COMMISSION DECISION

of 21 December 1979

authorizing Denmark and the Federal Republic of Germany to apply special health guarantees for the prevention of leucosis in the case of bovine animals imported for breeding or production

(Only the Danish and German texts are authentic)

(80/235/EEC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting trade in bovine animals and swine (1), as last amended by Directive 79/580/EEC (2), and in particular Article 8 (2) thereof,

Whereas in accordance with Article 8 (2) of Council Directive 64/432/EEC a Member State may be authorized, under the procedure laid down in Article 12 and the conditions fixed under that procedure, to apply for intra-Community trade, health guarantees equivalent at most to those required by that Member State within the framework of a national programme for the prevention of a contagious or infectious bovine or swine disease which is not referred to in Annex E to the abovementioned Directive;

Whereas by Commission Decision 77/331/EEC of 22 April 1977 (3), as last amended by Decision 79/80/EEC (4), Denmark has already, by this procedure, been authorized to apply special health guarantees for the prevention of leucosis in the case of bovine animals imported for breeding or production;

Whereas by Commission Decision 73/30/EEC of 23 January 1973 (5), as last amended by Decision 79/80/EEC, the Federal Republic of Germany has also, by this procedure, been authorized to apply special health guarantees for the prevention of leucosis in the case of bovine animals imported for breeding or production;

Whereas Denmark and the Federal Republic of Germany by communications of 20 and 5 November 1979 respectively, have requested permission to apply special health guarantees in respect of leucosis for bovine animals imported for breeding or production originating in a Member State and intended in particular for combining with bovine stock not under suspicion for leucosis;

Whereas Denmark and the Federal Republic of Germany have submitted national plans for the accelerated eradication of leucosis which were approved by Commission Decisions 78/481/EEC (6) and 79/48/EEC (7); whereas these plans are currently implemented;

Whereas the special health guarantees which Denmark and the Federal Republic of Germany wish to apply in intra-Community trade in bovine animals for breeding or production are equivalent at most to those which these Member States apply within the framework of their national programmes for the prevention of leucosis; whereas their requests can therefore be granted;

Whereas Denmark and the Federal Republic of Germany have requested that the authorizations take effect on 1 January 1980; whereas the authorizations should be given for a limited period so as not to prejudice harmonization at a later stage;

Whereas the measures provided for in this Decision are in accordance with the opinion of the Standing Veterinary Committee,

HAS ADOPTED THIS DECISION :

Article 1

From 1 January to 31 December 1980, Denmark and the Federal Republic of Germany are authorized to

(1) OJ No 121, 29. 7. 1964, p. 1977/64.
(2) OJ No L 158, 26. 6. 1979, p. 17.
(3) OJ No L 116, 7. 5. 1977, p. 13.
(4) OJ No L 17, 21.1. 1979, p. 22.
(5) OJ No L 77, 26. 3. 1973, p. 40.
(6) OJ No L 152, 8. 6. 1978, p. 23.
(7) OJ No L 13, 19. 1. 1979, p. 63.

require the application of the health guarantees laid down in Article 2 on entry into their territory of bovine animals for breeding or production, originating from the territory of another Member State and intended for combining with bovine stock not under suspicion of leucosis.

Article 2

The health guarantees referred to in Article 1 shall consist of the production of a certificate issued on the day of embarkation by an official veterinarian, drawn up, as a minimum requirement, in the language of the country of destination and certifying:

1. that the veterinarian has no official knowledge of facts which would lead him to conclude that a case of leucosis has occurred within the three preceding years in the bovine herd stock of origin, and that the owner of this herd had declared in writing that he has no knowledge of such facts and that he has also, in writing, further declared that the animal or animals intended for intra-Community trade have either been born and bred in the said herd or have remained an integral part of it for the previous 12 months;
2. that during the past 12 months all the bovine animals over 12 months of age forming part of the bovine herd stock of origin have reacted negatively to a serological test carried out in accordance with Annex A or a haematological test carried out in accordance with Annex B.

Article 3

This Decision is addressed to the Kingdom of Denmark and the Federal Republic of Germany.

Done at Brussels, 21 December 1979.

For the Commission

Finn GUNDELACH

Vice-President

ANNEX A

A. **Agar gel immuno diffusion test**

1. The antigen to be used in the test shall contain bovine leucosis virus glyco proteins. The antigen shall be standardized against a standard serum (EI 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 EEC standard serum (EI serum) provided by the State Veterinary Serum Laboratory, Copenhagen.

   (a) Germany: Bundesforschungsanstalt für Viruskrankheiten der Tiere — Tübingen;

   (b) Denmark: Statens Veterinaere Serum Laboratorium, Copenhagen.

