Translation from Bulgarian

Regulation No. 18 of 10 June 2004 on the requirements and procedures for testing, processing and storage of blood and blood components, and the quality of imported blood

Issued by the Minister of Health, promulgated in State Gazette (SG) Issue 58 of 6 July 2004, effective as of 6 July 2004, amended in Issue 64 of 19 August 2011, effective as of 1 July 2011

Chapter One
GENERAL PROVISIONS

Article 1. This Regulation lays down the requirements and procedure for testing, processing and storage of blood and blood components, and the quality of imported blood.

Chapter Two
IMMUNO-HAEMATOLOGIC TESTING OF BLOOD AND BLOOD COMPONENTS

Article 2. Immuno-haematologic testing includes a set of tests conducted to determine the blood type characteristics of the tested blood. The tests include the following operations:
1. determination of blood cell antigens;
2. detection of antibodies, specifically directed to blood cell antigens;
3. analysis of the reactions between blood cell antigens and their specific antibodies.

Article 3. (1) All immuno-haematologic tests of blood and blood components should be conducted in compliance with the requirements of this regulation.
(2) Testing of units of donated blood or blood components should be performed in blood transfusion establishments.

Article 4. (1) The container with the blood sample from the donated blood unit should be labeled by the healthcare professional collecting the blood.

(2) The container with the blood sample from the donated blood unit should be labeled with a reference number.
(3) The final testing of blood samples should be conducted not later than 5 days after blood collection.

Article 7. Containers with blood samples should be stored at +2 ° to +8°C.
Article 8. (1) Containers with blood samples should be accepted by a healthcare professional or laboratory technician who should verify that the details on the container label are identical with the details contained in the associated documentation.
(2) Containers with blood samples should be submitted to the laboratory with a delivery report containing the following information:
1. delivery report reference number;
2. blood establishment collecting the blood or blood components;
3. unique identification number of the donated units (barcode);
4. name and signature of the person inspecting the containers;
5. name and signature of the person accepting the containers.

(3) No blood sample containers should be accepted for testing, if the specific requirements referred to in Article 4, 5, 6 and 7 are not met.
(4) No blood sample containers should be accepted for testing, if there is obvious evidence of haemolysis. Such containers should be destroyed by the laboratory which accepted the blood samples.

Article 9. Testing of blood samples should be carried out with agents authorised for use in the country and meeting the requirements of Annex No. 1.
Article 10. All test results should be included in the documents accompanying the blood sample container, at each level of the registry under Article 36 of the Blood, Blood Donation and Blood Transfusion Act (SG Issue 102 of 2003) (BBDBTA) and stored as a hard copy in the laboratory that conducted the tests.
Article 11. (1) The final determination of blood types under the AB0 system should be carried out using a cross method comprising:

1. Testing of antigens according to the AB0 blood group system with test reagents anti-A, anti-B, anti-A+B, or anti-AB;

2. Testing of anti-A and anti-B antibodies with test erythrocytes A, A, B

1 2 and 0.

(2) During the first two consecutive donations from a single donor, blood groups under the AB0 system should be determined in parallel using two different sets of test reagents and test erythrocytes as follows:

1. Test reagents (anti-A, anti-B and anti-AB) obtained from two different sources;

2. Test erythrocytes A, A, B and 0 - two sets from two different

1 2 sources.

(3) In subsequent blood collections, blood groups should be tested with one set of test reagents and test erythrocytes. The obtained results should be compared with those of previous collections of blood and stored in the registry referred to in Article 36 of BBDBTA at levels one and two. In the event of discrepancies in the results, a new blood sample should be taken. Blood group retesting should be carried out with a different set of test reagents and test erythrocytes.

(4) The sub-groups of the A antigen (A and A) should be determined when blood

1 2 should be selected for a patient whose serum revealed presence of clinically significant anti-A antibodies.

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(5) Anti-A and anti-B antibodies titre should be tested only when the specific unit of blood or blood components will not be transfused as an iso group under the AB0 system. The results are only valid for the specific unit and can not be used in subsequent collections of blood from the same donor.

Article 12. (1) The Rh (D) antigen should be tested for each collected blood unit.

(2) During the first two consecutive donations, such tests should be carried out in parallel with anti-D test reagents obtained from two different sources.

(3) In all subsequent blood collections, tests should be carried out with an anti-D test reagent from only one source. The result should be compared to the results of the previous donations.

(4) In case of a discrepancy in the results, a new blood sample should be taken from the donated unit. The Rh (D) antigen should be tested with test reagents from a different source.
(5) Each donated blood unit defined as Rh (D) negative should be tested additionally for presence of weak Rh (D) antigen (D).

Article 13. (1) Each Rh (D) negative blood unit collected in the first two consecutive blood collections should be tested for C and E antigens of the Rhesus system. The tests should be performed with monospecific anti-C and anti-E test reagents or with polyspecific anti-C+D+E, or anti-C + D and anti-D + E test reagents obtained from two different sources.

(2) All subsequent blood collections should use the results obtained in the previous two donations stored in the registry referred to in Article 36 of BBDBTA at levels one and two.

