INgezim® IBR gE

R.12.IBE.K.3

Blocking ELISA for the detection of specific antibodies to gE glycoprotein of the Bovine Herpesvirus (BHV-1) in bovine serum, milk and whey samples.

TECHNICAL INFORMATION

LAST REVISION: 12/07/2023

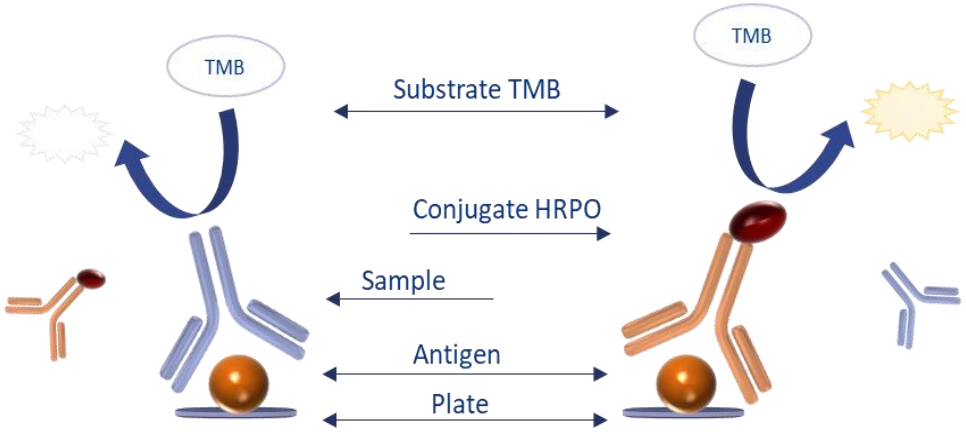
INDEX OF CONTENTS

1 PRODUCT APPLICATION

INgezim® IBR gE kit, has been designed for the detection of antibodies specific to gE protein of BHV (Bovine Herpesvirus) in bovine serum, plasma, milk (individual and tank) and whey samples. Since the kit detects antibodies specific to gE protein, it allows to differentiate between infected animals and animals vaccinated with a delected vaccine.

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 BHV semipurified and inactivated antigen. On these wells, samples are added and incubated.
2. If serum samples contain specific antibodies to BHV-1, 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 to gE glycoprotein of BHV, conjugated with HRPO) is added, only if there are no antibodies blocking the antigen (negative or vaccinated 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

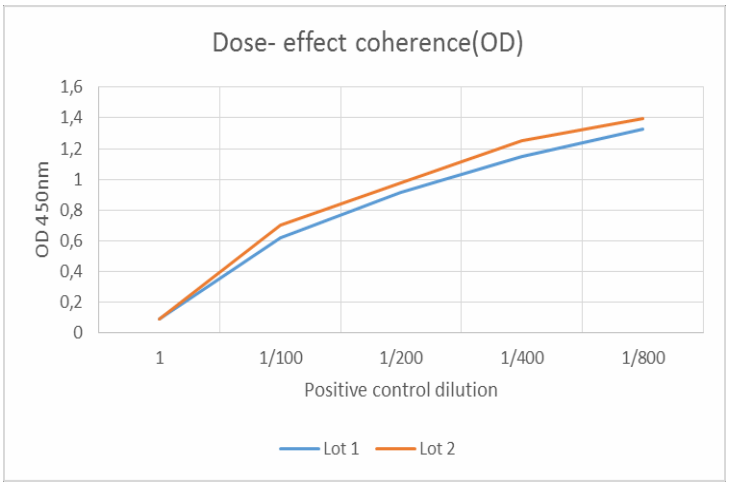
The optimal performance of the assay is mainly due to the quality of the key reagents, which are briefly described below:

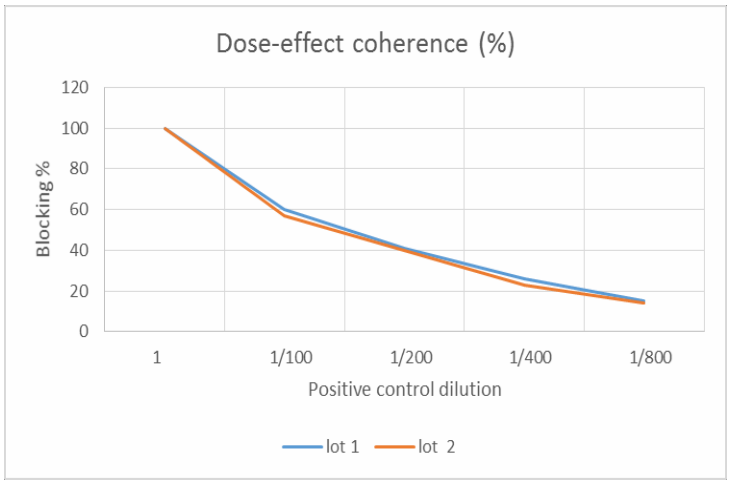
- **IBR Antigen:** protein extract of Bovine Herpesvirus type 1 obtained from cell cultures, inactivated and semi-purified.
- **Monoclonal antibody:** specific for gE glycoprotein of BHV, conjugated with peroxidase; used as conjugate.

