# COMMISSION OF THE EUROPEAN COMMUNITIES

COM(78) 614 final

Brussels, 21 November 1978

Proposal for a

COUNCIL DIRECTIVE

amending Directive 64/432/EEC in respect of enzootic leukosis among cattle

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(submitted to the Council by the Commission)

Enzootic bovine leukosis (E.B.L.) is an infectious virus disease inducing proliferation of leukocytes (white blood cells) and causing a blood cancer. 1 - 2% of cows in infected herds are lost each year due to these tumours.

The disease has been recognised in most parts of the world including Europe it's spread being by way of trade in live animals from North Eastern Europe. In some regions 10 - 20% of herds have been demonstrated to be infected. In certain Member States E.B.L. is under eradication by national control systems all breeding herds being monitored by means of tests. Surveillance is now facilitated by a more efficient test (serological). Other Member States have not yet taken decisions concerning eradication although they have observed the disease.

While Community guarantees concerning E.B.L. in cattle traded between Member States have not until now been provided in Directive 64/432/EEC, the mechanism of Article 8.2 of that Directive has been availed of by five Member States to obtain health guarantees when breeding or production cattle are traded. It is now considered essential that community rules be adopted and it is proposed that Member States which have set up E.B.L. free herds should be afforded protection when animals are traded into such herds from other Member States.

Because of the fragmentary nature of rules in the Member States concerning E.B.L. at present this proposal should be seen as a first step towards more elaborate control systems which in the future should lead to the eradication of the disease within the Community.

. Proposal

Council Directive

of

amending Directive 64/432/EEC in respect of enzootic leukosis among cattle

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community, and in particular Articles 43 and 100 thereof,

Having regard to the proposal from the Commission,

Having regard to the opinion of the European Parliament,

Having regard to the opinion of the Economic and Social Committee,

Whereas one of the tasks of the Community in the veterinary field is to improve the state of health of livestock, and thus to make stock-breeding more profitable;

Whereas there is a need to protect the Community against enzootic leukosis among cattle; whereas the Community, by Council Directive 77/391/EEC[1] and 78/52/EEC[2], has already taken measures to eradicate this disease;

Whereas such measures must contribute to the abolition of barriers to trade in fresh meat or live animals between Member States, which are due to differences in health situations;

Whereas the measures for protection against enzootic leucosis among cattle should accordingly be incorporated in Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra-Community trade in bovine animals and swine[3], as last amended by Directive [4];

Whereas provision should be made for certain special temporary measures in order to facilitate the introduction of such protection measures;

Whereas, with regard to enzootic leucosis among cattle, the risk of the disease spreading must be assessed according to categories of animals; whereas provision should therefore be made for derogations applicable to animals for meat production, and animals for slaughter should not be included in the system provided for,

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[1] OJ No L 145, 13.6.1977, p. 44.

[2] OJ No L 15, 19.1.1978, p. 34.

[3] OJ No 121, 29.7.1964, p. 1977/64.

[4] OJ No

HAS ADOPTED THIS DIRECTIVE :

Article 1

Directive 64/432/EEC is amended as follows:

1. In Article 2 the following point (p) is added:

"(p) Enzootic bovine leukosis free herd : means a bovine herd which satisfies the conditions laid down in Annex G.

Article 2

2. In Article 3 the following point (e) is added to paragraph 3:
to paragraph 3:

(e) in the case of animals consigned to an enzootic bovine leukosis free herd, come from an enzootic bovine leukosis free herd and have reacted negatively to a serological test carried out during the 30 days before loading and in accordance with the provisions of Annex H."

3. In Article 7 the following point (c) is added to sub-paragraph B of paragraph 1 :

" (c) those animals which, by way of derogation from Article 3(3) (e) do not come from an enzootic bovine leukosis free herd. However these animals must come from a bovine herd all of whose animals have reacted negatively to a serological test carried out during the 30 days before loading and in accordance with Annex H.

This provision shall apply until 31 December 1981."

