

INgezim® BTV Compac 2.0

R.12.BTV.K.3

Blocking ELISA for the detection of specific antibodies to protein vp7 of the Blue Tongue Virus (BTV).
Ruminant serum samples.

TECHNICAL INFORMATION

LAST REVISION: 17/05/2023

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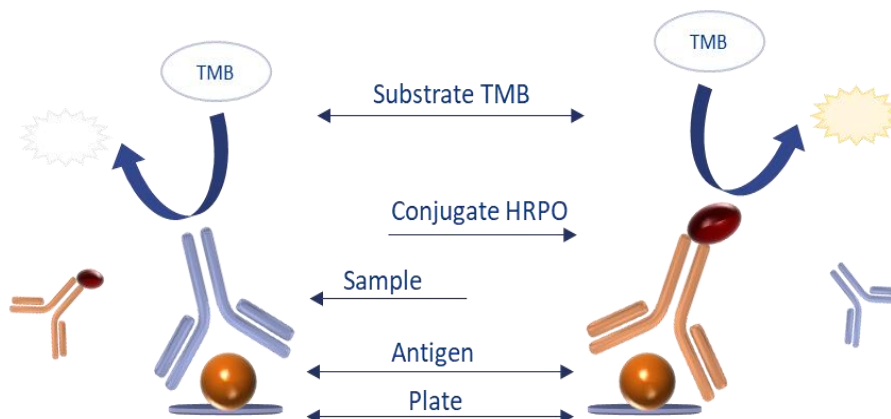
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1 PRODUCT APPLICATION

INgezim® BTV Compac kit, has been designed for the detection of antibodies specific to vp7 protein of BTV (Blue Tongue Virus) in bovine, goat and ovine serum, plasma individual samples.

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 BTV vp7 recombinant protein antigen. On these wells, samples are added and incubated.
2. If serum samples contain specific antibodies to BTV, 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 vp7 of BTV, conjugated with HRPO) is added, only if there are no antibodies blocking the antigen (negative 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:

- **BTV Antigen:** protein vp7 of BTV obtained as recombinant protein.
- **Monoclonal antibody:** specific for vp7 of BTV, conjugated with peroxidase; used as capture antibody.

4 VALIDATION

4.1 AGID TECHNIQUE

In order to determine the sensitivity and specificity of the assay with respect to the AGID technique, a panel of 225 collection sera previously typified by this technique was analyzed, carried out according to the protocol described by the OIE for the diagnosis of Bluetongue.

The results obtained are shown below:

✓ **Bovine sera (95)**

(AGID)	INgezim® BTV COMPAC 2.0			
		POSITIVES	NEGATIVES	DOUBTFUL
	POSITIVES	61	3	0
	NEGATIVES	0	31	0

Specificity: True Negatives / True Negatives + False Positives (31/ 31) = 100%

Sensibility: True positives/ True Negatives + False Negatives (61/ 61+3) = 95%

✓ **Ovine sera (33)**

(AGID)	INgezim® BTV COMPAC 2.0			
		POSITIVES	NEGATIVES	DOUBTFUL
	POSITIVES	6	0	0
	NEGATIVES	1 ^a	25	1

a. These sera have DO values very close to the cut-off point.

