

# INGEZIM BLV Compac 2.0

- Blocking ELISA**
- Detection of antibodies specific to gp51 of BLV**
- Bovine samples (serum or milk) individually assayed**
- Bovine samples (serum) assayed in pools of up to 10**
- Milk samples individually assayed**



1 PRODUCT APPLICATION

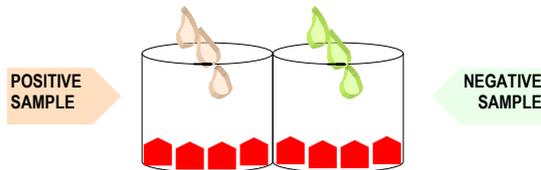
The INGEZIM BLV 2.0 kit, has been designed for the detection of specific antibodies specific of BLV in samples (**serum or milk**) from cattle, assayed **individually or in mixtures of up to 10 sera**.

Using this technique it is possible to detect any immunoglobulin specific for gp51 of the BLV.

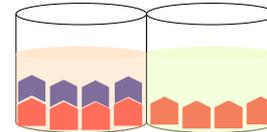
2 TECHNICAL BASIS OF THE PRODUCT

This technique is a blocking enzymatic immunoassay (Blocking Elisa).

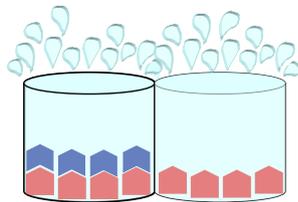
1.- Plates are supplied with semipurified extract of BLV. On these wells, sample sera are added and incubated:



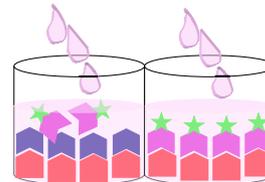
2.- If sera samples contains specific antibodies to gp51 protein of BLV, they will bind the antigen:



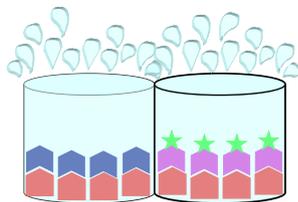
3.- At this point a washing step is needed to remove all non specifically fixed materials:



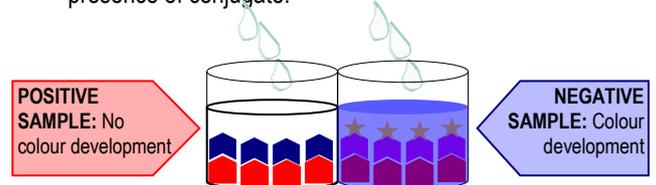
4.- After adding the Mab specific for gp51, it will bind to the protein just only in case that there were not antibodies against gp51 blocking the antigen, in the sample. If there were antibodies in the sample, the Mab couldn't bind to the antigen. This Mab is conjugated to Peroxidase in order to detect its presence later..



4.- Again it is required a washing step to remove all non specifically fixed conjugate:



5.- If we add a substrate for the enzyme labelling the conjugate, a colorimetric reaction will be detected in presence of conjugate:





### 3 KEY REAGENTS USED

The optimum performance of the test is partially due to the high quality of the key reagents used in its formulation, which basically are:

- Plates coated with: Semipurified extract of BLV.
- Conjugate: Is a monoclonal antibody to gp51 protein, labelled with HRPO (horseradish peroxidase). This monoclonal antibody has been also developed by us and has been choose to be the most adequate just for this assay due to its characteristics of specificity, affinity, etc...

