INgezim IBR COMPAC 2.0

R.12.BHV.K.3

Blocking ELISA for the detection of specific antibodies to the gB glycoprotein of BoHV.
Bovine milk and serum samples.

TECHNICAL INFORMATION

LAST REVISION: 09/06/2023

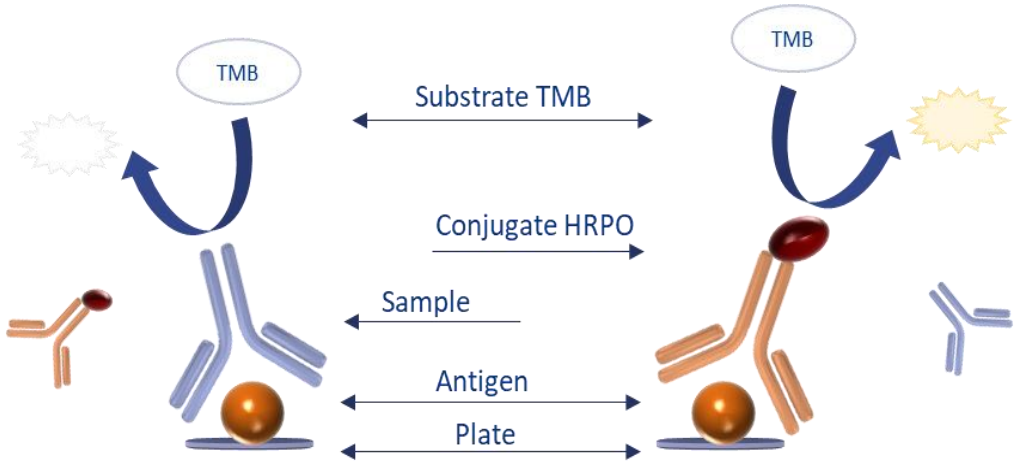
INDEX OF CONTENTS

1 PRODUCT APPLICATION

INgezim® IBR COMPAC 2.0 kit, has been designed for the detection of antibodies specific to gB protein of Bovine Rinotracheitis virus (BoHV-1) in bovine serum, plasma, milk (individual and tank) and whey samples. Since the kit detects antibodies specific of gB protein, it does not allow to differentiate between infected and vaccinated animals.

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 BoHV-1 semipurified and inactivated antigen. On these wells, samples are added and incubated.
2. If serum samples contain specific antibodies to BoHV-1, they will bind the antigen.
3. At this point, a washing step is necessary to remove any non-specifically bound material.
4. When a monoclonal antibody specific to gB protein of the BoHV-1 is added, it will bind to the coated antigen if the serum is negative. The monoclonal antibody has been conjugated with HRPO for future detection.
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 Infectious Bovine Rhinotracheitis virus (IBRV) or Bovine Herpesvirus type 1 (BoHV-1) obtained from cell cultures, inactivated and semi-purified.
- **Monoclonal antibody:** specific for gB glycoprotein of BoHV-1, conjugated with peroxidase; used as conjugate.

4 VALIDATION

4.1 ANALYTICAL SENSITIVITY

To determine the analytical sensitivity, OIE reference sera (EU1, EU2) positive against BHV-1 by Seroneutralization (SN), two sera weakly positive by SN for both gE and gB antibodies, from FLI (Friedich-Loeffler-Institut, Riems, Germany) (R1C and R2C with titers of SN 6 and 4 respectively), 2 positive sera and one reference milk from ANSES (Laboratorire de Ploufragan-Plouzané_Niort) (SRF2 and EFI).
The results obtained indicated 100% correspondence with the expected results:

	Lote 1		Lote 2		Result	Expected Value OIE/FLI/ANSES
	Average	% Blocking	Average	% Blocking		
C. POS	0,120		0,088			
C. NEG	1,568		1.633			
EU1	0,380	82,04%	0,347	83,28%	Positive	Positive
EU2	0,927	44,25%	0,860	50,06%	Positive	Positive
R1C	0,813	52,12%	0,592	67,37%	Positive	Positive
R2C	1,051	35,70%	0,971	42,90%	Positive	Positive

4.2 ANALYTICAL SPECIFICITY

In order to determine the analytical specificity, several sera corresponding to:

- Negative reference serum of the OIE (EU3)
- Reference sera of the FLI (R31B y R32B).

Classification	Serum	Lote 1		Lote 2	
		DO 450nm	% Blocking	DO 450nm	% Blocking
C. POS		0,120	pos	0,088	
C. NEG		1,568	neg	1,633	
Negative OIE	**EU3**	2.,15	-36	1,474	10,31
FLI	**R31B**	1,518	3,43	1,521	7,28
	R32C	1,75	-12,6	1,413	14,26

4.3 CROSS-REACCIÓN STUDIES

In order to determine if there is a cross-reaction in the detection of specific antibodies against other unrelated agents, sera from animals infected with BTV were analyzed. The results obtained indicated that there is no cross-reaction, all results being negative.