Article 14. (1) Testing of antigens C, c, E, e of the Rhesus system of a donated blood unit should be carried out in the cases where blood or blood components should be selected for transfusions according to the Rh phenotype.
(2) The antigens C, c, E of the Rhesus system should be tested regardless of the Rh (D) type of the donated blood unit.
(3) The tests of the first two consecutive donations should be carried out with monospecific anti-C, anti-c, anti-E and anti-e test reagents obtained from two different sources.
(4) All subsequent blood collections should use the results obtained from the first two donations stored in the registry referred to in Article 36 of BBDBTA at levels one and two.
Article 15. (1) The erythrocyte antigens outside the AB0 and Rhesus systems should be tested in the cases where selection of blood or blood components is required for transfusion according to the antigenic formula.
(2) In the first two consecutive blood donations, erythrocyte antigens from other blood group systems like KEL, MNS, Duffy, Kidd, Lewis, P, Lutheran, etc. should be tested with the specific test reagents.
(3) All subsequent blood collections should use the results obtained from the first two donations stored in the register under Article 36 of BBDBTA at levels one and two.
Art. 16. (1) Each collected blood unit should be tested by the aid of a set of methods for presence of anti-erythrocyte antibodies. The tests include:

1. direct antiglobulin test (DAT) with polyspecific antiglobulin serum (AGS);

2. indirect antiglobulin test (IAT - Coombs test) with polyspecific AGS;

3. agglutination and enzyme test or another test of equivalent sensitivity.

(2) In case of positive test results for anti-erythrocyte antibodies additional immuno-haematologic tests should be conducted to determine their specificity and titre. If it is impossible to determine the specificity of the anti-erythrocyte antibodies, the sample should be sent for conclusive testing to the National Haematology and Transfusiology Centre (NHTC).

(3) Each collected blood unit with a positive test result for anti-erythrocyte allo-antibodies should be used in accordance with the table in Annex No. 2.

(4) Collected blood units with a positive DAT should not be accepted for clinical use.

(5) Packed red blood cells and platelet concentrates derived from blood with positive DAT results should not be accepted for clinical use.

(6) Blood donors whose test results for anti-erythrocyte antibodies are positive should be notified and referred to their GP for further testing and consulting. New blood samples may be taken from the same person, if there are no contraindications and after negative results from testing for anti-erythrocyte antibodies conducted at least 6 months after the first test.

Article 17. The label of each collected blood unit should contain the following details from the immuno-haematologic tests:

1. blood group antigens under the AB0 system; according to the blood group, the labels of the units of blood or components are coloured as follows:

a) blood group A - blue;

b) blood group B - red;

c) blood group 0 - white;

d) blood group AB - yellow;

2. Rh (D) antigen:

a) blood units with positive results for Rh (D) antigen should be labeled as Rh (D) (+) positive; the text on the label should be written in black letters on white background;

b) blood units with negative results for Rh (D) antigen should be labeled as Rh (D) (-) negative; the text on the label should be written in white letters on black background; and

c) blood units with positive results for weak Rh (D) antigen (D) should be labeled as Rh (D) (+) positive;

3. when tested, the label should be marked with the A antigen subgroups (A or A), the Rh phenotype, the antigens from the other blood group

1 2 systems, the titre of the anti-A and anti-B antibodies.

Chapter Three

TESTING OF BLOOD AND BLOOD COMPONENTS FOR TRANSMISSIBLE INFECTIONS

Article 18. Testing of donated blood and blood components for markers of transmissible infections should take place in specific blood establishments according to the rules of good laboratory practice and in compliance with the requirements of this regulation.

Article 19. Testing of donated blood should be carried out with reagents which are authorized for use in Bulgaria. Each delivered batch of tests should be accepted together with a certificate of quality.

Article 20. (1) Each donated blood unit should be tested for anti-HIV 1, 2 antibodies, surface antigen of hepatitis B, anti-HCV antibodies and anti-treponemal antibodies. The Minister of Health may order the performance of other mandatory tests.

(2) The donors referred to in Article 6 of BBDBTA should be tested for all markers under paragraph 1 up to 10 days prior to blood collection.

(3) Donors of blood components obtained by plasmapheresis and cytopheresis should be tested for all markers under paragraph 1 up to 10 days prior to inclusion in the aphaeresis programmes.

(4) Donors of preoperatively taken autologous donations should be tested for all markers under paragraph 1 before inclusion in the autologous donation programmes. In case of a positive result, the decision for inclusion in the programmes should be taken by the attending physician.

Article 21. (1) Donors of blood for immunization should be tested for all markers referred to in Article 20, paragraph 1, before donation.

(2) The blood donated for immunization should be frozen and stored for a period of not less than six months. At the expiration of this period, donors should be retested for all markers referred to in Article 20, paragraph 1.

(3) In case of negative test results, the donated blood is released for immunization. In case of one or more positive test results, the blood should not be allowed for use.

Article 22. (1) Containers with samples from donated blood and blood components should meet the requirements of Article 4, 6 and 7.

(2) Containers with samples from donated blood and blood components should be submitted to the laboratory with acknowledgment of receipt containing the following information:

1. number of delivery report;
2. healthcare facility collecting the blood or blood components;
3. unique identification number of the units (barcode);
4. name and signature of the person inspecting the containers;
5. name and signature of the person accepting the containers.

(3) Tests should be carried out within 5 days from the date on which the blood is taken. If tests need to be delayed, a certain quantity of serum or plasma should be separated and stored frozen until the next tests, especially when sending samples to laboratories carrying out confirmatory tests.

(4) Each donor and/or donated blood unit should be tested for all markers referred to in Article 20, paragraph 1. In case of negative results, the unit of blood or blood components derived from it should be released for clinical use or processing. The results from the tests should be stored in the registry referred to in Article 36 of BBDBTA at levels one and two, and as a hardware copy kept at the laboratory performing the tests.

(5) If the test results for each marker are positive (primary positive result) the blood or blood components should be blocked and quarantined until the final result is received. Such blood and blood components should be clearly marked as blocked. The material should be retested twice.

(6) If retesting gives two negative results, the blood or blood components can be released for clinical use or processing.

(7) If retesting gives two positive results or one positive and one negative result, the sample should be considered as positive. If the test is conducted with a sample from the donor, it should be repeated with material derived from the donated unit to confirm the result. In case of conflicting results, the conclusive result is the one obtained by testing the material derived from the donated unit. Upon confirmation of a positive result, the blood or blood components and the control tests should be documented, discarded and destroyed.