### 4 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 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), diluted 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 en sera	0,072	POS
E4 1/100 en sera	0,293	POS
E4 1/250 en sera	0,681	POS
E4 1/500 en sera	1,094	NEG
E05 1/10 en sera	0,096	POS
E05 1/100 en sera	0,255	POS
E05 1/250 en sera	0,591	POS
E05 1/500 en sera	0,886	POS
E05 1/2500 en sera	1,446	NEG
E4 1/250 en milk	0,90	POS
E4 1/500 en milk	1,228	NEG
E05 1/250 en milk	0,682	POS
E05 1/500 en milk	1,065	DOUBTFUL
NEG OIE	1,206	NEG
C. POS	0,162	POS
C.NEG	1,682	NEG

Cut off neg: 1,074

Cut off pos: 0,922



## 4.2 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.

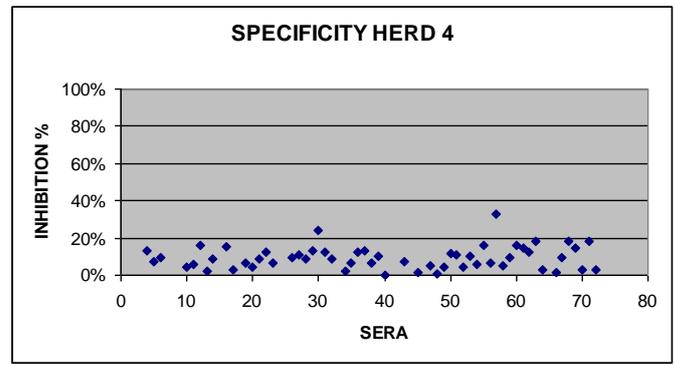
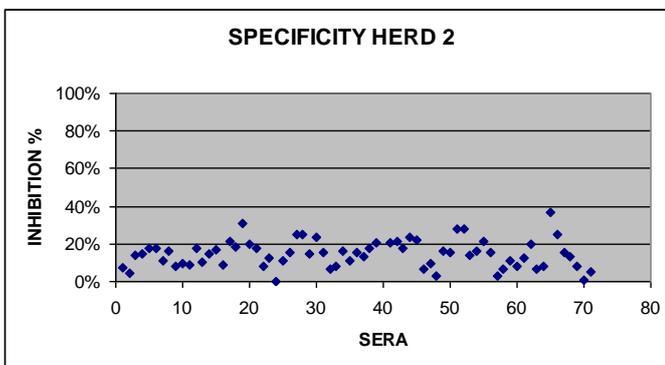
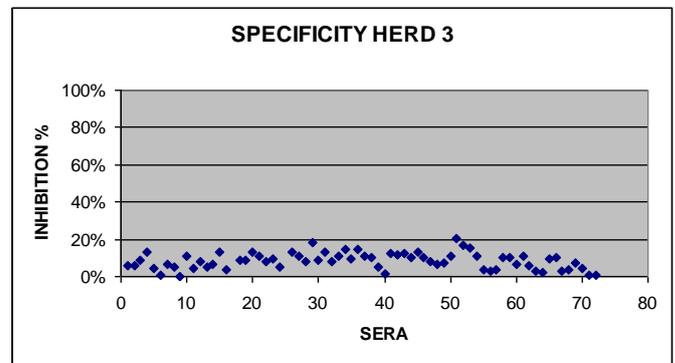
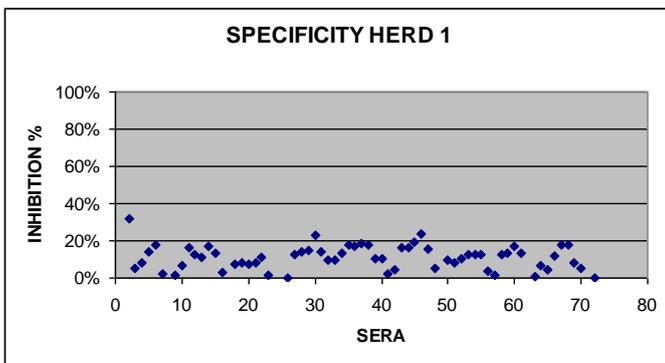
SERUM	INGEZIM BLV COMPAC		IMMUNODIFFUSION
	OD VALUE	RESULTS	RESULTS
1	0.713	-	-
2	0.842	-	-
3	1.019	-	-
4	0.934	-	-
5	0.800	-	-
6	1.164	-	-
7	0.766	-	-
8	0.763	-	-
9	0.752	-	-
10	0.651	-	-
11	0.071	+	+
12	0.060	+	+
13	0.060	+	+
14	0.061	+	+
15	0.062	+	+
16	0.058	+	+
17	0.059	+	+
18	0.061	+	+
19	0.066	+	+
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



