COUNCIL DIRECTIVE

of 11 November 1980

amending Directive 64/432/EEC with regard to enzootic bovine leukosis

(80/1102/EEC)

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 (1),

Having regard to the opinion of the European Parliament (2),

Having regard to the opinion of the Economic and Social Committee (3),

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 bovine leukosis; whereas the Community, by Council Directives 77/391/EEC (4) and 78/52/EEC (5), has already taken measures to eradicate this disease;

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

Whereas the measures for protection against enzootic bovine leukosis 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 (6), as last amended by Directive 80/1098/EEC (7);

Whereas provision should be made for certain special temporary measures and for the possibility of applying these measures on a regional basis in order to facilitate the introduction of such protection measures;

Whereas the special health guarantees applicable in intra-Community trade in bovine animals for breeding or production must be equivalent at most to those which the Member States apply within the framework of their national programmes for the prevention of enzootic bovine leukosis;

Whereas the risk of the spread of enzootic bovine leukosis must be assessed according to categories of animals; whereas provision should therefore be made for derogations in the case of animals for meat production, and whereas animals for slaughter should not be included in the system provided for,

HAS ADOPTED THIS DIRECTIVE:

Article 1

Directive 64/432/EEC is hereby amended as follows:

1. the following point shall be added to Article 3 (2):

'(j) in the case of pure-bred breeding bovine animals, as defined in Article 1 of Directive 77/504/EEC, which are intended solely for reproductive purposes and are highly valuable, come from a herd:

(i) in which no facts have been brought to the notice of the official veterinarian which would lead him to conclude that a case of enzootic bovine leukosis has occurred within the three preceding years;

(ii) the owner of which has declared that he has no knowledge of such facts and has further declared in writing that the animal or animals intended for intra-Community trade have either been born and reared in the said herd or have remained an integral part of it for the previous 12 months.';

2. the following subparagraph shall be added to Article 3 (3):

'(e) come from a herd in which there has been no evidence of enzootic bovine leukosis during the preceding three years and, if they are more than 12 months of age, have reacted negatively to a

(1) OJ No C 86, 2. 4. 1979, p. 1.
(2) OJ No C 140, 5. 6. 1979, p. 128.
(3) OJ No C 133, 28. 5. 1979, p. 30.
(4) OJ No L 145, 13. 6. 1977, p. 44.
(5) OJ No L 15, 19. 1. 1978, p. 34.
(6) OJ No 121, 29. 7. 1964, p. 1977/64.
(7) See page 11 of this Official Journal.

serological test carried out in accordance with Annex G during the 30 days before they are loaded.

However, this test will not be required in the case of male bovine animals and bullocks less than 30 months of age intended for meat production, provided that such animals are indentified by a special mark when they are loaded and that the Member State takes all measures to prevent contamination of indigenous herds.';

3. the following point shall be added to Article 7 (1):

'G. female bovine animals less than 30 months of age intended for meat production which, by way of derogation from Article 3 (3) (e), have not been subjected to a serological test. Such animals must bear a special mark. The Member State of destination shall take all measures to prevent contamination of indigenous herds.';

4. the following paragraph shall be added to Article 8 (2):

'With particular regard to enzootic bovine leukosis and in the case of the animals referred to in Article 3 (2) (j), Member States are authorized to require in addition, subject to compliance with the general provisions of the Treaty, that all members of the herd from which the animals come and more than 24 months of age at the date of the test have in the previous 12 months reacted negatively to a serological test carried out in accordance with Annex G. However, such guarantees shall not be required upon the introduction of animals from a Member State which, in accordance with the procedure laid down in Article 12, is recognized as providing adequate guarantees as regards enzootic bovine leukosis.';

5. the following Article shall be inserted:

'*Article 8 a*

1. Member States which, on the date of implementation of this Directive, are applying a compulsory national programme for the eradication of enzootic bovine leukosis, may make the introduction into their territory of bovine animals for breeding or production intended for combining with bovine herds not suspected of having leukosis, conditional upon the production of a certificate issued on the day of loading by a competent official veterinarian and drawn up, as a minimum requirement, in the language or languages of the country of destination, certifying:

(a) that the veterinarian has no knowledge of facts which would lead him to conclude that a case of enzootic bovine leukosis has occurred within the three preceding years in the herd from which they come, and that the owner of this herd has declared that he has no knowledge of such facts and that he has further declared, in writing, that the animal or animals intended for intra-Community trade have been born and reared in the said herd or have remained an integral part of it for the previous 12 months;

(b) that during the previous 12 months all the bovine animals over 24 months of age on the date of the test and forming part of the herd from which they come have reacted negatively to a serological test carried out in accordance with Annex G.