Specificity True Negatives / True Negatives + False Positives (25/ 25+1) = 96%

Sensibility True positives/ True Negatives + False Negatives (6/ 6) = 100%

✓ **Goat sera (97)**

(AGID)	INgezim® BTV COMPAC 2.0			
		POSITIVES	NEGATIVES	DOUBTFUL
	POSITIVES	5	0	0
	NEGATIVES	0	92	0

Specificity True Negatives / True Negatives + False Positives (92/ 92) = 100%

Sensibility True positives/ True Negatives + False Negatives = 100%

4.2 REFERENCE SERA

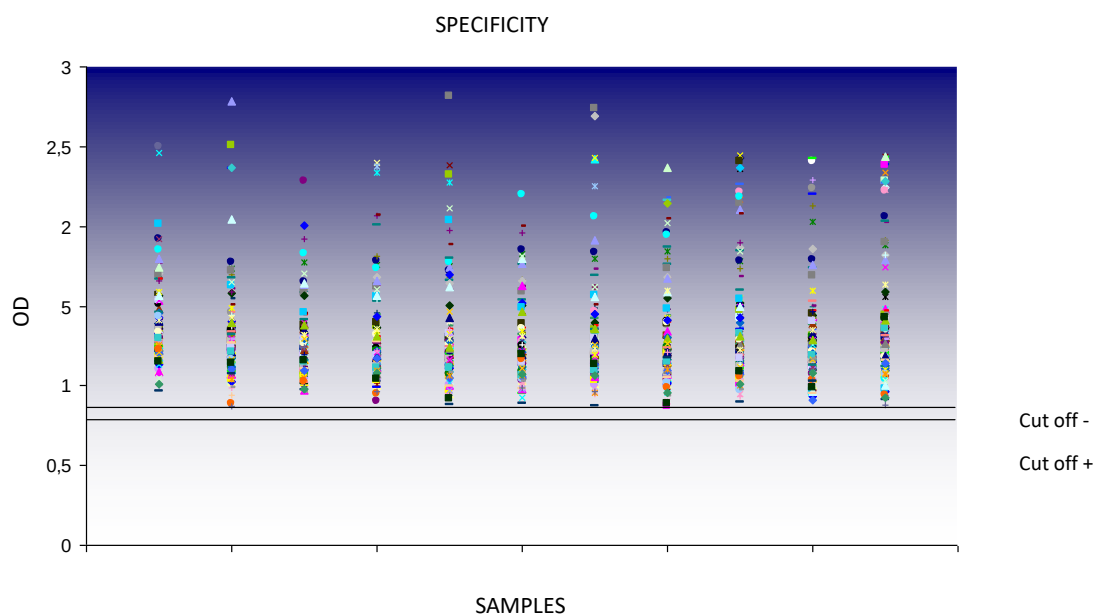
Sera from animals infected with all 24 BTV serotypes have been analyzed. These sera have been obtained from the different Reference Laboratories for BTV (Central Veterinary Laboratory of Algete, Pirbright Laboratory, USDA and Onderstepoort) and the obtained results have been compared with the two possible incubation options in the kit.

A slight increase in sensitivity was observed when using the option of incubation 18-24 hours at room temperature in those cases of sera with minimal antibody titers.

SERO TYPE	1.2.BTV.K3 2.0 (ON RT)								1.2.BTV.K3 2.0 (2,5 hours at 37°C)							
	SOURCE								SOURCE							
	Pirbright		USDA		Onderstepoort*		Ref. Algete		Pirbright		USDA		Onderstepoort*		Ref. Algete	
	OD	RESULT	OD	RESULT	OD	RESULT	OD	RESULT	OD	RESULT	OD	RESULT	OD	RESULT	OD	RESULT
1	0,994	POS			0,118	POS			1,11	POS			0,172	POS		
2	0,561	POS			0,398	POS			0,802	POS			0,596	POS		
3	0,857	POS					0,68	POS	1,445	NEG					1,161	POS
4	0,511	POS							0,746	POS						
5	1,514	DUD	0,469	POS					1,948	NEG	0,746	POS				
6	0,483	POS							0,666	POS						
7	0,88	POS							1,257	POS						
8	0,315	POS							0,554	POS						
9	0,741	POS							0,805	POS						
10	1	POS	0,286	POS			0,802	POS	1,267	POS	0,361	POS			0,996	POS
11	0,848	POS							1,02	POS						
12	0,997	POS							1,292	POS						
13	0,186	POS			0,519	POS			0,253	POS			0,754	POS		
14	0,448	POS			0,539	POS			0,581	POS			0,713	POS		
15	0,256	POS							0,365	POS						
16	0,28	POS							0,464	POS						
17	0,145	POS							0,17	POS						
18	0,064	POS							0,091	POS						
19			1,356	POS			0,158	POS			1,851	NEG			0,382	POS
19 VK12	0,701	POS							0,747	POS						
19 RY19	0,146	POS							0,186	POS						
19 VH59	1,515	NEG							1,844	NEG						
20	0,987	POS	0,643	POS					1,184	POS	0,858	POS				
21	0,16	POS							0,432	POS						
22	0,1	POS			0,518	POS			0,212	POS			0,755	POS		
23	0,065	POS			0,589	POS			0,125	POS			0,859	POS		
24	0,187	POS							0,306	POS						
C+	2,362	Cut off (+)		C+ x 0,6	1,4289				2,329	Cut off (+)		C+ x 0,6	1,3164			
C+	2,401	Cut off (-)		C+ x 0,65	1,548				2,059	Cut off (-)		C+ x 0,65	1,4261			

4.3 SPECIFICITY ASSAY

In order to determine the specificity of the test in field samples, 1262 sera (92 sheep, 92 goats and 1078 cattle) from Spanish farms free of BTV have been analyzed. The results indicate a specificity of the assay >99.9%.