Article 23. (1) All samples with positive results for the markers referred to in Article 20, paragraph 1, should be subjected to confirmatory tests as follows:

1. when sending samples to laboratories for confirmatory testing, all requirements for transport of biological materials should be met;

2. the accompanying documentation should contain information about the identification of the samples, the values of the primary and repeated positive reactions (or the degree of the positive reaction under the 4 + system and titres in specific agglutination tests for syphilis);

3. in case of a positive test result for anti-HIV antibodies, the primary sample and a samples from the collected blood should be sent to the specific reference laboratory and:

a) if the confirmatory tests show a negative result, the donor is not removed from future donations;

b) if retesting of the same donor gives a positive result in screening and a negative result in a confirmatory test, the donor is removed from future donations;

c) in case of inconclusive confirmatory test results, the confirmatory tests should be repeated with a new sample provided by the Dermatology and Venerology Clinics and Dispensaries (DVCD); in case of a negative result from screening and confirmatory tests, the donor is not removed from future donations; in case of repeated inconclusive results, the donor is removed from future donations;

d) in case of a positive result from the confirmatory tests, the donor should be removed from future donations;

4. in case of a positive result for hepatitis B surface antigen, the positive sample from the collected blood should be tested with a neutralization test at a BTE and:

a) in case of a negative result from the confirmatory tests, the donor is not removed from future donations;

b) if retesting of the same donor gives a positive result in screening and a negative result in a confirmatory test, the donor is removed from future donations;

c) in case of a positive result from the confirmatory tests, the donor is removed from future donations;

5. In case of a positive result for anti-HCV antibodies, the primary sample and the sample from the collected blood should be sent to the National Haematology and Transfusiology Centre (NHTC) and:

a) if the confirmatory tests show a negative result, the donor is not removed from future donations;

b) if retesting of the same donor gives a positive result in screening and a negative result in a confirmatory test, the donor is removed from future donations;

c) in case of inconclusive confirmatory test results, the confirmatory tests should be repeated with a new sample taken from the donor or provided by the Institute of Hygiene and Epidemiology; in case of a negative result of screening and confirmatory tests, the donor is not removed from future donations; in case of repeated inconclusive results, the donor is removed from future donations;

d) in case of a positive result from the confirmatory tests, the donor should be removed from future donations;

6. In case of a positive result for anti-treponemal antibodies, the positive sample taken from the donated unit should be further tested using a specific test and:

a) in case of a negative result of the further tests, the donor is not removed from future donations;

b) if retesting of the same donor gives a positive result in screening and a negative result in a confirmatory test, the donor is removed from future donations;

c) in case of a positive result of the further tests, the donor should be removed from future donations.

(2) All details about the donors who are permanently removed from future donations should be recorded in the registry referred to in Article 36 of BBDBTA, at all levels.

(3) All data about the donors with confirmed positive results for hepatitis B surface antigen and antibodies against Hepatitis C should be communicated to the Institute of Hygiene and Epidemiology.

(4) All data about donors who test positive for anti-treponemal antibodies should be communicated to DVCD.

Chapter Four

PROCESSING OF BLOOD AND BLOOD COMPONENTS

Article 24. Processing of collected blood and blood components is a technological process using standard methods for separation and/or further processing to obtain:

1. blood and blood components for clinical use;
2. starting materials for the production of drugs derived from human plasma.

Article 25. (1) Blood and blood components are processed in processing units at blood transfusion establishments subject to the rules of the Good Manufacturing Practice and in accordance with modern scientific and technical achievements.

(2) All procedures used in the processing of blood and blood components should be carried out in accordance with the standard operating protocols.

Article 26. The processing of blood should be documented as a hardware copy and electronically.

Article 27. The processing of a standard donated blood unit yields the following blood components:

1. packed red blood cells;
2. packed red blood cells with added solution;
3. packed red blood cells with removed buffy-coat;
4. packed red blood cells with removed buffy-coat and added solution;
5. leukocyte-depleted packed red blood cells;
6. leukocyte-depleted packed red blood cells with added solution;
7. washed packed red blood cells;
8. frozen packed red blood cells;
9. fresh frozen plasma (FFP);
10. plasma with reduced labile coagulation factors;
11. platelet concentrate;
12. washed platelet concentrate;
13. leukocyte-depleted platelet concentrate;

14. frozen platelet concentrate.

Article 28. (1) The blood or blood components obtained by aphaeresis should be taken in accordance with the existing requirements from donors who meet the established selection criteria.

(2) Blood and blood components should be collected by using a closed system of sterile plastic bags with various configurations, containing blood conserving solution (added solution too, in some configurations) authorised for use in Bulgaria.

Article 29. Unit of blood and blood components should be accepted in the processing unit with acknowledgment of receipt which contains the following details:

1. healthcare facility collecting the blood;

2. reference number;

3. identification number of the unit;

4. initial blood typing results;

5. date and time of blood collection;

6. quantity in milliliters;

7. blood collection duration in minutes;

8. temperature conditions of storage and transport until processing;

9. name, surname, position and signature of the delivering and receiving official.

Article 30. (1) The donated units of blood or blood components should be referred for processing and the following individual parameters of each unit should be reported:

1. blood collection duration;

2. type and specification of the closed system of plastic bags in which the blood was collected;

3. storage temperature, transportation, and period of time until processing;

4. manufacturing program of the blood transfusion establishment consistent with the needs of the inpatient healthcare facilities and dispensaries of a certain type of blood components, and the need of plasma for drug manufacturing.

(2) Each standard blood unit taken in accordance with its individual parameters under paragraph 1 should be included in one of the technological chains specified in Annex No. 3.

