II

(Acts whose publication is not obligatory)

COUNCIL

COUNCIL DIRECTIVE

of 14 June 1988

amending Directive 64/432/EEC as regards enzootic bovine leukosis and repealing Directive 80/1102/EEC

(88/406/EEC)

THE COUNCIL OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community, and in particular Article 43 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 Directives 77/391/EEC (4) and 78/52/EEC (5), and by Decision 87/58/EEC (6) 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 to that end measures for protection against enzootic bovine leukosis were introduced until 31 December 1987, by Council Directive 64/432/EEC of 26 June 1964 on animal health problems affecting intra-Community trade in bovine animals and swine (7), as last amended by Regulation (EEC) No 3805/87 (8);

Whereas such measures should be extended, whilst recognizing new methods of detection of enzootic bovine leukosis;

Whereas, at the end of a transitional period, special rules laid down by the current arrangements for countries which applied national programmes for the combat of this disease, should be cancelled;

Whereas rules should be laid down for the qualification of herds with regard to enzootic bovine leukosis;

Whereas Member States must, in order to qualify their herd, implement a programme such that their herds undergo one of the tests for detecting leukosis laid down in Annex G to Directive 64/432/EEC;

Whereas, with the exception of animals for slaughter of less than 30 months, animals intended for intra-Community trade must come from a tested herd and have undergone an individual test,

HAS ADOPTED THIS DIRECTIVE:

Article 1

As from 1 July 1988, Directive 64/432/EEC is hereby amended as follows:

(1) OJ No C 5, 9. 1. 1988, p. 5.
(2) OJ No C 49, 22. 2. 1988, p. 164.
(3) OJ No C 80, 28. 3. 1988, p. 34.
(4) OJ No L 145, 13. 6. 1977, p. 44.
(5) OJ No L 15, 19. 1. 1978, p. 34.
(6) OJ No L 24, 27. 1. 1987, p. 51.
(7) OJ No 121, 29. 7. 1964, p. 1977/64.
(8) OJ No L 357, 19. 12. 1987, p. 1.

1. the following is added to Article 2:

'(s) 'Enzootic bovine leukosis free herd' means: a herd in which:

(i) there is no evidence, either clinical or as a result of tests carried out in accordance with Annex G, of any case of enzootic bovine leukosis nor has any such case been confirmed in the previous two years;

(ii) the animals over 24 months of age have, during the previous 12 months reacted negatively to two tests carried out in accordance with Annex G, at intervals of at least four months or, in the case of a herd which has already satisfied that requirement, have reacted negatively to a single test carried out in accordance with that Annex;

(iii) from the date of the first inspection, there are only animals born in the herd, or which have come from an enzootic bovine leukosis free herd.';

2. the following is 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 preceding two 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.';

3. the following is added to Article 3 (3):

'(d) come from a herd in which there has been no evidence of enzootic bovine leukosis during the preceding two years and, if they are more than 12 months of age, have reacted negatively to an individual 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 identified by a special mark when they are loaded and that the Member State takes all measures to prevent contamination of indigenous herds.';

4. the following is 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) (d), have not been subjected to an individual test. Such animals must bear a special mark. The Member State of destination shall take all measures to prevent contamination of indigenous herds.';

5. the following is 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 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.';

6. the following Article is inserted:

'Article 8a

1. Member States which since 1980 have been 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 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 Decision 87/58/EEC, or if it can be proved that on the date on which the matter is referred to the Standing Veterinary Committee they have been applying therein for at least two years a minimum eradication programme including the following minimum 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 that Annex,

— 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 supervision until such time as it has shown a negative reaction to at least two tests carried out at at least four-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.

