whole blood collection[17,18];
2. Blood Cycle: fractionation of blood units collected in blood components, serological and immunohematological screening, storage, distribution and transfusion[17,18].

The Resolution also establishes Regional Blood Centers, Hemonuclei, Collection and Transfusion Units and Transfusion Agencies. The latter only performs the transfusion activity of the collected blood and, in this study, services of this type were not considered[36].

The Hemotherapy Service Register (Hemocad) was used to select HS and is available on Anvisa's website. At the time, it had 2,351 registered services, distributed in the 27 states of the federation. Of this total, 1,777 services corresponded to transfusion agencies, so they were excluded. Therefore, there was a total of 574 services, including Coordinating Blood Bank, Blood Banks (private and/or SUS services), Regional Blood Centers, Hemonuclei and Collection and Transfusion Units.

For the purpose of this study, 72 HS were selected from the country's five macroregions: Center-West, Northeast, North, Southeast (except for the state of São Paulo, which did not join the project) and South, based on confirmation of the availability of each state to participate in this evaluation, which was duly accompanied by a local health surveillance representative.

The selection of HS included services located in border areas, like Boa Vista (Roraima), Rio Branco (Acre), Porto Velho (Rondônia) and other municipalities of the following states: Amapá, Roraima, Amazonas, Acre, Rondônia, Mato Grosso, Mato Grosso do Sul, Paraná, Santa Catarina and Rio Grande do Sul[37].

Analysis of quality indicators by Donabedian Triad

For the analysis of the indicators, we used the Donabedian Triad model, according to the approach of only two specific attributes: Processes and Results[35,38].

The Process is part of the monitoring activity. It is a set of activities done in the relations of production, as well as between professionals and patients. It is also considered the most direct manner to evaluate the quality and results obtained from the desirable characteristics of the products or services, without errors, imperfections or harmfulness. In addition to improving the workplace, it is an indicator of indirect quality assessment[35,38]. Process indicators comprised equipment available in the HS to support the production, freezing and storage of FFP units, like power generators, refrigerated centrifuges and freezers.

The indicators of the process of FFP units production were also analyzed, including validation of the freezing process, time and temperature adopted by the HS between the collection of whole blood and the freezing of FFP units. These parameters are considered critical control points[17,18]. As for the results, the average monthly productivity of the FFP units and the factor VIII content dosage in the units collected during the technical visits were analyzed.

Regarding the analysis of the Results attribute, the monthly productivity of FFP and CP units produced by the HS was evaluated.

Collection, packaging and transport of FFP units for factor VIII analysis

The collection of five FFP samples per HS visited was done by researchers accompanied by local VISA professionals. This included the fulfillment of the Sample Collection Term or similar document, evidencing that it is Contamination-Free Biological Material. The samples were individually placed in plastic bags with hermetic closure and arranged in boxes of rigid material and recyclable ice, in order to preserve the samples.

The procedure adopted for the transportation of FFP units was Anvisa/SAS Joint Ordinance n. 370, of May 7, 2014, which provides for the Technical-Sanitary Regulation of blood component transportation. Upon receipt of the samples, each bag was visually inspected for solid state, since thawed samples could impair the results of factor VIII content analysis[39].

Analysis of factor VIII content of FFP units collected during technical visits to HS from 2013 to 2015

The procedure adopted for factor VIII content analysis involved the following steps[28]:

1. preparation of FFP units for analysis: thawing in water bath at 37 +/- 1ºC, with strictly controlled temperature;
2. after thawing, each FFP unit was divided into three different aliquots: two of approximately 100 mL each in polypropylene bottles and immediately frozen and a 3 mL aliquot in test tubes intended for factor VIII dosing;
3. for factor VIII analysis we used STA Compact by Stago, with MS-DOS operating system. Because it is an automated device, the v. 107.10 software directly calculated the calibration curve as well as the result of tests, which are given in IU/mL;
4. The reagents were purchased commercially from Stago and chosen because of the following parameters: they were suitable for that particular device; authorized by the in vitro Diagnostic Product Management of Anvisa's General Management for Technology and Health Products GEVIT/GGTPS; and used in the laboratory for over 15 consecutive years.