(3) Each donated blood unit with an unusual blood:preservative ratio (quantities from 300 to 404 ml taken in blood conserving solution for 450 +/- 45 ml) should only be processed to packed red blood cells with reduced volume and reduced shelf life.

(4) Each donated blood unit or blood component should be separated, withdrawn and discarded, if:

1. it is not stored at temperatures consistent with Annex No. 4 until acceptance;

2. its pressurization is compromised;

3. there is evidence of indicators deviating from the requirements of Article 29.

(5) Each donated unit of blood or blood component should be tagged with a technological code indicating the technological referral for processing.

(6) The platelet concentrate obtained through cytopheresis can be:

1. applied directly for clinical use;

2. subjected to:

a) further centrifugation;

b) in-depth filtration with leukocyte filters;

c) washing.

Article 31. (1) The processing of donated blood can begin with in-depth filtering to remove leukocytes, or with initial centrifugation.

(2) Filtration equipment should be standardized in each processing unit for all parameters that influence the effectiveness of the procedure, so that the obtained blood components meet the benchmarks set out in Table 1 and 2 of Annex No. 5.

Article 32. (1) Initial centrifugation used to obtain various blood components should be performed using one of the methods specified in Annex No. 6.

(2) Platelet concentrate can be derived from donated blood units by any of these two methods:

1. from platelet-rich plasma;

2. from buffy coat.

(3) Secondary centrifugation used to obtain finished components should be performed in accordance with parameters recommended by the manufacturer.

(4) The speed and duration of each method are selected and standardized for each centrifuge in such a way as to get blood components whose composition meets the parameters set out in Table 1, 2, 3 and 4 of Annex No. 5.

(5) Prior to loading, each centrifuge should be adjusted to reach the lower limit of the working temperature range:

1. the primary temperature range for all methods is +4°C to +8°C;

2. when the centrifugation method (I, II or III) as specified in Annex No. 6 is part of the technological chain for obtaining platelet concentrate, the centrifugation mode temperature range is 20-24°C.

Article 33. Blood components should be separated after each initial or further centrifugation.

Article 34. (1) The separated cellular blood components should be resuspended in a closed system.

(2) The buffy coat should be carefully resuspended in plasma or a suitable culture solution when it will be included as an intermediate product in the technological chain for obtaining platelet concentrate.

(3) The packed red blood cells should be resuspended in plasma or a suitable culture solution when the hematocrit levels are ? 0.76.

Article 35. Satellite bags with buffy coat from 20 to 60 ml should be removed and destroyed, if not subject to further processing.

Article 36. (1) The plasma obtained during the processing of donated blood units or aphaeresis donations may be preserved as fresh frozen plasma.

(2) The time in which the bag with plasma should be placed in a shock freezer for obtaining fresh frozen plasma is determined by the time and temperature of storage after collection:

1. within the first hour after collection of plasma by aphaeresis, at room temperature;

2. within the first six hours after collection, at a temperature of 2-8°C;

3. within the first twelve hours after collection, at a temperature of 20-24°C.

(3) Before each loading, the shock freezer should be adjusted to the temperature recommended by the manufacturer.

Article 37. (1) Intermediate blood and blood components should be quarantined until completion of the entire production cycle and release of the final results of the laboratory and quality control tests.

(2) Finished blood and blood components should be quarantined until all results from the mandatory laboratory and control tests established by this regulation come out.

(3) The storage conditions of intermediate and finished blood and blood components during quarantine are listed in Annex No. 4.

Article 38. (1) Plasma derived from the processing of non-standard blood units as specified in Article 30, paragraph 3 may be used to produce stable plasma products.

(2) Intermediate blood and blood components which at a certain stage of the laboratory and quality control tests show results that do not comply with the requirements of this regulation should be withdrawn from the technological process, marked and discarded.
(3) Finished blood and blood components which do not meet the requirements of the quality control tests should be marked and discarded.
(4) Blood and blood components discarded under paragraph 2 and 3 should be destroyed or released for training or scientific medical purposes as specified in the regulation under Article 43, paragraph 1 of BBDBTA.

Article 39. (1) Finished blood and blood components that have passed all stages of laboratory testing and quality control, and have parameters consistent with the ones set out in Annex No. 7, should be labelled as blood and blood components for clinical use, or as starting materials for the manufacture of medicines from plasma.
(2) The different types of blood components should be labelled in accordance with the blood transfusion standards.
(3) Each label should contain at least the following information:
1. identity of the manufacturer;
2. unique identification number;
3. AB0 and Rh (D) group;
4. collection date;
5. name of the anticoagulant;
6. name of the component;
7. additional information about the component (irradiated, leukocyte-depleted, etc.);
8. expiration date and time;
9. size or weight of the component;
10. storage temperature;
11. phenotype (if determined);
12. the component should not be transfused in case of evidence of haemolysis or other changes;
13. the component should be transfused through a 170-200? m filter.
(4) The finished blood and blood components should be released from quarantine immediately after labelling.

(5) All labelled blood and blood components that are released from quarantine should be moved and stored at the depot for starting materials for the production of medicines from plasma and the depot for blood or blood components for clinical use under the conditions laid down in Annex No. 4.

Article 40. (1) The distribution of starting materials for the production of drugs from plasma to drug manufacturers and of blood and blood components for clinical use to inpatient hospitals and dispensaries should be agreed in contracts signed with consumers.

(2) The transportation of blood and blood components for clinical use and starting materials for the production of drugs from plasma should be carried out in special containers in which the temperature conditions specified in Annex No. 4 are maintained.