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.';

7. the following is added at the end of (a) of Annex E:

'— enzootic bovine leukosis.';

8. Annex F, Model I:

(a) V:

(aa) the following is added after (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, during the preceding three years (5) to the knowledge of the undersigned and as declared by their owner, no cases of enzootic bovine leukosis have been diagnosed (2) (12),

— they come from a herd in which there has been no evidence of enzootic bovine leukosis during the preceding three years (2),

— on the date of their examination, all the animals more than 24 months old had within the previous 12 months (5) undergone (2) (12) a test (13) the result of which proved negative,

— within the prescribed 30 days (5), they have given a negative reaction (2) (5) (11) to an individual test (13) for enzootic bovine leukosis,

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

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

(b) the following are 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 individual test was carried out in accordance with Annex G to Directive 64/432/EEC.';

9. Annex G annexed to this Directive is added.

Article 2

As from 1 July 1990, Directive 64/432/EEC is hereby amended as follows:

1. Article 3 (2) (j) is deleted;

2. Article 3 (3) (d) is replaced by the following and (e) to (f) are added:

'(d) come from an enzootic bovine leukosis free herd within the meaning of Article 2 (s);

(e) in addition to the condition under (d) where they are over 12 months of age, have reacted negatively to an individual test carried out in accordance with Annex G during the 30 days before they were loaded;

(f) not be subject to the requirements of (d) and (e) if they are under 30 months of age and intended for meat production, if they:

(i) come from a herd in which no case of enzootic bovine leukosis has been notified and confirmed within the previous 2 years;

(ii) they are identified by a special mark when they are loaded and they remain under supervision until they are slaughtered;

provided that the Member State of destination takes all the measures necessary to prevent contamination of indigenous herds.';

3. Article 7 (1) G is deleted;

4. Article 8 (2), the last subparagraph is deleted;

5. Article 8a is deleted;

6. in Model I of Annex F:

— V (e) is replaced by the following:

'(e) — the previous 12 months (5) or, if less than 12 months of age (5), since birth, they have been kept in an enzootic bovine leukosis free herd (2) (11),

— within the prescribed 30 days (5) they have given a negative reaction to an individual test (12) for the detection of enzootic bovine leukosis (2) (11),

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

— footnote (11) is replaced by the following:

'(11) This exception is permitted only in the case of bovine animals less than 30 months of age intended for fattening, and provided that the animals:

— come from a herd in which no case of enzootic bovine leukosis has been notified and confirmed during the preceding two years,

— are marked in a different manner and undergo a special check in the country of destination.';

— footnote (12) is deleted,

— footnote (13) becomes footnote (12).

Article 3

1. In order to qualify their herds as enzootic bovine leukosis free, Member States shall implement a programme, such that their herds undergo one of the tests referred to in Annex G to Directive 64/432/EEC.

2. Member States shall notify the Commission at least once a year of the progress of their programmes, and the results obtained.

Article 4

The Council, acting on a proposal from the Commission, shall, before 1 January 1990, lay down the criteria permitting a Member State or part of the territory of a Member State to be recognized as being free from enzootic bovine leukosis and the conditions to be implemented to guarantee the maintenance of such status, as well as the rules applicable to trade from enzootic bovine leukosis free regions or Member States.

Article 5

Member States shall bring into force the necessary provision to comply with the requirements of:

— Articles 1 and 3 not later than 1 July 1988,

— Article 2 not later than 1 July 1990.

They shall forthwith inform the Commission thereof.

Article 6

Directive 80/1102/EEC (1) is hereby repealed.

Article 7

This Directive is addressed to the Member States.

Done at Luxembourg, 14 June 1988.

For the Council

The President

I. KIECHLE

(1) OJ No L 325, 1. 12. 1980, p. 18.

ANNEX

'ANNEX G

TESTS FOR ENZOOTIC BOVINE LEUKOSIS

Tests for enzootic bovine leukosis shall be carried out by the immuno-diffusion test under the conditions described in points A and B below or by the enzyme-linked immunosorbent assay (Elisa) under the conditions described in point C below. The immuno-diffusion method may only be used for individual tests.

If test results are the subject of a duly-substantiated challenge, an additional check shall be carried out by means of the immuno-diffusion test.

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 (El 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 (El 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 national de pathologie bovine, Lyon
(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
(j)	Spain:	Laboratorio de Sanidad y Produccion Animal de Barcelona
(k)	Portugal:	Laboratório Nácional de Investigação Veterinária, Lisboa

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.