### 4.3 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 analyzed. 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 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:

- Inter-plate reproducibility
- Intra-plate reproducibility
- 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 Intra- plate reproducibility

The same serum is assayed in all wells of the plate. To these assay reagents from others validated batches are used.

Once performance the assay, the OD is measured and the variation coefficient calculated. If the variation coefficient is higher than 10%, the batch is rejected.

The Variation Coefficient values  $[(\text{standard deviation} / \text{average}) * 100]$  obtained with the batches produced have been always lower than 7%

---

#### 6.1.2 Inter- plate reproducibility

The variation existing between plates is determined by assaying a serum described in the previous section in 1 strip out of 10 plates. The inter-CV should be below 10%. The Variation Coefficient values  $[(\text{standard deviation} / \text{average}) * 100]$  obtained with the batches produced have been always lower than 9%



## INGEZIM BLV 2.0 (12.BLV.K3)

### 6.1.3 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%

### 6.1.4 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 was 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

### 6.3 SPANISH AUTHORITIES VALIDATION

Every batch of INGEZIM BLV 2.0 produced in INGENASA are sent to the Spanish Reference Laboratory for validation (Laboratorio Central de Veterinaria 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.

### 6.4 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 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.

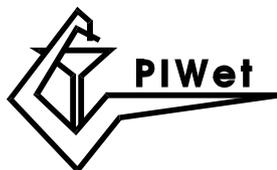
The number of determination produced is shown in the following table:

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 FOLOWING 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.



**NATIONAL VETERINARY RESEARCH INSTITUTE  
DEPARTMENT OF BIOCHEMISTRY**

Al. Partyzantów 57 24-100 Pulawy Poland  
tel. 0048 81 8863051 fax 0048 81 8862595 tlx 0642401

**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<sup>+</sup> - Polish substandard to reference serum E1

**Interpretation of results:**

The laboratory achieved the correct results with all samples

Jacek Kuzmak DVM, PhD

Head of National Reference Laboratory  
for Enzootic Bovine Leukosis

## Institute of Virology

---

Prof. Dr. Dr. Thomas W. Vahlenkamp  
OIE Reference Laboratory for EBL  
Email: thomas.vahlenkamp@uni-leipzig.de  
An den Tierkliniken 29  
04103 Leipzig (Germany)

☎ +49(0) 0341-97 38200  
Fax: +49(0) 341-97 38219  
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:**

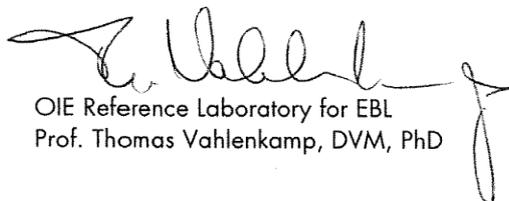
1) INGEZIM BLV COMPAC 2.0 ELISA (Prod Ref: 12.BLV.K3)

Sample	Mean OD	Result
Positive control	0,12	<b>positive</b>
Negative control	1,96	negative
E05 dilution 1:2	0,07	<b>positive</b>
E05 dilution 1:10	0,10	<b>positive</b>
E05 dilution 1:50	0,15	<b>positive</b>
E05 dilution 1:100	0,17	<b>positive</b>
E05 dilution 1:1000	0,35	<b>positive</b>
E05 dilution 1:10.000	0,69	<b>positive</b>
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