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Contents

I *Acts whose publication is obligatory*

.....

II *Acts whose publication is not obligatory*

Council

87/67/EEC:

★ Council Decision of 26 January 1987 accepting on behalf of the Community the European Agreement on the Exchange of Therapeutic Substances of Human Origin	1
European Agreement on the Exchange of Therapeutic Substances of Human Origin	2
Protocol to the European Agreement on the Exchange of Therapeutic Substances of Human Origin	4
Additional Protocol to the European Agreement on the Exchange of Therapeutic Substances of Human Origin	29

87/68/EEC:

★ Council Decision of 26 January 1987 accepting on behalf of the Community the European Agreement on the Exchange of Blood-grouping Reagents	30
European Agreement on the Exchange of Blood-grouping Reagents the signatory Governments of the member States of the Council of Europe	31
Protocol to the European Agreement on the Exchange of Blood-grouping Reagents	33
Additional Protocol to the European Agreement on the Exchange of Blood-grouping Reagents	44

II

(Acts whose publication is not obligatory)

COUNCIL

COUNCIL DECISION

of 26 January 1987

**accepting on behalf of the Community the European Agreement on the Exchange of
Therapeutic Substances of Human Origin**

(87/67/EEC)

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community, and in particular Article 28 thereof,

Having regard to the proposal from the Commission,

Whereas Article 5 (1) of the European Agreement on the Exchange of Therapeutic Substances of Human Origin provides that the Contracting Parties shall take all necessary measures to exempt from all import duties the therapeutic substances of human origin placed at their disposal by the other Parties;

Whereas any derogation from the Common Customs Tariff, whether autonomous or conventional, falls within the sole competence of the Community;

Whereas the entry into force of an additional Protocol to the Agreement enabling the European Economic Community to become a Contracting Party to that Agreement allows the Community to exercise its competence in this matter; whereas the derogations provided for in the Agreement are already granted by Community rules of relief from customs duty;

Whereas the Community ought therefore to become a Contracting Party to the Agreement,

HAS DECIDED AS FOLLOWS:

Article 1

The European Agreement on the Exchange of Therapeutic Substances of Human Origin is hereby accepted on behalf of the European Economic Community.

The text of the Agreement is attached to this Decision.

Article 2

The President of the Council is hereby authorized to designate the persons empowered to sign the Agreement in order to bind the Community.

Done at Brussels, 26 January 1987.

*For the Council
The President*

L. TINDEMANS

EUROPEAN AGREEMENT
on the Exchange of Therapeutic Substances of Human Origin

THE GOVERNMENTS SIGNATORY HERETO, BEING MEMBERS OF THE COUNCIL OF EUROPE,

Considering that therapeutic substances of human origin are by their very nature the result of an act of the human donor and therefore not available in unlimited quantities;

Considering that it is most desirable that member countries, in a spirit of European solidarity, should assist one another in the supply of these therapeutic substances, should the need arise;

Considering that such mutual assistance is only possible if the character and use of such therapeutic substances are subject to rules laid down jointly by the member countries and if the necessary import facilities and exemptions are granted,

HAVE AGREED AS FOLLOWS:

Article 1

For the purposes of this Agreement, the expression 'therapeutic substances of human origin' refers to human blood and its derivatives.

The provisions of this Agreement may be extended to cover other therapeutic substances of human origin by exchange of letters between two or more of the Contracting Parties.

Article 2

The Contracting Parties undertake, provided that they have sufficient stocks for their own needs, to make therapeutic substances of human origin available to other Parties who are in urgent need of them and to charge only those costs involved in the collection, processing and carriage of such substances.

Article 3

Therapeutic substances of human origin shall be made available to the other Contracting Parties subject to the express condition that no profit is made on them, that they shall be used solely for medical purposes and shall be delivered only to bodies designated by the governments concerned.

Article 4

The Contracting Parties shall certify that the minimum requirements with regard to the properties of the therapeutic substances, and the regulations on labelling, packing and dispatch, as laid down in the Protocol to this Agreement, have been observed.

They shall also comply with any rules to which they have subscribed with regard to international standardization in this field.

All consignments of therapeutic substances of human origin shall be accompanied by a certificate to the effect that they were prepared in accordance with the specifications in the Protocol. This certificate shall be based on the model to be found in Annex 1 to the Protocol.

The Protocol and its Annexes may be amended or supplemented by the Governments of the Parties to this Agreement.

Article 5

The Contracting Parties shall take all necessary measures to exempt from all import duties the therapeutic substances of human origin placed at their disposal by the other Parties.

They shall also take all necessary measures to provide for the speedy delivery of these substances, by the most direct route, to the consignees referred to in Article 3 of this Agreement.

Article 6

The Contracting Parties shall forward to one another, through the Secretary-General of the Council of Europe, a list of the bodies empowered to issue certificates as provided in Article 4 of this Agreement.

They shall also forward a list of bodies empowered to distribute imported therapeutic substances of human origin.

Article 7

The present Agreement shall be open to the signature of Members of the Council of Europe, who may become Parties to it either by:

- (a) signature without reservation in respect of ratification; or
- (b) signature with reservation in respect of ratification followed by ratification.

Instruments of ratification shall be deposited with the Secretary-General of the Council of Europe.

Article 8

The present Agreement shall enter into force on the first day of the month following the date on which three Members of the Council shall, in accordance with Article 7, have signed the Agreement without reservation in respect of ratification or shall have ratified it.

In the case of any Member of the Council who shall subsequently sign the Agreement without reservation in respect of ratification, or who shall ratify it, the Agreement shall enter into force on the first day of the month following such signature or deposit of the instrument of ratification.

Article 9

The Committee of Ministers of the Council of Europe may invite any non-member State to accede to the present Agreement. Such accession shall take effect on the first day of the month following the deposit of the

instrument of accession with the Secretary-General of the Council of Europe.

Article 10

The Secretary-General of the Council of Europe shall notify Members of the Council and acceding States:

- (a) of the date of entry into force of this Agreement and of the names of any Members who have signed without reservation in respect of ratification or who have ratified it;
- (b) of the deposit of any instrument of accession in accordance with Article 9;
- (c) of any notification received in accordance with Article 11 and its effective date;
- (d) of any amendment to the Protocol or its Annexes under Article 4, paragraph 4.

Article 11

The present Agreement shall remain in force indefinitely.

Any Contracting Party may terminate its own application of the Agreement by giving one year's notice to that effect to the Secretary-General of the Council of Europe.

In witness whereof the undersigned, duly authorized thereto by their respective Governments, have signed the present Agreement.

Done at Paris, this 15th day of December 1958, in the English and French languages, both texts being equally authoritative, in a single copy which shall remain deposited in the archives of the Council of Europe. The Secretary-General shall transmit certified copies to each of the signatory and acceding Governments.

PROTOCOL TO THE EUROPEAN AGREEMENT**on the Exchange of Therapeutic Substances of Human Origin****PART I****GENERAL PROVISIONS****A. LABELLING**

A label printed in English and French, based on the appropriate model to be found in Annexes 2 to 10 to the Protocol, shall be affixed to each container or giving-set.

B. PACKING AND DISPATCH

Whole human blood shall be dispatched in containers in which a temperature of 4 to 6°C is maintained throughout the period of transport.

This condition is not required for the derivatives mentioned in the Protocol.

C. PRODUCTS AND APPARATUS

The products and apparatus referred to in Part II of this Protocol shall be sterile, non-pyrogenic and non-toxic.

It is recommended that the giving-set, as well as the solvents required for the dried products, be sent with each consignment.

D. FREEDOM FROM TOXICITY OF PLASTIC BLOOD TRANSFUSION EQUIPMENT

Equipment shall comply with the provisions set out in Annex 11 to this Protocol.

PART II**SPECIFIC PROVISIONS****1. WHOLE HUMAN BLOOD**

Whole human blood is blood which has been mixed with a suitable anticoagulant, after collection from a human subject in normal health.

The blood shall not be obtained from a human subject:

- (a) who is known to be suffering from or to have suffered from syphilis; or
- (b) whose blood has not been tested with negative results for evidence of syphilitic infection; or
- (c) who is not, as far as can be ascertained after medical examination and the study of his antecedents, free from disease transmissible by blood transfusion.

The blood shall be withdrawn aseptically through a closed system of sterile tubing into a sterile container in which the anticoagulant solution has been placed before the container is sterilized. The equipment used must be pyrogen-free. When withdrawal is complete the container shall be immediately sealed and cooled to 4 to 6°C and not opened thereafter until immediately before the blood is to be used.

The blood will be collected into a citrate solution of acid reaction containing dextrose. No antiseptic or bacteriostatic substance shall be added. The volume of the anticoagulant solution must not exceed 220 ml per litre of the whole human blood and the haemoglobin concentration must not less than 97 grams per litre.

Blood group

The blood group under the AB0 system shall have been determined by examination of both corpuscles and serum and that under the Rh system by examination of the corpuscles, using a separate sample of the donor's blood. When there is a national standard, or nationally recommended technique of blood grouping, that technique shall be used.

The term Rh negative is only to be used when specific tests have shown the absence of the antigens C, D, D^u and E. All other blood must be labelled Rh positive.

Blood exchange under this agreement should only be used for recipients of the corresponding AB0 group.

Storage

Whole human blood shall be kept in a sterile container sealed so as to exclude micro-organisms and stored at a temperature of 4 to 6°C until required for use, except during any period necessary for examination and transport at higher temperatures, any such period not to exceed 30 minutes after which the blood must immediately be cooled again to 4 to 6°C.

Labelling

The label on the container shall give all the information shown on the model label (Annex 2). The Rhesus group shall be written as 'Positive' or 'Negative' or, in abbreviated form, 'POS' or 'NEG'.

1a. HUMAN RED CELL CONCENTRATE

A human red cell concentrate is a unit of whole human blood from which most of the plasma has been removed.

It contains most of the red cells of the unit from which it has been prepared; other cell components may be present or may have been partially removed.

The liquid content of the concentrate will consist either of the residual plasma, or of an appropriate isotonic artificial aqueous solution added after the plasma was removed. The volume of red cells should constitute between 65 and 75 % of the total volume of the product, but if a greater red cell concentration is applied the approximate percentage of erythrocyte volume (haematocrit) shall be indicated on the label.

All operations required in the preparation shall be carried out under aseptic conditions: decantation shall be carried out using a sterile, closed system and by compression only. No antiseptic or bacteriostatic agents should be added.

Blood group and storage

as for whole human blood.

Labelling

The label on the container shall give all the information shown on the model label (Annex 2a). The Rhesus group shall be written as 'Positive' or 'Negative' or, in abbreviated form, 'POS' or 'NEG'. If an artificial aqueous solution has been added, the label shall also indicate its volume and composition.

2. DRIED HUMAN PLASMA

Dried human plasma is prepared by drying the supernatant fluids which are separated by centrifuging or by sedimentation from quantities of whole human blood.

During preparation no antiseptic or bacteriostatic or other substance shall be added. Dried human plasma shall be obtained by freeze-drying or by any other method which will avoid denaturation of proteins. The dried product shall be readily soluble in a quantity of water equal to the volume of the liquid from which the substance was prepared. The protein concentration of the solution thus obtained must not be less than 45 grams per litre, and must not show visible evidence of the products of haemolysis. The haemagglutinin titre shall not be greater than 1 : 32.

Dried human plasma prepared from one or two donations of blood

Donations shown to contain dangerous levels of iso-haemolysins (determined using a sample of fresh serum) or any immune haemagglutinins shall be excluded. Unless the plasma is pooled and frozen within 48 hours of collecting the blood, the sterility of each unit shall be tested by culturing not less than 10 ml.

Dried human plasma prepared from pools of more than two donations

Pools shown to contain dangerous levels of immune haemagglutinins or of iso-haemolysins shall be excluded. To avoid untoward effects due to the products of bacterial growth in the plasma no individual donation shall be used if there is any evidence of bacterial contamination, and the sterility of each pool shall be tested by culturing not less than 10 ml. To minimize the risk of transmitting serum hepatitis, plasma should be prepared from pools which should contain not more than 12 donations, or by any other method that has been shown to diminish the risk in comparable manner.