Chapter Five

STORAGE OF BLOOD AND BLOOD COMPONENTS

Article 41. Donated blood units should be stored and transported until processed into blood components under the following conditions:

1. at temperatures from +2°C to +6°C, when:

a) blood processing may begin during a period of time which allows receipt of fresh frozen plasma within 6 hours from blood collection;

b) blood processing can not begin within 18 hours from blood collection;

2. under conditions ensuring the storage of units at temperatures from +20°C to +24°C for a period of up to 18 hours, when the intention is to obtain packed red blood cells, platelet concentrate and fresh frozen plasma.

Article 42. The possibilities for obtaining various blood components according to the duration of blood collection and the duration and temperature of storage of the donated blood are determined in accordance with Annex No. 3.

Article 43. Blood components should be stored in conditions which ensure the maintenance of their optimal viability and functions.

Article 44. Untested blood and blood components should be quarantined until completion of all tests specified in this regulation under the conditions specified in Annex No. 4.

Article 45. Finished blood components which meet the requirements of Article 39, paragraph 1 should be kept for the duration and under the conditions laid down in Annex No. 4.

Chapter Six

PROPERTIES OF IMPORTED BLOOD

Article 46. (1) Blood and blood components may be imported in the country in exceptional circumstances in which the quantities of blood and blood components available in the country are not sufficient to ensure the protection of public health.

(2) Such insufficiency of the available quantities of blood and blood components should be determined by the Minister of Health based on the report of the Director of NHTC.

Article 47. The Bulgarian Red Cross should research the possibilities of importation of blood and blood components under the conditions set out under Article 46, define which countries are suitable, and submit a proposal to the Minister of Health for the initiation of negotiations and import.

Article 48. (1) Subject to import can only be blood and blood components that are collected, tested, processed, labelled and secured by an institution that is legally recognised in compliance with the established procedures in the country to provide such services.

(2) Importation is permitted only in the event that the institution under paragraph 1 has an implemented quality management system ensuring the safety of blood and blood components.

Article 49. The existence of the circumstances under Article 48 can be certified by:

1. licenses, permits or other documents certifying that the competent authority in the country of origin of the blood and blood components has duly authorised the said institution to provide services related to the collection, processing and storage of such products;

2. documents certifying the implementation of a quality management system for the safety of blood and blood components;

3. export license, if such is required by the laws of the country of origin of the blood and blood components;

4. documents certifying that the institution collecting, testing, processing and labelling the blood and blood components undergoes regular audits (inspections; other measures to control quality) conducted by the competent authority in the country.

Article 50. (1) Subject to importation can only be blood and blood components whose properties meet the requirements of this regulation.

(2) No blood or blood components can be imported if they do not comply with the requirements of Annex No. 7.

(3) No blood or blood components can be imported from countries in which there are registered infections that can be transmitted through blood and in which no measures are taken to ensure the safety of blood.

Article 51. The circumstances under Article 50, paragraph 1 and 2 should be certified by documents that contain the identification number of the units, the tests, diagnostic methods, processing and shelf life.

Article 52. (1) The documents referred to in Article 49 and Article 50, paragraph 3 and 4 should be submitted by the institution providing the blood or blood components, or the Bulgarian Red Cross at the Ministry of Health.

(2) The Minister of Health should appoint a commission to investigate the submitted documentation and assess the quality of the blood and blood components.

(3) The Commission should consist of not less than seven members and include two representatives of NHTC and two representatives of the Bulgarian Drug Agency and a representative of the Bulgarian Red Cross.

(4) If necessary, the Commission may require additional information for consideration of the circumstances under Article 49 and 50.

(5) The Commission should submit a proposal to the Minister of Health to issue an import authorization, indicating the distribution of the blood and blood components to the blood transfusion centres.

Art. 53. (1) The blood transfusion establishment that receives the imported blood components should verify the conformity of the imported blood and blood components with the documents specified in Article 51 and the information specified in the delivery report.

(2) The imported blood and blood components should be accepted at the blood transfusion establishments with documentation proving compliance with the storage conditions during transportation.

ADDITIONAL PROVISIONS

§ 1. For the purposes of this regulation:

1. 'Two successive collections' will mean collections of blood from one and the same person carried out within one year at the same blood transfusion establishment or ward. The results of these donations should be entered in the registry under Article 36 of BBDBTA.

2. 'Test reagents from two different sources' are:

a) monoclonal test reagents obtained from two hybridoma lines;

b) polyclonal test reagents obtained from two different manufacturing batches.

3. 'Test erythrocytes from two different sources' are the test erythrocytes obtained from two blood donors.

4. 'Small amounts of blood for testing purposes' are the blood donations taken from a donor which may not exceed 160 ml per month.

5. 'Standard blood units are units in which the blood: blood conserving solution ratio meets the recommendations of the manufacturer of plastic bags for blood collection.

6. 'Intermediate blood and blood components' are blood and blood components awaiting further processing.

7. 'Finished blood and blood components' are blood and blood components in which the technological processes have been completed and which are ready for clinical use.

§ 2. Healthcare facilities that store information at the levels of the registry referred to in Article 36, paragraph 1 of BBDBTA KK should be defined in the regulation under Article 37 of BBDBTA.

FINAL PROVISION

§ 3. This regulation is issued pursuant to Article 20, paragraph 2 of BBDBTA and becomes effective on the day of its promulgation in the State Gazette.

FINAL PROVISIONS to the regulation amending Regulation No. 18 of 2004 on the requirements and procedures for testing, processing and storage of blood and blood components, and the quality of imported blood
(SG, Issue 64 of 2011, effective as of 1 July 2011)

§ 2. This regulation transposes the provisions of Directive 2011/38/EO of the European Commission of 11 April 2011 amending Annex V to Directive 2004/33/EC with regards to maximum pH values for platelets concentrates at the end of the shelf life (OJ, L 97/28 of 12 April 2011).