4. The following Article 7a is inserted:

"

Article 7a

Countries of destination shall grant to exporting countries general authorisations for the introduction into their territories of bovine animals for meat production, under 30 months of age, which by way of derogation from Article 3(3)(e), do not come from an enzootic bovine leukosis free herd. Such animals shall at loading for transportation to the country of destination bear a special identification mark. The Member States of destination shall take all necessary measures to ensure that the animals shall at no time come into contact with enzootic bovine leukosis free herds in their territories."

5. The following is added to point (a) of Annex E:
"Enzootic bovine leukosis in all its forms."

6. 1. In point V of the Health Certificate, Model 1, in Annex F, there is inserted after paragraph (d) a new paragraph ($d_1$) as follows:

($d_1$) - they come from an enzootic bovine leukosis free herd (2)

- they do not come from an enzootic bovine leukosis free herd (2)

The result of the serological test carried out within the prescribed 30 days time limit (5) was negative (2).

- they are bovine animals intended only for meat production and do not come from a bovine herd which is enzootic bovine leukosis free (2) (11).

2. The following is added to the footnotes to Model 1 in Annex F.

(11) This derogation applies only in the case of bovine animals for meat production on condition that they bear a special mark and are specially supervised in the country of destination.

Article 7

"The following Annexes G and H are added:"

ANNEX G

ENZOOTIC BOVINE LEUKOSIS FREE HERD

A. A bovine herd is considered to be enzootic bovine leukosis free if :

(a) within the last three years no leukotic tumour cases or leukotic infiltrations associated with bovine leukosis virus in live or dead animals have been officially detected and no other facts have been reported which might give rise to suspicion of enzootic bovine leukosis in the herd.

(b) all the bovine animals over 12 months of age have reacted negatively to at least two official serological tests carried out in accordance with Annex H at intervals of at least 4 months and at most 12 months.

(c) in the interval between the first and second serological tests described under paragraph (b), no bovine animal has been introduced into the herd without a certificate from an official veterinarian declaring that the animal comes from,

- an enzootic bovine leukosis free herd,

  or

- a bovine herd in which all animals have reacted negatively to a serological test carried out in accordance with Annex H during the 30 days before it was taken into the herd.

B. A bovine herd considered to be enzootic bovine leukosis free according to paragraph A shall continue to be so considered if :

(a) since the herds approval as enzootic bovine leukosis free no leukotic tumour cases or leukotic infiltration associated with bovine leukosis virus in live or dead animals have been officially detected and no other facts have been reported which might give rise to suspicion of enzootic bovine leukosis in the herd.

(b) all the bovine animals over 12 months of age have reacted negatively to serological check-tests carried out in accordance with Annex H at intervals of at least 12 months and at most 3 years after the approval of the herd as enzootic bovine leukosis free.

(c) no bovine animal has been introduced into the herd without a certificate from an official veterinarian declaring that the animal comes from :

- an enzootic bovine leukosis free herd,

  or

- a bovine herd in which all animals have reacted negatively to a serological test carried out in accordance with Annex H during the 30 days before it was taken into the herd.

C. (a) Until 31 March 1980 and notwithstanding the provisions of Article 2 (p) a bovine herd may also be considered to be enzootic bovine leukosis free provided that,

(i) within the last three years no leukotic tumour cases or leukotic infiltrations in live or dead animals have been officially detected and no other facts have been reported which might give rise to suspicion of enzootic bovine leukosis in the herd.

(ii) before 1 January 1979 every animal over two years of age forming part of the bovine herd has been the subject of two blood tests for leukosis at intervals of at least 12 months which have given no indication of an abnormal increase in the number of lymphocytes.

(iii) the herd conforms to the national rules of the Member State concerned and is deemed to be enzootic bovine leukosis free according to those rules on 31 December 1978.