Solubility in water

Add a quantity of water equal to the volume of the liquid from which the sample was prepared; the substance dissolves completely within 10 minutes at 15 to 20 °C.

Identification

Dissolve a known quantity of the product in a volume of water equal to the volume of the liquid from which it was prepared; the solution passes the following tests:

- (i) by precipitation tests with specific antisera, it must be shown to contain only human plasma proteins;
- (ii) to 1 ml add a suitable amount of thrombin or calcium chloride; coagulation occurs, which can be accelerated by incubation at 37 °C.

Loss of mass on drying

When dried over phosphorus pentoxide at a pressure not exceeding 0,02 mm of mercury for 24 hours, dried human plasma must not lose more than 0,5% of its weight.

Sterility

The final product, after reconstitution, shall be sterile when examined by a suitable bacteriological method.

Storage

Dried human plasma must be kept in an atmosphere of nitrogen or in a vacuum in a sterile container sealed so as to exclude micro-organisms and, as far as possible, moisture, protected from light and stored at a temperature below 20°C.

Labelling

The label on the container shall give all the information shown on the model label (Annex 3).

3. HUMAN ALBUMIN AND HUMAN PLASMA PROTEIN FRACTION

Human albumin and human plasma protein fraction are preparations of that protein component which forms about 60 % of the total protein mass in the plasma of whole human blood.

The method of preparation used shall be one which produces a material meeting the requirements herein described. Regardless of whether the final product is liquid or dried, the preparation, after the addition of a suitable stabilizing agent or agents, must have been heated in the liquid state in the final container at $60^{\circ}\text{C} \pm 0,5^{\circ}\text{C}$ for 10 hours, in order to inactivate the agent causing serum hepatitis. During preparation no antiseptic or bacteriostatic substance shall be added.

In preparations of human albumin, not less than 95% of the mass of the proteins present shall be albumin. In preparations of human plasma protein fraction, not less than 85% of the protein mass shall be albumin. In both preparations, more than 10 milligrams of immunoglobulin G per gram of product shall be present.

When the final product is freeze-dried, it must contain not less than 950 milligrams of protein per gram of product.

When human plasma protein fraction is prepared as a solution it shall have a total protein concentration of between 45 and 50 grams per litre.

When human albumin is prepared as a solution it shall have a total protein concentration of not less than 45 grams per litre.

Solubility of the dried product

Add water to the recommended volume; the dried preparation must be completely soluble.

Stability

By comparison of the solutions before and after heat treatment no evidence of significant denaturation of the proteins in solution shall have been detected as estimated by viscosity and turbidity measurements, ultracentrifugation and electrophoresis. The solution shall be substantially free from visible particles after

heating at 57°C and after agitation in a mechanical shaker for six hours at this temperature.

Identification

- i) By precipitation tests with specific antisera, both preparations must be shown to contain only human plasma proteins.
- ii) By electrophoresis, using the moving boundary technique under acceptable and appropriate conditions, it must be shown that the protein fraction having the mobility of the albumin component of normal human plasma, is not less than 95% of the protein mass in preparations of human albumin, or not less than 85% of the protein mass in preparations of human plasma protein fraction.

Sodium content and sodium concentration

The sodium content of salt-poor human albumin must not exceed 0,61 millimoles per gram of albumin. In other preparations of human albumin and in human plasma protein fraction, the sodium concentration must not exceed 0,15 moles per litre of solution or reconstituted dried product.

Potassium concentration

The potassium concentration of human plasma protein fraction must not exceed 2 millimoles per litre of solution or reconstituted dried product.

Acidity

The pH of either preparation shall be $6,8 \pm 0,2$ when measured at a temperature of 15 to 25°C in a solution diluted to a protein concentration of 10 grams per litre by means of a solution containing 0,15 moles sodium chloride per litre.

Loss of mass on drying

Dried preparations, when dried over phosphorus pentoxide at a pressure not exceeding 0,02 mm of mercury for 24 hours, must not lose more than 0,5% of their weight.

Sterility

The final product shall be sterile when examined by a suitable bacteriological method.

Storage

Dried human albumin must be kept in an atmosphere of nitrogen or in a vacuum in a sterile container, sealed so as to exclude micro-organisms and, as far as possible, moisture, protected from light and stored at a temperature below 20°C.

Solutions of human albumin and human plasma protein fraction must be kept in sterile containers, sealed so as to exclude micro-organisms, protected from light and stored at a temperature of 4 to 6°C.

Labelling

The label on the container shall give all the information shown on the appropriate model label (Annex 4). For solutions, the date of preparation is the date of heat treatment in the final container.

4. HUMAN NORMAL IMMUNOGLOBULIN

Human normal immunoglobulin is a preparation of the plasma proteins prepared from whole human blood, containing the antibodies of normal adults. It is obtained from pooled liquid human plasma from not less than 1 000 donors.

The method of preparation used should be one which produces a material meeting the requirements herein prescribed and which prevents the transmission of serum hepatitis by the final product. In addition the method of preparation shall be such that the antibodies contained in the starting material shall be concentrated in an adequate amount in the final product. The procedure shall be shown, for each final preparation, to be satisfactory in this respect by titrating in the starting material and in the final product antibodies to at least one virus and one bacterial toxin. The antibodies chosen shall be those for which there are recognized methods of titration.

During preparation no antiseptic or bacteriostatic substance shall be added; a suitable preservative and a stabilizing agent may be added to the final preparation to maintain bacterial sterility and stability of the final product.

The final product is issued as a solution in which the immunoglobulin concentration shall be between 100 and 170 grams per litre.

Identification

- (i) By precipitation tests with specific antisera, it must be shown to contain only human plasma proteins.
- (ii) By electrophoresis, using the moving boundary technique under acceptable and appropriate conditions, not less than 90% of the mass of the proteins have the mobility of the gamma component of the globulins of normal human plasma.

Stability

Both before and after heating the final solution at 37°C for seven days there should be no visible evidence of precipitation or turbidity. It is advisable also to carry out tests using an ultracentrifugation

method to determine the extent of degradation of the product to smaller molecular weight components. The method used should be one approved by the national control authority.

Acidity

The pH of the final solution shall be $6,8 \pm 0,4$ when measured at a temperature of 15 to 25°C in a solution diluted to a protein concentration of 10 grams per litre by means of a solution containing 0,15 moles sodium chloride per litre.

Sterility

The final product shall be sterile when examined by a suitable bacteriological method.

Storage

Human immunoglobulin solution must be kept in a sterile container, sealed so as to exclude micro-organisms, protected from light and stored at a temperature of 4 to 6°C.

Labelling

The label on the container shall give all the information shown on the model label (Annex 5). The date of preparation is the date of filling the final container.

5. HUMAN SPECIFIC IMMUNOGLOBULINS

Human specific immunoglobulins contain antibodies against designated viral or bacterial agents. Therefore they may be prepared from pools of a limited number of donations.

The following human specific immunoglobulins are included in these requirements:

- Human immunoglobulin anti-tetanus
- Human immunoglobulin anti-vaccinia.

Other specific immunoglobulins may be developed and when the appropriate international standard is in existence, they should be assayed in relation to that standard and their potency expressed in international units.

Human immunoglobulin anti-vaccinia shall contain not less than 500 IU per ml of vaccinia antibody as determined by a neutralization test on chorio-allantoic membranes or in tissue culture. Human immunoglobulin anti-tetanus shall contain not less than 50 IU per ml of tetanus antitoxin as determined by a neutralization test in animals.

Human specific immunoglobulins must further meet the requirements as described in section 4, Human normal immunoglobulin.

Depending on the antibody content, the immunoglobulin concentration of the final solution may vary between 100 and 170 grams per litre.

Labelling

The label on the container shall give all the information shown on the model label (Annex 5). In addition the label shall state the potency in international units in terms of the appropriate International Standard or International Reference Preparation.

6. DRIED HUMAN FIBRINOGEN

Dried human fibrinogen is a dried preparation which contains the soluble constituent of liquid human plasma which, on the addition of thrombin, is transformed to fibrin. The method of preparation used should be one which produces a material meeting the requirements herein prescribed and which minimizes the risk of transmitting serum hepatitis. Plasma pools used in the preparation of fibrinogen should contain as few donations as possible.

During preparation no antiseptic or bacteriostatic substance shall be added. The final product shall be freeze-dried.

Solubility

Add water to the recommended volume; the dried preparation must be completely soluble. No precipitation shall occur within 60 minutes of reconstitution.

Identification

- (i) By precipitation tests with specific antisera, it must be shown to contain only human plasma proteins.
- (ii) The freshly reconstituted product has the property of clotting on the addition of thrombin. When thrombin is added to a solution of human fibrinogen of the same concentration as that in fresh normal plasma, clotting shall occur in not more than twice the time taken for clotting to occur in fresh normal plasma after the addition of thrombin.
- (iii) Clottable protein. Not less than 50% of the total protein shall be clottable by thrombin.

Loss of mass on drying

Preparations, when dried over phosphorus pentoxide at a pressure not exceeding 0,02 mm of mercury for 24 hours, must not lose more than 0,3% of their weight.

Sterility

The final product after reconstitution shall be sterile when examined by a suitable bacteriological method.

Storage

Human fibrinogen shall be kept in an atmosphere of nitrogen or in a vacuum in a sterile container, sealed so as to exclude micro-organisms and, as far as possible, moisture, protected from light and stored at the temperature recommended.

Labelling

The label on the container shall give all the information shown on the model label (Annex 6). The date of preparation is the date of placing into final solution before freeze-drying.

7. DRIED OR FROZEN HUMAN COAGULATION FACTOR VIII

I. Requirements applying to donors

Donors must be in good health and, in particular, free of any communicable disease, in accordance with the criteria adopted for dried human plasma.

II. Requirements applying to preparations

Sterility and atoxicity

The final product must be sterile and pyrogen-free. Where cryoprecipitation is performed in plastic bags, the product must not contain organic solvent or other foreign substances present in the freezing mixture. The passage of such products through the walls of the plastic bag can be prevented by placing the bag in a second impermeable bag during the whole period of immersion. The risk of the plastic bag tearing during storage in the frozen state can be reduced by keeping each bag in a protective box.

Erythrocytes, leukocytes and platelets

Centrifuging should be such as to eliminate the formed elements of the blood as soon and as completely as possible after its collection.

Solubility

The addition of the indicated quantity of appropriate solvent must result in the complete solution of the dry product in less than 30 minutes at

37°C. Small and easily separable aggregates of fibrinogen may persist.

Stability

The preparation conserved at 20°C, must not show any sign of precipitation within three hours after it has been dissolved.

Potency

The reconstituted preparation should contain the indicated minimum quantity of factor VIII, one unit corresponding to the potency of 1 ml of average normal fresh plasma, the potency being determined by a method approved by the competent national authority.

Absence of irregular antibodies and, if the preparation is intended for patients of any ABO group, a titre of anti-A and anti-B antibodies not exceeding 32.

Identification

Precipitation tests with specific antisera shall show that the product contains only human plasma proteins.

Loss of mass on drying

Freeze-dried preparations, when dried over phosphorus pentoxide at a pressure not exceeding 0,02 mm of mercury for 24 hours must not lose more than 1,5 % of their weight.

Storage

Human factor VIII shall be stored in the deep-frozen state at a temperature under -30°C, and in the freeze-dried state below 5°C, and protected from light. The dried preparation shall be kept in an atmosphere of nitrogen or *in vacuo*, in a sterile vial, stoppered so as to exclude all micro-organisms and, as far as possible, all humidity. Storage in the frozen state shall not exceed six months, in the dried state one year, unless the preparation has been retested for minimum required potency.

III. Labelling

The label on the preparation shall give all the information shown on the model label (Annex 7).

8. DRIED HUMAN COAGULATION FACTOR IX

I. Requirements applying to donors

Donors must be in good health and, in particular, free from any communicable disease in accordance with the criteria adopted for dried human plasma.