The methodology adopted for factor VIII content was One Stage (2.7.4) Coagulometric, a method described in the European Pharmacopoeia ed. 6.0. We used Reference Serum from the National Institute for Biological Standards and Control (NIBSC) n. 07/316 - 6th International Standard 2009 - Factor VIII and von Willebrand in plasma. Data obtained from the performed analyses were treated with analysis tools available in Microsoft Excel v. 2013 - Statistical Analysis. The following parameters were analyzed: mean; standard deviation; coefficient of variation and confidence interval.

At the same time, the project was submitted to the Research Ethics Committee, since it involves the collection of five units of FFP surplus from therapeutic use and stored in HS. Opinion n. 198.914 was favorable to the conduction of the project.

RESULTS AND DISCUSSION

Questionnaire

The questionnaire was based on RDC n. 34/2014 and prepared in Excel v. 2013. It addressed nine items that referred to the quality indicators of interest in this study. It was filled out manually and qualitatively, at the time of the visit to the HS, with the following answers: Yes - corresponds to positive information; No - for negative information; Not applicable - for questions that were not applicable to the evaluated HS setting.

The 72 completed questionnaires from the HS visited between 2013 and 2015 were transcribed into Excel v.2013 spreadsheets.

Selected and visited Hemotherapy Services

The 72 HS selected were scattered about Brazil's five macroregions: 27% in the Northeast (20 HS), 24% in the Center-West (17 HS), 17% in the North (12 HS), 17% in the South (12 HS) and 15% in the Southeast (11 HS). The Northeast region was the most representative, with its nine states. However, it is worth mentioning the absence of the Southeastern state of São Paulo, which did not join the project. This fact is also occurred in the 6th Hemotherapy Production Bulletin (Hemoprod), issued by Anvisa[40].

The sampling was completely random, including public and private services, high-complexity HS, like the Coordinating Blood Centers, and even less complex services, like Collection and Transfusion Units, located in smaller municipalities, especially in border areas[37].

Analysis of quality indicators by Donabedian Triad

Processes executed in HS

In the Processes attribute analysis, according to the Donabedian Triad, quality indicators or critical control points of importance to obtain FFP units for the recovery of factor VIII were analyzed, including power generators, refrigerated centrifuges, freezers and blast freezers. The presence or absence of such equipment was evaluated.

Other critical control points evaluated were: validation of the freezing process; freezing temperature and waiting time between whole blood collection and the fractionation of the FFP units.

Power generators are intended for the maintenance of the cold network in a reliable and continuous fashion, thus providing stability and quality to the product. Of the 72 services we visited, 84.7% (61) had power generators to supply the facilities and 15.3% (11) did not yet have such equipment. This fact is contrary to item 5 of article 8 of RDC n. 34/2014, in which the HS must have an emergency power source with capacity compatible with critical activities and equipment. However, the absence of power generators was observed in less complex HS, usually located in municipalities distant from the state capitals[17,18].

Refrigerated centrifuges are intended for fractionation of whole blood into FFP and other blood components. It was found that 93.0% (67) of the HS had this equipment, whereas 7.0%, (five) did not. This suggests that these HS use a procedure called spontaneous sedimentation. This procedure is intended for the therapeutic use of packed red blood cells and allocated to hard-to-reach municipalities of the hinterland. Nevertheless, the precepts of Blood Cycle Good Practices still need further encouragement[17].

Regarding the freezers available for freezing and storing FFP units, 91.6% (66) of the services had the equipment in their facilities and 8.4% (six) did not yet have it, resulting in the loss of coagulation factors of this blood component. These six services are located in smaller municipalities, but they also need to

implement Blood Cycle Good Practices[17]. Regarding the presence or absence of blast freezers - devices used for rapid freezing - the study highlights that 44.4% (32) of the services had this piece of equipment, while 55.6% (40) did not.