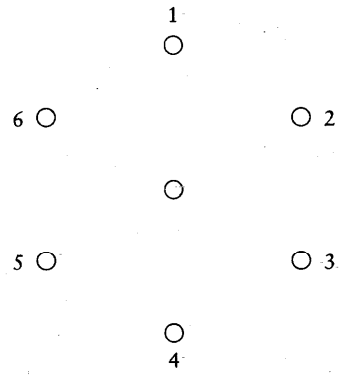
5. A test pattern of seven moisture-free wells be cut in the agar to the bottom of the plate; the pattern must consist of one central well and six wells in a circle around it.

   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 conclusive 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 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:

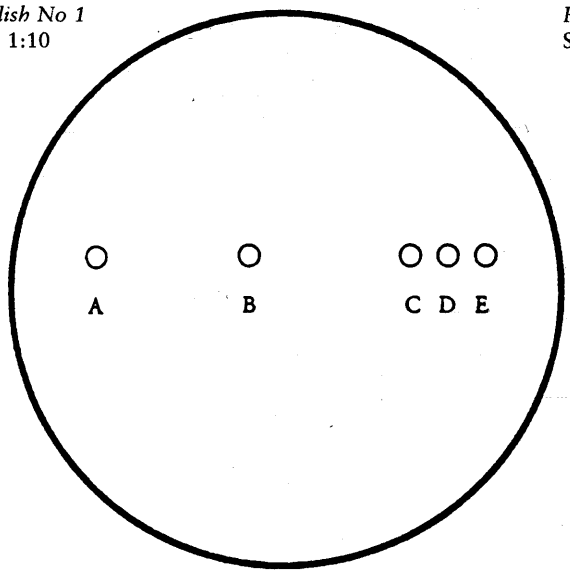
Dissolve the agarose (1,6 %) in the Tris/HCl buffer by carefully heating to 100 °C. Place in 56 °C water bath for approximately one hour. Also, place the bovine leukosis serum dilutions in 56 °C water bath.

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.

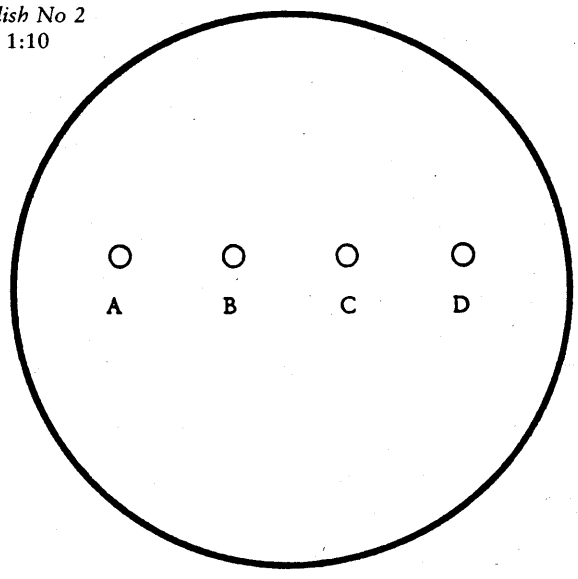
Repeat this procedure with the bovine leukosis serum diluted 1:5.