(b) The blood test referred to in paragraph (a) (ii) shall comply with the following requirements :

(i) account shall be taken of the absolute number of leukocytes and the proportion of lymphocytes. The total number of lymphocytes per cubic millimetre shall be the determining factor and shall be calculated according to the following formula :

$$\frac{\text{total number of leukocytes per cubic millimetre} \times \text{percentage of lymphocytes}}{100}$$

(ii) the presence of an abnormal increase in the number of lymphocytes shall be concluded in the light of the following data :

for bovine animals :

of more than two years and not more than three years - more than 10 500 lymphocytes per cubic millimetre;

of more than three years and not more than four years - more than 9 500 lymphocytes per cubic millimetre;

of more than four years and not more than five years - more than 8 500 lymphocytes per cubic millimetre;

of more than five years and not more than six years - more than 8 000 lymphocytes per cubic millimetre;

of more than six years - more than 7 500 lymphocytes per cubic millimetre;

(iii) if the result of the blood test referred to in paragraph (a) (ii) indicates a relatively high lymphocyte count i.e. a number of lymphocytes less than 2 000 below the figures mentioned in (b) (ii), the same sample must be immediately given a fresh test, the result of which shall form the basis of the final evaluation.

ANNEX H

A. Agar gel immuno diffusion test

1. The antigen to be used in the test shall contain bovine leukosis virus glyco proteins. The antigen shall be standardised against a standard serum (E.I. serum) supplied by the State Veterinary Serum Laboratory, Copenhagen.

2. The official institutes indicated below shall be made responsible for calibrating the working laboratory standard antigen against the official E.E.C. standard serum (E.I. serum) provided by the State Veterinary Serum Laboratory, Copenhagen.

| | | |
|---|---|---|
| (a) | Germany | Bundesforschungsanstalt für Virus-krankheiten der Tiere - Tübingen |
| (b) | Belgium | Institut national de recherches vétérinaires, Bruxelles |
| (c) | France | Laboratoire des Médicaments Vétérinaires, Fougères |
| (d) | Grand Duchy of Luxembourg | - |
| (e) | Italy | Instituto Zooprofilattico Sperimentale, Perugia |
| (f) | Netherlands | Centraal Diergeneeskundig Instituut, Afdeling Rotterdam |
| (g) | Denmark | Statens Veterinaere Serum Laboratorium, Copenhagen |
| (h) | Ireland | Veterinary Research Laboratory, Abbotstown, Dublin |
| (i) | United Kingdom | The Central Veterinary Laboratory, Weybridge, England. |

3. The working laboratory standard antigens shall be submitted for testing against the official E.E.C. standard serum at least once a year to the E.E.C. reference laboratories listed in paragraph 2 above. Besides this standardisation the antigen in use can be calibrated according to paragraph B.

4. The reagents for the test shall consist of :

a) antigen : the antigen shall contain specific glycoproteins of enzootic bovine leukosis virus which has been standardised against the official E.E.C. serum.

b) the test serum

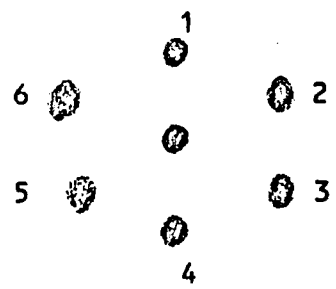
c) known positive control serum

d) Agar-gel
0.8% agar
8.5% NaCl
0.05 M Tris-buffer pH 7.2
15 ml of this agar shall be filled into a Petri-dish of 85 mm diameter, resulting in a depth of 2.6 mm Agar.

5. A test pattern of sever moisture free wells shall be cut in the agar to the bottom of the plate; the pattern shall consist of one centre well and six wells in a circle around it.

Diameter of central well : 4 mm
Diameter of peripheral wells : 6 mm
Distance between central and peripheral wells : 3 mm

6. The central well shall be filled with the standard antigen. The peripheral wells 1 and 4 (according to scheme) are filled with known positive serum, the wells 2, 3, 5 and 6 are filled with test sera. The wells shall be filled until the meniscus disappears.