Validation of the freezing process according to Good Manufacturing Practices should consist of documented evidence, which assures - with a high degree of confidence - that a specific process will consistently generate products that meet predetermined specifications and quality characteristics[41]. It was observed that 52.7% (38) of the HS validated the freezing process and 47.3% (34) of the HS had not yet validated it. That is contrary to the provisions of article 9 of RDC n. 34/2014: "The Hemotherapy Service must validate processes considered critical to the quality assurance of products and services prior to their introduction and revalidate them whenever they change." Again, the HS that had not yet performed the FFP unit freeze validation process corroborated the need to implement the principles of Blood Cycle Good Practices[17] (Figure 1).

Regarding the freezing temperature of the FFP units adopted by the HS, this study revealed that 42.0% (30) of the HS use a freezing temperature between -26 and -30ºC, 30.0% (22) used temperatures below -60ºC, 14.0% (ten) used temperatures between -31ºC and -40ºC and 14.0% (ten) used freezing temperatures from -20 to -25ºC. Temperatures below -60ºC were found in HS that had a blast freezer available[17,18] (Figure 2).

These results corroborate the studies by Sidhu et al.[42] and Naghadeh et al.[43], which described the freezing temperature of -30°C for FFP units. The rapid freezing procedure of FFP units was also discussed by Bloom et al.[44], who compared fast and slow freezing and found that both recovered 70% of factor VIII, agreeing with other authors.

International literature has also shown that rapid freezing achieves a heavier amount of factor VIII, but with low activity, further recommending that the complete freezing of the FFP unit should be 30 min[45]. Myllyla et al. stated that freezing should be rapid when the freezing temperature is below the eutectic point of the solution, thereby achieving complete and homogeneous plasma freezing. The recommended freezing temperature in the study is equal to or lower than -30ºC[44,45,46].

As for the waiting time between collection and fractionation of whole blood into a FFP unit and other blood components, the following results were obtained: 91.6% (66) of the HS had a waiting time of up to 8 h, 2.8 % (two) of the HS had from 18 to 24 h and 5.6% (four) of the HS had a waiting time longer than 24 h (Figure 3).

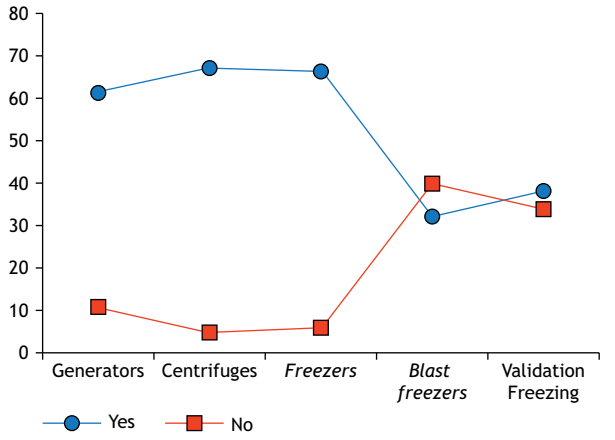

Figure 1. Processes done in Hemotherapy Services.

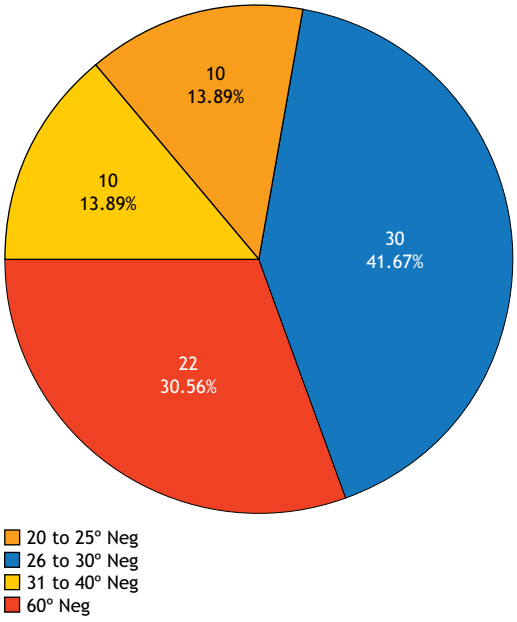

Figure 2. Freezing Temperature of FFP Units.