II. Requirements applying to the concentrate

Sterility and atoxicity

The final product, tested by appropriate methods must be sterile, pyrogen-free and free from undesirable vaso-depressor or respiratory effects. The test for absence of vaso-depressor effects should be performed on a dog or cat.

Solubility

The addition of the indicated quantity of the solvent must result in complete solution in 10 minutes at 37°C.

Thromboplastin activity and absence of free thrombin

The recalcification time of a normal plasma measured at 37°C in the presence of an equal volume of various dilutions of the reconstituted product, must not be less than 40 seconds. The reconstituted product, with an equal volume of fibrinogen (3 g/l) added to it, must not coagulate within six hours at 37°C.

Potency

The reconstituted preparation must contain the indicated minimum quantity of factor IX, one unit corresponding to the potency of 1 ml of average normal fresh plasma, the potency being determined by a method approved by the competent national authority.

Yield and stability in vivo

The method of preparation must be such that the injection of a dose of 50 units per kg body weight, rapidly administered intravenously, using several batches of material given to several patients, shall cause, in 15 minutes, in the absence of a specific inhibitor and in basal conditions, an average rise of not less than 300 units per litre of plasma, and of the persistence, after 24 hours; of an average rise of not less than 60 units per litre of plasma.

Identification

Precipitation tests with specific antisera shall show that the product contains solely human plasma proteins.

Loss of mass on drying

When dried over phosphorus pentoxide at a pressure not exceeding 0,02 mm of mercury for 24 hours, the product must not lose more than 1,5 % of its weight.

Storage

The preparations must be stored dry at a temperature below 5°C. The period of storage

must not exceed two years, unless the potency of the preparation has been retested.

III. Labelling

The label on the preparation shall give all the information shown on the model label (Annex 8).

ANNEXE 1 AU PROTOCOLE
ANNEX 1 TO THE PROTOCOL

CONSEIL DE L'EUROPE
 COUNCIL OF EUROPE

ACCORD EUROPÉEN RELATIF À L'ÉCHANGE DE SUBSTANCES
 THÉRAPEUTIQUES D'ORIGINE HUMAINE

EUROPEAN AGREEMENT ON THE EXCHANGE OF THERAPEUTIC
 SUBSTANCES OF HUMAN ORIGIN

CERTIFICAT

(Article 4)

CERTIFICATE

À NE PAS DÉTACHER DE L'ENVOI
 NOT TO BE SEPARATED FROM THE SHIPMENT

	 19
	(lieu) (date)
	(place)
Nombre de colis Number of packages	Le soussigné déclare que l'envoi spécifié en marge The undersigned certifies that the shipment in the margin
.....	
.....	
Désignation Marked	préparé sous la responsabilité de
	prepared under the responsibility of
.....	
.....	
N° des lots Batch No	organisme visé à l'article 6 de l'accord, est conforme aux spécifications du protocole à l'accord et qu'il peut être délivré immédiatement au destinataire
.....	(nom et lieu)
.....	one of the bodies referred to in Article 6 of the Agreement, is in conformity with the specifications of the Protocol to the Agreement and can be delivered imme- diately to the consignee
	(name and place)
	
	
	(cachet) (signature) (titre)
	(stamp) (signature) (title)

10. Ne pas utiliser en cas de signe visible quelconque d'altération.
Not to be used if there is any visible evidence of deterioration.

ANNEXE 2 bis AU PROTOCOLE
ANNEX 2a TO THE PROTOCOL

CONSEIL DE L'EUROPE
 COUNCIL OF EUROPE

ACCORD EUROPÉEN RELATIF À L'ÉCHANGE DE SUBSTANCES
 THÉRAPEUTIQUES D'ORIGINE HUMAINE

EUROPEAN AGREEMENT ON THE EXCHANGE OF THERAPEUTIC
 SUBSTANCES OF HUMAN ORIGIN

1. Nom et adresse du producteur:
 Name and address of the producer:
2. Concentré de globules rouges humains
 Human red cell concentrate
3. Numéro de référence:
 Reference number:
4. Groupe sanguin:
 Blood group:
5. Groupe Rh:
 Rh group:
6. ml préparé à partir de ml de sang.
 ml prepared from ml of blood.
7. Volume et composition de l'anti-coagulant utilisé:
 Volume and composition of anti-coagulant used:
8. Date de prélèvement:
 Date of collection:
 Date de préparation:
 Date of preparation:
 Date de péremption:
 Date of expiry:
9. Conserver de 2 à 6 °C.
 Store at 2 to 6 °C.
10. Soluté artificiel ajouté volume:
 Artificial aqueous solution added composition:

ANNEXE 3 AU PROTOCOLE
ANNEX 3 TO THE PROTOCOL

CONSEIL DE L'EUROPE
 COUNCIL OF EUROPE

ACCORD EUROPÉEN RELATIF À L'ÉCHANGE DE SUBSTANCES
 THÉRAPEUTIQUES D'ORIGINE HUMAINE

EUROPEAN AGREEMENT ON THE EXCHANGE OF THERAPEUTIC
 SUBSTANCES OF HUMAN ORIGIN

1. Nom et adresse du producteur:
 Name and address of the producer:
 2. Plasma humain desséché:
 Dried human plasma:
 3. Numéro de référence:
 Reference number:
 4. Reconstituer avec ml d'eau distillée, stérile et apyrogène.
 Reconstitute with ml sterile, pyrogen-free, distilled water.
 5. Le plasma reconstitué contient:
 The reconstituted plasma contains:
 g glucose/l
 mole citrate disodique/l
 disodium citrate/l
 g/l concentration de protéines (au moins)
 protein concentration (at least)
 6. Nombre de prélèvements individuels dans le mélange:
 Number of individual donations in pool:
 7. Date de préparation:
 Date of preparation:
 Date de péremption:
 Date of expiry:
8. Protéger de la lumière et conserver à une température inférieure à 20 °C.
 Store, protected from light, below 20 °C.
 9. À utiliser immédiatement après la reconstitution.
 To be used immediately after reconstitution.

ANNEXE 4 AU PROTOCOLE
ANNEX 4 TO THE PROTOCOL

CONSEIL DE L'EUROPE
COUNCIL OF EUROPE

ACCORD EUROPÉEN RELATIF À L'ÉCHANGE DE SUBSTANCES
THÉRAPEUTIQUES D'ORIGINE HUMAINE

EUROPEAN AGREEMENT ON THE EXCHANGE OF THERAPEUTIC
SUBSTANCES OF HUMAN ORIGIN

1. Nom et adresse du producteur:

Name and address of the producer:

2. Albumine humaine desséchée
Dried human albumin

3. Numéro du lot:

Batch number:

4. Albumine: g
Albumin:

Stabilisateur: nature, g/l (en solution reconstituée)
Stabilizer: (in reconstituted solution)

Sodium mmol/g (d'albumine)
(albumin)

5. Date de préparation:

Date of preparation:

Date de péremption:

Date of expiry:

6. Reconstituer avec ml d'eau distillée, stérile et apyrogène.

Reconstituted with ml sterile, pyrogen-free, distilled water.

7. Protéger de la lumière et conserver à une température inférieure à 20°C.
Store, protected from light, below 20°C.

8. À injecter immédiatement après reconstitution.
To be used immediately after reconstitution.

ANNEXE 4 (suite 1)
ANNEX 4 (continued 1)

CONSEIL DE L'EUROPE
COUNCIL OF EUROPE

ACCORD EUROPÉEN RELATIF À L'ÉCHANGE DE SUBSTANCES
THÉRAPEUTIQUES D'ORIGINE HUMAINE

EUROPEAN AGREEMENT ON THE EXCHANGE OF THERAPEUTIC
SUBSTANCES OF HUMAN ORIGIN

1. Nom et adresse du producteur:
Name and address of the producer:
 2. Solution d'albumine humaine ml
Human albumin solution
 3. Numéro du lot:
Batch number:
 4. Albumine: g/l
Albumin:

Stabilisateur: nature g/l
Stabilizer:

Sodium: mmol/g (d'albumine)
(albumin)
 5. Date de préparation:
Date of preparation:

Date de péremption:
Date of expiry:
6. Protéger de la lumière et conserver de 4 à 6 °C.
Store, protected from light, at 4 to 6 °C.
 7. À injecter seulement si le liquide est clair et sans dépôt.
Not to be used unless clear and free from deposits.

ANNEXE 4 (suite 2)
ANNEX 4 (continued 2)

CONSEIL DE L'EUROPE
 COUNCIL OF EUROPE

ACCORD EUROPÉEN RELATIF À L'ÉCHANGE DE SUBSTANCES
 THÉRAPEUTIQUES D'ORIGINE HUMAINE

EUROPEAN AGREEMENT ON THE EXCHANGE OF THERAPEUTIC
 SUBSTANCES OF HUMAN ORIGIN

1. Nom et adresse du producteur:
 Name and address of the producer:
2. Solution stable de protéines plasmatiques humaines: ml
 Plasma protein fraction:
3. Numéro du lot:
 Batch number:
4. Albumine: g/l
 Albumin:
 Stabilisateur: nature, g/l
 Stabilizer:
 Sodium: mmol/l
5. Date de préparation:
 Date of preparation:
 Date de péremption:
 Date of expiry:

- | |
|--|
| <ol style="list-style-type: none"> 6. Protéger de la lumière et conserver de 4 à 6°C.
 Store, protected from light, at 4 to 6°C. 7. À injecter seulement si le liquide est clair et sans dépôt.
 Not to be used unless clear and free from deposits. |
|--|

ANNEXE 5 AU PROTOCOLE
ANNEX 5 TO THE PROTOCOL

CONSEIL DE L'EUROPE
COUNCIL OF EUROPE

ACCORD EUROPÉEN RELATIF À L'ÉCHANGE DE SUBSTANCES
THÉRAPEUTIQUES D'ORIGINE HUMAINE

EUROPEAN AGREEMENT ON THE EXCHANGE OF THERAPEUTIC
SUBSTANCES OF HUMAN ORIGIN

1. Nom et adresse du producteur:

Name and address of the producer:

2. Immunoglobuline humaine normale
Human normal immunoglobulin

3. Numéro du lot:

Batch number:

4. Protéines totales: g/l
Total protein:

Autres substances ajoutées: nature, g/l
Other material introduced:,

Volume total: ml
Total volume:

5. Date de préparation:

Date of preparation:

Date de péremption:

Date of expiry:

6. Protéger de la lumière et conserver de 4° à 6°C.
Store, protected from light, at 4 to 6°C.

7. Ne pas injecter par voie intraveineuse.
Not for intravenous injection.

ANNEXE 6 AU PROTOCOLE
ANNEX 6 TO THE PROTOCOL

CONSEIL DE L'EUROPE
 COUNCIL OF EUROPE

ACCORD EUROPÉEN RELATIF À L'ÉCHANGE DE SUBSTANCES
 THÉRAPEUTIQUES D'ORIGINE HUMAINE

EUROPEAN AGREEMENT ON THE EXCHANGE OF THERAPEUTIC
 SUBSTANCES OF HUMAN ORIGIN

1. Nom et adresse du producteur:
 Name and address of the producer:
 2. Fibrinogène humain desséché
 Dried human fibrinogen
 3. Numéro du lot:
 Batch number:
 4. Protéine coagulable: g
 Clottable protein: g
 Autres substances ajoutées: nature, g/l de la solution reconstituée.
 Other material introduced: g/l reconstituted solution.
 5. Date de préparation:
 Date of preparation:
 Date de péremption:
 Date of expiry:
 6. Reconstituer avec ml d'eau distillée, stérile et apyrogène.
 Reconstitute with ml sterile, pyrogen-free, distilled water.
 7. Nombre de prélèvements individuels dans le mélange:
 Number of individual donations in pool:
8. Protéger de la lumière et conserver à une température inférieure à 20°C.
 Store, protected from light, below 20°C.
 9. À injecter immédiatement après la reconstitution.
 To be used immediately after reconstitution.