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Annex No. 1

To Article 9

Requirements for the test reagents and test erythrocytes used in immuno-haematologic testing:

1. Test reagents for the testing of antigens of the ABO system may be monoclonal or polyclonal with anti-A, anti-B, anti-A+B, or anti-A,B specificity. They must possess the following properties:

a) activity – when undiluted, the test reagent should give 3 to 4 pluses reaction in the tube agglutination test with 3% erythrocyte suspension at room temperature;

b) specificity – the test reagent should have a clear response to the erythrocytes carrying the respective antigen without evidence of haemolysis, false positive or false negative reactions;

c) titre – it is tested by the method specified by the manufacturer; titres should not be lower than 128 for anti-A, anti-B and anti-AB with A and B

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test erythrocytes, and not lower than 64 with A and A B test erythrocytes; this

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parameter does not apply to monoclonal reagents.

2. In terms of origin, test reagents for testing antigens of the Rhesus system may be monoclonal or polyclonal, monospecific with anti-D, anti-C, anti-c, anti-E and anti-e specificity or polyspecific anti-C+D, anti-D+E and anti-C+D+E specificity. They must possess the following properties:

a) activity – when undiluted, the test reagent should give 3 to 4 pluses reaction with the test erythrocytes carrying the respective antigen by a method specified by the manufacturer;

b) specificity – the test reagent should have a clear response to erythrocytes carrying the respective antigen without evidence of haemolysis, false positive or false negative reactions;

c) titre - not lower than 16 with test erythrocytes with the following RH phenotype:

ca) for anti-D test reagents - ccDee and CcDee;

cb) for anti-C test Reagents - CcDee or Ccdee;

cc) for anti-E test reagents - ccDEe or ccdEe;

cd) for anti-c test reagents - CcDee or Ccdee;

ce) for anti-e test reagents - ccDEe or ccdEe.

3. The test reagents for testing antigens from other blood group systems, different from the ABO and Rh system, may be monoclonal or polyclonal. They must possess the following properties:

a) activity – when undiluted, the test reagent should give 3 to 4 pluses reaction with the test erythrocytes carrying the respective antigen by a method specified by the manufacturer;

b) specificity – the test reagent should have a clear response to the erythrocytes carrying the respective antigen, without evidence of haemolysis, false positive or false negative reactions.

4. Test erythrocytes:

a) the antigen formula of the test erythrocytes used in immuno-haematologic testing should be defined in advance with two sets of test reagents for each specificity;

b) the test erythrocytes should have the following properties:

ba) reactivity – test erythrocytes should have clear agglutination with a specific test reagent using a test method specified by the manufacturer;

bb) specificity - except for the erythrocytes sensitized with IgG and C3, all test erythrocytes should have a negative reaction in direct antiglobulin testing with a polyspecific antiglobulin test reagent;

c) the test erythrocytes used in the screening for antierythrocyte antibodies should contain antigens discovering clinically significant antibodies -

a b a b a b

C, c, D, E, e, Kк, Fy , Fy , Jk , Jk , S, s, M, N, P , Le , Le :

1

ca) it is recommended that one type of the test erythrocytes should be with an Rh haplotype cDE;

a b

cb) the test erythrocytes used should carry antigens D, E, Fy, Fy,

a b

Jk, Jk, S, in a homozygous form;

cc) mixing (pooling) of test erythrocytes from different sources should be avoided; each type of test erythrocytes should be used separately.

5. The polyspecific antihuman antiglobulin serum (AGS) contains anti-IgG and anti-C3/C3d antibodies with the following properties:

a) activity – the polyspecific AGS agglutinates erythrocytes sensitized with IgG class antibodies and complement;

b) specificity – the polyspecific AGS should not have haemolytic activity and agglutinate non-sensitized erythrocytes.

6. The saline solution is an isotonic sodium chloride solution (0.154 M/l = 9 g/l) with pH 7±0.2 at temperatures + 22°±1°C.

7. The saline solution with low ionic strength (LISS) is 0.03 M solution of NaCl, 0.003 M Na HPO: NaH PO, pH 6.7 at temperatures of +22° ±

2 4 2 4

1°C and 0.24 M Glycin. The final solution has a pH of 6.7 (from 6.5 to 7) and conductivity of 3.7 ms/cm at +22°±1°C (from 3.44 to 3.75).

8. Proteases – they should be used, controlled and stored according to the manufacturer's instructions.

9. Control of the test reagents may be carried out in the immuno-haematologic laboratory upon receipt of each delivery of test reagents and test erythrocytes, and before each series of tests.

10. Control tests for test reagents and test erythrocytes upon receipt of a delivery:

a) control of test reagents - depending on the size of the delivery, not less than 1% of the individual containers of each reagent should be tested, or at least one individual container of each test reagent; such control tests include:

aa) testing the activity of test erythrocytes from 3 different sources;

ab) testing the specificity of test erythrocytes from 3 different sources;

ac) testing the titre with test erythrocytes from 3 different sources;

b) control of test erythrocytes - the number of tested containers should be determined in accordance with the size of the delivery, but it should not be less than one individual container; the control tests include:

ba) testing the reactivity with test reagents from two sources;

bb) testing the specificity with test reagents from two sources;

c) these tests should be carried out in compliance with the methods specified by the manufacturers;

d) the results should be documented in a dedicated logbook;

e) one container of each test reagent should be stored for one month after the expiry date of the respective batch.

11. Control of test reagents and test erythrocytes prior to each series of tests:

a) the control of test reagents includes:

aa) testing their activity - with test erythrocytes from a single source;

ab) testing their specificity - with test erythrocytes from a single source;

b) the control of test erythrocytes includes:

ba) testing their activity - with test reagents from a single source;

bb) testing their specificity - with test reagents from a single source;

c) these tests should be carried out in compliance with the methods specified by the manufacturer;

d) the results should be documented in a dedicated logbook.