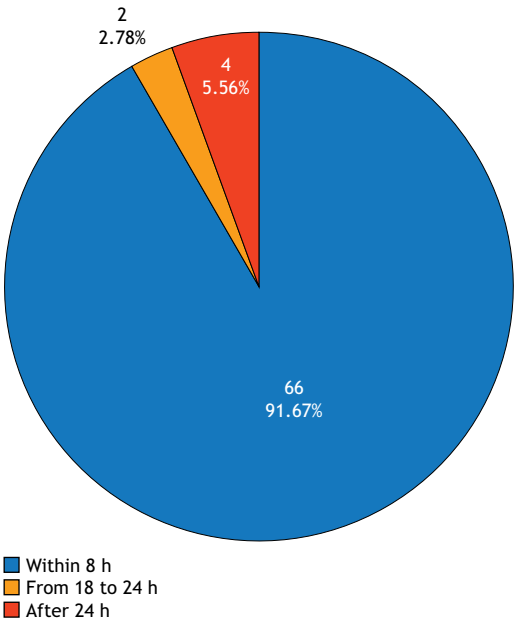

Figure 3. Waiting time between whole blood collection and fractionation.

The data obtained are compatible with the studies by Serrano et al. from 2010, when the time described was 8 h and 24 h, and Cardigan et al., who worked with FFP units with waiting time between 18 and 24 h.

If the sum of the waiting times between collection and fractionation of whole blood up to 24 h is considered, 94.4% (68) of the HS that obtained FFP units had this waiting time. However, 5.6% (four) of the HS still had waiting times longer than 24 h, indicating that they need improvement in their work processes to properly obtain FFP units.

Although these services produce only CP, as defined in Consolidation Ordinance n. 05/2017, that is an important pharmaceutical ingredient that is rich in two proteins of therapeutic importance: human albumin and immunoglobulin[17,18,27].

When analyzing the attribute of Processes performed in HS, it was observed that HS do not pose risks to patients, corroborating the 6th Hemotherapy Production Bulletin[40], but they still need improvement in their processes, as well as the implementation of Blood Cycle Good Practices[17].

Results obtained in HS

In this attribute, the monthly production of FFP units in the 72 HS visited was analyzed, with an average monthly production of 17,715 FFP units and 1,324 CP units. These data are corroborated by Soares, when 12 months are considered, totaling 212,580 L/plasma obtained per year[32].

It is worth highlighting the information from the 6th Hemotherapy Production Bulletin of 2018, which presents 2017 Hemoprod data. According to this document, Brazil discarded 1,831,355 bags of FFP and 386,129 bags of CP[40], not including the disposal done in the state of São Paulo. Considering that each FFP and CP unit contains at least 150 mL in volume, these values would correspond to 274,703 L of FFP and 57,919 L of CP, respectively.

According to Soares, if the price of 1 L of FFP is assumed at USD 70.00, it is possible to estimate a loss of approximately USD 19,000,000.00 in discarded FFP. This value does not include storage costs for these units until their expiration date, which is 12 to 24 months, depending on the storage temperature[18]. Thus, these products that could be used as pharmaceutical ingredients remain stored in HS and are discarded after their expiration, awaiting a government decision about what to do with them, since Hemobrás is not yet fully operational.

Collection, packaging and transport of FFP units for factor VIII content analysis

For factor VIII content analysis, 360 FFP units were collected, received and registered for factor VIII content analysis, which corresponds to five FFP units collected in the 72 HS we visited. In the laboratory, these FFP units were considered samples and thawed at the time of analysis.

In the visual inspection of the 360 FFP units received, 20 units were discarded for being damaged. Therefore, the factor VIII content analysis was done in 340 samples collected.

