INgezim® IBR 2.0

R.12.BHV.K.1

Indirect Elisa for the specific detection of antibodies to *Bovine herpesvirus type I* (BoHV-1) causing Infectious Bovine Rhinotracheitis.
Bovine samples (serum, plasma and milk).

TECHNICAL INFORMATION

LAST REVISION: 09/06/2023

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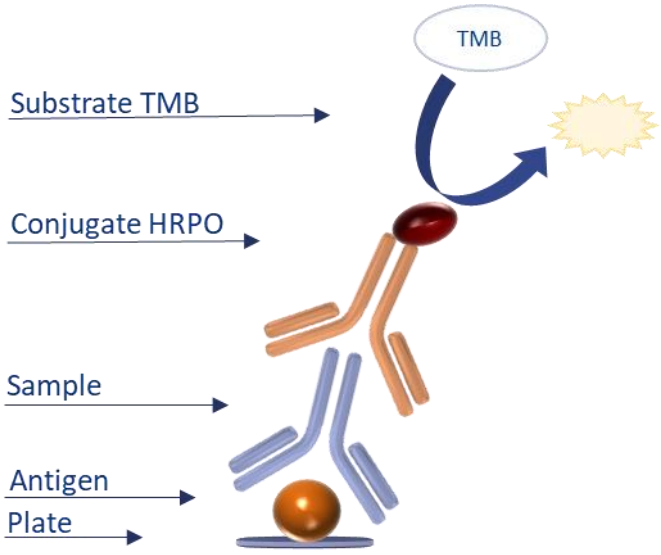
1 PRODUCT APLICATION

The INgezim® IBR 2.0 kit, has been designed for the detection of antibodies specific gB protein of BoHV-1 in bovine serum, plasma, milk (individual and tank) and whey samples.

2 TECHNICAL BASIS OF THE PRODUCT

The assay is based on an indirect ELISA method, the scheme of which is briefly described hereunder:



1. The plates are supplied with antigenated with semipurified and inactivated BoHV antigen type 1.
2. The samples are added and incubated 45min. at 37ºC.
3. Wash three times to remove non-adhered material.
4. Add the specific conjugate and incubate 45 min at 37ºC.
5. Wash four times to remove non-adhered material.
6. Substrate is added and read after 15 min (serum) or 5 min (milk).

3 KEY REAGENTS

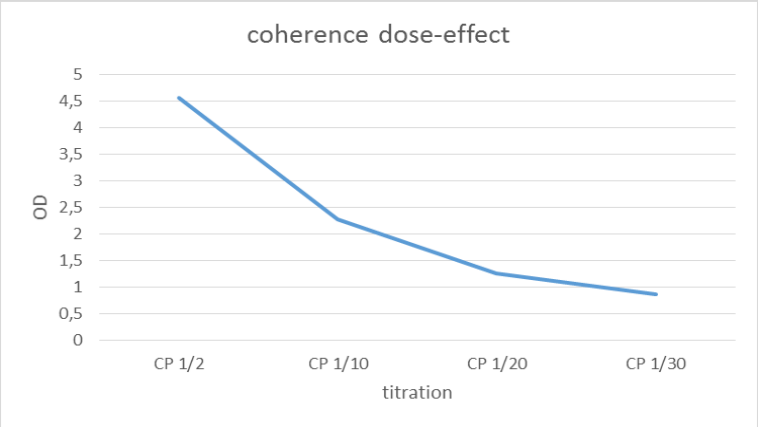
The basic reagents which compose the INgezim® IBR 2.0 kit are:

- IBR Antigen: Protein extract of Infectious Bovine Rhinotracheitis virus (IBRV) or Bovine Herpesvirus type 1 (BoHV-1) obtained from cell cultures, inactivated and semi-purified.
- Monoclonal antibody: specific of bovine IgG, conjugated with peroxidase; used as conjugate.

4 VALIDATION

4.1 COHERENCE DOSE-EFFECT

In order to determine the dose-effect coherence, the positive control of the kit was used. The assay was made following the instructions described in the insert of this product. The blocking percentage of each dilution was calculated. The pictures show the results obtained.