3. The working laboratory standard antigens shall be submitted for testing against the official EEC standard serum at least once a year to the EEC reference laboratories listed in paragraph 2 above. Besides this standardization 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 leucosis virus which has been standardized against the official EEC 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.

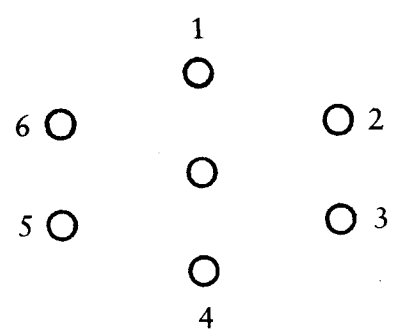
5. A test pattern of seven moisture-free wells shall be cut in the agar to the bottom of the plate; the pattern shall consist of one centre well and six wells in a circle around it.

   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 to 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 BLV 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 BLV 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 BLV 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 utilized.

B. **Method for antigen standardization**

Necessary solutions and materials :

1. 40 ml of 1·6 % agarose in 0·05 M Tris/H l buffer, pH 7·2 with 8·2 % NaCl;

2. 15 ml of a bovine leucosis serum, having antibody only to bovine leucosis 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 leucosis serum, having antibody only to bovine leucosis virus glycoproteins, diluted 1 : 5 in 0·5 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 to 6 mm;

6. a reference antigen;

7. the antigen which is to be standardized;

8. a waterbath (56 °C).

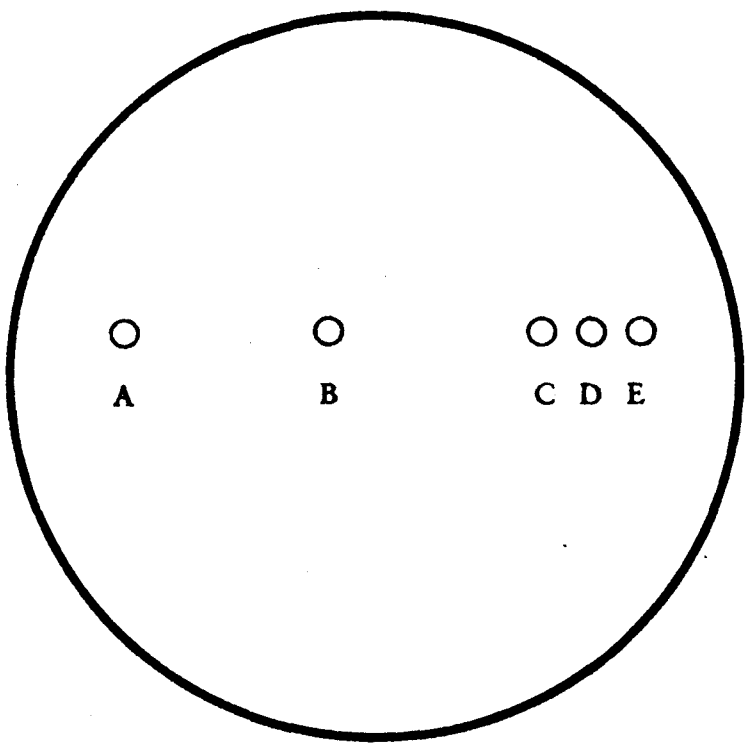
Procedure

Dissolve the agarose (1·6 %) in the Tris/HCl buffer by carefully heating to 100 °C. Place in 56 °C water bath for approximately one hour. Also, place the bovine leucosis serum dilutions in 56 °C water bath.

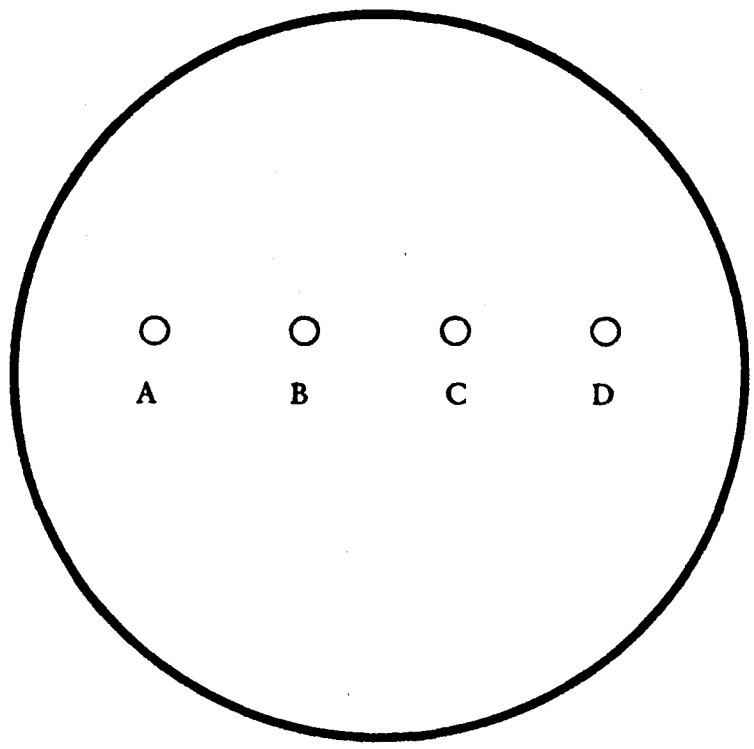
Now mix 15 ml of the 56 °C agarose solution with the 15 ml bovine leucosis serum (1 : 10), quickly shake and pour 15 ml into two petri dishes each. Repeat this procedure with the bovine leucosis serum diluted 1 : 5.

