STUDIES ON BOVINE LEUKOSIS. VI. ENZYME LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF ANTIBODIES TO BOVINE LEUKOSIS VIRUS

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STUDIES ON BOVINE LEUKOSIS

VI. ENZYME LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF ANTIBODIES TO BOVINE LEUKOSIS VIRUS

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Résumé

ÉTUDES SUR LA LEUCOSE BOVINE. VI. UTILISATION DU TEST ELISA POUR LA DETECTION DES ANTICORPS CONTRE LE VIRUS DE LA LEUCOSE BOVINE. — Les résultats de l'examen de 2784 échantillons de sérum par le test d'immunodiffusion en gel d'agarose (AGIDT-gp) ont été comparés à ceux du test ELISA. Une discordance a été enregistrée dans 0,5 % des cas. On peut en conclure que la technique ELISA est une autre méthode sérologique intéressante à ajouter à la liste des techniques sérologiques actuellement disponibles pour la détection des anticorps contre le BLV. Les avantages de la technique sont discutés.

A diverse array of immunologic tests are known to-date for the serological diagnosis of enzootic bovine leukemia (EBL). Because of its simplicity, reproducibility and sensitivity the agar gel immunodiffusion test (AGIDT), using bovine leukemia virus glycoprotein (BLV gp) as antigen, has been selected as tool to be used for the serodiagnosis of EBL in the nine European community countries. The search for more sensitive techniques for large screening purposes is, however, continuing. A radioimmunoassay (RIA) is less suitable as it requires expensive equipment. Its application is therefore restricted to central facilities. Immuno-fluorescence as practised in this Institute (Ressang et al, 1978) is helpful if the condition of the test sera is optimal. The evaluation of its end-results, especially with low-titer sera, is sometimes dependent on subjective assessment. The question arises if the enzyme linked immunosorbent assay as described by Engvall and Perlmann (1972) can serve as an alternative. The adaption of an indirect ELISA for the serology of EBL in this Institute was made possible through the work of one of us (Gielkens; manuscript in preparation).

This paper reports the results of a comparative study employing the ELISA and the AGIDT to examine the presence of antibodies to BLV in bovine sera. A small number of sera tested previously in the RIA (Dr. Levy and Dr. Devare) were also examined in both tests.
Materials and Methods

Antigen

Bat cells were obtained through courtesy of Prof. J.F. Ferrer, New Bolton Center, Univ. of Pennsylvania. The cells were kept in stationary cultures in 75 cm² disposable plastic flasks. The medium consisted of MEM Hanks' solution to which was added 10 % inactivated foetal calf serum, 0.042 % NaHCO₃, 1 % glutamine, 1 % non-essential amino acids and 0.1 % lactalbumine. The cells were passaged every 4-5 days and medium was collected and stored at 4° C until used.

Two liter of medium was clarified by centrifugation at 2 000 rpm for 15 minutes and the virus was pelleted by centrifugation at 9 000 rpm for 16 hours in a JA-14 rotor of a Beckman centrifuge. The pellet was resuspended in 10 mM Tris-HCl pH 7.4, 100 mM NaCl and 1 mM EDTA (TNE) and BLV was isolated after spinning through a cushion of 10 % sucrose (w/v) in TNE at 20,000 rpm (fixed rotor 40) for two hours. The isolated virus preparation was treated with an equal volume of ether for five minutes. Quick evaporation of the ether was accomplished by flushing with N₂. The antigen was divided in small aliquots (25-50 µl) and stored at −20° C.

Sera

A total of 2 784 sera were examined. These could be sub-divided as follows:

a) 433 sera were obtained from the same number of cows known to be free from EBL as evidenced by clinical, hematological and AGIDT gp examinations. These sera were examined in the ELISA to determine the extinction values in normal sera;
b) 15 sera were courteously given by Dr. Daniel Levy (Lab. d'Immunologie et de Virologie des Tumeurs, Hopital Cochin, Paris) and 25 sera were a generous gift from Dr. S.G. Devare, N.I.H., Bethesda, Maryland, USA). Both groups of sera were examined in the RIA using labelled BLV p24 and BLV 24 plus gp 51 respectively;
c) 2 311 serum samples were derived from 17 farms that have been infected with EBL by import of cattle in the past. The sera came from the same number of animals.

AGIDT

The test was carried out as described (Res-
PBS, was diluted to give a final protein concentration of 2 mg x ml⁻¹. This antigen preparation was added to an equal volume of test serum, diluted 1 : 5 in PBS and the mixture was incubated for one hour at 37°.

