No. 7910

BELGIUM, DENMARK, FEDERAL REPUBLIC OF GERMANY, FRANCE, GREECE, etc.

European Agreement on the exchange of blood-grouping reagents (with Protocol and annex). Done at Strasbourg, on 14 May 1962

Official texts: English and French.

Registered on 2 September 1965 by the Council of Europe acting on behalf of the Contracting Parties, in accordance with Resolution 54 (6) of the Committee of Ministers of the Council of Europe, adopted on 3 April 1954.

BELGIQUE, DANEMARK, RÉPUBLIQUE FÉDÉRALE D'ALLEMAGNE, FRANCE, GRÈCE, etc.

Accord européen relatif à l'échange des réactifs pour la détermination des groupes sanguins (avec Protocole et annexe). Fait à Strasbourg, le 14 mai 1962

Textes officiels anglais et français.

Enregistré le 2 septembre 1965 par le Conseil de l'Europe agissant au nom des Parties contractantes, conformément à la résolution 54 (6) du Comité des ministres du Conseil de l'Europe, adoptée le 3 avril 1954.

No. 7910. EUROPEAN AGREEMENT[1] ON THE EXCHANGE OF BLOOD-GROUPING REAGENTS. DONE AT STRASBOURG, ON 14 MAY 1962

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 :

[1] In accordance with article 8, the Agreement entered into force on 14 October 1962, one month after the date on which three Members of the Council of Europe had signed the Agreement without reservation in respect of ratification or approval. Subsequently, for the Members of the Council who, having signed the Agreement subject to ratification or approval, deposited their instruments of ratification, the Agreement came into force one month after the date of deposit of the instrument. Following is the list of States in respect of which the Agreement entered into force, indicating in respec tof each State either the date of signature without reservation as to ratification or the date of deposit of the instrument of ratification with the Secretary-General of the Council of Europe, and the date of entry into force :

State	*Signature or deposit of instrument of ratification (r) or approval (a)*	*Date of entry into force*
Norway	14 May 1962	14 October 1962
Sweden	14 May 1962	14 October 1962
Denmark	13 September 1962	14 October 1962
France	5 February 1964 (*a*)	21 January 1964*
Turkey	27 November 1964 (*r*)	28 December 1964
United Kingdom of Great Britain and Northern Ireland**	8 December 1964 (*r*)	9 January 1965
Netherlands***	20 May 1965 (*r*)	21 June 1965

* Date specified in the notification of approval.

** Declaration made by the Government of the United Kingdom at the time of deposit of the instrument of ratification :

" The ratification of the Agreement is in respect of the United Kingdom only and does not extend to any other territory for the international relations of which the Government of the United Kingdom are responsible. "

*** The Government of the Kingdom of the Netherlands reserves the right to extend the implementation of the Agreement with Protocol and annexes to Surinam when the Government of that country expresses the wish thereto.

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 in 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 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 standardisation 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 moste 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.[1]

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 or such signature or the date of deposit of the instrument of ratification or approval.

[1] United Nations, *Treaty Series*, Vol. 351, p. 159; Vol. 363, p. 409; Vol. 388, p. 386; Vol. 406, p. 336; Vol. 407, p. 284; Vol. 473, p. 349, and Vol. 514, p. 287.

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.

IN WITNESS WHEREOF the undersigned, duly authorised 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.

For the Government
of the Republic of Austria :

Pour le Gouvernement
de la République d'Autriche :

For the Government
of the Kingdom of Belgium :

with reservation in respect of ratification or approval

Pour le Gouvernement
du Royaume de Belgique :

sous réserve de ratification ou d'approbation

P. H. SPAAK

For the Government
of the Republic of Cyprus :

Pour la Gouvernement
de la République de Chypre :

For the Government
of the Kingdom of Denmark :

Pour le Gouvernement
du Royaume de Danemark :

Strasbourg, le 13 septembre 1962

M. WARBERG

For the Government
of the French Republic :

At the time of signature, the Government of the French Republic hereby declares, in pursuance of Article 1, that the application of this Agreement shall be limited to blood-grouping reagents of human origin.

with reservation in respect of ratification or approval

Pour le Gouvernement
de la République française :

Au moment de la signature, le Gouvernement de la République française déclare, conformément à l'article 1er, limiter l'application de l'Accord aux réactifs pour la détermination des groupes sanguins d'origine humaine.

sous réserve de ratification ou d'approbation

G. GORSE

For the Government
of the Federal Republic of Germany :

with reservation in respect of ratification or approval

Pour le Gouvernement
de la République Fédérale
d'Allemagne :

sous réserve de ratification ou d'approbation

Strasbourg, le 26 juin 1962

Felician PRILL

For the Government
of the Kingdom of Greece :

with reservation in respect of ratification or approval

Pour le Gouvernement
du Royaume de Grèce :

sous réserve de ratification ou d'approbation

N. CAMBALOURIS

For the Government
of the Icelandic Republic :