TITRATION	OD
POS 1/2	4.57
POS 1/10	2.278
POS 1/20	1.26
POS 1/30	0.871

4.2 SERA

4.2.1 Analytical sensitivity

In order to determine the analytical sensitivity 2 OIE reference sera positive to BHV-1 (EU1 & EU2) by Seroneutralization (SN) and two FLI (Friedich-Loeffler-Institut, Riems, Germany) weak positive sera by SN (R1C & R2C with titers of SN 6 & 4 respectively) were tested.

The obtained results indicated 100% correspondence with the expected results:

	Lot 1		Lot 2		Result	Expected value OIE/FLI
	Mean OD	M/P	Mean OD	M/P		
C. POS	1.928		2.058			
C. NEG	0.119		0.185			
EU1	1.348	0.70	2.076	1.009	Positive	Positive
EU2	0.412	0.213	0.615	0.298	Positive	Positive
R1C	0.554	0.287	0.681	0.331	Positive	Positive
R2C	0.387	0.200	0.483	0.234	Positive	Positive

4.2.2 Analitycal specificity

In order to determine the analytical specificity, 3 sera were used:

- OIE negative reference serum (EU3)
- FLI SN negative sera (R31B y R32B)

	Lot 1		Lot 2		Result	Expected result OIE/FLI
	Mean OD	M/P	Mean OD	S/P		
C. POS	1.928		2.058			
C. NEG	0.119		0.185			
EU3	0.15	0.077	0.219	0.106	Positive	Positive
R31B	0.206	0.106	0.204	0.099	Positive	Positive
R32B	0.191	0.099	0.245	0.119	Positive	Positive

4.2.3 Comparison between 2 comercial kits using sera:

Positive and negative sera from different sources were used. These será were classified by a blocking ELISA (INgezim® IBR compac) and by an indirect ELISA (Svanovir® IBR Ab).

Results obtained in this study indicated a correspondence between assays as the described in the table below:

	INgezim® IBR Compac	Svanovir® IBR Ab
INgezim® IBR 2.0	93,7%	94,3%
INgezim® IBR Compac		93,7%

4.3 MILK

4.3.1 Diagnostic specificity

4.3.1.1 In comparison with Svanovir® IBR Ab

In order to determine the diagnostic specificity of the assay using milk as a kind of sample, 39 milk tank samples previously classified by Svanovir® IBR Ab as negative, were analyzed. Results obtained in this study indicated 100% relative specificity respect to Svanovir® IBR Ab.

4.3.1.2 In comparison with INgezim® IBR COMPAC 2.0

To determine the performance of the assay using milk samples, 51 milk tank samples previously classified by INgezim® IBR COMPAC 2.0 as negative were analyzed. The obtained results indicated a relative specificity to INgezim® IBR COMPAC 2.0 of 98% (50/51). These results are due to the fact that blocking assays such as INgezim® IBR COMPAC 2.0 are more specific because of the use of MAb as conjugates.

4.3.2 Diagnostic sensitivity

4.3.2.1 In comparison with Svanovir® IBR Ab

To determine the sensitivity of the assay using milk samples, 39 milk tank samples were tested for Svanovir® IBR Ab. The obtained results indicated a relative sensitivity to Svanovir® IBR Ab of 90%.

The obtained results indicated a correspondence between the different tests as shown in the table:

	INgezim® IBR Compac	**Svanovir® IBR Ab**
INgezim® IBR 2.0	94,4%	95,5%
INgezim® IBR Compac		95,5%

4.3.2.2 In comparison with INgezim® IBR COMPAC 2.0

To determine the sensitivity of the assay using milk samples, 38 milk tank samples previously classified by INgezim® IBR COMPAC 2.0 as positive were analyzed. The obtained results indicated a relative sensitivity to INgezim® IBR COMPAC 2.0 of 90%.

		INgezim® IBR Compac 2.0		
INgezim® IBR 2.0		POSITIVE	NEGATIVE	TOTAL
	POSITIVE	34	0	34
	NEGATIVE	4	0	4
	TOTAL	38	0	38