4.4 SENSITIVITY OF THE ASSAY

In order to determine the sensitivity of the test, a panel of samples from 8 positive farms was analyzed. The results were compared with those obtained by another commercially available immunoassay (commercial EIA).

The following tables show a summary of the study conducted.

- FARM 1 (88 sera)**

EIA	INgezim® BTV COMPAC 2.0			
		POSITIVES	NEGATIVES	DOUBTFUL
	POSITIVES	37	0	0
	NEGATIVES	8 ^a	42	1

a. 5 of these sera gave results close to the cut-off point by INgezim BTV COMPAC 2.0

- FARM 2 (86 sera))**

EIA	INgezim® BTV COMPAC 2.0			
		POSITIVES	NEGATIVES	DOUBTFUL
	POSITIVES	29	0	0
	NEGATIVES	4 ^a	53	0

a. 2 of these sera gave results close to the cut-off point of the external kit

- FARM 3 (87 sera))

EIA	INgezim® BTV COMPAC 2.0			
		POSITIVES	NEGATIVES	DOUBTFUL
	POSITIVES	19	0	0
	NEGATIVES	3 ^a	64	1

a. 1 serum with OD value equal to the cut-off point for the external kit.

- FARM 4 (86 sera))

EIA	INgezim® BTV COMPAC 2.0			
		POSITIVES	NEGATIVES	DOUBTFUL
	POSITIVES	12	1	0
	NEGATIVES	3 ^a	70	0

a. 2 sera have DO values close to the cut-off point for the external kit.

- FARM 5 (76 sera))

EIA	INgezim® BTV COMPAC 2.0			
		POSITIVES	NEGATIVES	DOUBTFUL
	POSITIVES	35	0	0
	NEGATIVES	10 ^a	31	0

a. 8 sera have OD values close to the cut-off point for the external test.

- FARM 6 (96 sera)

EIA	INgezim® BTV COMPAC 2.0			
		POSITIVES	NEGATIVES	DOUBTFUL
	POSITIVES	52	1	0
	NEGATIVES	0	43	0

- FARM 7 (53 sera)

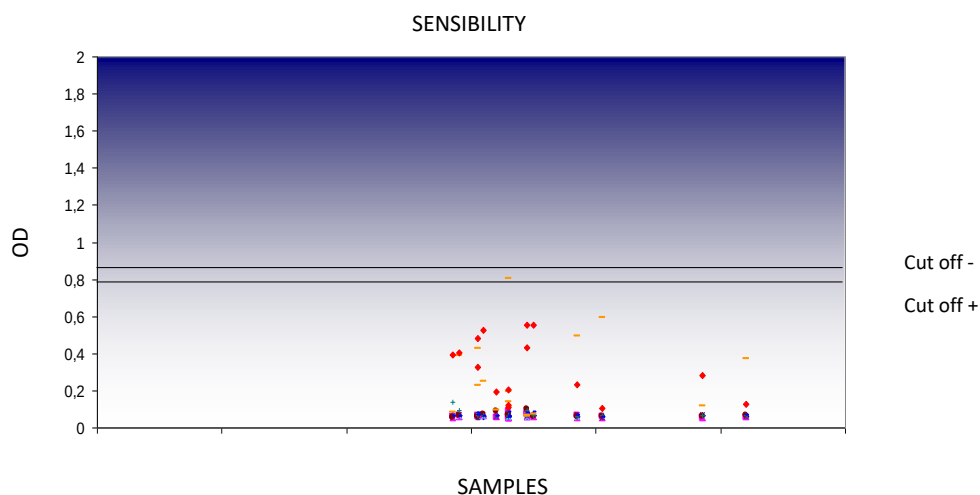
EIA (comercial)	INgezim® BTV COMPAC 2.0			
		POSITIVES	NEGATIVES	DOUBTFUL
	POSITIVES	26	2	0
	NEGATIVES	4	21	0

- FARM 8 (92 sera))

EIA	INgezim® BTV COMPAC 2.0			
		POSITIVES	NEGATIVES	DOUBTFUL
	POSITIVES	13	0	0
	NEGATIVES	0	79	0

4.4.1 Sensitivity with field samples

A total of 172 samples previously characterized as positive were analyzed. Of all of them, only one gave a doubtful result, the rest being positive. Therefore, the sensitivity of the assay with field samples is 99.4%.