2. In accordance with the procedure laid down in Article 12, Member States other than those referred to in paragraph 1 may be authorized to apply the same requirements for their territory or, in the case of the United Kingdom, for Northern Ireland, if a plan for the eradication of enzootic bovine leukosis is being implemented therein pursuant to Directive 77/391/EEC, or if it can be proved that on the date on which the matter is referred to the Standing Veterinary Committee, the minimum conditions laid down in Article 2 of Directive 80/1102/EEC have been complied for at least two years.

The additional conditions to which this extension to each Member State or part thereof concerned may be subject may be specified in the decision provided for in the first subparagraph.';

6. the following words shall be added at the end of point (a) of Annex E:

— enzootic bovine leukosis;';

7. Annex F, Model I:

(a) point V:

(aa) the following point shall be added after point (d):

'(e) — for the previous 12 months (5) or, if less than 12 months of age, since birth, they have been kept in a herd in which, to the knowledge of the undersigned and as declared by their owner, no cases of enzootic bovine leukosis have been diagnosed,

— on the date of their examination, all the animals more than 24 months old had undergone (2) (12) a serological test (13) within the

previous 12 months (5) the result of which proved negative,

— within the prescribed 30 days (5), they have given a negative reaction (8) (11) (2) to a serological test for enzootic bovine leukosis,

— they are intended for fattening (2) (11).';

(bb) points (e) to (i) shall become (f) to (j) respectively;

(b) the following footnotes shall be added after footnote (10):

'(11) This exception is permitted only in the case of male animals less than 30 months of age intended for fattening, and provided that the animals are marked in a different manner and undergo a special check in the country of destination.

(12) This is unnecessary except in the case of pure-bred breeding animals intended solely for reproductive purposes and which are highly valuable.

(13) The serological test was carried out in accordance with Annex G to Directive 64/432/EEC.';

8. the following Annex shall be added:

'*ANNEX G*

A. Agar gel immuno diffusion test for enzootic bovine leukosis

1. The antigen to be used in the test must contain bovine leukosis virus glycoproteins. The antigen must be standardized against a standard serum (EI serum) supplied by the State Veterinary Serum Laboratory, Copenhagen.

2. The official institutes indicated below must be made responsible for calibrating the standard working antigen of the laboratory 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)	Belgium:		Institut national de recherches vétérinaires, Bruxelles
(c)	France:		Laboratoire des Médicaments Vétérinaires, Fougères
(d)	Grand Duchy of Luxembourg:		—
(e)	Italy:		Istituto Zooprofilattico Sperimentale, Perugia
(f)	Netherlands:		Centraal Diergeneeskundig Instituut, Afdeling Rotterdam
(g)	Denmark:		Statens Veterinære Serum Laboratorium, Copenhagen
(h)	Ireland:		Veterinary Research Laboratory, Abbotstown, Dublin
(i)	United Kingdom:	1. Great Britain:	The Central Veterinary Laboratory, Weybridge, England
		2. Northern Ireland:	The Veterinary Research Laboratory, Stormont, Belfast

3. The standard antigens used in the laboratory must be submitted at least once a year to the EEC reference laboratories listed in paragraph 2 above for testing against the official EEC standard serum. Apart from this standardization the antigen in use can be calibrated in accordance with B.

4. The reagents for the test shall consist of:

(a) antigen: the antigen must contain specific glycoproteins of enzootic bovine leukosis 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 must be introduced into a Petri dish of 85 mm diameter, resulting in a depth of 2·6 mm of agar.

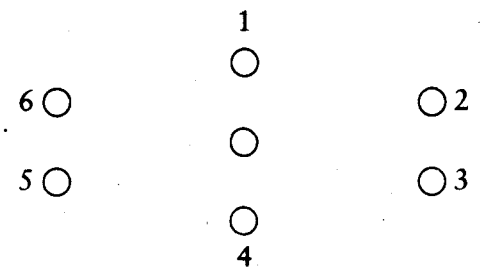
5. A test pattern of seven moisture-free wells must be cut in the agar to the bottom of the plate; the pattern must consist of one central well and six wells in a circle around it.

Diameter of central well: 4 mm

Diameter of peripheral wells: 6 mm

Distance between central and peripheral wells: 3 mm

6. The central well must be filled with the standard antigen. The peripheral wells 1 and 4 (see diagram below) are filled with the known positive serum, the wells 2, 3, 5 and 6 with the test sera. The wells must be filled until the meniscus disappears.