Analysis of factor VIII content of FFP units collected during technical visits to the services from 2013 to 2015

The study was designed to demonstrate the worst case or critical points in factor VIII recovery in the collected FFP units. In this case, we considered the waiting time of 24 h between the collection of whole blood until the freezing of the FFP units provided for in the current legislation, RDC n. 34/2014. The level of complexity of the HS was not considered. We included both samples collected in the Coordinating Blood Centers and samples collected at the Collection and Transfusion Units, which are services with a lower level of complexity.

Analysis of factor VIII content (IU/mL) was done 15 min after thawing of the FFP units and produced the following results: mean of 0.68 IU/mL, standard deviation of 0.32 IU/mL, coefficient of variation of 47.1%, confidence interval from 0.64 to 0.71. Therefore, it can be seen that the factor VIII content of the units sent for analysis achieved results within the specification for therapeutic use, above 0.50 IU/mL[48,49].

However, according to the guidelines of the European Pharmacopoeia and Brazilian Pharmacopoeia, which recommend - in the Monograph for Fresh Frozen Plasma or Fractionation Plasma - that the FFP unit, considered as a pharmaceutical input for the production of blood products, should have at least 0.7 IU/mL of factor VIII[22,28,48]. This study has shown that 38.5% (131) of the analyzed FFP units can be considered a pharmaceutical input for the production of blood products, with results equal to or greater than 0.7 IU/mL of factor VIII recovered.

It should be noted that when analyzing the results of factor VIII content in the collected FFP units, these were properly grouped into three categories: higher than 0.70 IU/mL, corresponding to 38.5% (131) of the samples; 0.69 to 0.50 IU/mL, corresponding to 32.1% (109) of the samples; and less than 0.49 IU/mL, corresponding to 29.4% (100) of the samples (Figure 4).

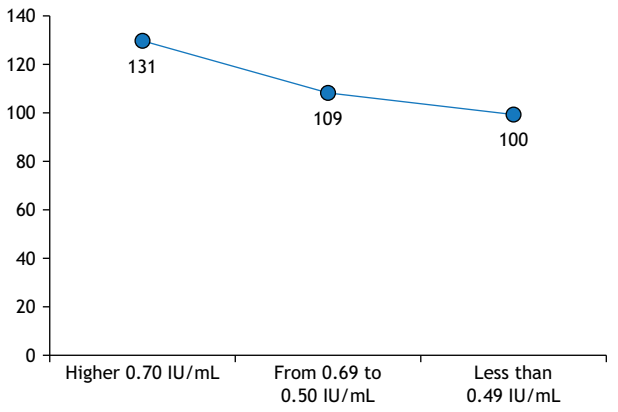

Figure 4. Dosing of factor VIII content in FFP units collected at Hemotherapy Services.

In this study it was possible to show that, regardless of the waiting time between collection, fractionation and freezing, as well as the freezing temperature, 38.5% (131) of the FFP units have shown results above or equal to 0.70 IU/mL of factor VIII content, in agreement with Naghadeh et al., who have found a level higher than 0.70 IU/mL in 34.8% of the samples[43].

The factor VIII content lower than or equal to 0.69 IU/mL obtained from the samples corresponded to 61.5% (209) of the samples. Although outside the specification of 0.70 IU/mL of factor VIII, these are fully capable of producing two other extremely important blood products: human immunoglobulin and albumin.

When analyzing the results obtained from the factor VIII content of the FFP units versus the information from the 6th Hemotherapy Production Bulletin, which declared a disposal of 1,831,355 FFP units in 2017, we observed that 274,703 L of FFP were lost.

This study has shown that 38.5% of the FFP units analyzed had values greater than or equal to 0.70 IU/mL of factor VIII content[40]. If we include the number of discarded FFP units, we have approximately 500 million IU/L of factor VIII. This means that, considering only what was discarded in terms of pharmaceutical input, Brazil could produce approximately 83% of the factor VIII concentrate drug that is imported, which would mean savings of about USD 100 million to the public treasury every year.