ANNEXE 7 AU PROTOCOLE
ANNEX 7 TO THE PROTOCOL

CONSEIL DE L'EUROPE
 COUNCIL OF EUROPE

ACCORD EUROPÉEN RELATIF À L'ÉCHANGE DE SUBSTANCES
 THÉRAPEUTIQUES D'ORIGINE HUMAINE

EUROPEAN AGREEMENT ON THE EXCHANGE OF THERAPEUTIC
 SUBSTANCES OF HUMAN ORIGIN

1. Nom et adresse du producteur:
 Name and address of the producer:
2. Facteur VIII de coagulation humain congelé ou
 Facteur VIII de coagulation humain desséché
 Frozen human coagulation factor VIII or
 Dried human coagulation factor VIII
 Méthode de préparation:
 Method of preparation:
3. Numéro du lot:
 Batch number:
4. Quantité minimale de facteur VIII, quantité de protéines totales, nature et quantité de toute
 substance ajoutée:
 Minimum quantity of factor VIII, quantity of total proteins, nature and quantity of any added
 substance:
5. Nature et volume du solvant:
 Nature and volume of solvent:
6. Nombre de donneurs par lot:
 Number of donors per batch:
7. Titre des hématagglutinines non supérieur à 1 : 32 ou Groupe sanguin AB0
 Haemagglutinin titre not greater than 1 : 32 or AB0 blood group
8. Date de préparation:
 Date of preparation:
9. Date de péremption:
 Date of expiry:
10. Protéger de la lumière et conserver congelé à une température inférieure à -30°C ou desséché à
 une température inférieure à 5°C .
 Store, protected from light and frozen at a temperature below -30°C or in the dry state at a
 temperature below 5°C .
11. Après reconstitution du produit, injecter immédiatement par voie intraveineuse ou au plus tard
 après 3 heures de conservation à 20°C .
 After reconstitution of the product, inject intravenously, immediately or at the latest after three
 hours of storage at 20°C .

ANNEXE 8 AU PROTOCOLE
ANNEX 8 TO THE PROTOCOL

CONSEIL DE L'EUROPE
COUNCIL OF EUROPE

ACCORD EUROPÉEN RELATIF À L'ÉCHANGE DE SUBSTANCES
THÉRAPEUTIQUES D'ORIGINE HUMAINE

EUROPEAN AGREEMENT ON THE EXCHANGE OF THERAPEUTIC
SUBSTANCES OF HUMAN ORIGIN

1. Nom et adresse du producteur:
.....
Name and address of the producer:
.....
 2. Facteur IX de coagulation humain desséché:
Autres facteurs de coagulation présents:
Dried human coagulation factor IX:
Other blood coagulation factors present:
Méthode de préparation:
Method of preparation:
 3. Numéro du lot:
Batch number:
 4. Quantité minimale de facteur IX, quantité de protéines totales, nature et quantité de toute substance ajoutée:
Minimum quantity of factor IX, quantity of total proteins, nature and quantity of any added substance:
 5. Nature et volume du solvant:
Nature and volume of solvent:
 6. Nombre de donneurs par lot:
Number of donors per batch:
 7. Date de préparation:
Date of preparation:
 8. Date de péremption:
Date of expiry:
9. Protéger de la lumière et conserver à une température inférieure à 5°C.
Store, protected from light, at a temperature below 5°C.
 10. Après reconstitution du produit, injecter immédiatement par voie intraveineuse.
After reconstitution of the product, inject immediately by the intravenous route.

ANNEXE 9 AU PROTOCOLE
ANNEX 9 TO THE PROTOCOL

CONSEIL DE L'EUROPE
COUNCIL OF EUROPE

ACCORD EUROPÉEN RELATIF À L'ÉCHANGE DE SUBSTANCES
THÉRAPEUTIQUES D'ORIGINE HUMAINE

EUROPEAN AGREEMENT ON THE EXCHANGE OF THERAPEUTIC
SUBSTANCES OF HUMAN ORIGIN

1. Nom et adresse du producteur:

Name and address of the producer:

2. Eau distillée, stérile et apyrogène
Sterile, pyrogen-free distilled water

Pour la reconstitution du plasma humain desséché
de l'albumine humaine desséchée
du fibrinogène humain desséché
ou des facteurs VIII et IX humains de coagulation desséchés

For the reconstitution of dried human plasma
of dried human albumin
of dried human fibrinogen
or dried human coagulation factors VIII and IX

3. Quantité: ml
Quantity:

ANNEXE 10 AU PROTOCOLE
ANNEX 10 TO THE PROTOCOL

CONSEIL DE L'EUROPE
COUNCIL OF EUROPE

ACCORD EUROPÉEN RELATIF À L'ÉCHANGE DE SUBSTANCES
THÉRAPEUTIQUES D'ORIGINE HUMAINE

EUROPEAN AGREEMENT ON THE EXCHANGE OF THERAPEUTIC
SUBSTANCES OF HUMAN ORIGIN

1. Nom et adresse du producteur:

Name and address of the producer:

2. Dispositif à injection
Giving-set

Dispositif pour l'administration du sang humain total, du plasma humain desséché reconstitué, de l'albumine humaine, des solutions stables de protéines plasmatiques humaines, du fibrinogène humain ou du facteur VIII de coagulation humain congelé ou desséché ou du facteur IX de coagulation humain desséché.

Giving-set for the administration of whole human blood, reconstituted dried human plasma, human albumin, human plasma protein fraction, human fibrinogen or of dried or frozen human coagulation factor VIII or dried human coagulation factor IX.

**ANNEX II TO THE PROTOCOL
COUNCIL OF EUROPE**

**EUROPEAN AGREEMENT ON THE EXCHANGE OF THERAPEUTIC
SUBSTANCES OF HUMAN ORIGIN**

FREEDOM FROM TOXICITY OF PLASTIC BLOOD-TRANSFUSION EQUIPMENT

I. CHEMICAL TESTS

The tests are intended to be applied to plastic blood-transfusion equipment. This equipment consists of two main categories:

1. plastic containers for the collection, separation and storage of blood and blood products;
2. plastic sets for taking and giving blood.

The tests shall be carried out on the materials after they have been sterilized by the method to be used in the final sterilization of the equipment. These materials shall include:

1. the plastics used to make the containers;
2. the tubing used in the containers; and
3. the blood-taking and -giving sets.

The tests on containers shall be carried out before the containers are filled with anticoagulant solution. However, if the tests are carried out on containers which have been filled with anticoagulant solution, the limit tests in Section III on the anticoagulant solution itself shall be taken into account when evaluating the results of the tests on the container.

The manufacturer of the transfusion equipment is required to disclose to the appropriate health authority the detailed formulations of the plastic material or materials and other materials used in the manufacture of the equipment, the source of the components of the material or materials and their methods of manufacture (or alternatively, the compound reference numbers), details of manufacture of the equipment, the nature of any processing additives and adhesives and the method of sterilization. No change shall be permitted in any of the foregoing without prior submission to and approval of the appropriate health authority.

Each batch of raw material used in the manufacture of the equipment shall be identified by a batch number, which shall be recorded by the manufacturer of the equipment together with the identification numbers of all batches of transfusion equipment made from it and the results of all tests relevant to these batches.

Every practicable precaution must be taken to reduce the risk of adventitious contamination at each stage of the manufacturing process.

A. Preparation of extract and blank

- (a) A total test as described below requires 1 250 cm² plastic (total surface area, both sides, of a plastic sample in sheet form with surface area of 625 cm²). The sample — without any printing or label on it — should be cut into pieces of not more than 10 cm².

For tubing the length (L) in cm is calculated as follows:

$$L = \frac{1\,250}{3,14 (D_1 + D_2)}$$

Where:

D_1 = inner diameter in cm,

D_2 = outer diameter in cm.

The tubing should be cut lengthwise into sections measuring approximately 10 cm. For the extraction 10 ml of water is used per 50 cm² of surface area.

- (b) The pieces of plastic film or tubing should be placed in a container of borosilicate glass with 250 ml pyrogen-free distilled water obtained from an efficient still having glass condensation surfaces and collecting tubes⁽¹⁾. The opening of the container is covered with an inverted beaker and the container is then heated in saturated steam at 110°C for 30 minutes (autoclaving) and then quickly cooled to room temperature and the volume adjusted to 250 ml with pyrogen-free distilled water. It is of no significance if the plastic specimens tend to stick together slightly.

Heat-sensitive plastic material, instead of being heated in an autoclave, may be heated at 70°C for 72 hours.

A blank preparation is made in a corresponding manner omitting the plastic.

⁽¹⁾ If the plastic has been in contact with an anticoagulant solution, the pieces should first be placed in a similar container with cold distilled water (100 ml) and shaken several times. This should be repeated once.

B. Tests on the extract**1. Oxidizable matter**

To 20 ml of the extract in an Erlenmeyer flask of borosilicate glass add 20 ml of 2 millimole potassium permanganate solution per litre and 1,0 ml of 1 mole sulphuric acid per litre and boil the mixture for three minutes. Cool the solution rapidly and add 0,1 g of potassium iodide and five drops of starch solution. Titrate with a solution containing 10 millimole sodium thiosulphate per litre. At the same time carry out a blank titration. The difference in the volume of thiosulphate used in the two titrations does not exceed 2,00 ml of a solution containing 10 millimole sodium thiosulphate per litre.

2. Chloride

The extract complies with a suitable limit test for chloride equivalent to not more than 11,2 μ mole chloride per litre.

3. Ammonia

The extract complies with a suitable limit test for ammonia equivalent to not more than 120 μ mole NH_3 per litre.

4. Phosphoric acid — phosphate

The extract complies with the limit test for phosphate.

Limit test for phosphate

Evaporate 25 ml of the extract almost to dryness in a Kjeldahl flask, cool the residue, add two drops sulphuric acid and 1 ml nitric acid, heat the mixture until white fumes appear, then cool. Add one drop of perchloric acid and heat gently for half an hour. Cool the residue and add water to 25 ml. Transfer 10 ml of the solution to a 25 ml titration flask, add 8 ml ammonium molybdate-sulphuric acid solution and 2 ml of freshly prepared solution of ascorbic acid, having a concentration of 100 g/l. Heat on a water bath at 50°C for 30 minutes, cool and dilute the mixture to 25 ml. The green or blue colour of the solution is not more intense than that obtained by treating 25 ml of the blank solution in the same manner.

5. Acidity or alkalinity

10 ml of the extract is not coloured red on the addition of two drops of phenolphthalein solution and requires not more than 0,4 ml solution containing 10 millimole sodium hydroxide per litre to produce a red colour. After removal of the colour by the addition of 0,08 ml solution containing 10 millimole hydrochloric acid per litre, the addition of five drops of methyl red solution produces a red or orange-red colour.

6. Residue on evaporation

Evaporate 100 ml of the extract to dryness on a water bath and dry at 105°C to constant weight. The residue weighs not more than 5,0 mg.

7. Clarity and colour

The extract when viewed through a thickness of 5 cm is clear and colourless when compared with the blank.

8. Taste and smell

The extract compared with the blank is odourless and tasteless.

9. Special elements

The extract complies with suitable limit tests for:

(i) any of the following elements: arsenic, chromium, copper, lead, silicon, silver and tin, equivalent to 1 μ g/g;

(ii) cadmium, equivalent to 0,1 μ g/g.

10. Residue on ignition

1,0 g of the plastic material when ignited to constant weight leaves not more than 1 mg of residue.

11. Heavy metals

Dissolve the residue on ignition in the minimum quantity of a solution of 2 mole hydrochloric acid per litre, heating if necessary. Carry out a suitable limit test for heavy metals. The plastic material complies with a limit not exceeding 5 micrograms per gram as calculated as Pb.

II. BIOLOGICAL TESTS

1. A test for undue toxicity shall be carried out in the initial evaluation of plastic formulations intended for the fabrication of containers and taking- and giving-sets, using extract A, and on each new batch of materials of the approved formulations, using extract B, by the procedure specified in the national pharmacopoeia or some other method approved by the national control authority. (Extracts A and B are defined in the note below.)