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

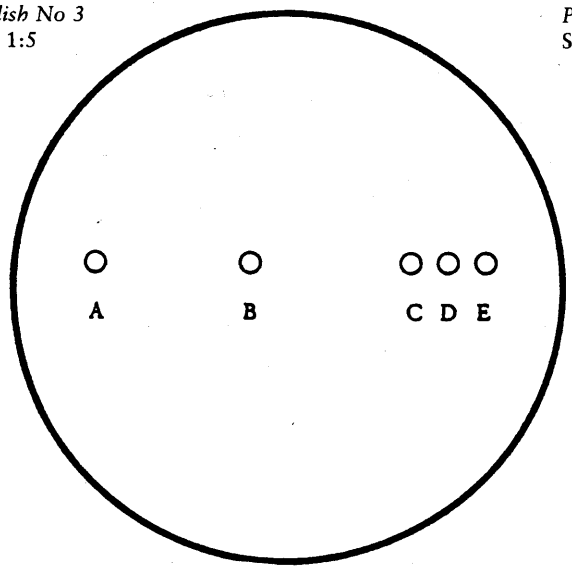
Petri dish No 1
Serum 1:10



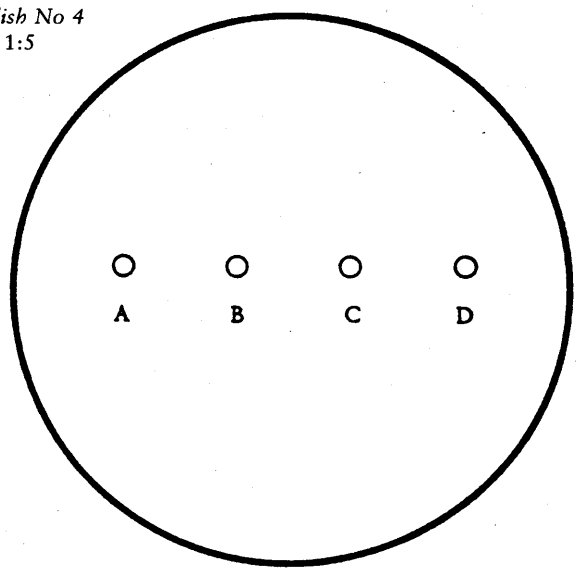
Petri dish No 2
Serum 1:10



Petri dish No 3
Serum 1:5



Petri dish No 4
Serum 1:5



Addition of antigen:

I. Petri dishes 1 and 3

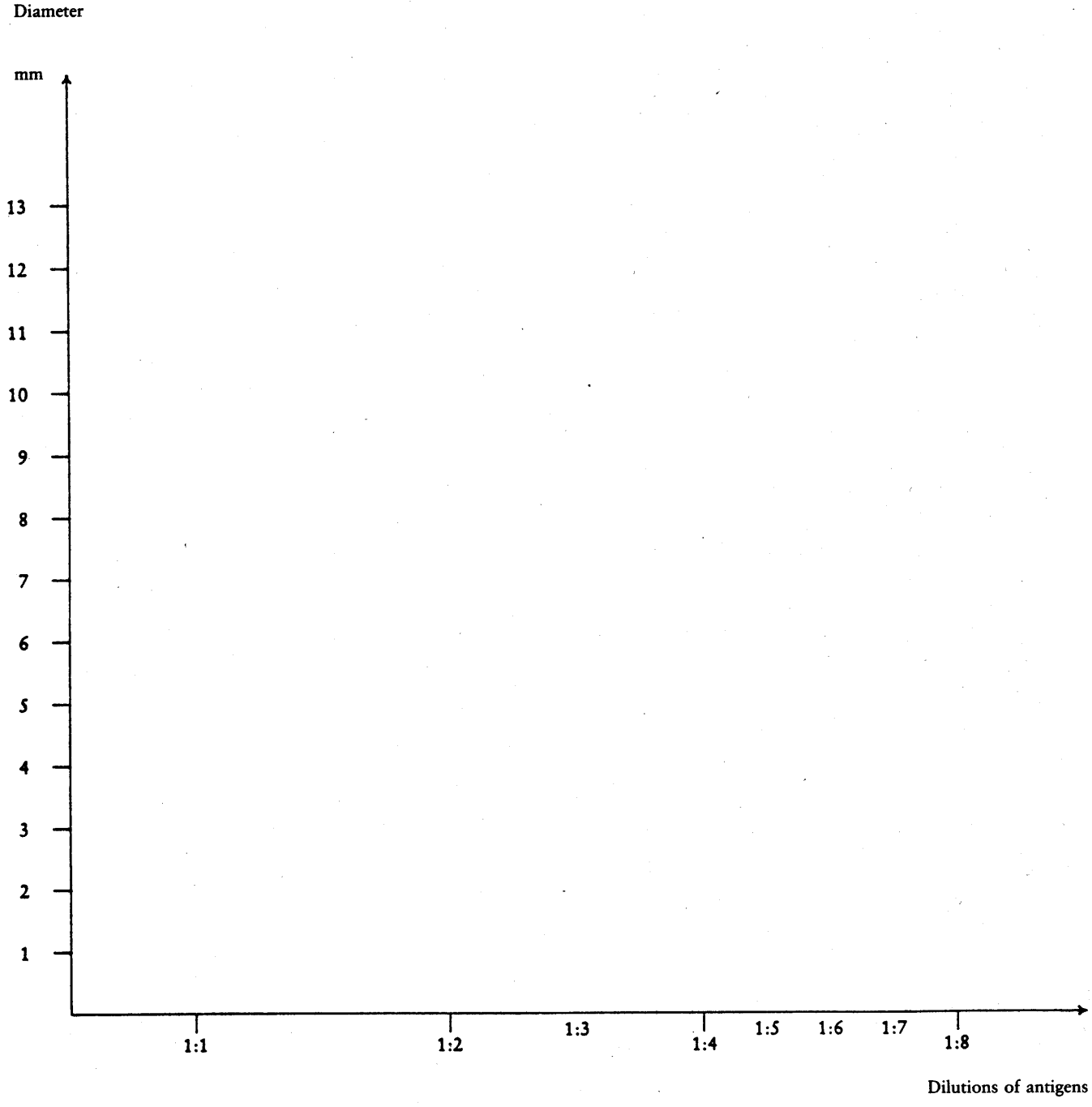
well A = undiluted reference antigen,
well B = 1:2 diluted reference antigen,
wells C and E = reference antigen,
well D = undiluted antigen to be tested.