CONCLUSIONS

Considering the evidence and results obtained in this study, it was found that, although the HS visited in the five regions of the country had equipment and work processes of medium and low risk, implementing the principles of Blood Cycle Good Practices is still necessary, as recommended by RDC n. 34/2014.

Regarding the factor VIII content analyzed in the collected FFP units, this study, outlined as the worst case, identified 38.5% of the FFP units with factor VIII content greater than or equal to 0.70 IU/mL, which corresponds to the specification of the plasma for fractionation recommended by the Brazilian Pharmacopoeia. These units can and should be used as pharmaceutical input for the production of blood products in the country.

Finally, it was possible to evidence the disposal of approximately 500 million IU/L of factor VIII, which accounts for about 83% of the annual procurement of factor VIII concentrate by the Ministry of Health. The use of this input can enable significant savings for the public treasury.

REFERENCES

1. Cairutas CM. O que corre em nossas veias fragmentos de sua história. Recife: Ebge; 2001.
2. Kameda K, Corrêa MCDV, Cassier M. A incorporação do teste diagnóstico baseado na amplificação de ácidos nuclêicos (NAT) para a triagem do sangue no SUS: arranjos tecnológicos para a nacionalização do "NAT brasileiro". Physis. 2018;28(1):1-21. https://doi.org/10.1590/s0103-73312018280108
3. Greenwalt TJ. A short history of transfusion medicine. Transfusion. 1997;37(5):550-63. https://doi.org/10.1046/j.1537-2995.1997.37597293889.x
4. Giangrande PLF. The history of blood transfusion. Br J Haematol. 2000;110(4):758-67. https://doi.org/10.1046/j.1365-2141.2000.02139.x
5. Junqueira PC, Rosenblit J, Hamerschlak N. História da hemoterapia no Brasil. Rev Bras Hematol Hemoter. 2005;27(3):201-7. https://doi.org/10.1590/S1516-84842005000300013
6. Brasil. Lei N° 1.075, de 27 de março de 1950. Dispõe sobre a doação voluntária de sangue. Diário Oficial União. 28 mar 1950.
7. Lordeiro MAM, Santos RO, Lapa AT, Leal MFFS, Lourenço VS. Evolução da história da doação de sangue no Brasil dentro do âmbito do SUS. Rev Rede Cuidados Saúde. 2017;11(3):1-4.
8. Santos LC, Moraes C, Coelho VSP. A hemoterapia no Brasil de 64 a 80. Physis. 1991;1(1):161-81. https://doi.org/10.1590/S0103-73311991000100008
9. Santos LC, Moraes C, Coelho VSP. Os anos 80: politização do sangue. Physis. 1992;2(1):107-49. https://doi.org/10.1590/S0103-73311992000100005
10. Brasil. Lei Nº 4.701, de 28 de junho de 1965. Dispõe sobre o exercício da atividade hemoterápica no Brasil e dá outras providências. Diário Oficial União. 9 set 1965.
11. Brasil. Lei Nº 6.360, de 23 de setembro de 1976. Dispõe sobre a vigilância sanitária a que ficam sujeitos os medicamentos, as drogas, os insumos farmacêuticos e correlatos, cosméticos, saneantes e outros produtos e dá outras providências. Diário Oficial União. 24 set 1976.
12. Brasil. Lei N° 6.437, de 20 de agosto de 1977. Configura infrações à legislação sanitária federal, estabelece as sanções respectivas, e dá outras providências. Diário Oficial da União. 24 ago 1977.
13. Ministério da Saúde (BR). Qualidade do sangue: sangue e hemoderivados. Brasília: Ministério da Saúde; 2000; 52p.
14. Brasil. Portaria Nº 1.376, de 19 de novembro de 1993. Normas técnicas para coleta, processamento e transfusão de sangue, componentes e derivados. Diário Oficial União. 2 dez 1993.
15. Brasil. Portaria Nº 121, de 24 de novembro de 1995. Normas para implementação, fiscalização e inspeção em unidades hemoterápicas. Diário Oficial União. 30 nov 1999.