Pour le Gouvernement
de la République islandaise :

For the Government
of Ireland :

Pour le Gouvernement
d'Irlande :

For the Government
of the Italian Republic :

with reservation in respect of ratification or approval

Pour le Gouvernement
de la République italienne :

sous réserve de ratification ou d'approbation

Attilio PICCIONI

For the Government of the Grand Duchy of Luxembourg :

with reservation in respect of ratification or approval

Pour le Gouvernement du Grand Duché de Luxembourg :

sous réserve de ratification ou d'approbation

Pierre WURTH

For the Government of the Kingdom of the Netherlands :

Pour le Gouvernement du Royaume des Pays-Bas :

sous réserve de ratification ou d'approbation[1]

Strasbourg, le 15 juillet 1964

W. J. D. PHILIPSE

For the Government of the Kingdom of Norway :

Pour le Gouvernement du Royaume de Norvège:

Einar LÖCHEN

For the Government of the Kingdom of Sweden :

Pour le Gouvernement du Royaume de Suède :

Gunnar LANGE

[1] With reservation in respect of ratification or approval.

For the Government of the Turkish Republic :

with reservation in respect of ratification or approval

Pour le Gouvernement de la République turque :

sous réserve de ratification ou d'approbation

Zeki KUNERALP

For the Government of the United Kingdom of Great Britain and Northern Ireland :

Pour le Gouvernement du Royaume-Uni de Grande-Bretagne et d'Irlande du Nord :

with reservation in respect of ratification or approval[1]

Strasbourg, 21st November 1963

I. F. PORTER

For the Government of the Swiss Confederation :

Pour le Gouvernement de la Confédération Suisse :

sous réserve de ratification ou d'approbation[2]

Strasbourg, le 15 avril 1964

H. VOIRIER

[1] Sous réserve de ratification ou d'approbation.
[2] With reservation in respect of ratification or approval.

PROTOCOL TO THE AGREEMENT

GENERAL PROVISION

1. Specificity

A blood-grouping reagent must agglutinate all blood samples tested which contain the agglutinogen homologous to the antibody or other agglutinating 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 agglutinating 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 appropriate medium. To each dilution is added an equal volume of red corpuscles in suspension. The titre is the reciprocal of the figure representing the highest serum dilution in which microscopically visible agglutination occurs, the dilution being calculated with 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 Organisation for Anti-A and Anti-B 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.[1]

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 per cent of its weight when tested by further drying over phosphorus pentoxide at a pressure not exceeding 0.02 mm of mercury for 24 hours.

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[1] 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) can be expressed in International Units* 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 serum contains 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 A_1 red corpuscles and A_2B red corpuscles.

	Serum S	*Reagent U*	*Serum S*	*Reagent U*
A_1 corpuscles	1 : 512	1 : 128	256	64
A_2B corpuscles	1 : 32	1 : 16	256	128
	titres (observed)	titres (observed)	Units (by definitions)	Units (by comparison)

* See *Bull.* Wld. Hlth. Org. 1954, *10*, 937, 941.
See *Bull.* Wld. Hlth. Org. 1950, *3*, 301.

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 affect the agglutinating properties.

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 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[1] to the present Protocol. Examples of labels[2] and leaflets[3] are attached to the present Protocol.

[1] See p. 76 of this volume.
[2] See p. 76 of this volume.
[3] See p. 77 of this volume.

SPECIFIC PROVISIONS

A. BLOOD-GROUPING SERA OF HUMAN ORIGIN

(a) *Blood-grouping sera of human origin for ABO group*

(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 immunised by Group A red corpuscles or group A specific substance. Anti-A serum agglutinates human red corpuscles containing A agglutinogens, 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 agglutinogens, i.e. those of blood groups O 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 5 % to 10 % suspension of A_1, A_2, and A_2B corpuscles, 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 immunised by group B red corpuscles or group B specific substance. Anti-B serum agglutinates human red corpuscles containing B agglutinogen, i.e. those of blood groups B and AB, and does not agglutinate human red corpuscles which do not contain B agglutinogen, i.e. those of blood groups O 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 pre-

paration 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 5 % to 10 % suspension of B corpuscles, 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 a reference standard of equivalent avidity.