2. A test for freedom from pyrogens shall be carried out in the initial evaluation of plastic formulations intended for the fabrication of containers and taking- and giving-sets, using extract A, and on each new batch of materials of the approved formulation, using extract C, and in the routine control of containers and taking- and giving-sets, using extract C, by the procedure specified in the national pharmacopoeia or some other method approved by the national control authority.

The incidence of pyrogen testing, using extract C, shall be decided by the national control authority. (Extracts A and C are defined in the note below.)

3. A test for haemolytic effects in buffered systems shall be performed in the initial evaluation of plastic formulations intended for the fabrication of containers and taking- and giving-sets and on each new batch of materials of the approved formulations using the extract described in para-

graph I. A above. (For method and acceptable limit, see Appendix to the present Annex.)

4. A test for the *in vivo* survival of red cells shall be carried out in the initial evaluation of plastic formulations intended for the fabrication of containers for blood. If any change is made in the agreed formulation, the test shall be repeated. (For suggested methods and acceptable limit, see Appendix to the present Annex.)

Note:

Extract A

is prepared by adding to the extract described in I. A above pyrogen-free sodium chloride to a final concentration of 9 grams per litre.

Extract B:

Transfusion set. Fill a transfusion set as completely as possible with sterile pyrogen-free solution containing 9 grams sodium chloride per litre, clamp the ends securely and immerse the filled set completely for one hour in water maintained at 85°C.

Plastic container. If the container is filled with anti-coagulant solution it should be emptied and rinsed twice with 250-ml portions of sterile pyrogen-free distilled water at a temperature of 20°C. Fill the container with 100 ml sterile pyrogen-free solution containing 9 grams sodium chloride per litre, close it securely and immerse it for one hour in a horizontal position in water maintained at 85°C. Collect the contents of the container.

Extract C:

Transfusion set. Pass 40-ml portions of sterile pyrogen-free sodium chloride solution of a concentration of 9 grams per litre, at room temperature through not less than 10 transfusion sets at a flow rate of approximately 10 ml per minute and pool the effluents. Test the solution obtained.

Plastic container. Empty. Pass 100-ml portions of sterile pyrogen-free solution containing 9,0 grams sodium chloride per litre, at room temperature through the collecting tubes of not less than four plastic containers, allow to remain in the containers for 10 minutes and pool the effluent by discharging through the transfer tubes. Test the solution obtained.

Plastic container with anticoagulant (See paragraph III).

III. REQUIREMENTS FOR ANTICOAGULANT SOLUTION IN PLASTICS CONTAINERS

Each container shall contain the quantity and formulation of anticoagulant solution indicated on the label for the volume of blood to be collected.

The anticoagulant solution and/or the ingredients used in its preparation shall satisfy the requirements of the national pharmacopoeia of the country concerned.

The anticoagulant solution shall satisfy the requirements of the national pharmacopoeia of the country concerned with regard to limits for heavy metals, the absence of particulate matter, freedom from toxicity and pyrogenicity.

Appendix

BIOLOGICAL TEST: LIMITS AND METHODS

A. Test for undue toxicity

(See Item II, 1 of Annex above): limit as specified in national pharmacopoeia.

B. Test for freedom from pyrogens

(See Item II, 2 of Annex above): limit as specified in national pharmacopoeia.

C. Test for haemolytic effects in buffered systems

(See Item II, 3 of Annex above):

(a) *Limit:*

A salt solution equivalent to a solution containing 5,0 grams NaCl per litre, in so far as electrolyte osmotic action is concerned, shall not produce a haemolysis value higher than 10% and a salt solution of 4,0 grams per litre shall not differ by more than 10% in haemolysis value from that caused by the corresponding control solution.

(b) *Method:*

From the primary buffer stock solution for haemolysis three solutions are prepared: 30 ml buffer stock solution and 10 ml water (solution a_0), 30 ml buffer stock solution and 20 ml water (solution b_0) and 15 ml buffer stock solution and 85 ml water (solution c_0).

To each of three centrifuge tubes (1, 2 and 3), 1,40 ml extract is added. To tube 1 is added 0,10 ml a_0 , to tube 2, 0,10 ml b_0 and to tube 3, 0,10 ml c_0 , thus obtaining salt solutions equivalent to solutions containing 5,0 (tube 1), 4,0 (tube 2) and 1,0 grams NaCl per litre (tube 3) in so far as electrolyte osmotic action is concerned. To each tube is added 20 μ l fresh, well mixed heparinized human blood. The tubes are put into a water bath at 30°C ($\pm 1^\circ$ C) for 40 minutes. Then three solutions containing 3,0 ml a_0 and 12,0 ml water (solution a_1), 4,0 ml b_0 and 11,0 ml water (solution b_1), and 4,75 ml b_0 and 10,25 ml water (solution c_1) are prepared.

To the first tube is added 1,50 ml of a_1 , to the second 1,50 ml of b_1 and to the third 1,50 ml of c_1 . The tubes are centrifuged for five minutes at 2 000 to 2 500 rpm in a swing-out centrifuge. Concurrently, control solutions, in which the extract is replaced with water, are prepared for each of the concentrations.

The extinction at 540 nm of the liquid layer is measured. Buffer stock solution for haemolysis is used as

blank. The haemolysis value in per cent is calculated according to the following formula:

$$\frac{E_{\text{exp}} \times 100}{E_{100\%}}$$

where

$E_{100\%}$ = extinction for the solution containing an equivalent of 1,0 grams salt per litre

and

E_{exp} = extinction for the solutions containing an equivalent of 4,0 and 5,0 grams salt per litre respectively

Buffer stock solution for haemolysis

90,0 g sodium chloride, 13,7 g anhydrous disodium phosphate and 1,90 g anhydrous monosodium phosphate are dissolved in distilled water and made up to 1 000,0 ml.

D. Test for the *in vivo* survival of red cells

(See Item II, 4 of Annex above):

(a) *Limit:*

Of the erythrocytes on whole human blood with ACD anticoagulant, which has been stored for 21 days at 4 to 6°C, at least 70% shall have a post-transfusion survival time of 24 hours. This can be determined according to one of the methods proposed in (b) below.

(b) *Suggested methods:*

1. Method of ISO/TC/76/WGD/3, App. E.
2. Ashby Technique — Ashby, W. The determination of the length of life of transfused blood corpuscles in man.
J. Exp. Med. 29: 267—82. 1919.

Young, L. E., Platzer, R. F., and Rafferty, J. A. Differential agglutination of human erythrocytes.
J. Lab. Clin. Med. 32: 489—501, 1947.
3. The Gibson-Scheitlin method — Gibson, J. G. and Scheitlin, W. A. A method employing radio-active chromium for assaying the viability of human erythrocytes returned to the circulation after refrigerated storage.
J. Lab. Clin. Med. 46: 679—88, 1955.
4. The Strumia method — Strumia, M. M., Taylor, L., Sample A. B., Colwell, L. S. and Dugan, A. Uses

and limitations of survival studies of erythrocytes tagged with Cr 51.

Blood 10: 429—40, 1955.

5. Cr⁵¹ — I¹²⁵ technique — Button, L. N., Gibson, J. G. and Walter, C. W. Simultaneous determination

of the volume of red cells and plasma for survival studies of stored blood.

Transfusion 5: 143—48, 1965.

6. Recommended method for radioisotope red cell survival studies Brit. J. Haemat. 21: 241, 1971.

Done at Strasbourg, this 19th day of April 1982.

Franz ARASEK
Secretary-General

Certified a true copy of the sole original document in English and in French, deposited in the Archives of the Council of Europe.

Erik HARREMOES
Director of Legal Affairs of the Council of Europe

ADDITIONAL PROTOCOL TO THE EUROPEAN AGREEMENT
on the exchange of therapeutic substances of human origin

THE MEMBER STATES OF THE COUNCIL OF EUROPE,

Contracting Parties to the European Agreement of 15 December 1958 on the exchange of therapeutic substances of human origin (hereinafter called 'the Agreement'),

Having regard to the provisions of Article 5, paragraph 1, of the Agreement, according to which 'The Contracting Parties shall take all necessary measures to exempt from all import duties the therapeutic substances of human origin placed at their disposal by the other Parties';

Considering that so far as the Member States of the European Economic Community are concerned, the undertaking to grant this exemption falls within the competence of the Community, which possesses the necessary powers in this respect by virtue of the treaty which instituted it;

Considering therefore that for the purpose of the implementation of Article 5, paragraph 1, of the Agreement, it is necessary for the European Economic Community to be able to become a Contracting Party to the Agreement,

HAVE AGREED AS FOLLOWS:

Article 1

The European Economic Community may become a Contracting Party to the Agreement by signing it. In respect of the Community, the Agreement shall enter into force on the first day of the month following such signature.

Article 2

1. This Additional Protocol shall be open for acceptance by the Contracting Parties to the Agreement. It shall enter into force on the first day of the month following the date on which the last of the Contracting Parties has deposited its instrument of acceptance with the Secretary-General of the Council of Europe.

2. However, this Additional Protocol shall enter into force on the expiration of a period of two years from the date on which it has been opened for acceptance, unless one of the Contracting Parties has notified an objection to the entry into force. If such an objection has been notified, paragraph 1 of this Article shall apply.

Article 3

From the date of its entry into force, this Additional Protocol shall form an integral part of the Agreement. From that date, no State may become a Contracting Party to the Agreement without at the same time becoming a Contracting Party to the Additional Protocol.

Article 4

The Secretary-General of the Council of Europe shall notify the member States of the Council of Europe, any State having acceded to the Agreement and the European Economic Community of any acceptance or objection made under Article 2 and of the date of entry into force of this Additional Protocol in accordance with Article 2.

The Secretary-General shall also notify the European Economic Community of any act, notification or communication relating to the Agreement.

Done at Strasbourg, the 29th day of September 1982, in English and in French, and opened for acceptance the 1st day of January 1983. Both texts are equally authentic and shall be deposited in a single copy in the archives of the Council of Europe. The Secretary-General of the Council of Europe shall transmit certified copies to each member State of the Council of Europe, to any State invited to accede to the Agreement and to the European Economic Community.

COUNCIL DECISION**of 26 January 1987****accepting on behalf of the Community the European Agreement on the Exchange of Blood-grouping Reagents****(87/68/EEC)**

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

HAS DECIDED AS FOLLOWS:

Having regard to the Treaty establishing the European Economic Community, and in particular Article 28 thereof,

Article 1

Having regard to the proposal from the Commission,

Whereas Article 5 (1) of the European Agreement on the Exchange of Blood-grouping Reagents provides that the Contracting Parties shall take all necessary measures to exempt from all import duties the blood-grouping reagents placed at their disposal by the other Parties;

The European Agreement on the Exchange of Blood-grouping Reagents is hereby accepted on behalf of the European Economic Community.

The text of the Agreement is attached to this Decision.

Whereas any derogation from the Common Customs Tariff, whether autonomous or conventional, falls within the sole competence of the Community;

Article 2

Whereas the entry into force of an Additional Protocol to the Agreement enabling the European Economic Community to become a Contracting Party to that Agreement allows the Community to exercise its competence in this matter; whereas the derogations provided for in the Agreement are already granted by Community rules of relief from customs duty;

The President of the Council is hereby authorized to designate the persons empowered to sign the Agreement in order to bind the Community.

Done at Brussels, 26 January 1987.

Whereas the Community ought therefore to become a Contracting Party to the Agreement,

*For the Council
The President*

L. TINDEMANS

EUROPEAN AGREEMENT
on the Exchange of Blood-grouping Reagents

THE SIGNATORY GOVERNMENTS OF THE MEMBER STATES
OF THE COUNCIL OF EUROPE,

Considering that blood-grouping reagents are not available in unlimited quantities;

Considering that it is most desirable that member countries, in a spirit of European solidarity, should assist one another in the supply of these blood-grouping reagents, should the need arise;

Considering that such mutual assistance is only possible if the character and use of such blood-grouping reagents are subject to rules laid down jointly by the member countries and if the necessary import facilities and exemptions are granted,

HAVE AGREED AS FOLLOWS:

Article 1

For the purposes of this Agreement, the expression 'blood-grouping reagents' refers to reagents of human, animal and plant and other origin, used for blood-grouping and for the detection of blood incompatibilities.