16. Brasil. Lei N° 10.205, de 21 de março de 2001. Regulamenta o parágrafo 4° do artigo 199 da Constituição Federal, relativo à coleta, processamento, estocagem, distribuição e aplicação do sangue, seus componentes e derivados, estabelece o ordenamento institucional indispensável à execução adequada dessas atividades, e dá outras providências. Diário Oficial da União. 22 mar 2001.
17. Agência Nacional de Vigilância Sanitária - Anvisa. RDC Nº 34, de 11 de junho de 2014. Dispõe sobre as boas práticas no ciclo do sangue. Diário Oficial União. 16 jun 2014.
18. Brasil. Portaria de consolidação Nº 5, de 28 de setembro de 2017. Consolidação das normas sobre as ações e os serviços de saúde do Sistema Único de Saúde. Diário Oficial União. 29 set 2017.
19. Farrugia A. Plasma for fractionation: safety and quality issues. Haemophilia. 2004;10(4):334-40. https://doi.org/10.1111/j.1365-2516.2004.00911.x
20. Said DMP. Registro sanitário de medicamentos: uma experiência de revisão [dissertação]. Rio de Janeiro: Fundação Oswaldo Cruz; 2004.
21. Food and Drug Administration - FDA. Revisions to the requirements applicable to blood, blood components, and source plasma: confirmation in part and technical amendment. Fed Regist. 2011;66(7):7463-4.
22. Blood Products Advisory Committee - BPAC. Current considerations on plasma for further manufacturing obtained from whole blood donors. Washington: Blood Products Advisory Committee; 2012.
23. American Association of Blood Banks - AABB. Circular of information for the use of human blood and blood componentes. Bethesda: American Association of Blood Banks; 2013.
24. Eder AF, Sebok MA. Plasma components: FFP, FP24 and thawed plasma. Immunohematology. 2007;23(4):150-7.
25. Council of Europe. Human plasma for fractionation. In: Council of Europe. European Pharmacopoeia 6.2. Estrasburgo: Council of Europe; 2008. p.3759-60.
26. World Health Organization - WHO. WHO Recommendations for the production, control and regulation of human plasma for fractionation. Geneva: World Health Organization; 2005.
27. Agência Nacional de Vigilância Sanitária - Anvisa. RDC N° 46, de 18 de maio de 2000. Normatiza os processos de produção e controle de qualidade, a aquisição e distribuição dos medicamentos hemoderivados para uso humano. Diário Oficial União. 19 maio 2000.
28. Agência Nacional de Vigilância Sanitária - Anvisa. Farmacopéia Brasileira. 5a ed. Brasília: Agência Nacional de Vigilância Sanitária; 2010.
29. Farrugia A. Studies on the procurement of blood coagulation factor VIII: effects of plasma freezing rate and storage conditions on cryoprecipitate quality. J Clin Pathol. 1985;38(4):433-7. https://doi.org/10.1136/jcp.38.4.433
30. Burnouf T. Factors affecting the quality/safety of hemophilia treatment products. Flórida: World Federation of Hemophilia; 2002.
31. Kasper CK, Silva MC. Register of clotting factor concentrates. Montreal: World Federation of Hemophilia; 2005.
32. Soares BMD. Política de hemoderivados no Brasil: desafios e perspectivas [dissertação]. Brasília: Centro de Desenvolvimento Sustentável; 2002.
33. Brasil. Lei Nº 10.972, de 2 de dezembro de 2004. Autoriza o poder executivo de criar a empresa pública denominada empresa brasileira de hemoderivados e biotecnologia - HEMOBRÁS e dá outras providências. Diário Oficial União. 3 dez 2004.
34. Brasil. Constituição da República Federativa do Brasil. Brasília: Senado Federal; 1988.
35. Marconi MA, Lakatos EM. Fundamentos de metodologia científica. 5a ed. São Paulo: Atlas; 2003.
36. Agência Nacional de Vigilância Sanitária - Anvisa. RDC Nº 151, de 21 de agosto de 2001. Aprova o regulamento técnico sobre níveis de complexidade dos serviços de hemoterapia. Diário Oficial União. 22 ago 2001.
37. Peiter PC. A geografia da saúde na faixa de fronteira continental do Brasil na passagem do milênio [tese]. Rio de Janeiro: Universidade Federal do Rio de Janeiro; 2005.
38. Silva LMV, Formigli VLA. Avaliação em saúde: limites e perspectivas. Cad Saude Publica. 1994;10(1):80-91. https://doi.org/10.1590/S0102-311X1994000100009.
39. Agência Nacional de Vigilância Sanitária - Anvisa, Secretaria de Atenção a Saúde - SAS. Portaria conjunta Anvisa/SAS Nº 370, de 07 de maio de 2014. Dispõe sobre o regulamento técnico sanitário para transporte de sangue e componentes. Diário Oficial União. 8 maio 2014.
40. Agência Nacional de Vigilância Sanitária - Anvisa. 6º Boletim de produção hemoterápica Hemoprod 2017. Brasília: Agência Nacional de Vigilância Sanitária; 2018.
41. Agência Nacional de Vigilância Sanitária - Anvisa. RDC Nº 17, de 16 de abril de 2010. Dispõe sobre as boas práticas de fabricação de medicamentos. Diário Oficial União. 17 abr 2010.
42. Sidhu RS, Le T, Brimhall B, Thompson H. Study of coagulation factor activities in apheresed thawed fresh frozen plasma at 1-6 degrees C for five days. J Clin Apher. 2006;21(4):224-6. https://doi.org/10.1002/jca.20095
43. Naghadeh HT, Roudkenar MH. A study of the quantity of some stable and labile coagulation factors in fresh-frozen plasma produced from whole blood stored for 24 hours in Iran. Blod Transfus. 2009;7(1):439-42. https://doi.org/10.2450/2008.0022-08
44. Bloom AL, Giddings JC, Bevan B, Letton M, Drummond RJ. Comparison of quick and slow thaw methods of producing cryoprecipitate antihaemoplilic fator from fresh and 24-hour-old blood. J Clin Path. 1969;22(4):447-52. https://doi.org/10.1136/jcp.22.4.447