n	DO	% Blocking
1	1,411	1,8
2	1,537	-7,5
3	1,318	8,7
4	1,753	-23,4
5	1,333	7,6
6	1,687	-18,6
7	1,682	-18,2
8	1,615	-13,2
9	1,702	-19,6
10	1,554	-8,7
11	1,659	-16,5
12	1,635	-14,7
13	1,286	11,0
14	1,536	-7,4
15	1,552	-8,6
16	1,805	-27,3
17	1,654	-16,1
18	1,437	-0,1
19	1,554	-8,8
20	1,707	-20,0
21	1,545	-8,0
22	1,546	-8,2
23	1,636	-14,8
24	1,651	-15,9
25	1,624	-13,9
26	1,345	6,7
27	1,689	-18,7
28	1,619	-13,5
29	1,658	-16,4
30	1,582	-10,8
31	1,589	-11,3
32	1,511	-5,6
33	1,714	-20,5
34	1,288	10,9
35	1,687	-18,5
36	1,646	-15,5
37	1,665	-16,9
38	1,532	-7,1
39	1,733	-21,9
40	1,714	-20,5
41	1,253	13,4
42	1,533	-7,2
43	1,679	-18,0
44	1,338	7,2
45	1,491	-4,1
46	1,657	-16,3
47	1,812	-27,7
48	1,836	-29,5
49	1,562	-9,3
50	1,560	-9,2
51	1,751	-23,3
52	1,608	-12,7
53	1,604	-12,4
54	1,610	-12,8
55	1,635	-14,7
56	1,541	-7,8
57	1,655	-16,2
58	1,441	-0,4
59	1,784	-25,7
60	1,726	-21,4
61	1,643	-15,3
62	1,731	-21,8
63	1,479	-3,2
64	1,637	-14,8
65	1,235	14,8
66	1,036	29,4
67	1,669	-17,2
68	1,413	1,6
69	1,506	-5,2

4.4 SERA

4.4.1 Diagnostic specificity with respect to Seroneutralization

In order to determine the performance of the assay, a study with field sera was performed. The study analyzed 81 sera from three dairy cattle herds and compared the results obtained by INgezim® IBR COMPAC 2.0 and Seroneutralization (SN).

The obtained results (OD not available because it is an external study) indicated a good correlation between INgezim® IBR COMPAC 2.0 and SN, being more sensitive than ELISA. SN negative and ELISA positive sera were weak positives. The tables show the results obtained by comparing the two techniques. The assay presented a specificity of 79% with respect to SN (it should be taken into account that the SN only detects neutralizing Ab while the ELISA detects all types of Ab).

	SN	
INgezim® IBR Compac 2.0		POSITIVE
	POSITIVE	15
	NEGATIVE	65
	DOUBTFUIL	2
	TOTAL	81

4.4.2 Negative experimental Sera

In order to determine the performance of the assay with respect to diagnostic specificity, a study was carried out with experimental sera from studies carried out with animals infected for other diseases (820). Therefore, negative to BHV-1. The obtained results indicated a specificity of 99.4%.

4.4.3 Field sera

185 field sera previously classified as negative by IDEXX IBR gB Ab test were analyzed. The results obtained with INgezim® IBR Compac 2.0 indicated a relative specificity or negative percentage agreement (NPA) of 99.5%.

4.5 DIAGNOSTIC SENSITIVITY

4.5.1 Respect Seroneutralization (SN)

In order to determine the performance of the assay, a study with field sera was performed. The study analyzed 103 sera from three dairy cattle herds and compared the results obtained by INgezim® IBR COMPAC 2.0 and Seroneutralization (SN).

The obtained results (OD not available because it is an external study) indicated a good correlation between INgezim® IBR COMPAC 2.0 and SN. The sensitivity shown was 100% in this particular study.

4.5.2 Regarding commercial tests available on the market

In order to determine the performance of the assay with respect to diagnostic sensitivity, a study with field sera was carried out. The study analyzed 207 sera from positive Spanish farms located in Extremadura and the Balearic Islands and the results obtained by INgezim® IBR COMPAC 2.0 and the blocking assay for detection of gB-specific Ab were compared, ID Screen® IBR gB competition. The obtained results indicated a relative sensitivity or percentage of positive agreement (PPA) of 99.5% with respect to this trial (206/207).

4.5.3 Use of serum pools

To determine the sensitivity of the assay using pools of 10 sera, samples of sera previously classified as positive and with different blocking percentages were selected and diluted 1/10 in negative serum. These results were also compared with those obtained by the ID Screen® IBR gB competition kit.

The obtained results indicated that the INgezim® IBR COMPAC 2.0 assay can detect positive sera of different degrees of positivity in pools of 10, while the ID Screen® IBR gB Competition assay failed to detect any of these pools.

		ID Screen IBR gB competition		
INgezim® IBR Compac 2.0		POSITIVE	NEGATIVE	TOTAL
	POSITIVE	54	14	68
	NEGATIVE	0	0	0
	TOTAL	54	14	68

4.6 MILK

4.6.1 Diagnostic sensitivity in milk tank

To determine the performance of the milk tank test, 232 samples of this type previously classified as positive by the Svanovir® IBR Ab kit were analyzed.
The results indicated a relative sensitivity or PPA of 99.9% for this study.

		ID Screen IBR gB competition		
		POSITIVE	NEGATIVE	TOTAL
INgezim® IBR Compac 2.0	POSITIVE	232	0	232
	NEGATIVE	0	0	0
	TOTAL	232	0	232

4.6.2 Diagnostic specificity in individual milks

In order to determine the diagnostic specificity of the assay, 849 samples of individual milk from Denmark, considered a free zone of this disease, were analyzed. The obtained results indicated 100% specificity.

4.6.3 Diagnostic specificity with tank milk

To determine the specificity of the assay using milk tank samples, 50 milk tank samples previously catalogued by Svanovir® IBR Ab were analyzed. The obtained results in this study indicated a NPA of 96% between both milk tank trials.

4.7 COHERENCE DOSE-EFFECT

To determine coherence dose-effect, positive kit control was used. A test was carried out following the instructions described in the insert. The percentage of blocking of each of the dilutions was determined. The figures show the results obtained.