INgezim® BLV Compac 2.0

R.12.BLV.K.3

Blocking immunoenzymatic assay for the detection of specific antibodies to bovine leukaemia virus.
Bovine milk and serum samples.

TECHNICAL INFORMATION

LAST REVISION: 04/07/2023

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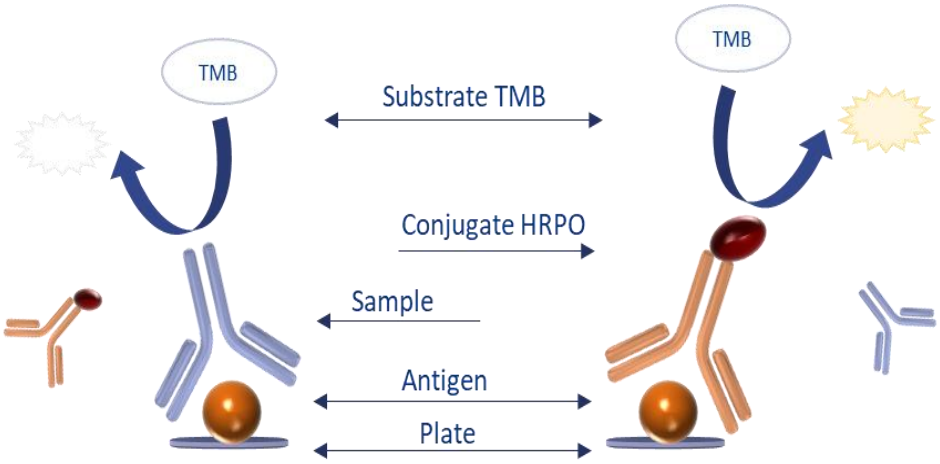
1 PRODUCT APPLICATION

INgezim® BLV Compac 2.0 kit, has been designed for the detection of specific antibodies against BLV in serum samples of cattle, assayed individually or in mixtures of up to 10 sera and in milk samples individually assayed.

2 TECHNICAL BASIS OF THE PRODUCT

The assay is based on a blocking ELISA method, which scheme is briefly described hereunder:



1. Plates are supplied coated with BLV antigen. On these wells, samples are added and incubated.
2. If serum samples contain specific antibodies to BLV, they will bind the antigen.
3. At this point, a washing step is necessary to remove any non-specifically bound material.
4. When the conjugate (monoclonal antibody specific of gp51 protein, conjugated with peroxidase, AcM-PO) is added, only if there are no antibodies in the sample blocking the antigen (negative animals), it will bind to the antigen. In case the sample contains antibodies blocking the antigen (positive animals), the conjugate will not be able to bind to it.
5. Again a washing step is necessary after incubation with conjugate to remove material not bound to the protein.
6. When adding a specific peroxidase substrate, if the serum is negative, colorimetric reaction will appear.

3 KEY REAGENTS USED

The optimal performance of the assay is mainly due to the quality of the key reagents, which are briefly described below:

- **Antigen:** BLV virus (Bovine Leukosis Virus) obtained from cell cultures.
- **Conjugate:** Monoclonal antibody specific against gp51 protein of the BLV virus, conjugated to peroxidase.

4 VALIDATION

4.1 SENSITIVITY AND SPECIFICITY OF THE TEST

With the object of adequately verifying the test using sera, both from collection as from the field, a total number of sera from different populations of animals were analysed.

4.1.1 Using 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 serum weakly positive), dilutes 1/100 in negative sera (as pool of 10 sera) and diluted 1/250 in negative milk (as individual sample of milk). As shown in the table of point 4.2, 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 Compac 2.0, maintains the level of sensitivity and specificity required by O.I.E legislation, so it can be used for the testing of serum or milk individual samples and mixtures of 10 sera.

SERA	OD	INTERPRETATION
E4 1/10 in sera	0,072	POS
E4 1/100 in sera	0,293	POS
E4 1/250 in sera	0,681	POS
E4 1/500 in sera	1,094	NEG
E05 1/10 in sera	0,096	POS
E05 1/100 in sera	0,255	POS
E05 1/250 in sera	0,591	POS
E05 1/500 in sera	0,886	POS
E05 1/2500 in sera	1,446	NEG
E4 1/250 in milk	0,90	POS
E4 1/500 in milk	1,228	NEG
E05 1/250 in milk	0,682	POS
E05 1/500 in milk	1,065	DOUBTFUL
NEG OIE	1,206	NEG
C. POS	0,162	POS
C.NEG	1,682	NEG