45. Allain PJ. What are the critical factors in the production and quality control of frozen plasma intended for direct transfusion on for fractionation to provide medically needed labile coagulation factors. Vox Sang. 1983;44(4):246-59. https://doi.org/10.1111/j.1423-0410.1983.tb01891.x

46. Myllyla G. Factors determining quality of plasma. Vox Sang. 1998;74(Supl.2):507-11. https://doi.org/10.1111/j.1423-0410.1998.tb05466.x

47. Serrano K, Scammell K, Weiss S, Culibrk B, Levin E, Gyöngyössy-Issa M et al. Plasma and cryoprecipitate manufatured from whole blood held overnight at room temperature meet quality standards. Transfusion. 2010;50(2):344-53. https://doi.org/10.1111/j.1537-2995.2009.02441.x

48. Cardigan R, Lawrie AS, Mackie IJ, Williamson LM. The quality of fresh-frozen plasma produced from whole blood stored at 4ºC overnight. Transfusion. 2005;45(6):1342-8. https://doi.org/10.1111/j.1537-2995.2005.00219.x

49. Cardigan R, Van der Meer PF, Pergande C, Cookson P, Baumann-Baretti B, Cancelas JA et al. Coagulation factor content of plasma produced from whole blood for 24 hours at ambient temperature: results from an international multicenter BEST Collaborative study. Transfusion. 2011;51(Supl.1);s50-7. https://doi.org/10.1111/j.1537-2995.2010.02963.x

Acknowledgement

To the professionals of the local Health Surveillance bodies. Support: National Health Surveillance Agency/United Nations Development Program (Anvisa/UNDP).

Conflict of interest

The authors report that there is no potential conflict of interest with peers and institutions, nor political or financial conflicts in this study.