7. This results in the following amounts of reagents :

| | |
|---|---|
| Antigen : | 32 microlitres |
| Control serum : | 73 microlitres |
| Test sera : | 73 microlitres |

8. Incubation shall be for 72 hours at room temperature (20-27°C) in a closed humid chamber.

9. The test may be read at 24 and 48 hours but a final result may not be obtained before 72 hours.
   a) a test serum is positive if it forms a specific precipitin line with the B.L.V. antigen and forms a complete line of identity with the control serum;

b) a test serum is negative if it does not form a specific line with the B.L.V. antigen and if it does not bend the line of the control serum;

c) the reaction would be considered inconclusive if it :

i) bends the line of the control serum towards the B.L.V. antigen well without forming a visible precipitin line with the antigen, or

ii) if it cannot be read either as negative or as positive.

In inconclusive reactions the test may be re-run and concentrated serum may be utilised.

B. Method for Antigen Standardisation

Necessary solutions and materials :

1. 40 ml of 1.6% agarose in 0.05 M Tris/HCL buffer, pH 7.2 with 8.5% NaCl.
2. 15 ml of a bovine leukosis serum, having antibody only to bovine leukosis virus glycoproteins, diluted 1 : 10 in 0.05 M Tris/HCl buffer, pH 7.2 with 8.5% NaCl.
3. 15 ml of a bovine leukosis serum, having antibody only to bovine leukosis virus glycoproteins, diluted 1 : 5 in 0.05 M Tris/HCl buffer, pH 7.2 with 8.5% NaCl.
4. Four plastic petri dishes with a diameter of 85 mm.
5. A punch with a diameter of 4 - 6 mm.
6. A reference antigen.
7. The antigen which is to be standardised.
8. A waterbath (56°C).

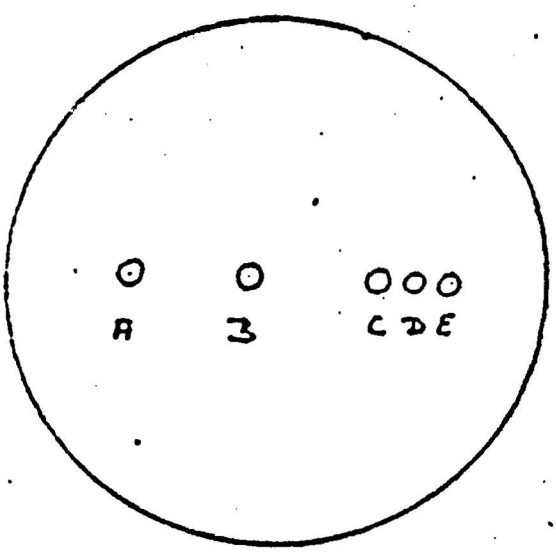
Procedure :

Dissolve the agarose (1.6%) in the Tris/HCl buffer by carefully heating to 100°C. Place in 56°C water bath for approximately 1 hour. Also, place the bovine leukosis serum dilutions in 56°C water bath.

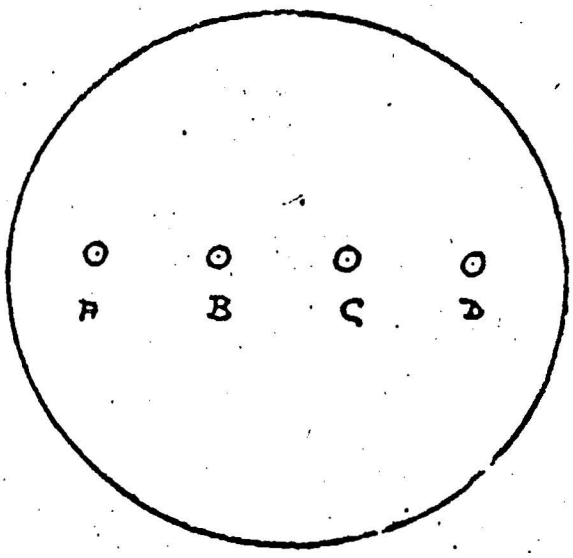
Now, mix 15 ml of the 56°C agarose solution with the 15 ml bovine leukosis serum (1 : 10), quickly shake and pour 15 ml into 2 petri dishes each. Repeat this procedure with the bovine leukosis serum diluted 1 : 5.