4.4.2 Sensitivity in experimental tests

In order to determine the sensitivity of the assay, different extractions from 6 animals not vaccinated and experimentally infected with BTV serotype 4 at day 0 were analyzed.

The results of INgezim BTV COMPAC 2.0 were compared with those obtained with INgezim® BTV COMPAC, showing an increase in sensitivity of version 2.0 in 3 of the 6 animals, with detection of antibodies one day earlier than with the previous version.

*Oveja = sheep

*%bloqueo = blocking %

Oveja 19 3590	% Bloqueo		Abs 450 nm	
	INGEZIM BTV COMPAC	INGEZIM BTV COMPAC 2.0	INGEZIM BTV COMPAC	INGEZIM BTV COMPAC 2.0
0	33,0	30,8	1,067	1,134
3	31,6	31,7	1,090	1,119
5	37,8	42,6	0,991	0,940
7	49,7	70,5	0,802	0,483
10	78,8	88,6	0,337	0,186
13	82,3	90,9	0,282	0,149
17	80,7	90,7	0,308	0,153

Bovino = bovine

*%bloqueo = %blocking

Bovino F10	% Bloqueo		Abs 450 nm	
	INGEZIM BTV COMPAC	INGEZIM BTV COMPAC 2.0	INGEZIM BTV COMPAC	INGEZIM BTV COMPAC 2.0
0	8,3	7,2	1,461	1,521
2	28,5	24,2	1,140	1,241
5	34,1	36,8	1,050	1,035
8	44,6	69,2	0,883	0,504
12	62,0	100,0	0,605	
15	76,8	91,6	0,370	0,138
19	83,1	92,1	0,269	0,130
21	83,1		0,270	

Oveja = sheep

*%bloqueo = %blocking

F21 OVEJA 77		% Bloqueo		Abs 450 nm	
		INGEZIM BTV COMPAC	INGEZIM BTV COMPAC 2.0	INGEZIM BTV COMPAC	INGEZIM BTV COMPAC 2.0
0		2,5	1,9	1,553	1,607
3		10,8	17,4	1,422	1,353
5		26,8	35,7	1,166	1,053
7		58,5	87,0	0,662	0,214
10		74,8	83,8	0,401	0,266
13		73,4	85,6	0,424	0,237
18		67,8	74,6	0,513	0,416
21		69,4	75,1	0,487	0,408
24		70,2	75,4	0,474	0,403
27		67,9	70,7	0,511	0,480

F17 OVEJA 2		% Bloqueo		Abs 450 nm	
		INGEZIM BTV COMPAC	INGEZIM BTV COMPAC 2.0	INGEZIM BTV COMPAC	INGEZIM BTV COMPAC 2.0
0		13,9	11,5	1,371	1,450
2		21,7	20,6	1,247	1,300
3		27,3	20,9	1,158	1,296
5		26,5	31,9	1,171	1,116
7		80,1	91,2	0,317	0,144
10		88,2	93,9	0,188	0,100
14		79,7	91,9	0,323	0,132
17		77,3	86,4	0,361	0,222
21		75,0	82,5	0,399	0,287
23		73,3	80,0	0,426	0,327

Oveja 19 3531		% Bloqueo		Abs 450 nm	
		INGEZIM BTV COMPAC	INGEZIM BTV COMPAC 2.0	INGEZIM BTV COMPAC	INGEZIM BTV COMPAC 2.0
0		11,0	14,5	1,418	1,401
3		16,0	23,5	1,338	1,254
5		64,8	74,5	0,561	0,419
7		72,5	77,5	0,438	0,369
10		75,3		0,393	
13		79,9	82,0	0,320	0,296
17		79,7	82,0	0,323	0,296

F17 OVEJA 17		% Bloqueo		Abs 450 nm	
		INGEZIM BTV COMPAC	INGEZIM BTV COMPAC 2.0	INGEZIM BTV COMPAC	INGEZIM BTV COMPAC 2.0
0		32,6	31,4	1,074	1,124
2		33,1	33,7	1,066	1,086
3		28,1	31,8	1,146	1,117
5		33,3	53,1	1,063	0,769
7		84,3	92,2	0,250	0,127
10		89,4	92,9	0,170	0,116
14		90,0	92,4	0,159	0,124
17		85,6	91,9	0,230	0,133
21		80,0	87,2	0,319	0,209
23		77,5	84,9	0,358	0,248

Cut off +: 55% blocking

Cut off -: 50% de blocking