INGENASA

DOSSIER DE REGISTRO

Ingezim BLV CONFIRMATION R.1.2.BLV.K1

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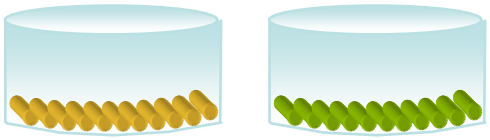
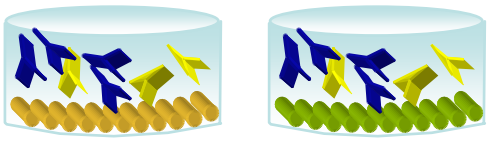
1. DESCRIPTION AND PRINCIPLES OF THE KIT INGEZIM BLV CONFIRMATION

The assay is based on the Indirect ELISA technique. The scheme is shown below:

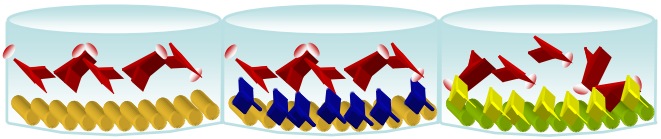


Two types of plates are supplied: coated with positive antigen ( ) and coated with negative antigen ( ).



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.

At this point a washing step is necessary to eliminate the not specific bindings.



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.

At this point a washing step is necessary to eliminate the not specific bindings.

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.

NEGATIVE

POSITIVE

2. SPECIFICATIONS

The product is for laboratory use.
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 immunoglobulines specific of BLV.

3. DATA FROM MANUFACTURER

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4. NAME OF THE PRODUCT

INGEZIM BLV CONFIRMATION

5. COMPOSITION OF THE PRODUCT

- **96-well microtiter plates:** coated with antigen (semipurified extract of BLV virus).
- **96-well microtiter plates:** coated with negative antigen.
- **Positive Control Serun**: Natural inactivated bovine serum from animals infected with BLV virus.
- **Negative Control Serum**: Natural inactivated bovine serum from animals free of BLV virus.
- **Specific conjugate**: Specific monoclonal antibody against ruminant IgG , in a stabilising solution (proprietary formula of the company).
- **Substrate**: Commercial one-step TMB solution.
- **Stop solution**: Diluted sulphuric acid.

6. APLICATION OF INGEZIM BLV CONFIRMATION

This is a laboraory tool to confirm presence of antibodies specific of BLV.
The samples to be analysed can be either fresh or frozen serum.
No special sample treatment is required prior to its analysis using this kit.
Bacterial or fungal contamination of the samples as well as a poor condition of the sample (haemolysis), may affect the result of the assay.

7. STABILITY OF THE PRODUCT INGEZIM BLV CONFIRMATION

The stability of INGEZIM BLV CONFIRMATION is 18 months at 4ºC since the date of manufacture.

	OD POSITIVE CONTROL		OD NEGTAIVE CONTROL	
	Positive plate	Ag pos-Ag neg	Positive plate	Ag pos-Ag neg
0	1,31	1,008	0,074	0,01
3 months	1,29	0,99	0,080	0,008
6 months	1,20	0,998	0,086	0,006
9 months	1,15	0,92	0,080	0,0098
12 months	1,09	0,891	0,078	0,002
15 months	1,01	0,823	0,084	0,003
18 months	0,997	0,895	0,07	0,005

Validation requirement indicates that:

- Positive control: Abs Ag POS – Abs Ag NEG > 0.3
- Negtaive Control: Abs Ag POS – Abs Ag NEG < 0.2

Hence, the product is stable for 18 months at 4ºC.

8. VALIDATION OF THE ASSAY

8.1. *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.

8.2. *USE OF FIELD SERA*

A total of 400 bovine sera previously classified by INGEZIM BLV Compac were analyzed by INGEZIM BLV Confirmation:

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

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

Correspondence between both assays was 98.7%.

9. VARIABILITY INTRA E INTER PLATE

9.1. *REPRODUCIBILITY INTRA AND INTER PLATE*

In order to determine the intra-plate variability, one plate of 96 wells of 3 different batches was used. One positive serum was analyzed in all the wells.

To determine the inter-plate variability, 1 strip of each 10 plates in each batch was tested. 3 batches were analyzed. Results obtained are shown below

	VARIATION COEFFICIENT (V.C.) = σ/×		
	Lot 1	Lot 2	Lot 3
INTRA PLATE VARIABILITY	3,83%	3,40%	3,10%
INTER PLATE VARIABILITY	8,2%	6,3%	5,98%

9.2. *INTER BATCH REPRODUCIBILITY*

To determine the inter batch reproducibility, 6 replica of a positive serum and 2 of a negative one were analyzed in 3 different batches. With the average of these values variation coeficients were calculated (V.C. = Standard Deviation / mean). Table shows the results obtained:

	LOTE A	**LOTE B**	**LOTE C**	
CONTROL+	1,941	1,717	1,641	
	1,937	1,833	1,655	
	1,933	1,891	1,747	
	1,997	2,046	1,877	
	2,006	2,063	1,831	
	1,921	1,888	1,85	
CONTROL -	0,151	0,134	0,149	
	0,162	0,134	0,17	
MEDIA C+	1,956	1,906	1,767	1,876
σ	0,036	0,131	0,102	0,124
CV	**1,85**	**6,87**	**5,77**	**6,59**
MEDIA C-	0,157	0,134	0,160	

9.3. *INTER DAY REPRODUCIBILITY*

3 replica of positive serum and 2 of a negative serum were analyzed in plates belonguing to the same batch. The assay was run 2 days. With the OD values, VC were calculated for the different plates. Table shows the results obtained:

	Day 1	Day 2
C.POS	2,084	2,176
	2,027	2,063
	2,04	2,061
C.NEG	0,097	0,096
	0,099	0,094
MEAN	2,050	2,100
ST. DEV.	0,098	0,095

INTERDAY		
MEAN	ST. DEV.	V.C.%
2,075	0,035	1,7
0,097	0,002	2,2