When the agarose has hardened, holes are punched according to the following scheme :

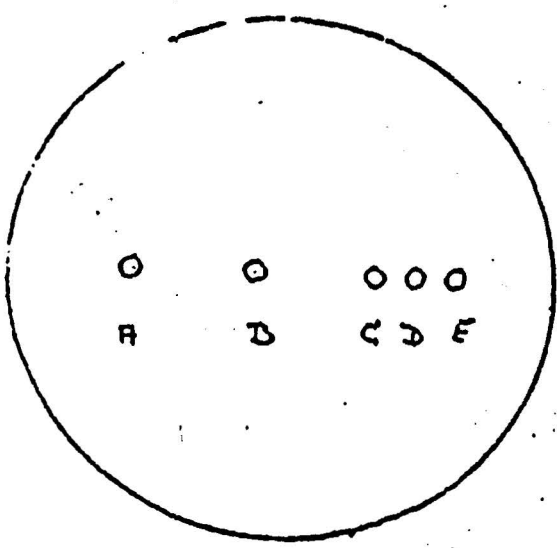
Petri dish No. 1
Serum 1:10



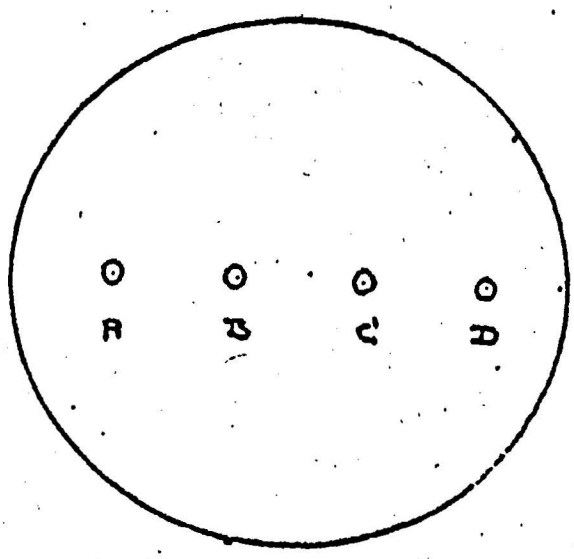

Petri dish No 2
Serum 1:10




Petri dish No. 3
Serum 1:5




Petri dish No. 4
Serum 1:5



Addition of antigen:

I. Petri dishes 1+3

Well A = Undiluted reference antigen
Well B = 1:2 diluted reference antigen
Well C+E = Reference antigen
Well D = Undiluted antigen to be tested

II. Petri dishes 2+4

Well A = Undiluted test antigen
Well B = 1:2 diluted test antigen
Well C = 1:4 diluted test antigen
Well D = 1:8 diluted test antigen