Neg cut off: 1,074
Pos cut off: 0,922

4.1.2 Comparing with reference technique

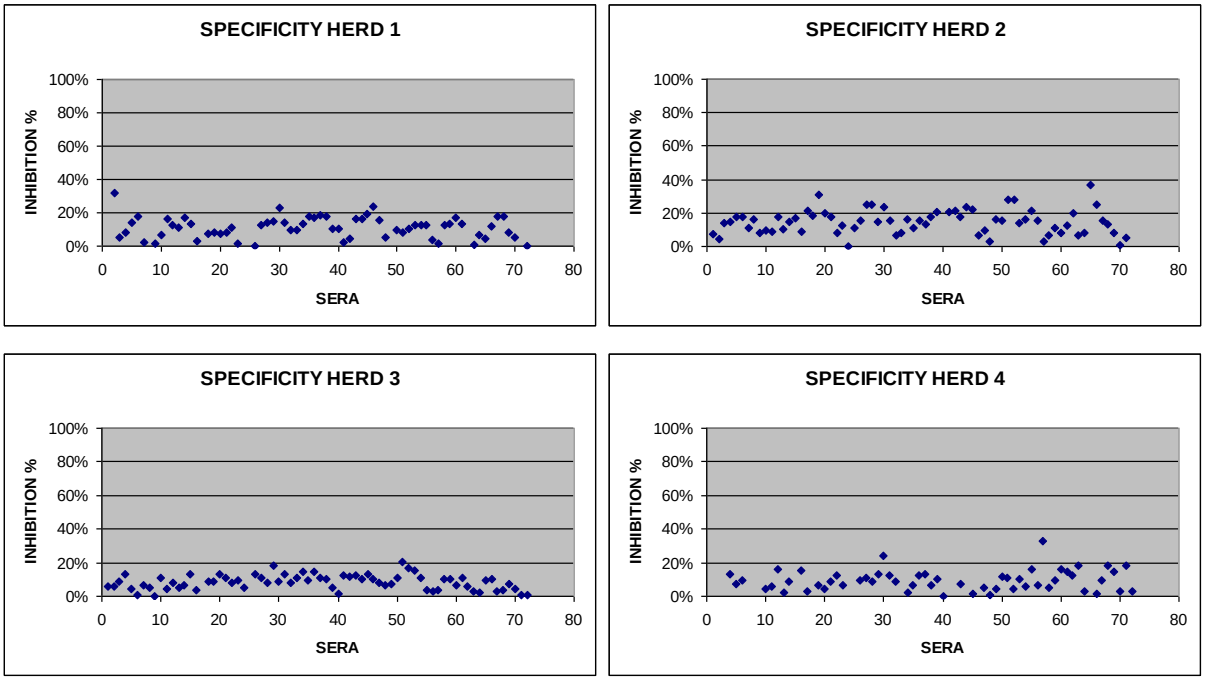
The specificity and the sensitivity of this diagnostic method has been assessed, comparing the results of the positive and negative sera, with those obtained with the gel diffusion technique (AGID), showing a 100% correlation. The results are given below.

SERUM	INGEZIM BLV COMPAC		INMUNODIFFUSION
	OD VALUE	RESULTS	RESULTS
1	0.713	-	-
2	0.842	-	-
3	1.019	-	-
4	0.934	-	-
5	0.800	-	-
6	1.164	-	-
7	0.766	-	-
8	0.763	-	-
9	0.752	-	-
10	0.651	-	-
11	0.071	+	+
12	0.060	+	+
13	0.060	+	+
14	0.061	+	+
15	0.062	+	+
16	0.058	+	+
17	0.059	+	+
18	0.061	+	+
19	0.066	+	+
20	0.082	+	+
21	0.850	-	-
22	0.951	-	-
23	0.868	-	-
24	0.782	-	-
25	1.082	-	-
26	0.705	-	-
27	0.922	-	-
28	0.891	-	-
29	0.900	-	-
30	1.020	-	-
E-4	0.072	+	+
E-4 (1/10 dilution) in negative serum	0.280	+	Doubtful
E-4 (1/10 dilution) in negative milk	0.270	+	Not determined

4.1.3 Specificity using field sera from negative herds

In order to evaluate the specificity of the assay, 354 bovine sera from four different negative and controlled herds were analysed. Data obtained indicated that all samples showed inhibition percentages lower than 40% which is the cut off for negative samples hence, the specificity of the assay was 100% in this situation.

- Herd 1 (72 sera)
- Herd 2 (91 sera)
- Herd 3 (93 sera)
- Herd 4 (98 sera)



5 ADVANTAGES

INgezim® BLV Compac 2.0 has been designed with several characteristics which make it to have some handling advantages:

- Very sort incubations (1 hour + 30 min. + 10 min.)
- Possibility to test pools of samples (serum)
- Dilution buffer ready to use
- The dilution of the sample can be made in the well directly

6 OTHER DATA

6.1 QUALITY CONTROL AND INTERNAL VALIDATION

Each batch of INGEZIM BLV is validated in INGENASA following the standard proceeding defined by our Quality Control System (ISO 9001:2000).

