COUNCIL OF EUROPE
CONSEIL DE L'EUROPE

*European Treaty Series - No. 84*

# European Agreement on the Exchange of Tissue-typing reagents

Strasbourg, 17.IX.1974

## Protocol *

---

### General provisions

1 Specificity

A *Tissue-typing reagents to be used in cytotoxic techniques on lymphocytes*

These reagents must, when used according to the technique recommended by the producer, react with all lymphocytes known to contain the antigen(s) corresponding to the specificity(ies) mentioned on the label. They must not react with any cell known not to contain this antigen (these antigens). If a sole reagent does not satisfy these conditions, a combination of four sera of the same specificity must be used together. In this case, at least three sera must react with each lymphocyte sample containing the corresponding antigen and, inversely, not more than one should react with cells not containing this antigen.

When these reagents are used according to the technique recommended by the producer there must be no evidence of any interfering serological phenomena such as:

a prozone effects,
b anticomplementarity.

B *Tissue-typing reagents for use in a complement fixation technique on platelets*

These reagents must, when used according to the technique recommended by the producer, give complement fixation with all platelets known to contain the antigen(s) corresponding to the specificity(ies) mentioned on the label. They must not give complement fixation with any platelets known not to contain this antigen (these antigens). If a sole reagent does not satisfy these conditions, a combination of four reagents of the same specificity must be used together. In this case, at least three sera must react with each platelet sample containing the corresponding antigen and, inversely, not more than one should react with cells not containing this antigen.

When these reagents are used according to the technique recommended by the producer there must be no evidence of any interfering serological phenomena such as:

a prozone effects,
b anticomplementarity.

---

(*) Revised text as approved by the Committee of Ministers at its 378th meeting (13 December 1984).

2 Potency

A *Tissue-typing reagents to be used in cytotoxic techniques on lymphocytes*

The titre of such a reagent is determined by making successive twofold dilutions of the serum under study in inactivated AB serum or in another appropriate medium from a donor who is negative for the antigen(s) corresponding to the antibody (antibodies) in the reagent and who should also not have been immunised against tissue antigens by transfusion, pregnancy or other means. Each dilution is then tested with lymphocytes known to contain the corresponding antigen(s) in the reagent, using the technique recommended by the producer. The titre is the reciprocal of the figure representing the highest serum dilution in which a significantly positive reaction occurs, the dilution being calculated without the inclusion of the volume of the corpuscular suspension or any other additive in the total volume.

B *Tissue-typing reagents for use in a complement fixation technique on platelets*

The titre of such a reagent is determined by making successive twofold dilutions of the serum under study in a solution containing inactivated AB serum in Veronal (R) buffer with a volume fraction of 0.01. Each serum is then tested with platelets known to contain the antigen homologous to the antibodies in the reagent, using the technique recommended by the producer. The titre is the reciprocal of the figure representing the highest serum dilution in which a significantly positive reaction occurs, the dilution being calculated without the inclusion of the volume of the corpuscular suspension or any other additive in the total volume.

Further provisions, for tissue-typing reagents to be used in cytotoxic techniques on lymphocytes as well as for reagents to be used in a complement fixation technique on platelets:

3 Preservation

Tissue-typing reagents may be preserved in the liquid or in the dried state. Liquid reagents shall be kept at a temperature not above - 40°C and dried reagents at a temperature not above + 4°C.

Thawing and refreezing of the reagents during the period of storage must be avoided as much as possible.

Dried reagents shall be kept in an atmosphere of inert gas or in vacuo in the 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 phosphorous pentoxide at a pressure not exceeding 0.02mm 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 producer may decide that an antiseptic and/or antibiotic shall be added to the reagent. In such cases the reagent must still fulfil the requirements for specificity and potency in the presence of the added substance.

The above also applies to any other additives such as anticoagulants. Reagents, after thawing or after reconstitution, should be transparent and should not contain any sediment, gel or visible particles.

4 Stability and expiry date

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

The expiry date of a reagent in the liquid state 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.

5 Dispensing and volume

Tissue-typing 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 as described in this Protocol.

6 Records and samples

Written records shall be kept by the producing laboratory of all steps in the production and control of tissue-typing 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.

7 Shipment

Frozen reagents must be shipped in such fashion that they remain frozen until arrival. Care must be taken to protect reagents against inactivation by the entry of CO2. Dried reagents may be shipped at ambient temperatures.

8 Labels, leaflets and certificates

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

a name and address of producer,

b the specificity of the reagent,

c name and amount of antiseptic and/or antibiotic, or indication of absence,

d the volume or, when the reagent is dried, the volume and composition of the fluid needed for reconstitution,

e expiry date,

f identification,

g conditions of storage,

h results of the test for HBs-Ag.

Moreover, the leaflet accompanying the containers shall include the following information:

a full name and address of producer,

b the recognised specificity of the reagent,

c the volume or, when the reagent is dried, the volume and composition of the fluid needed for reconstitution,

d date of last potency test,

e expiry date (if any),

f identification and (if possible) the name of the reagent,

g adequate description of the method of use recommended by the producer including technique, volume and dilution to be used,

h conditions of storage of unopened ampoules and precautions to be taken after opening,

i exact composition, including antiseptic and/or antibiotic if any,

j statement whether the product contains or does not contain material of human origin,

k the reaction score ++, -+, +-, --, and the values of coefficient r (serum/antigen).

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 label and leaflet are attached to the present Protocol.

**Specific provisions**

To be completed under Article 4, paragraph 4, of the European Agreement on the Exchange of Tissue-typing Reagents.