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

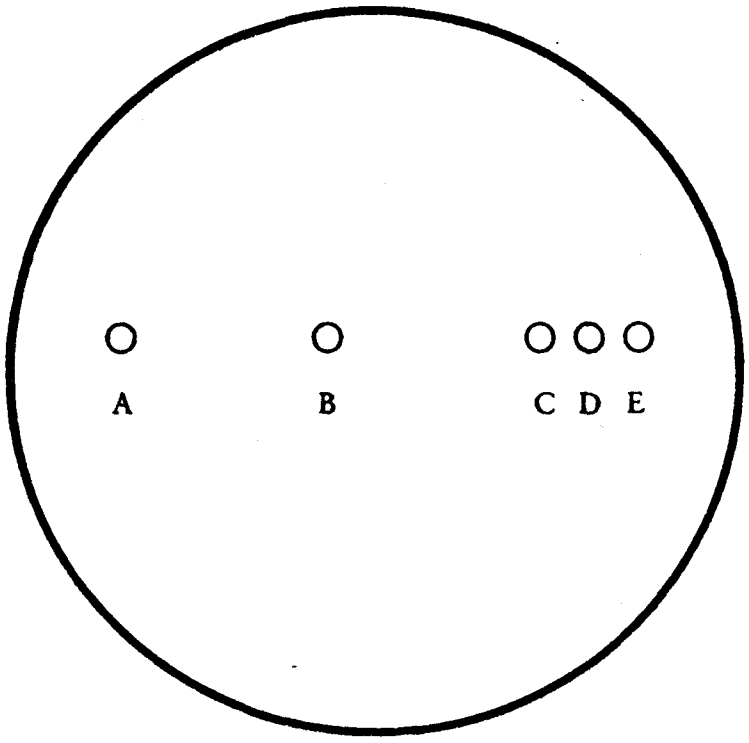
Petri dish No 1
Serum 1 : 10



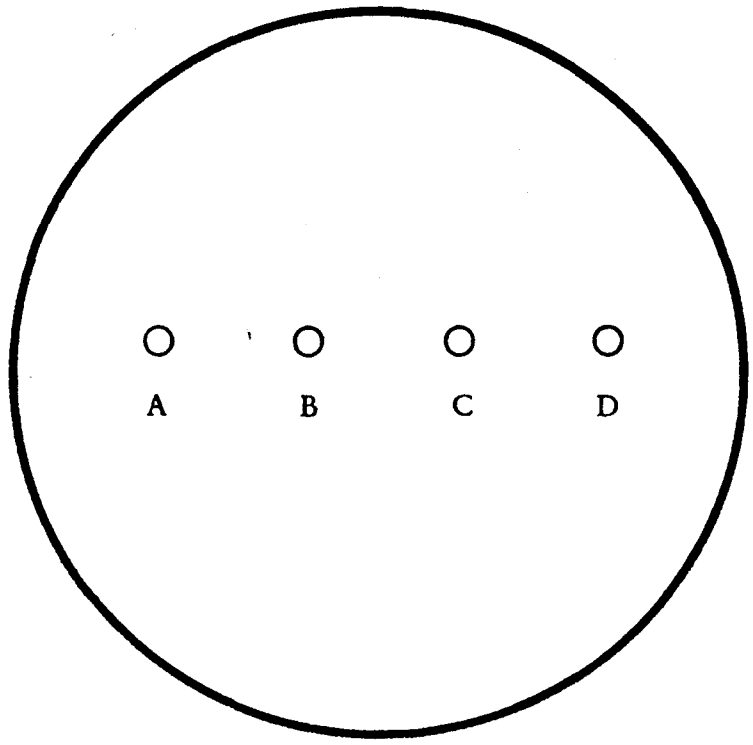
Petri dish No 2
Serum 1 : 10



Petri dish No 3
Serum 1 : 5



Petri dish No 4
Serum 1 : 5



Addition of antigen:

I. Petri dishes 1 and 3:

well A — undiluted reference antigen,
well B — 1 : 2 diluted reference antigen,
well C and E — reference antigen,
well D — undiluted antigen to be tested.