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

Anti-A + anti-B (group O) serum is derived from the blood of selected group O persons who may or may not have been immunised by group A and group B red corpuscles or group A and group B specific substances. Anti-A + anti-B (group O) serum agglutinates human red corpuscles containing A or B agglutinogens or both, i.e. those of group A including sub-groups A_1 and A_2, group B and group AB including sub-groups 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 O. It agglutinates human red corpuscles containing the A_x agglutinogen (which are not, in general, agglutinated by anti-A serum derived from group B donors).

POTENCY

Titration

An anti-A + anti-B (group O) 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 equialent 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 O) blood-grouping serum used undiluted shall also give readily detectable agglutination of group A_x corpuscles.

Determination of avidity

When anti-A + anti-B (group O) serum is mixed on a slide with equal volumes of 5 % to 10 % suspensions of A_1, A_2 and A_2B corpuscles 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 O) serum is mixed on a slide with an equal volume of a 5 % to 10 % suspension of B corpuscles, 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 an anti-A + anti-B (group O) serum is mixed on a slide with an equal volume of a 5 % to 10 % suspension of A_x corpuscles, agglutination shall first appear in not more than 5 minutes at a temperature between 18° C and 25° C.

(b) *Blood-grouping sera of human origin for Rh groups*

Anti-Rh blood-grouping sera, whatever their specificity, may be of either of two varieties differing in the conditions under which they agglutinate homologous corpuscles. Certain sera commonly known as "complete" agglutinate corpuscles in a saline medium. Others, commonly known as "incomplete" agglutinate only 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.

Most "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 preparation become available.

(i) Anti-D (anti-Rh_0) blood-grouping serum (human)

Anti-D serum is derived from the blood of one or more persons immunised by the D agglutinogen of the Rh system. It agglutinates suspensions of human red corpuscles containing the D agglutinogen, but not human red corpuscles which do not contain the D agglutinogen.

POTENCY

Titration

"Complete" anti-D sera shall have a titre not less than 32 against CcDee (R_1r) corpuscles in 0.9 % solution of sodium chloride.

"Incomplete" anti-D sera shall have a titre not less than 128 against CcDee (R_1r) corpuscles under the conditions specified by the laboratory preparing them and besides agglutinating all corpuscles containing the D antigen they should, as far as possible, agglutinate all corpuscles containing the D^u antigen.

Determination of avidity

Anti-D sera intended for use on slides should, when mixed on a slide with an equal volume of a 40 % to 50 % suspension of CcDee (R_1r) 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 immunised by the C agglutinogen of the Rh system. It agglutinates suspensions of human red corpuscles containing the C agglutinogen, but not human red corpuscles, which do not contain the C agglutinogen. In this connection the C agglutinogen is regarded as including the C^w agglutinogen.

Most diagnostic anti-Ci sera contain "complete" anti-C together with "incomplete" anti-D. These sera are therefore specific for the C agglutinogen only when the corpuscles under test are suspended in a 0.9 % solution of sodium chloride.

POTENCY

Titration

Anti-C sera should have a titre not less than 8 against Ccddee (r'r) corpuscles.

Determination of avidity

Anti-C sera intended for use on slides (and which must not contain any form of anti-D) should, when mixed on a slide with an equal volume of a 40 % to 50 % suspension of Ccddee (r'r) corpuscles, 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 immunised by the E agglutinogen of the Rh system. It agglutinates suspensions of human red corpuscles containing the E agglutinogen, but not human red corpuscles which do not contain the E agglutinogen.

POTENCY

Titration

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

Determination of avidity

Anti-E sera intended for use on slides (and which must not contain any form of anti-D) should,when mixed on a slide with an equal volume of a 40 %

to 50 % suspension of ccddEe (r''r) corpuscles at approximately 40° C, show visible agglutination within 30 seconds, and agglutination should be complete within 120 seconds.

(iv) Anti-D + C (anti-Rh_0rh') blood-grouping serum (human)
Anti-D + E (anti-Rh_0rh'') blood-grouping serum (human)

Sera of specificity anti-D + C and of specificity anti-D + E may be obtained directly from the blood of immunised 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 agglutinogen. 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 immunised persons) it is desirable that the anti C titre should not be less than 32. Where a serum is intended for use in slide tests, the times of agglutination for all reacting types of red corpuscles should not be 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 immunised by group A red corpuscles or group A specific substances. Anti-A serum agglutinates human red corpuscles containing A agglutinogens, 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 agglutinogens, i.e. those of blood groups O and B.

POTENCY

Titration

An anti-A serum shall be titrated separately against suspensions of A_1, A_2 and A_2B 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.