Any Contracting Party may, by a declaration addressed to the Secretary-General of the Council of Europe, when signing this Agreement or depositing its instrument of ratification or approval, or accession, limit the application of this Agreement to blood-grouping reagents of human origin. This declaration may be withdrawn at any time, by notification addressed to the Secretary-General of the Council of Europe.

Article 2

The Contracting Parties undertake, provided that they have sufficient stocks for their own needs, to make blood-grouping reagents available to other Parties who are in urgent need of them and to charge only those costs of collection, processing and carriage of such substances and the cost (if any) of their purchase.

Article 3

Blood-grouping reagents shall be made available to the other Contracting Parties subject to the condition that no profit is made on them, that they shall be used solely for medical purposes and shall be delivered only to bodies designated by the Government concerned.

Article 4

The Contracting Parties shall certify that the provisions as laid down in the Protocol to this Agreement have been observed.

They shall also comply with any rules to which they have subscribed with regard to international standardization in this field.

All consignments of blood-grouping reagents shall be accompanied by a certificate to the effect that they were prepared in accordance with the specifications in the Protocol. This certificate shall be based on the model to be found in the Annex to the Protocol.

The Protocol and its Annex constitute an administrative arrangement and may be amended or supplemented by the Governments of the Parties to this Agreement.

Article 5

The Contracting Parties shall take all necessary measures to exempt from all import duties the blood-grouping reagents placed at their disposal by the other Parties.

They shall also take all necessary measures to provide for the speedy delivery of these substances, by the most direct route, to the consignees referred to in Article 3 of this Agreement.

Article 6

The Contracting Parties shall forward to one another, through the Secretary-General of the Council of Europe, a list of the bodies empowered to issue certificates as provided in Article 4 of this Agreement.

They shall also forward a list of bodies empowered to distribute imported blood-grouping reagents. Wherever possible these bodies should be the same as those referred to in Article 6 of the European Agreement on the Exchange of Therapeutic Substances of Human Origin.

Article 7

The present Agreement shall be open to the signature of Members of the Council of Europe, who may become Parties to it either by:

- (a) signature without reservation in respect of ratification or approval; or
- (b) signature with reservation in respect of ratification or approval, followed by ratification or approval.

Instruments of ratification or approval shall be deposited with the Secretary-General of the Council of Europe.

Article 8

The present Agreement shall enter into force one month after the date on which three Members of the Council shall, in accordance with Article 7, have signed the Agreement without reservation in respect of ratification or approval or shall have ratified or approved it.

In the case of any Member of the Council who shall subsequently sign the Agreement without reservation in respect of ratification or approval or who shall ratify or approve it, the Agreement shall enter into force one month after the date of such signature or the date of deposit of the instrument of ratification or approval.

In witness whereof the undersigned, duly authorized thereto by their respective Governments, have signed the present Agreement.

Done at Strasbourg, this 14th day of May 1962, in English and French, both texts being equally authoritative, in a single copy which shall remain deposited in the archives of the Council of Europe. The Secretary-General shall transmit certified copies to each of the signatory and acceding Governments.

Article 9

After the entry into force of this Agreement, the Committee of Ministers of the Council of Europe may invite any non-member State to accede to the present Agreement. Such accession shall take effect one month after the date of deposit of the instrument of accession with the Secretary-General of the Council of Europe.

Article 10

The Secretary-General of the Council of Europe shall notify Members of the Council and acceding States:

- (a) of the date of entry into force of this Agreement and of the names of any Members who have signed without reservation in respect of ratification or approval or who have ratified or approved it;
- (b) of the deposit of any instrument of accession in accordance with Article 9;
- (c) of any declaration or notification received in accordance with the provisions of Article 1, paragraph 2;
- (d) of any notification received in accordance with Article 11 and its effective date;
- (e) of any amendment of the Protocol and of its Annex under Article 4, paragraph 4.

Article 11

The present Agreement shall remain in force indefinitely.

Any Contracting Party may terminate its own application of the Agreement by giving one year's notice to that effect to the Secretary-General of the Council of Europe.

PROTOCOL TO THE EUROPEAN AGREEMENT

on the Exchange of Blood-grouping Reagents

GENERAL PROVISIONS

1. Specificity

A blood-grouping⁽¹⁾ reagent must react with all blood samples tested which contain the antigen homologous to the antibody or other substance mentioned on the label.

When a reagent is used according to the technique recommended by the producer there must be no evidence of any of the following factors or phenomena:

- (a) haemolytic properties;
- (b) antibodies or other substances besides those mentioned on the label;
- (c) bacterial products liable to cause false positive or false negative reactions;
- (d) pseudo-agglutination through the formation of rouleaux;
- (e) prozone phenomena.

2. Potency

Titre is measured by making successive two-fold dilutions of the reagent under study in an appro-

priate medium. To each dilution is added an equal volume of a suspension of red corpuscles. The titre is the reciprocal of the figure representing the highest serum dilution in which a reaction occurs, the dilution being calculated without the inclusion of the volume of the corpuscular suspension in the total volume.

In the case of anti-A, anti-B and other reagents intended for use on slides, avidity is expressed by means of the time required for agglutination on a slide.

3. International Standards and International Units

International Standards have been established by the World Health Organization for anti-A and anti-B and incomplete anti-D blood-grouping reagents and are in process of being established for blood-grouping reagents of other specificities. An International Standard Preparation contains, by definition, a certain number of International Units per mg or ml and this definition is independent of the titres observed against particular red corpuscle preparations⁽²⁾.

- (1) At the time of approving the present version of the Protocol and its Annexes, it was understood by the representatives of the Contracting Parties that when in the English text of the Agreement the expression 'blood incompatibilities' was mentioned, 'blood grouping incompatibilities' was implied.

It was also agreed that the expression 'blood-grouping' with a hyphen in the English text on the Agreement and of the Protocol should read as 'blood grouping' without a hyphen.

- (2) The potency of blood-grouping reagents of most specificities is expressed as the agglutination titre observed in a dilution series, against a suspension of red cells. The titre indicates the dilution of reagent in the last mixture of the series which shows agglutination microscopically visible.

The potency of blood-grouping reagents for which International Standard Preparations exist (at present anti-A and anti-B and incomplete anti-D) can be expressed in International Units (see Bull. Wld. Hlth. Org. 1954, 10, 937, 941—1950, 3, 301) on the basis of the titration of the unknown reagent in comparison with the International Standard, or a national sub-standard.

The International Standard Preparations of blood-grouping sera are dispensed in ampoules containing dried human serum. When reconstituted to the volume of 1 ml, the anti-A and anti-B sera contain by definition 256 International Units per ml. They can be obtained free of charge, from the International Laboratory for Biological Standards of WHO, Statens Seruminstitut, Copenhagen.

The following table shows an example of a comparative titration of the International Standard anti-A Serum (S) and an 'unknown' anti-A reagent (U) against A₁ red corpuscles and A₂B red corpuscles.

	Serum S	Reagent U	Serum S	Reagent U
A ₁ corpuscles	1 : 512	1 : 128	256	64
A ₂ B corpuscles	1 : 32	1 : 16	256	128
	titres (observed)	titres (observed)	Units (by definition)	Units (by comparison)

4. Stability and expiry date

Each reagent, when kept under the conditions of storage recommended by the manufacturer, should retain the requisite properties for at least one year.

The expiry date of a reagent in the liquid form as given on the label shall be not more than one year from the date of the last satisfactory potency test. The expiry date can be extended for further periods of one year by repetition of potency tests.

The expiry date of reagents in the dried form as given on the label shall be in accordance with evidence obtained from experiments on stability and shall be approved by the national control authorities.

5. Preservation

Blood-grouping reagents may be preserved in the liquid or dried state. Dried reagents shall be kept in an atmosphere of an inert gas or *in vacuo*, in the glass container in which they were dried and which shall be closed so as to exclude moisture. A dried reagent must not lose more than 0,5% of its weight when tested by further drying over phosphorus pentoxide at a pressure not exceeding 0,02 mm of mercury for 24 hours.

Reagents shall be prepared with aseptic precautions and shall be free from bacterial contamination. In order to prevent bacterial growth the competent national authority may decide that an antiseptic and/or antibiotic shall be added to the reagent (or to any solvent issued with dried reagents), provided that, in the presence of the added substance, the reagent still fulfils the requirements for specificity and potency.

Blood-grouping sera of human origin must contain at least 2,5 mg of protein nitrogen per ml of liquid or reconstituted serum.

Reagents whether in the liquid state or after reconstitution should be transparent and should not contain any sediment, gel or visible particles.

6. Coloration

Blood-grouping reagents for international exchanges should preferably not be artificially coloured at least until an international agreement is reached on a uniform system. Any added colouring matter must not interfere with the specific reaction.

7. Dispensing and volume

Blood-grouping reagents shall be dispensed in such a way and in such volumes that the reagent in one container is sufficient for the performance of tests

with positive and negative control corpuscles in addition to the performance of tests with the unknown corpuscles. The volume in one container shall be such that the contents can if necessary be used for the performance of the appropriate tests for potency described in this Protocol.

8. Records and samples

Written records shall be kept by the producing laboratory of all steps in the production and control of blood-grouping reagents. Adequate samples of all reagents issued shall be retained by the laboratory until it can be reasonably assumed that the batch is no longer in use.

9. Classification of reagents

Reagents used for blood-grouping may contain substances of human, animal, vegetable (or mineral) origin, of which some constitute the active principle and others are adjuvants for enhancing the activity or maintaining the stability of the reagent.

For technical reasons these reagents have been divided into three categories according to the origin of their active principle. This does not mean that reagents of human origin contain exclusively substances of human origin or that animal or vegetable reagents cannot contain substances of human origin.

10. Labels, leaflets and certificates

A label printed in English and French, in black on white paper, shall be affixed to each final container and shall contain the following information:

1. Name and address of producer;
2. Name of the reagent as it appears in the heading of the relevant specification;
3. Name and amount of antiseptic and/or antibiotic, if present, or indication of absence;
4. The volume or, where the reagent is dried, the volume and composition of the fluid needed for reconstitution;
5. Expiry date;
6. Batch number.

Moreover, this label or the label of the carton enclosing several final containers, or the leaflet accompanying the containers, shall contain the following information:

1. Full name and address of producer;
2. Name of the reagent as it appears in the heading of the relevant specification;

3. The volume, or, where the reagent is dried, the volume and composition of the fluid needed for reconstitution;
4. Date of last potency test;
5. Expiry date (if any);
6. Batch number;
7. Adequate description of the method of use recommended by the producer;
8. Conditions of storage of unopened ampoules and precautions to be taken after opening;
9. Exact composition, including antiseptic and/or antibiotic if any;
10. Statement whether the product contains or does not contain material of human origin.

Each consignment shall be accompanied by a certificate as provided in Article 4 of the Agreement and the Annex to the present Protocol. Examples of labels and leaflets are attached to the present Protocol.

SPECIFIC PROVISIONS

A. BLOOD-GROUPING SERA OF HUMAN ORIGIN

(a) SERA OF HUMAN ORIGIN FOR AB0 GROUPING

(i) Anti-A blood-grouping serum (human)

Anti-A serum is derived from the blood of selected group B persons, who may or may not have been immunized by group A red corpuscles or group A specific substance. Anti-A serum agglutinates human red corpuscles containing A antigen, i.e. those of blood groups A and AB, including sub-groups A_1 , A_2 , A_1B and A_2B , and does not agglutinate human red corpuscles which do not contain A antigen, i.e. those of blood groups 0 and B.