The quality Control is applied on the reagents composing each new batch, and consists in the following test:

- Control of the Internal Reference sera OD values
- Control of the OIE Reference sera OD values
- Control of the kit control sera OD values

6.1.1 Quality control using internal reference sera

In order to determine the validity of each batch, two internal sera are analysed:

- TOL 1/10 Positive sera (secondary patron)
- Problematic Negative

The inhibition percentage range of these sera in the batches produced since 2002 to 2013 have been within the range of:

TOL 1/10	70-88%
Neg. problematic	15-25%

6.1.2 Quality control using OIE reference sera

In Order to determine the validity of each batch, the E4 1/10 (E05 since the change of the Reference Sera) OIE Reference sera were analysed in each batch.

The inhibition percentage range of this serum in the batches produced per year was between 80-90%.

6.2 QUALITY CONTROL AND EXTERNAL VALIDATION

6.2.1 Spanish authorities' validation

Every batch of INgezim® BLV Compac 2.0 produced in EUROFINS-INGENASA are sent to the Spanish Reference Laboratory for validation (Laboratorio Central de Vaterinaria de Algete) as indicate the Real Decreto 2611/1996.

All these batches sent have been certified as VALID by this Reference Laboratory in test done at different dates until the expiry date.

6.2.2 Other countries validation

In order to evaluate the kit out of the original country, INGENASA took part in a Ring Test organized by the Poland National Reference Laboratory for Enzootic Bovine Leukosis (National Veterinary Research Institute Department of Biochemistry, Al. Partyzantów 57, 24-100 Pulawy, Poland) in 2005. The results obtained indicated a total correspondence with the expected ones and with the reference technique, AGID. The results are attached at the end of this report.

7 EXPERIENCE

7.1 EXPORTATION

Product INgezim® BLV Compac 2.0 is exported to the following countries:

- ITALY
- RUMANIA
- POLAND
- PORTUGAL
- AUSTRIA
- HUNGARY
- SLOVAKIA

7.2 NATIONAL TENDERS

This product has been used in tenders of Spanish Administration Authorities in years 2001, 2002, 2003, 2005, 2009, 2012 and 2013.

The number of determinations produced is shown in the following table:

2001	2002	2003	2005	2009	2012	2013
340.000	535.000	2.300.000	400.000	2.000.000	200.000	100.000

7.3 REGISTRATION

This product has been registered in the following countries:

- SPAIN, with registration number 808 RD
- POLAND, with registration number PRWET.FARM 4610 / op-63 / 00
- ROMANIA, with registration number 81867
- GERMANY, with registration number FLI-C 033

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Al. Partyzantów 57 24-100 Pulawy Poland
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NATIONAL REFERENCE LABORATORY FOR ENZOOTIC BOVINE LEUKOSIS

Distribution: 2005 Date of distribution: 20.09.2005

Laboratory:

INGENASA, Madrid, Spain

ENZOOTIC BOVINE LEUKOSIS QUALITY ASSESSMENT RESULTS

SAMPLE No	RESULTS			
	EXPECTED		RECEIVED	
	AGID	ELISA	AGID	ELISA Batch # 271104, 180505
05/01+	+	+	NT	+
05/02*	+	+	NT	+
05/03	-	-	NT	-
05/04	+	+	NT	+
05/05	+	+	NT	+
05/06	+	+	NT	+
05/07	-	-	NT	-
05/08	+	+	NT	+
05/09	+	+	NT	+

NT - not tested

05/02* - Polish substandard to reference serum E4 diluted 1:10

$05/01^{+}$ - Polish substandard to reference serum E1

Interpretation of results:

The laboratory achieved the correct results with all samples

Jacek Kuzmak DVM, PhD

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for Enzootic Bovine Leukosis

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Leipzig 08.03.2017

Report: **INgezim BLV COMPAC 2.0 ELISA**

Delivery address: INGENASA
Hermanos García Noblejas, 39
28037 MADRID SPANIEN

Order number: -

Material: INgezim BLV COMPAC 2.0 ELISA (Prod Ref: 12.BLV.K3)
Lot: 110416; Expiry: 10/2017; Registration: 0808 RD

Request: BLV-ELISA with OIE reference serum for EBL 'E05'

Results:

1) INGEZIM BLV COMPAC 2.0 ELISA (Prod Ref: 12.BLV.K3)

Sample	Mean OD	Result
Positive control	0,12	**positive**
Negative control	1,96	negative
E05 dilution 1:2	0,07	**positive**
E05 dilution 1:10	0,10	**positive**
E05 dilution 1:50	0,15	**positive**
E05 dilution 1:100	0,17	**positive**
E05 dilution 1:1000	0,35	**positive**
E05 dilution 1:10.000	0,69	**positive**
E05 dilution 1:20.000	1,13	doubtful
E05 dilution 1:40.000	1,84	negative
E05 dilution 1:80.000	2,16	negative

Cut off negative: 1,2246; Cut off positive: 1,0408

The test is valid. Using a dilution of 1:10.000 of OIE reference serum for EBL 'E05', the INGEZIM BLV COMPAC 2.0 ELISA (Prod Ref: 12.BLV.K3) ELISA displays a positive result.

Kind regards

OIE Reference Laboratory for EBL
Prof. Thomas Vahlenkamp, DVM, PhD