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

Determination of avidity

When anti-A serum is mixed on a slide with an equal volume of 5 % to 10 % suspension of A_1, A_2 and A_2B corpuscles, 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 immunised by group B red corpuscles or group B specific substances. Anti-B serum agglutinates human red corpuscles containing B agglutinogen, i.e. those of blood groups B and AB, and does not agglutinate human red corpuscles which do not contain B agglutinogen, i.e. those of blood groups O 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 5 % to 10 % suspension of B corpuscles, 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]

In view of the present state of uncertainty regarding the precise nature of the proteins involved in the antiglobulin reaction and of the diverse components present in antiglobulin sera of different origins, the specificity of antiglobulin sera can at present only be defined in terms of their reactions with red corpuscles coated with a variety of antibodies.

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

Coombs, R.R.A., Mourant, A.E. and Race, R.R. (1945), Lancet, ii, 15

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

Definition

Anti-human-globulin serum is derived from the blood of animals immunised by the injection of human serum proteins. Anti-human globulin serum agglutinates all human red corpuscles coated with human globulins, whether actively as the result of an antigen-antibody reaction, or passively following treatment of the red corpuscles with tannic acid. Under the conditions specified by the manufacturer, it does not agglutinate uncoated human red corpuscles to whatever group they may belong.

POTENCY

Titration

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

It shall also, at the same or a different dilution, as specified on the label, agglutinate human red corpuscles coated with an incomplete antibody such as anti-Le^a for the demonstration of which the presence of fresh human serum is needed.

It shall not agglutinate uncoated human red corpuscles at either of these dilutions.

For routine clinical use it is desirable that the coating with all the types of incomplete antibody mentioned 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 agglutinogens, 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 agglutinogens, i.e. those of blood groups O and B.

POTENCY

Titration

An anti-A reagent 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.[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 a equal volume of a 5 % to 10 % suspension of A_1, A_2 and A_2B corpuscles, 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 agglutinogen, i.e. those of blood groups B and AB, and does not agglutinate human red corpuscles which do not contain B agglutinogen, 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 5 % to 10 % suspension of B corpuscles, 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.

ANNEXES TO THE PROTOCOL — ANNEXES AU PROTOCOL

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. N_3Na 0,1 % 4. 5 ml 5. 7 septembre 1965 6. N° 1 2 3 4	(a) *fluid serum* 1. .... Laboratory, Amsterdam 2. Anti-A serum (human) 3. Sodium Azide 0,1 % 4. 5 ml 5. 7th September, 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° 4321	(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. 31st December, 1968 6. N° 4321

EXEMPLE DE NOTICE
EXAMPLE OF LEAFLET

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

EUROPEAN AGREEMENT ON THE EXCHANGE OF BLOOD-GROUPING REAGENTS

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 0,9 %. Une suspension d'environ 3 % est préparée ensuite en mélangeant un volume ou une goutte de culot globulaire avec 30 volumes ou gouttes de solution saline. 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 une 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 0,01 ml). 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

1. Central Blood Transfusion Laboratory, 1 Main Street, Metropolis, Westland
2. Anti E (anti-rh") serum (human)
3. 10 ml
4. Date of last potency test : 30th May 1961
5. Expiry Date: 30th May 1962
6. No. 5432
7. The red corpuscles to be tested are washed one or more times with 0.9 % saline. An approximately 3 % suspension is prepared by mixing one volume or drop packed red corpuscles with 30 volumes or drops of saline. 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 corpuscle suspension from pipettes marked at a volume of 0.01 ml). 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

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 10%.

9. Sérum humain anti-E (anti-rh") : 5 ml
Albumine bovine à 30% : 5 ml

10. Ce réactif contient une substance d'origine humaine.

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 10 % solution of sodium azide.

9. Human anti-E (anti-rh") serum : 5 ml,
30 % solution of bovine albumin : 5 ml

10. This product contains material of human origin.

CONSEIL DE L'EUROPE

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ACCORD EUROPÉEN RELATIF À L'ÉCHANGE DES RÉACTIFS POUR LA DÉTERMINATION DES 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	Le soussigné déclare que l'envoi spécifié en marge . . . .
colis	The undersigned certifies that the shipment specified in the margin
Number of	. . . .
packages	. . . .
. . . . .	préparé sous la responsabilité de . . . .
. . . . .	prepared under the responsibility of . . . .
Désignation	. . . .
Marked	. . . .
. . . . .	organisme visé à l'article 6 de l'Accord, est conforme aux spéci-
. . . . .	one of the bodies referred to in Article 6 of the Agreement, is in
Nº des lots	fications du Protocole à l'Accord et qu'il peut être délivré immédiate-
Batch No.	conformity with the specifications of the Protocol to the Agree-
. . . . .	ment au destinataire (nom et lieu) . . . .
. . . . .	ment and can be delivered immediately to the consignee (name
. . . . .	. . . .
. . . . .	and place) . . . .

(cachet) (signature) (titre)
(stamp) (signature) (title)