Potency

Titration

An anti-A serum shall be titrated separately against suspensions of A_1 , A_2 , and A_2B corpuscles, in parallel with the reconstituted but undiluted International Standard Preparation of anti-A blood-grouping serum or an equivalent reference preparation. The potency of the serum shall in each case be not less than 64 International Units per ml.

Determination of avidity

When anti-A serum is mixed on a slide with an equal volume of a suspension of A_1 , A_2 and A_2B cells with a volume fraction of 0,05 to 0,1, agglutination of each suspension should first appear in not more than twice the time taken when the same test is performed with the reconstituted but undiluted International Standard Preparation of anti-A blood-grouping serum or with a reference standard of equivalent avidity.

(ii) Anti-B blood-grouping serum (human)

Anti-B serum is derived from the blood of selected group A persons, who may or may not have been immunized by group B red corpuscles or group B specific substance. Anti-B serum agglutinates human red corpuscles containing B antigen, i.e. those of blood groups B and AB, and does not agglutinate human red corpuscles which do not contain B antigen, i.e. those of blood groups 0 and A.

Potency

Titration

An anti-B serum shall be titrated against a suspension of group B corpuscles in parallel with the reconstituted but undiluted International Standard Preparation of anti-B blood-grouping serum or an equivalent reference preparation. The potency of the serum shall be not less than 64 International Units per ml.

Determination of avidity

When anti-B serum is mixed on a slide with an equal volume of a suspension of B cells with a volume fraction of 0,05 to 0,1, agglutination should first appear in not more than twice the time taken when the same test is performed with the reconstituted but undiluted International Standard Preparation of anti-B blood-grouping serum or with reference standard of equivalent avidity.

(iii) Anti-A + Anti-B (group 0) blood-grouping serum (human)

Anti-A + anti-B (group 0) serum is derived from the blood of selected group 0 persons who

may or may not have been immunized by group A and group B red corpuscles or group A and group B specific substances. Anti-A + anti-B (group 0) serum agglutinates human red corpuscles containing A or B agglutinogens or both, i.e. those of group A including subgroups A_1 and A_2 , group B and group AB including subgroups A_1B and A_2B , and does not agglutinate human red corpuscles which do not contain A or B agglutinogens, i.e. those of group 0. It agglutinates human red corpuscles containing the A_x (A_y or A_o) antigen (which are not, in general, agglutinated by anti-A serum derived from group B donors).

Potency

Titration

An anti-A + anti-B (group 0) serum shall be titrated separately against suspensions of A_1 and A_2 corpuscles in parallel with the reconstituted but undiluted International Standard Preparation of anti-A blood-grouping serum or an equivalent standard preparation. It shall also be titrated against a suspension of group B corpuscles in parallel with the reconstituted but undiluted International Standard Preparation of anti-B blood grouping serum or an equivalent standard preparation.

The potency of the serum shall in every case be not less than 64 International Units per ml.

Anti-A + anti-B (group 0) blood-grouping serum used undiluted shall also give readily detectable agglutination of group A_x (A_y or A_o) corpuscles.

Determination of avidity

When anti-A + anti-B (group 0) serum is mixed on a slide with equal volumes of suspensions of A_1 and A_2 cells with a volume fraction of 0,05 to 0,1, agglutination shall first appear in not more than twice the time taken when the same tests are performed with the reconstituted but undiluted International Standard Preparation of anti-A blood-grouping serum or with a reference standard of equivalent avidity. When anti-A + anti-B (group 0) serum is mixed on a slide with an equal volume of a suspension of B cells with a volume fraction of 0,05 to 0,1, agglutination shall first appear in not more than twice the time taken when the same test is performed with the reconstituted but undiluted International Standard Preparation of anti-B blood-grouping serum or a reference preparation of equivalent avidity. When anti-A + anti-

B (group 0) serum is mixed on a slide with an equal volume of a suspension of A_x (A_y or A_o) cells with a volume fraction of 0,05 to 0,1, agglutination shall first appear in not more than five minutes at a temperature between 18 and 25 °C.

(b) SERA OF HUMAN ORIGIN FOR Rh GROUPING

Anti-Rh blood-grouping sera, whatever their specificity, may be of either of two varieties differing in the conditions under which agglutination of homologous corpuscles is obtained. Certain sera commonly known as 'complete' agglutinate corpuscles suspended in saline. With others, commonly known as 'incomplete', agglutination can only be obtained in the presence of certain colloids such as bovine albumin or by means of other special techniques. The sera should be used under the conditions specified by the laboratory preparing them.

Some 'incomplete' sera will also agglutinate homologous red corpuscles suspended in their own serum or plasma on slides.

The following requirements of potency for Rh grouping sera may need to be revised when International Standard Preparations become available.

(i) **Anti-D (anti-Rh_o) blood-grouping serum (human)**

Anti-D serum is derived from the blood of one or more persons immunized by the D antigen of the Rh system. It reacts with human red corpuscles containing the D antigen, but not with human red corpuscles which do not contain the D antigen.

Potency

Titration

'Complete' anti-D sera shall have a titre of not less than 32 against CcDee cells in a solution containing 9 grams sodium chloride per litre.

An 'incomplete' anti-D serum shall be titrated against CcDee corpuscles in parallel with the reconstituted but undiluted International Standard Preparation of Incomplete Anti-D (anti-Rh_o) or an equivalent reference preparation. It shall have a potency of not less than 32 International Units. Besides reacting with all red corpuscles containing the D antigen, the serum should, as far as possible, react with corpuscles containing the D^u antigen.

Determination of avidity

Anti-D sera intended for use in the slide test of Diamond and Abelson should, when mixed on

a slide with an equal volume of a 40 to 50% suspension of CcDee corpuscles at approximately 40°C, show visible agglutination within 30 seconds, and agglutination should be complete within 120 seconds.

(ii) Anti-C (anti Rh) blood-grouping serum (human)

Anti-C serum is derived from the blood of one or more persons immunized by the C agglutinin of the Rh system. It agglutinates suspensions of human red corpuscles containing the C antigen, but not with human red corpuscles which do not contain the C antigen. In this connection the C antigen is regarded as including the C^w antigen.

Most diagnostic anti-C sera contain 'complete' anti-C together with 'incomplete' anti-D. These sera are therefore specific for the C antigen only when the cells under test are suspended in a solution containing 9 grams sodium chloride per litre.

Potency

Titration

Anti-C sera ('complete' or 'incomplete') should have a titre of not less than 8 against Ccdee corpuscles.

Determination of avidity

Anti-C sera intended for use in the slide test of Diamond and Abelson (and which must not contain any form of anti-D) should, when mixed on a slide with an equal volume of a suspension of Ccdee cells with a volume fraction of 0,4 to 0,5, at approximately 40°C, show visible agglutination within 30 seconds, and agglutination should be complete within 120 seconds.

(iii) Anti-E (anti-rh⁻) blood-grouping serum (human)

Anti-E serum is derived from the blood of one or more persons immunized by the E antigen of

the Rh system. It reacts with human red corpuscles containing the E antigen.

Potency

Titration

Anti-E sera ('complete' or 'incomplete') should have a titre of not less than 8 against ccdee corpuscles.

Determination of avidity

Anti-E sera intended for use in the slide test of Diamond and Abelson (and which must contain any form of anti-D) should, when mixed on a slide with an equal volume of a suspension of ccdee cells with a volume fraction of 0,4 to 0,5, at approximately 40°C, show visible agglutination within 30 seconds, and agglutination should be complete within 120 seconds.

(iv) Anti-D + C (anti-Rh₀rh⁺) blood-grouping serum (human)

Anti-D + E (anti-Rh₀rh⁻) blood-grouping serum (human)

Sera of specificity anti-D + C and of specificity anti-D + E may be obtained directly from the blood of immunized individuals or may be prepared by mixing anti-D with anti-C or anti-E serum. In a given serum both antibodies must be simultaneously active under the conditions of reaction specified by the producer. Each serum must react with all types of red corpuscles which would react with either of the component antibodies, and must fail to react with red corpuscles which contain neither the C nor D antigen in the case of anti-D + C and neither D nor E antigen in the case of anti-D + E. The titres should not be less than those specified for the component antibodies, but in the case of anti-D + C (which is a frequent combination in the serum of immunized persons) it is desirable that the anti-C titre should be not less than 32 and in the case of anti-D + E it is desirable that the anti-E titre should be not less than 8. Where a serum is intended for use in the slide test of Diamond and Abelson, the times of agglutination for all reacting types of red corpuscles should be not less than those specified for the component antibodies.

B. REAGENTS OF NON-HUMAN ORIGIN

(a) SERA OF ANIMAL ORIGIN

(i) Anti-A blood-grouping serum (animal)

Anti-A serum is derived from the blood of animals which may or may not have been immunized by group A red corpuscles or group A specific substances. Anti-A serum agglutinates human red corpuscles containing A antigen, i.e. those of blood groups A and AB, including sub-groups A₁, A₂, A₁B and A₂B, and does not agglutinate human red corpuscles which do not contain A antigen, i.e. those of blood groups 0 and B.

*Potency**Titration*

An anti-A serum shall be titrated separately against suspensions of A₁, A₂, and A₂B red corpuscles, in parallel with the reconstituted but undiluted International Standard Preparation of anti-A blood-grouping serum or an equivalent reference preparation⁽¹⁾. The potency of the serum shall in each case be not less than 64 International Units per ml.

Determination of avidity

When anti-A serum is mixed on a slide with an equal volume of a suspension of A₁, A₂ and A₂B cells with a volume fraction of 0,05 to 0,1, agglutination of each suspension shall in each case first appear in not more than twice the time taken when the same test is performed with the reconstituted but undiluted International Standard Preparation of anti-A blood-grouping serum or with a reference standard of equivalent avidity.

(ii) Anti-B blood-grouping serum (animal)

Anti-B serum is derived from the blood of animals which may or may not have been immunized by group B red corpuscles or group B specific substances. Anti-B serum agglutinates human red corpuscles containing B antigen, i.e. those of blood groups B and AB, and does not agglutinate human red corpuscles which do not contain B antigen, i.e. those of blood groups 0 and A.

*Potency**Titration*

An anti-B serum shall be titrated against a suspension of group B corpuscles in parallel with the reconstituted but undiluted International Standard Preparation of anti-B blood-grouping serum or an equivalent reference preparation⁽¹⁾. The potency of the serum shall be not less than 64 International Units per ml.

Determination of avidity

When anti-B serum is mixed on a slide with an equal volume of a suspension of B cells with a volume fraction of 0,05 to 0,1, agglutination shall first appear in not more than twice the time taken when the same test is performed with the reconstituted but undiluted International Standard Preparation of anti-B blood-grouping serum or with a reference standard of equivalent avidity.

(iii) Anti-human-globulin serum (animal)⁽²⁾

Anti-human globulin serum for use in blood group serology must contain agglutinating antibodies against IgG globulin and agglutinating antibodies against complement factors. It is derived from the blood of animals immunized by the injection of human serum protein. It must agglutinate all human red corpuscles coated with human IgG and/or complement factors. Under the conditions specified by the manufacturers it does not agglutinate uncoated human red corpuscles, to whatever group they may belong.

Specificity

The specificity of an anti-human globulin serum for use in blood group serology must be tested with human red corpuscles coated with a variety of antibodies i.e. red corpuscles sensitized with human incomplete antibodies anti-D, anti-K and anti-Fy^a, red corpuscles sensitized with complement-binding incomplete antibodies anti-Le^a in the presence of fresh human serum, and red corpuscles sensitized with so-called 'incomplete cold antibodies'

⁽¹⁾ The International Standard Preparation is of human origin; an equivalent reference preparation, if used, may be of human or non-human origin.

⁽²⁾ Coombs, R. R. A., Mourant, A. E. and Race, R. R. (1945), *Lancet*, iii 5
Coombs, R. R. A., Mourant, A. E. and Race, R. R. (1945), *Brit. J. exp. Path.*, 26, 255.

and with tanned red corpuscles sensitized with human IgG and, finally, with 10 different samples of non-coated human red corpuscles with and without A and B antigens.