After preincubation, blocking of virus-specific antibodies was measured by using the indirect ELISA.

As a control, positive and negative reference sera preincubated in the presence or absence of BLV gp antigen, was included in this assay.

### Results

The distribution of the extinction values in the indirect ELISA of 433 sera from clinically normal, non-EBL affected cows are given in Table 1.

The majority (63.05%) of normal sera had absorbance values between 0.2 - 0.4 and only 3.23% were in the 0.6 - 0.8 range.

It was therefore decided to consider sera with an E 499 value higher than 0.8 to be positive.

The outcome of the ELISA and AGIDT gp with 2311 sera is presented in Table 2.

There was a good agreement between the results of both tests. A discrepancy was seen in twelve (0.5%) cases.

The forty sera which were tested in the RIA had results identical to those of the ELISA and the AGIDT employing gp. All the positive sera (26 samples) had E 449 values higher than 1.0. Negative samples scored below 0.5.

### Discussion

Based on the results obtained with the ELISA it can be stated that this technique is another useful serological method that can be added to the list of established tests presently available for the demonstration of antibody to BLV. Its results compared very well with those of the AGIDT gp. A discrepancy in the outcome of both tests was found in only 0.5%.

The question whether the ELISA is an alternative to the RIA for the demonstration of antibodies to BLV can only be answered by a comparative test preferably with samples collected in an EBL infected farm (for sensitivity) and in an EBL free one (for specificity). The impression exists that for the detection of antibodies to BLV, the ELISA equals the indirect FAT as regards sensitivity and specificity. The results of the former can, however, be read more objectively by spectrophotometry. Besides the ELISA can be mechanized.

We are presently testing other methods of antigen preparation and it seems that simple, less laborious techniques such as precipitation with polyethylene glycol are equally suitable. A crucial part of the ELISA is the background staining in some negative sera. This can be greatly overcome by thorough washing, right choice of microtiter plates and an optimal working dilution of the conjugate. The latter can be found out by checkerboard titration. The addition of 1% lamb serum in the dilution fluid of the serum and the conjugate is also of much help. The overnight incubation at 4° C with serum can be reduced to 2-3 hours incubation at 37° C with equal results.

An explanation for the discrepancy in 12 samples (0.5%) is difficult to find. The sera from the six cows positive in the AGIDT but negative in the ELISA could not be retested as the animals were already slaughtered. From the six ELISA positive, AGIDT negative sam-

### Table 1: Absorbance values of 433 sera from clinically normal, non EBL affected cows at 449 nm.

<table>
<thead>
<tr>
<th>E 449</th>
<th>Number of sera</th>
<th>Percentage of Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 - 0.2</td>
<td>48</td>
<td>11.09</td>
</tr>
<tr>
<td>0.2 - 0.4</td>
<td>273</td>
<td>63.05</td>
</tr>
<tr>
<td>0.4 - 0.6</td>
<td>98</td>
<td>22.63</td>
</tr>
<tr>
<td>0.6 - 0.8</td>
<td>14</td>
<td>3.23</td>
</tr>
</tbody>
</table>

### Table 2: Results of examination of 2311 bovine test sera in the ELISA and AGIDT gp.

<table>
<thead>
<tr>
<th>ELISA</th>
<th>AGIDT gp</th>
<th>Number of Sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>53</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>2 246</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>6</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>6</td>
</tr>
</tbody>
</table>
amples only three sequent sera could be reexami-
med. One of these became positive in the
AGIDT. Of the remaining two sera the extinc-
tion values were reduced over 50 % as measu-
red in the ELISA competition test, after prein-
cubation with BLV gp antigen. This indicated
that the ELISA reaction with the discrepant
sera was specific.

It is quite possible that the discrepancy was
due to the different class of antibody demon-
strated in both tests.

Another possibility of the cause of the dis-
crepancy was the difference in the representa-
tion of gp-51 and p24 in the BLV preparation
used for the ELISA as compared with the Pit-
man Moore antigen.

Summary

The results of examination of 2 784 sera in the AGIDT-gp were compared with those of the
ELISA. A discrepancy was seen in 0.5 % of the cases. It was concluded that the ELISA was an-
other useful serological method added to the list of serological techniques presently available for
the detection of antibodies to BLV. The advantages of the technique are discussed.

References

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and the micro complement fixation test for the detection of antibodies to bovine leukemia virus. Tijdschr.
Diergeneesk., 103, 758.