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 using two different batches of kits and following the instructions described in the insert of this product. The blocking percentage of each dilution was calculated. The pictures show the results obtained.





4.2 SERA

4.2.1 Analytical sensitivity

In order to determine the analytical sensitivity and specificity of the assay, OIE Reference samples (EU1, EU2, and EU3) as well as samples from the Friedrich-Loeffler-Institut (FLI) (R1, R2, R3, R31, and R32) were tested.

- R1: Neutralisation titre 1/6. gB positive by ELISA. gE positive by ELISA.
- R2: Neutralisation titre 1/4. gB positive by ELISA. gE positive by ELISA.
- R3: Neutralisation titre <1/2-1/2. gB positive by ELISA. gE doubtful/negative by ELISA
- R31: Neutralisation titre <1/2. gB negative by ELISA. gE negative by ELISA.
- R32: Neutralisation titre <1/2. gB negative by ELISA. gE negative by ELISA. gE negative by ELISA

Serum	Expected result	Obtained result	
		blocking %	interpretation
OIE EU1	Positive	61,3	POSITIVE
OIE EU2	Weak positive	68,6	POSITIVE
OIE EU3	Negative	-10,1	NEGATIVE
FLI R1	Positive	81,0	POSITIVE
FLI R2	Positive	60,3	POSITIVE
FLI R3	Doubtful o negative	49,9	NEGATIVE
FLI R31	Negative	-5,4	NEGATIVE
FLI R32	Negative	2,2	NEGATIVE

The obtained results confirm that the analytical sensitivity and specificity of the assay meet the requirements of the OIE and the Friedrich-Loeffler-Institut (FLI).

4.2.2 Analytical specificity

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

- Animals infected with other different agents (BVD y BTV)
- Serum of animals vaccinated with IBR deleted vaccine (gE negative)

Results obtained indicate that there is not cross-reaction with antibodies developed against other different agents, neither against vaccine gE negative.

Classification	Serum	OD 450nm	Blocking %
Positive BTV	**H5**	1,839	**-7,7**
	D6	1,645	**3,6**
	H3	1,59	**6,9**
	B4	1,947	**-14,1**
	A9	1,805	**-5,7**
	D12	1,63	**4,5**
	B8	2,191	**-28,4**
	D3	1,917	**-12,3**
	G3	1,806	**-5,8**
	G5	1,771	**-3,7**
	D5	1,869	**-9,5**
	B3	1,995	**-16,9**
	H4	1,726	**-1,1**
	D9	2,114	**-23,8**
	G4	1,71	**-0,2**
	C7	1,623	**4,9**
Positive BVD	**G2**	1,364	**13,0**
	H2	1,518	**3,2**
	A3	2,121	**-35,3**
	B3	2,714	**-73,1**
	C3	1,537	**1,9**
	D3	1,513	**3,5**
	E3	1,657	**-5,7**
	F3	1,765	**-12,6**
	G3	2,23	**-42,3**
	H3	1,515	**3,3**
	A4	1,628	**-3,9**
	B4	1,425	**9,1**
	C4	1,611	**-2,8**
	D4	1,984	**-26,6**
	E4	1,808	**-15,3**
Vaccinated IBR (delected)	**B4**	1,192	**-31,6**
	C4	1,115	**-23,1**
	D4	1,2	**-32,5**
	E4	1,201	**-32,6**
	F4	1,196	**-32,1**
	G4	0,905	**0,1**

*OD Negative control of BTV-positive = 1.702; OD Negative control of BVD-positive = 1.567
OD Negative control of gE-vaccinated = 0.905; OD Negative control of EU3 = 1.177.

4.2.3 Diagnostic specificity

In order to assess the relative specificity of the assay, sera from different origins and previously catalogued by the IDEXX IBR gE Ab kit were analysed by INgezim® IBR gE:

- 111 negative sera from Cáceres and Gijón.
- 176 true negative sera from Gijón.

The obtained results indicated that the assay has a relative specificity or NPA (negative percent of agreement) of 98.3%.

4.2.4 Diagnostic sensitivity

In order to determine the relative sensitivity, 309 sera from Spanish farms were analyzed. The sera were classified as positive by the IDEXX assay, IBR gE Ab (as "gold standard"). The results obtained indicated a relative sensitivity or PPA (positive percent of agreement) of 98.1%.

4.3 USE OF MILK

254 tank milk samples (80-200 samples / tank) were analysed. These samples were obtained from vaccinated (delected vaccine) herds and with far history of vaccination with not delected vaccine. All samples were analysed in parallel with INgezim® IBR gE and INgezim® IBR Compac (not differentiation between vaccinated and infected). All positive samples by INgezim® IBR gE were positive by INgezim® IBR Compac and there not exist negative samples by INgezim® IBR Compac and positive by INgezim IBR gE.

		INgezim® IBR gE			
INgezim® IBR Compac		POS	NEG	DOUB	TOTAL
	POS	18	152	1	171
	NEG	0	65	0	65
	DOUB	0	16	0	16
	TOTAL	18	233	1	252