Additional Instructions

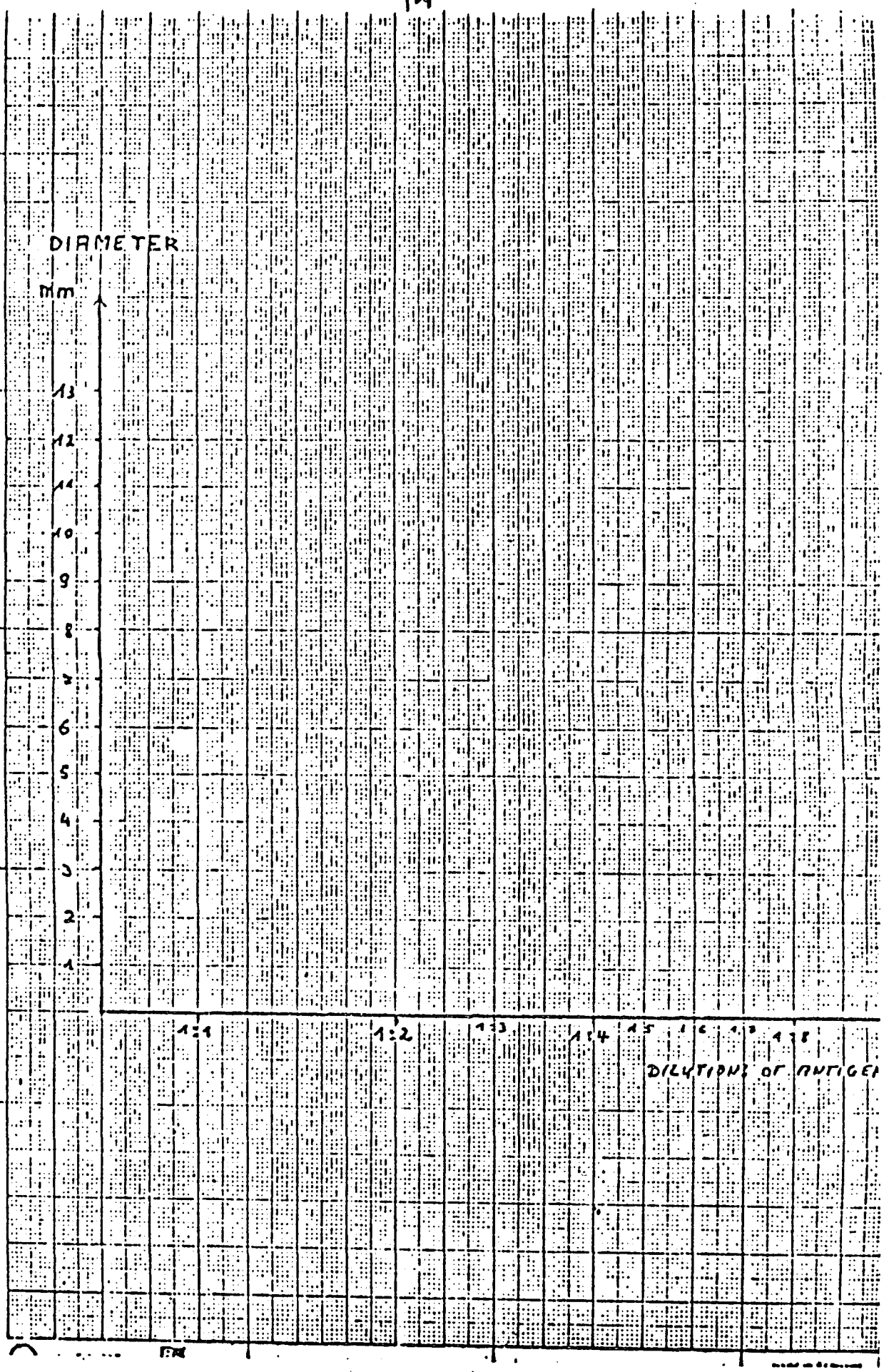
1. The experiment shall be carried out with two serum dilutions (1:5 and 1:10) in order to achieve optimal precipitation.

2. If the precipitation diameter is too small with both dilutions, then the serum shall be further diluted.

3. If the precipitation diameter in both dilutions is too large and faint, then a lower serum dilution shall be chosen.

4. The final concentration of the agarose shall be 0.8%; that of the sera 5% and 10% respectively.

5. Plot the measured diameters in the following coordinate system. The dilution of the antigen to be tested with the same diameter as the reference antigen is the working dilution.


DIAMETER
Mm
13
12
11
10
9
8
7
6
5
4
3
2
1
1:1
1:2
1:3
1:4
1:5
1:6
1:7
1:8
DILUTION OF ANTIGEN

Article 2

This Directive shall be implemented from 1st January 1979.

Article 3

This Directive is addressed to the Member States.

Done at Brussels,

For the Council,

The President