II. Petri dishes 2 and 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

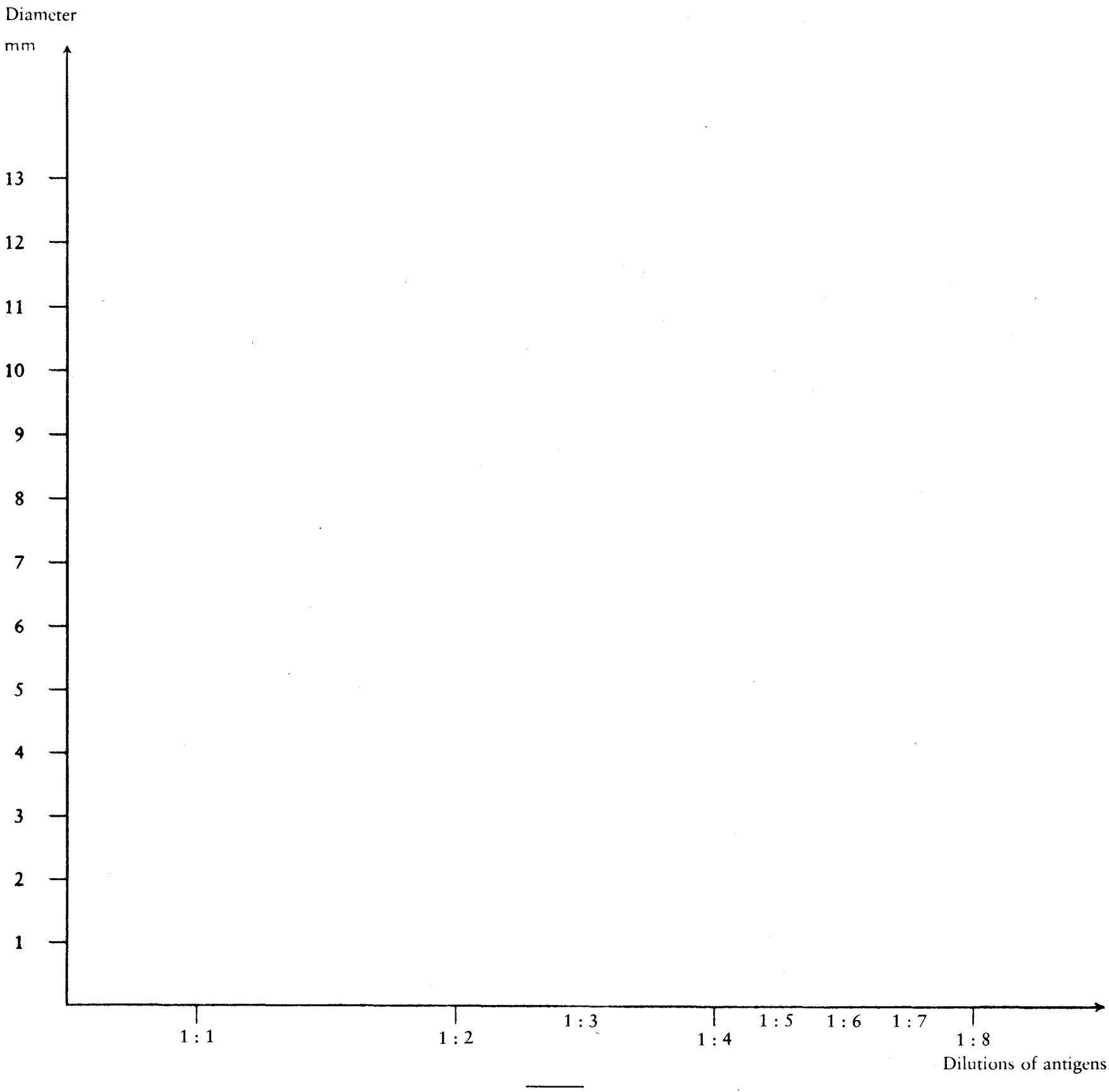
1. The experiment shall be carried out with two serum dilutions (1 : 5 and 1 : 10) in order to achieve optimal precipitation.

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.



ANNEX B

The blood test referred to in Article 2 (2) shall comply with the following requirements:

(a) 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:

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

(b) 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;

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