Diagnosis of enzootic bovine leucosis in single and pooled samples

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Summary: Diagnosis of enzootic bovine leucosis is based on detection of antibodies against bovine leukemia virus, BLV. Some ELISA modifications have proved sensitive enough for use in the examination of pooled blood samples from slaughterhouses, milk and pooled milk samples. Suggestions for the standardisation of different ELISA modifications using a common reference serum are presented.

KEYWORDS: BLV - ELISA - Pooled samples - Standardisation.

INTRODUCTION

The agar gel immunodiffusion (AGID) test has for many years served as the reference test for detection of antibodies against bovine leukaemia virus (BLV).

The AGID test used with undiluted serum samples has proved to be adequately sensitive to declare a herd free from infection with BLV when the herd sera are negative.

The higher sensitivity of some ELISA modifications (1) have their greatest advantages when samples with low antibody content are tested; e.g., pooled serum samples from slaughterhouses, milk or pooled milk samples.

Standardisation of the different available ELISA modifications using a common reference serum has been recommended.

Reference serum E-4 from the National Veterinary Laboratory in Copenhagen has been chosen as the reference serum.

The goal of the standardisation procedure is to ensure that an antibody-positive animal will be detected with the same probability, independent of whether the screening is performed on individual serum or milk samples, or on pooled serum or milk samples.

Standardisation does not take into consideration the different sensitivities between a herd milk test and a herd blood test.

The questions of how many milk tests will be equivalent to a blood test, and that of how to test non-milking cattle in dairy or mixed herds, have to be solved separately.

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STANDARDISATION OF TESTS

Standardisation of tests used on individual samples

AGID serum

Reference serum E-4 diluted 1:10 in negative bovine serum should always be clearly positive in the test without any re-test procedure.

ELISA serum

Reference serum E-4 diluted 1:10 in negative bovine serum should be positive with the cut-off used in the routine procedure.

The reference serum E-4 diluted 1:10 in negative bovine serum should always be diluted in the same buffer and tested in the same dilution as the field sera.

ELISA milk

The antibody content in milk averages 25 times less than in serum; therefore, for an antibody-positive animal to be detected independent of whether the analysis is performed on serum or on milk, an ELISA for testing milk samples needs to be at least 25 times more sensitive.

Reference serum E-4 diluted 1:10 in negative bovine serum, and then diluted 1:25 in milk from a seronegative herd must score positive in the test (this serum must also be tested in the same buffer and in the same dilutions, with the cut-off used in the routine procedure).

Standardisation of tests used on pooled samples

The titre of reference serum E-4 determines how many samples can be included in the pool.

The sensitivity standard set by reference serum E-4 is the minimum that should be observed for every test, independent of analysis variation of that particular ELISA.

The variation in test results of repeated examinations of reference serum E-4 dilutions, together with known negative samples, should be considered when a decision is taken about how many samples can be included in the pool.

ELISA serum

Reference serum E-4 must score positive when diluted ten times more than the dilution obtained from individual sera when these are included in pools; for example, pool size 50. E-4 diluted 1:10 × 50 times in negative serum must score positive in the test (once again tested in the buffer dilution, with the cut-off used in the routine procedure).

ELISA milk

Reference serum E-4 must also score positive when diluted 250 times more than the dilution of individual samples when these are included in pools; for example, pool size 20. E-4 diluted 250 × 20 in negative serum (tested in the buffer dilution with the cut-off used in the routine procedure), must score positive in the test. If reference serum E-4 [(diluted 1:10):25]:20 is negative, less than 20 samples can be tested in a pool.
DETERMINATION OF THE TITRE OF REFERENCE SERUM E-4

Determination of the titre of reference serum E-4 in indirect ELISA

The cut-off, or threshold, for positive samples can be defined as the mean test result of representative negative samples (at least 100 samples from the relevant category herd samples, slaughterhouse samples, milk samples, tank milk samples from herds with different numbers of milking cows) plus n times the standard deviation (n being 3 or more). The titration curve of reference serum E-4 is determined as the mean value in each dilution point, minus n times the standard deviation (n being 3) (see Fig. 1).

![Diagram showing titration curve of E-4](image)

**FIG. 1**

Determination of the titre of reference serum E-4
Indirect ELISA

**Example 1**

<table>
<thead>
<tr>
<th>Cut-off negative samples in indirect ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OD</strong></td>
</tr>
<tr>
<td>Mean of samples</td>
</tr>
<tr>
<td>Mean of samples n = 100</td>
</tr>
</tbody>
</table>

* Optical density (OD) determined on different plates and on different days
Example 2

Titration curve of reference serum E-4 in indirect ELISA

<table>
<thead>
<tr>
<th>Dilution of reference serum E-4 in negative milk</th>
<th>OD * Mean of 20 examinations</th>
<th>OD Standard deviation (SD)</th>
<th>OD Mean - 3SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: 8,194</td>
<td>1.335</td>
<td>0.208</td>
<td>0.711</td>
</tr>
<tr>
<td>1: 16,384</td>
<td>0.665</td>
<td>0.166</td>
<td>0.167</td>
</tr>
<tr>
<td>1: 32,768</td>
<td>0.332</td>
<td>0.088</td>
<td>0.068</td>
</tr>
</tbody>
</table>

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Determination of the titre of reference serum E-4 in blocking ELISA

The cut-off, or threshold, for positive samples can be defined as the mean test result of representative negative samples, minus n times the standard deviation (n being 3 or more). The titration curve of reference serum E-4 is determined as the mean value in each dilution point, plus n times the standard deviation (n being 3) (see Fig. 2).

**FIG. 2**

Determination of the titre of reference serum E-4

Blocking ELISA

Résumé: Le diagnostic de la leucose bovine enzootique s'effectue en recherchant la présence d'anticorps contre le virus de la leucose bovine (VLB). Certains tests ELISA modifiés se sont révélés suffisamment sensibles pour servir à l'examen du sang (sous forme de mélanges) provenant d'animaux abattus ou du lait (prélèvements individuels ou sous forme de mélanges). L'auteur propose de normaliser les différents tests ELISA utilisant le même sérum de référence.

MOTS-CLÉS : ELISA - Mélanges - Normalisation - VLB.

DIAGNÓSTICO DE LEUCOSIS BOVINA ENZOÓTICA EN MUESTRAS INDIVIDUALES Y MEZCLAS. – R. Hoff-Jørgensen.

Resumen: El diagnóstico de la leucosis bovina enzoótica se basa en la detección de anticuerpos contra el virus de leucosis bovina (VLB). Algunas pruebas ELISA modificadas han demostrado ser lo bastante sensibles como para servir en el examen de muestras de sangre (mezclas), procedentes de animales sacrificados, o de leche (muestras individuales o mezclas). El autor propone estandarizar las diferentes pruebas ELISA utilizando el mismo suero de referencia.

PALABRAS CLAVE: ELISA - Estandarización - Mezclas - VLB.

REFERENCE