INgezim® BLV CONFIRMATION

R.12.BLV.K1

Indirect ELISA for detection of antibodies to Bovine Leukemia Virus (BLV) in bovine serum.

TECHNICAL INFORMATION

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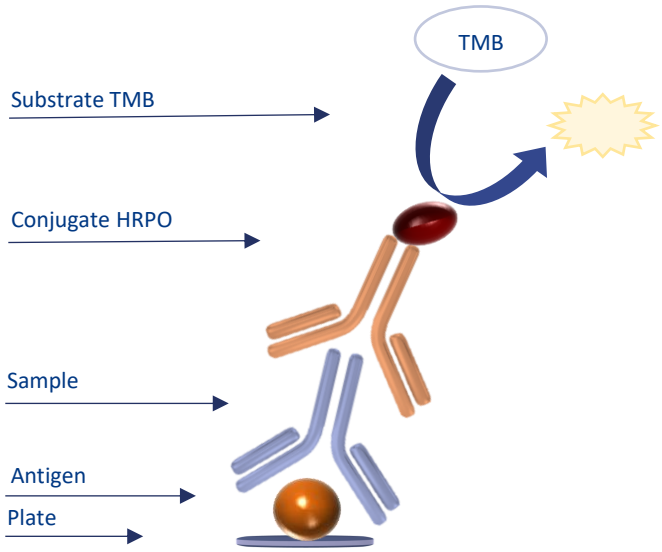
INDEX OF CONTENTS

1 PRODUCT APPLICATION

The kit INgezim® BLV CONFIRMATION has been developed to detect and confirm presence of antibodies specific of BLV in bovine sera samples.
This technique is able to detect every kind of immunoglobulins specific of BLV.

2 TECHNICAL BASIS OF THE PRODUCT

The assay is based on the Indirect ELISA technique.



1. Two types of plates are supplied: coated with positive antigen and coated with negative antigen.
2. Sera are added to both kind of plates and incubated for 30 min. at 37ºC. If sera contain antibodies specific of BLV, they will bind to the positive antigen.
3. At this point a washing step is necessary to eliminate the not specific bindings.
4. After washing, a monoclonal antibody specific of ruminant IgG and conjugated with HRPO is added and incubated 30 min. at 37ºC. The conjugate will recognize the ruminant IgG present in the wells.
5. At this point a washing step is necessary to eliminate the not specific bindings.
6. At the end, a substrate specific of HRPO is added to develop color after 10 min in positive wells and in some cases to the negative wells due to unspecific binding. The subtraction of the values obtained in the positive and negative plates will determine if the sample is positive to BLV or not.

3 KEY REAGENTS USED

- **Coated plate 1:** Semipurified extract of BLV virus.
- **Coated plate 2**: Negative antigen.
- **Conjugate:** Specific monoclonal antibody against ruminant' s IgG, conjugated with peroxidas.

4 VALIDATION

4.1 USE OF THE OIE REFERENCE SERA

In order to verify the sensitivity of the assay, E-4 and E05 Community Reference Sera were requested, and, following O.I.E. legislation, they were assayed at a 1/10 dilution in negative serum as positive serum and diluted 1/100 in negative sera (as pool of 10 sera). Positive results were obtained in all cases. The OIE Negative Reference Serum showed negative results in the same assay. Therefore, we conclude that INgezim® BLV Confirmation, maintains the level of sensitivity and specificity required by O.I.E legislation, so it can be used as confirmation assay.

4.2 USE OF FIELD SERA

A total of 400 bovine sera previously classified by I INgezim® BLV Compac 2.0 were analyzed by INgezim® BLV Confirmation:

- 29 positive sera: European (n=7) and Canadian (n=22)
- 371 negatives from free áreas in Holand (n=192), Spain (n=174), Poland (n=3) and Canada (n=2)

Classified	n	Pos	Doub	Neg
Negative	371	4	0	367
Positive	29	28	0	1

Correspondence between both assays was 98.7%.