Annex No. 2

To Article 16, paragraph 3

Use of blood and blood components with positive screening for anti-erythrocyte antibodies

Blood / Blood component	Result after dilution		Use
	1:50		
Blood	Positive	Positive	Not to be transfused
	Negative	To be transfused but not in infants	
Packed red blood cells	Positive	Positive	Not to be transfused
	Negative	To be transfused, but not in infants	
Fresh frozen plasma	Positive	Positive or negative	Not to be transfused
	Negative	Transfused, but not in infants	
Platelet concentrate	Positive	Positive or negative	Not to be transfused
	Negative	Transfused, but not in infants	
Plasma for fractionation	Positive	Positive	No to be fractionated
	Negative	To be fractionated	

Annex No. 3

To Article 30, paragraph 2

Technological chains for standard blood sample processing depending on the duration of blood collection and the duration and temperature of storage of the donated blood before processing

Duration of blood collection in minutes	Storage and transport temperature prior to processing	Commencement of processing in hours	Blood components that may be derived
Up to 15 min	2-6°C	Within 6 hours	Packed red blood cells

16 min or more			Packed red blood cells
Up to 12 min	20-24°C		Packed red blood cells
13-15 min	Within 18 hours		Packed red blood cells
16 min or more			Packed red blood cells

Annex No. 4

To Article 30, paragraph 4, point 1

Storage of Blood Components

Blood / Blood component	Storage temperature	Storage period	Transportation temperature	Transportation period
Fresh frozen plasma	-18°C - -25°C	3 months	Similar to storage temperature	
	24 months			
Thawed plasma	Thawed at +30°C - +37°C	To be transfused immediately		

		after thawing		
Platelet concentrates – from one donated unit, aphaeresis pool	+20°C - +24°C	5 days (with continuous careful shaking) Less than 6 hours (in an open system)	Similar to storage temperature (with continuous careful shaking)	
Aphaeresis frozen platelet concentrates	Frozen platelet concentrates should be stored at: - 80°C (in an electrical freezer) -150°C (in liquid nitrogen vapour) Thawed platelet concentrates should be stored at: +20°C - +24°C with adequate shaking, where short-term storage is required	Up to 12 months More than 12 months Used immediately after thawing	Similar to storage temperature	
Packed red blood cells	+2°C - +6°C	Up to 35 days (with anticoagulant with added adenine)	+2°C - +10°C	Less than 24 hours

Packed red blood cells with additive solution	+2°C - +6°C	Depending on the coagulant and the additive solution	+2°C - +10°C	Less than 24 hours
Packed red blood cells with added solution and removed buffy-coat	+2°C - +6°C	Depending on the anticoagulant and the additive solution	+2°C - +10°C	Less than 24 hours
Packed red blood cells with reduced leukocyte count	+2°C - +6°C	Up to 35 days (with anticoagulant with added adenine)	+2°C - +10°C	Less than 24 hours
Packed red blood cells frozen by using a method with low glycerol level	-140°C - -150°C in liquid nitrogen vapour	10 years	Similar to storage temperature	Frozen packed red blood cells: as short-term as possible
	Less than 24 hours; to be used as soon as possible after thawing			
Packed red blood cells frozen by using a method with high glycerol level	-60°C - -80°C in an electrical freezer	10 years	Similar to storage temperature	Frozen packed red blood cells: as short-term as possible
	Less than 24			

	hours; to be used as soon as possible after thawing			
Washed packed red blood cells	+2°C - +6°C	Less than 24 hours and prepared at low temperatures	+2°C - +6°C	Limited by the respective storage period
Blood (to be transfused)	+2°C - +6°C	Up to 35 days (with anticoagulant with added adenine)	+2°C - +10°C	Less than 24 hours
Blood (for preparation of blood components)	+1°C - +6°C +20°C - +24°C (if used for preparation of platelets)	Up to 8 hours prior to use Up to 24 hours prior to use		

Annex No. 5

To Article 31, paragraph 2

Approximate composition of plasma derived by one of the four methods of initial centrifugation under Annex No. 6

Table 1

	I	II	III	IV	V – after filtration
Quantity in	220 – 280	220 – 280	270 – 320	270 – 330	240 – 290

ml					
Platelets	70-80%	70-80%	10-20%	10-20%	<1%
Leukocytes	5-10%	5-10%	2-5%	2-5%	<0.01%

Approximate composition of packed red blood cells derived by one of the four methods of initial centrifugation under Annex No. 6

Table 2

	I	II	III	IV	V – after filtration
Hematocrit	0.75 – 0.80	0.65 – 0.75	0.85 – 0.90	0.80 – 0.90	0.80 – 0.90
Platelets	5-15%	20-30%	10-20%	80-90%	<1%
Leukocytes	25-45%	90-95%	25-45%	95-98%	<0.01%

Approximate composition of the layer of leukocytes and platelets derived by one of the four methods of initial centrifugation under Annex No. 6

Table 3

	I	II	III	IV	V – after filtration
Hematocrit	0.50 – 0.70		0.40 – 0.60		
Erythrocytes	10-15%		10-15%		
Platelets	10-25%		80-90%		
Leukocytes	60-70%		50-70%		

Approximate composition of the platelet concentrate derived from platelet-rich plasma by further centrifugation

Table 4

Volume of suspension in ml	50-60
Platelet count	$45 – 85 \times 10^9$
Leukocyte count	$< 1.0 \times 10^9$
Erythrocyte count	$< 1.0 \times 10^9$

Annex No. 6

To Article 32, paragraph 1

Methods of initial centrifugation of blood from standard donation

(450 ml ± 10% in 63 ml starting blood conservation solution)