7. This results in the following quantities being obtained:

antigen: 32 µl;

control serum: 73 µl;

test serum: 73 µl.

8. Incubation must 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 precipitin line with the BLV antigen and if it does not bend the line of the control serum;

(c) the reaction cannot be considered conlusive if it:

(i) bends the line of the control serum towards the BLV antigen well without forming a visible pricipitin line with the antigen; or

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

In inconclusive reactions the test may be repeated and concentrated serum utilized.

B. Method for antigen standardization

Solutions and materials required:

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 to 6 mm.
6. A reference antigen.
7. The antigen which is to be standardized.
8. A water bath (56 °C).

Procedure:

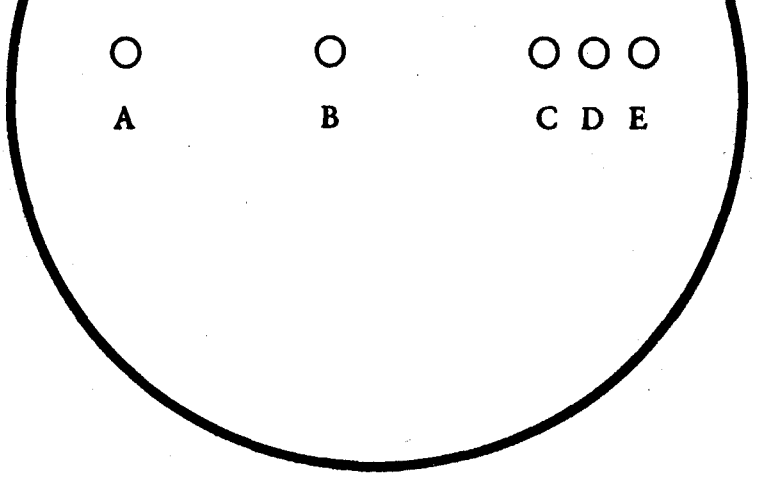
Dissolve the agarose (1·6 %) in the Tris/HCl buffer by carefully heating to 100 °C. Place in 56 °C water bath for approximately one hour. Also, place the bovine leukosis serum dilutions in 56 °C water bath.

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 each of two petri dishes.

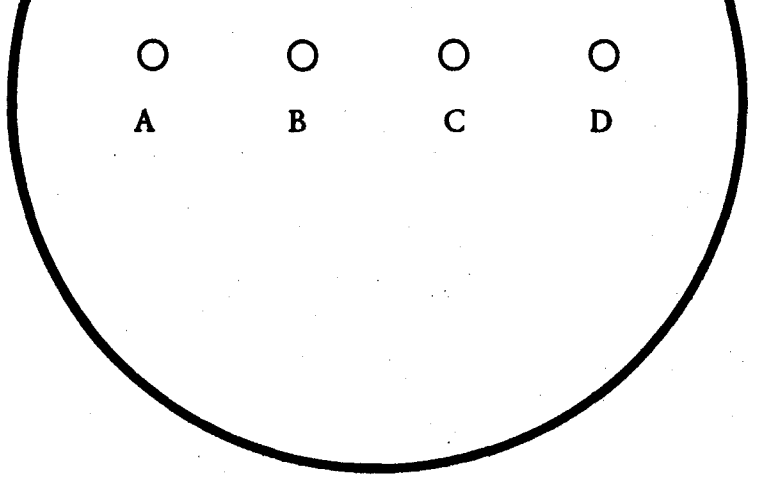
Repeat this procedure with the bovine leukosis serum diluted 1 : 5.

When the agarose has hardened, holes are made in it as follows:

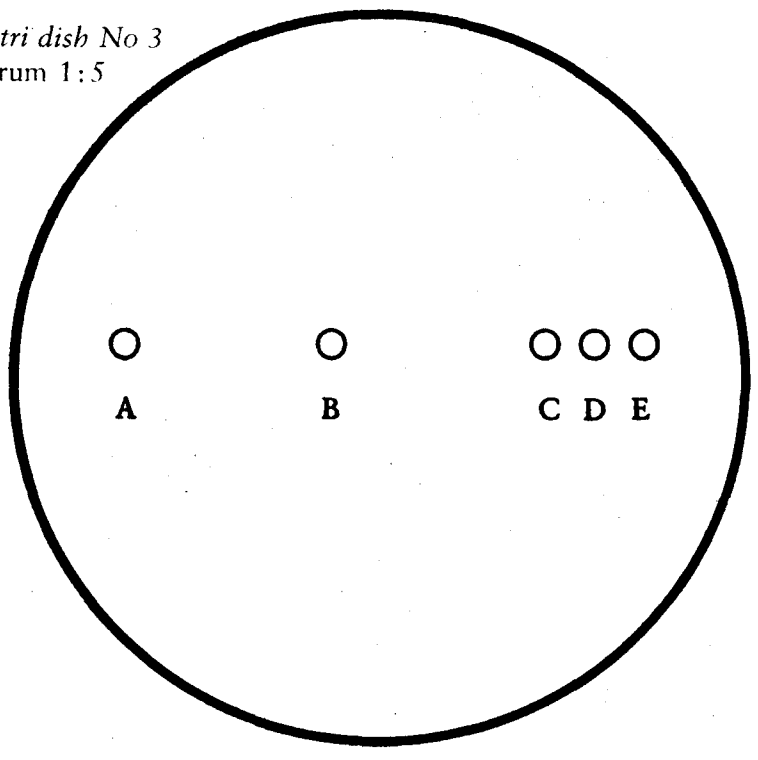
Petri dish No 1
Serum 1 : 10



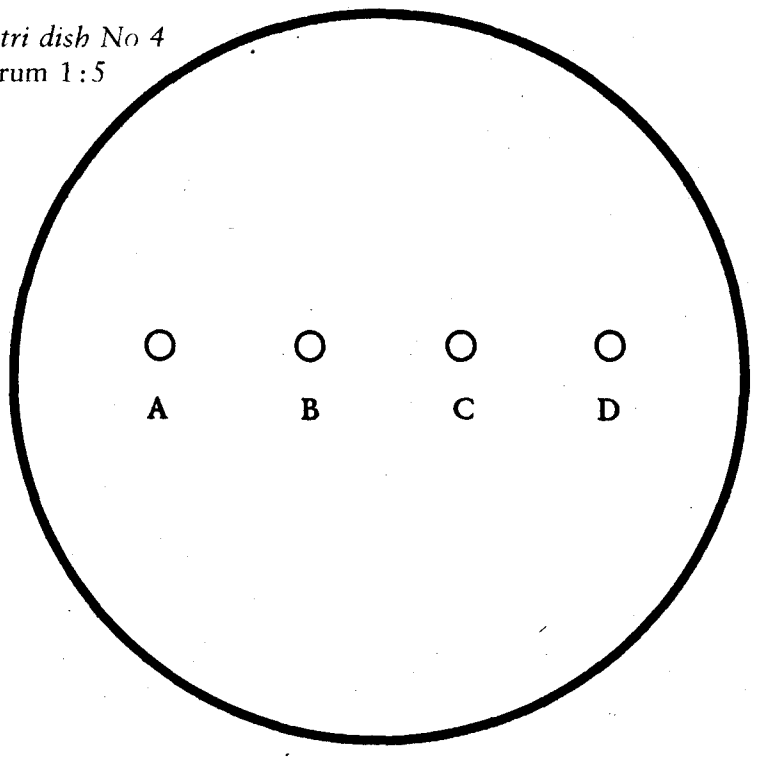
Petri dish No 2
Serum 1 : 10



Petri dish No 3
Serum 1 : 5



Petri dish No 4
Serum 1 : 5



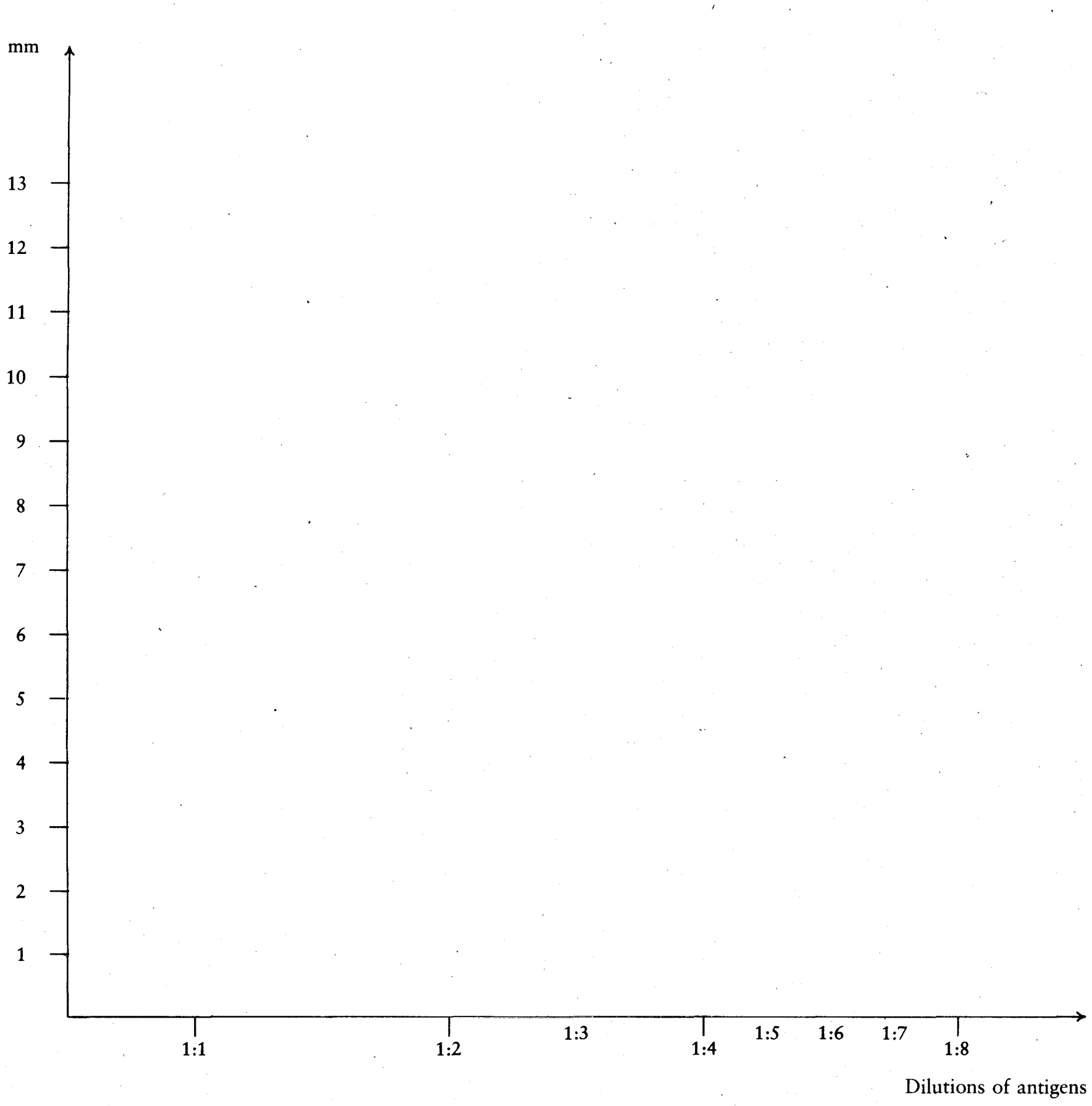
Addition of antigen:

I. Petri dishes 1 and 3
well A = undiluted reference antigen,
well B = 1:2 diluted reference antigen,
wells 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 must be further diluted.
3. If the precipitation diameter in both dilutions is too large and faint, then a lower serum must be chosen.
4. The final concentration of the agarose must 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.



Article 2

Member States which, on the date of implementation of this Directive, are not applying a compulsory national or regional programme for the prevention of enzootic bovine leukosis, shall introduce a minimum programme for the eradication of this disease including at least the following requirements:

— all tumours in the organs and the lymphatic system of bovines must be notified and must be examined histologically by a veterinary laboratory which is directly supervised by a laboratory mentioned in Annex G,

— all cattle in herds which have had contagious contact with an animal found to be suffering from leukotic tumours shall be subjected to a test for enzootic bovine leukosis carried out in accordance with the requirements of Annex G and in a laboratory directly supervised by a laboratory mentioned in Annex G,

— in a herd in which an animal is found to be afflicted with a leukotic tumour and the diagnosis of enzootic bovine leukosis has been confirmed, infected animals may be removed only for slaughter under the supervision of the veterinary authorities. The herd should remain under official control until such time as it has shown a negative reaction to at least three tests carried out at six-monthly intervals on all the cattle over 24 months of age and in accordance with the requirements of Annex G in a laboratory directly supervised by a laboratory mentioned in Annex G.

Article 3

Article 1 shall apply until 31 December 1985.

The Commission shall submit to the Council before 31 December 1984 a report on the application of this Directive accompanied by proposals concerning the definitive arrangements.

The Council shall take a decision on these proposals before 1 July 1985.

Article 4

Member States shall bring into force the laws, regulations and administrative provisions necessary to comply with this Directive not later than 1 January 1981. They shall forthwith inform the Commission thereof.

Article 5

This Directive is addressed to the Member States.

Done at Brussels, 11 November 1980.

For the Council

The President

C. NEY