Potency

Titration

An anti-human globulin serum, as supplied, or at the dilution recommended on the label, shall strongly agglutinate human red corpuscles coated with a human incomplete anti-D serum, having a titre of 4 (or less) against D-positive corpuscles, when the titration is performed by the albumin replacement method. At the same dilution it shall agglutinate K-positive human red corpuscles sensitized with selected weak anti-K antibodies and Fy^a positive red corpuscles sensitized with selected weak anti-Fy^a antibodies.

It shall also, at the same or a different dilution, as specified on the label, agglutinate human red corpuscles sensitized with weak complement-binding incomplete anti-Le^a antibodies in the presence of fresh serum.

For clinical use it is desirable that the coating of all the types of incomplete antibodies above shall be detectable with a single dilution of the anti-human globulin serum.

(b) BLOOD-GROUPING REAGENTS OF VEGETABLE ORIGIN

(i) Anti-A blood-grouping reagent (vegetable)

Anti-A reagent is prepared by extraction from the seeds or other parts of a suitable plant, followed, if necessary, by purification. Anti-A reagent agglutinates human red corpuscles containing A antigens, i.e. those of blood groups A and AB, including sub-groups A₁, A₂, A₁B and A₂B, and does not agglutinate human red corpuscles which do not contain A antigens, i.e. those of blood groups O and B.

Potency

Titration

An anti-A reagent shall be titrated separately against suspensions of A₁, A₂ and A₂B corpuscles, in parallel with the reconstituted but un-

diluted International Standard Preparation of anti-A blood-grouping serum or an equivalent reference preparation (¹).

The potency of the reagent shall in each case be not less than 64 International Units per ml.

Determination of avidity

When anti-A reagent is mixed on a slide with an equal volume of a suspension of A₁, A₂ and A₂B cells with a volume fraction of 0,05 to 0,1, agglutination of each suspension shall first appear in not more than twice the time taken when the same test is performed with the reconstituted but undiluted International Standard Preparation of anti-A blood-grouping serum or with a reference standard of equivalent avidity.

(ii) Anti-B blood-grouping reagent (vegetable)

Anti-B reagent is prepared by extraction from the appropriate part of a suitable plant, followed, if necessary, by purification. Anti-B reagent agglutinates human red corpuscles containing B antigen, i.e. those of blood groups B and AB, and does not agglutinate human red corpuscles which do not contain B antigen, i.e. those of blood groups O and A.

Potency

Titration

An anti-B reagent shall be titrated against a suspension of group B corpuscles in parallel with the reconstituted but undiluted International Standard Preparation of anti-B blood-grouping serum or an equivalent reference preparation (¹). The potency of the reagent shall not be less than 64 International Units per ml.

Determination of avidity

When anti-B reagent is mixed on a slide with an equal volume of a suspension of B cells with a volume fraction of 0,05 to 0,1, agglutination shall first appear in not more than twice the time taken when the same test is performed with the reconstituted but undiluted International Standard Preparation of anti-B blood-grouping serum or with a reference standard of equivalent avidity.

(¹) The International Standard Preparation is of human origin; an equivalent reference preparation, if used, may be of human or non-human origin.

EXEMPLES D'ÉTIQUETTE
EXAMPLES OF LABEL

CONSEIL DE L'EUROPE
COUNCIL OF EUROPE

ACCORD EUROPÉEN RELATIF À L'ÉCHANGE DES RÉACTIFS POUR LA DÉTERMINATION
DES GROUPES SANGUINS

EUROPEAN AGREEMENT ON THE EXCHANGE OF BLOOD-GROUPING REAGENTS

a) sérum liquide

1. Laboratoire X, Amsterdam
2. Sérum anti-A (humain)
3. N₃Na 0,1%
4. 5 ml
5. 7 septembre 1965
6. N° 1 2 3 4

b) sérum desséché

1. Laboratoire X, Amsterdam
2. Sérum anti-B (animal)
3. Mersalate 0,1%
4. Reconstituer avec 5 ml d'eau distillée
5. 31 décembre 1968
6. N° 4 3 2 1

(a) fluid serum

1. ... Laboratory, Amsterdam
2. Anti-A serum (human)
3. Sodium azide 0,1%
4. 5 ml
5. 7 September 1965
6. No 1 2 3 4

(b) dried serum

1. ... Laboratory, Amsterdam
2. Anti-B serum (animal)
3. Mersalate 0,1%
4. To be reconstituted with 5 ml of distilled water
5. 31 December 1968
6. No 4 3 2 1

EXEMPLE DE NOTICE

CONSEIL DE L'EUROPE

ACCORD EUROPÉEN RELATIF À L'ÉCHANGE DES RÉACTIFS POUR LA DÉTERMINATION
DES GROUPES SANGUINS

1. Laboratoire central de transfusion sanguine, 1 Main Street, Metropolis, Westland.
 2. Sérum anti-E (anti-rh ") (humain).
 3. 10 ml.
 4. Date du dernier contrôle d'activité: 30 mai 1961.
 5. Date de péremption: 30 mai 1962.
 6. N° 5432.
 7. Les globules rouges à examiner doivent être lavés une ou plusieurs fois avec une solution saline de 9 g/l. Une suspension de globules rouges d'une fraction de volume d'environ 0,03 est préparée ensuite en mélangeant un volume ou une goutte de culot globulaire avec 30 volumes ou gouttes de solution saline isotonique. Avec un peu d'habitude, la concentration d'une suspension peut être évaluée de façon satisfaisante à l'œil nu.

Une petite goutte de sérum est déposée dans un tube à hémolyse (6 mm × 30 mm) à l'aide d'une pipette Pasteur. On ajoute ensuite une petite goutte de suspension de globules rouges. (Avec un peu d'habitude, on peut réaliser une économie considérable en distribuant le sérum et la suspension globulaire à l'aide de pipettes graduées à µl). Le contenu du tube est mélangé et mis à incuber deux heures à 37°C. Le contenu du tube est alors transporté et étalé avec précaution sur une lame de microscope. Si l'agglutination n'est pas clairement visible à l'œil nu, la lame est examinée au microscope pour établir si l'agglutination s'est produite et déterminer son intensité.
 8. Conserver à une température inférieure ou égale à -20°C. Si le produit n'est pas utilisé le jour même de l'ouverture, ajouter 0,1 ml d'une solution de N_3Na à concentration de 100 g/l.
 9. Sérum humain anti-E ("anti-rh"): 5 ml,
Albumine bovine à 300 g/l: 5 ml.
 10. Ce réactif contient une substance d'origine humaine.
-

EXAMPLE OF LEAFLET

COUNCIL OF EUROPE

EUROPEAN AGREEMENT ON THE EXCHANGE OF BLOOD-GROUPING REAGENTS

1. Central Blood Transfusion Laboratory, 1 Main Street, Metropolis, Westland
 2. Anti-E (anti-rh ") serum (human)
 3. 10 ml
 4. Date du dernier co test: 30th May 1961
 5. Expiry Date, 30th May 1962
 6. No. 5432
 7. The red blood cells to be tested are washed one or more times with a NaCl solution of 9 g/l. An erythrocyte suspension with a volume fraction of approximately 0.03 is prepared by mixing one volume or drop of packed red cells with 30 volumes or drops of isotonic NaCl-solution. With practice the strength of a suspension can be judged adequately by inspection.

A small drop of serum is delivered into a precipitin tube (6 mm × 30 mm) from a Pasteur pipette, and a similar drop of red corpuscle suspension is added. (With practice considerable economy can be achieved by delivering the serum and cell suspension from pipettes marked at a volume of 10 µl). The contents of the tube are mixed and incubated at 37 °C for two hours. The contents of the tube are then cautiously transferred to a microscope slide and gently spread upon it. Unless agglutination is unmistakable to the unaided eye the slide is examined for the presence and degree of agglutination under the microscope.
 8. Store at -20 °C or below. If to be used after day of opening, add 0,1 ml of a solution containing 100 gram sodium azide per litre.
 9. Human anti-E ("anti-rh") serum: 5 ml; solution containing 300 gram bovine albumin per litre: 5 ml.
 10. This product contains material of human origin.
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ANNEXE AU PROTOCOLE
ANNEX TO THE PROTOCOL

CONSEIL DE L'EUROPE
 COUNCIL OF EUROPE

ACCORD EUROPÉEN RELATIF À L'ÉCHANGE DES RÉACTIFS POUR LA DÉTERMINATION
 DE GROUPES SANGUINS

EUROPEAN AGREEMENT ON THE EXCHANGE OF BLOOD-GROUPING REAGENTS

CERTIFICAT

(Article 4)

CERTIFICATE

À NE PAS DÉTACHER DE L'ENVOI
 NOT TO BE SEPARATED FROM THE SHIPMENT

	 19
	(lieu) (date)
	(place)
Nombre de colis Number of packages	Le soussigné déclare que l'envoi spécifié en marge The undersigned certifies that the shipment specified in the margin
.....	
Désignation Marked	préparé sous la responsabilité de prepared under the responsibility of
.....	
.....	
N° des lots Batch No	organisme visé à l'article 6 de l'accord, est conforme aux spécifications du protocole à l'accord et qu'il peut être délivré immédiatement au destinataire
.....	(nom et lieu)
.....	one of the bodies referred to in Article 6 of the Agreement, is in conformity with the specifications of the Protocol to the Agreement and can be delivered im- mediately to the consignee (name and place)
	
	
	(cachet) (signature) (titre)
	(stamp) (signature) (title)

ADDITIONAL PROTOCOL TO THE EUROPEAN AGREEMENT
on the Exchanges of Blood-grouping Reagents

THE MEMBER STATES OF THE COUNCIL OF EUROPE,

Contracting Parties to the European Agreement of 14 May 1962 on the exchanges of blood-grouping reagents (hereinafter called 'the Agreement');

Having regard to the provisions of Article 5, paragraph 1, of the Agreement, according to which 'The Contracting Parties shall take all necessary measures to exempt from all import duties the blood-grouping reagents placed at their disposal by the other Parties';

Considering that so far as the Member States of the European Economic Community are concerned, the undertaking to grant this exemption falls within the competence of the Community, which possesses the necessary powers in this respect by virtue of the Treaty which instituted it;

Considering therefore that for the purpose of the implementation of Article 5, paragraph 1, of the Agreement, it is necessary for the European Economic Community to be able to become a Contracting Party to the Agreement,

HAVE AGREED AS FOLLOWS:

Article 1

The European Economic Community may become a Contracting Party to the Agreement by signing it. In respect of the Community, the Agreement shall enter into force on the first day of the month following such signature.

Article 2

1. This Additional Protocol shall be open for acceptance by the Contracting Parties to the Agreement. It shall enter into force on the first day of the month following the date on which the last of the Contracting Parties has deposited its instrument of acceptance with the Secretary-General of the Council of Europe.

2. However, this Additional Protocol shall enter into force on the expiration of a period of two years from the date on which it has been opened for acceptance, unless one of the Contracting Parties has notified an objection to the entry into force. If such an objection

has been notified, paragraph 1 of this Article shall apply.

Article 3

From the date of its entry into force, this Additional Protocol shall form an integral part of the Agreement. From that date, no State may become a Contracting Party to the Agreement without at the same time becoming a Contracting Party to the Additional Protocol.

Article 4

The Secretary-General of the Council of Europe shall notify the member States of the Council of Europe, any State having acceded to the Agreement and the European Economic Community of any acceptance or objection made under Article 2 and of the date of entry into force of this Additional Protocol in accordance with Article 2.

The Secretary-General shall also notify the European Economic Community of any act, notification or communication relating to the Agreement.

Done at Strasbourg, the 29th day of September 1982, in English and in French, and opened for acceptance the 1st day of January 1983. Both texts are equally authentic and shall be deposited in a single copy in the archives of the Council of Europe. The Secretary-General of the Council of Europe shall transmit certified copies to each Member State of the Council of Europe, to any State invited to accede to the Agreement and to the European Economic Community.