Parameters	Method				
	II	III	IV	V	
Primary filtration	No	No	No	No	Platelet-leukocyte filter
Centrifugation speed	Low	Low	High	High	High
Centrifugation time	4-15 minutes	4-15 minutes	4-15 minutes	4-15 minutes	4-15 minutes
Temperature	6°/22°C	6°/22°C	6°/22°C	6°	6°
Derived components	Plasma + Buffy coat + Erythrocytes	Plasma + Erythrocytes	Plasma + Buffy coat + Erythrocytes	Plasma + Erythrocytes	Plasma + Leukocyte-depleted erythrocytes

Annex No. 7

To Article 39, paragraph 1

(Amended in SG, Issue 64 of 2011, effective as of 1 July 2011)

Quality parameters of finished blood and blood components for clinical use

No.	Blood and blood components	Definition	Control parameter	Quality requirements	Control frequency	Control carried out by	
	2	3	4	5	6		
	All units of blood or blood components		ABO, Rh(D)	Blood typing	All units	Blood typing laboratory	
			Blood typing laboratory				
			Screening laboratory				
			Screening laboratory				
			Screening laboratory				
			Screening laboratory				

1.	Blood	Blood for transfusion is the blood collected from a suitable donor in a sterile non-pyrogenic container (bag) with anticoagulant	Quantity	450 ml +/- 10% quantity without anticoagulant	1% of all units with a minimum of 4 units per month	Processing unit	
		4 units per month	Control laboratory				
		4 units per month	Control laboratory				
2.	Packed red blood cells	Component derived by removal of one part of the plasma from the blood, without further processing	Quantity	280 +/- 50 ml	1% of all units	Processing unit	
			Control laboratory				
			Control laboratory				
			Control laboratory				
3.	Packed red blood cells with removed buffy coat (erythrocyte and BCR)	Component derived by removal of one part of the plasma and the buffy coat from the packed red blood cells	Quantity	250 +/- 50 ml	1% of all units	Processing unit	
			Control laboratory				
			Control laboratory				
			Control laboratory				

			Control laboratory				
4.	Packed red blood cells with additive solution (erythrocytes AS)	Component derived from blood by centrifugation and separation of the plasma with subsequent addition of a suitable culture solution to the erythrocytes	Quantity	Defined according to the used system	1% of all units	Processing unit	
			Control laboratory				
			Control laboratory				
			Control laboratory				
5.	Packed red blood cells with additive solution and removed buffy coat (erythrocytes AS-BCR)	Component derived from blood by centrifugation, separation of the plasma and the buffy coat, with subsequent re-suspension of erythrocytes in a suitable culture solution	Quantity	Defined according to the used system	1% of all units	Processing unit	
			Control laboratory				
			Control laboratory				
			Control laboratory				
			Control laboratory				
6.	Packed red blood cells, washed	Component derived from blood by	Quantity	Defined according to the used	All units	Processing unit	

		centrifugation and separation of the plasma, with subsequent washing of erythrocytes in an isotonic solution		system			
			Control laboratory				
			Control laboratory				
			Control laboratory				
			Control laboratory				
7.	Packed red blood cells, leukocyte-depleted	Component derived by removal of most leukocytes from the packed red blood cells	Residual leukocytes*	$<1 \times 10^6$ per unit	1% of all units, with a minimum of 10 units per month	Processing unit	
			Control laboratory				
			Control laboratory				
8.	Packed red blood cells, frozen	Component derived from blood by freezing the erythrocytes, preferably within 7 days from blood collection, using a cryoprotectant,	Quantity	> 185 ml	All units	Processing laboratory	
			Control laboratory				
			Control laboratory				
			Control laboratory				
			Processing laboratory				
			Control				

		stored at temperatures of -80°C or less. Prior to use, the cells should be thawed, washed and suspended in a physiological solution or an additive culture solution for packed red blood cells	laboratory				
			Control laboratory				
9.	(Amended in SG, Issue 64 of 2011, effective as of 1 July 2011) Platelet concentrate from a single blood unit	Component derived from a single blood units containing in an effective therapeutic formulation most of the starting composition of platelets	HLA or HPA (where required)	Typing	Where required	HLA laboratory	
			Processing laboratory				
			Control laboratory				
			Control laboratory				
			Control laboratory				
			Control laboratory				

10.	(Amended in SG, Issue 64 of 2011, effective as of 1 July 2011) Aphaeresis platelet concentrate	Component derived from a single blood donor by platelet aphaeresis, using automatic cell separation equipment	Quantity	> 40 ml for 60 x 10^9 platelets	All units	Processing laboratory	
			Control laboratory				
			Control laboratory				
			Control laboratory				
			HLA laboratory				
11.	Fresh frozen plasma	Component derived from blood or plasma derived by aphaeresis and frozen for a certain period, at a temperature allowing adequate maintenance of the functions of the labile clotting factors	Quantity	Specified quantity +/- 10%	All units	Processing laboratory	
			Control laboratory				
			Control laboratory				
			Processing laboratory				
			Laboratory receiving the product				

*These requirements are considered to be met, when 90% of the controlled units fall within the specified ranges.

**This quantity of total albumin guarantees that the content of Ig A is lower than 0.2 mg/ unit.

***Final suspension solution.

****It is preferable that the pH measurement should be carried out in a closed system to avoid loss of CO_2.

These measurements may be carried out at different temperature and their values recalculated and reported for pH at +22°C.

I, the undersigned Maria Georgieva Eneva, hereby certify that this is a full, true and accurate translation from Bulgarian into English of the attached document: Regulation No. 18.
The translation consists of 36 (thirty-six) pages.
Translator: Maria Georgieva Eneva