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.



C. **Enzyme-linked immunosorbent assay (Elisa) for enzootic bovine leukosis**

1. For the Elisa method, the materials and reagents to be used are as follows:

   (a) solid phase microplates, cuvettes or any other solid phase;

   (b) the antigen is fixed to the solid phase with or without the aid of polyclonal or monoclonal catching antibodies. If antigen is coated directly to the solid phase all test samples giving positive reactions have to be retested against control antigen. The control antigen should be identical to the antigen except for the BLV antigens. If catching antibodies are coated to the solid phase the antibodies must not react to other antigens other than BLV antigens;

   (c) the bilogical fluid to be tested (serum or milk);

   (d) a positive and negative control;

   (e) conjugate — an antibovine immunoglobulin biotinylated or enzyme conjugated or an anti-BLV immunoglobulin biotinylated or enzyme conjugated;

   (f) avidin — enzyme for assays using biotynilated immunoglobulin preparations;

   (g) a substrate adapted to the enzyme used;

   (h) a stopping solution;

   (i) buffered solutions for the dilution of the test samples for preparations of the reagents and for washing;

   (j) a reading system with appropriate filters corresponding to the substrate used.

2. *Standardization and sensitivity of test*

   The sensitivity of the Elisa assay must be of such a level that E4 serum is scored positive when diluted 10 times (serum samples) or 250 times (milk samples) more than the dilution obtained of individual samples when these are included in pools.

   In assays where samples (serum and milk) are tested individually E4 serum diluted 1 to 10 (in negative serum) or 1 to 250 (in negative milk) must be scored positive when tested in the same assay dilution as used for the individual test samples.

   The E4 serum will be supplied by the National Veterinary Laboratory, Copenhagen.

3. *Conditions for use of the Elisa test*

   The Elisa method may be used on a sample of milk taken from the milk collected from a farm with at least 30 % of dairy cows in milk on condition that the sample comes from the milk produced by less than 50 cows, and from a whey concentration if collected from between 20 and 50 cows maximum, and that, if the milk collected comes from more than 50 cows, the number of samples is increased proportionally.

   The Elisa method may also be used on a blood sample taken from a maximum of 50 animals.

   If use is made of one of these abovementioned possibilities, measures must be taken to ensure that the samples taken can be identified with the animals from which the milk or sera examined were taken.

   If one of the samples scores positive, the herd must remain under official supervision until a negative result has been recorded for at least two individual tests carried out, at a minimum interval of four months, on all cattle aged more than six months, in accordance with the abovementioned provisions and in a laboratory that is directly supervised by a laboratory mentioned in point A.'