Internal validation report

ID Screen® Bluetongue Competition

Competitive ELISA for the detection of antibodies against the BTV VP7 protein in serum or plasma from multiple species

- **Proven specificity and sensitivity** and widespread use in recent outbreaks.
- **Detection of antibodies against all BTV serotypes**, at least 7 days post infection, thanks to the use of a monoclonal antibody against the highly conserved VP7 protein.
- **Easy-to-use**: only one wash step, with results in 90 minutes.

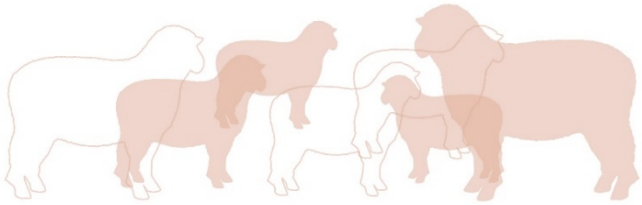
Introduction

Bluetongue (BT) is an infectious vector-borne viral disease that affects domestic and wild ruminants such as sheep. The disease is caused by the Bluetongue virus (BTV), of which 26 serotypes exist.

Laboratory detection of BTV may be achieved by detecting antibodies directed against the VP7 protein. The VP7 is a major core protein possessing the serogroup-specific antigens common to the 26 serotypes.

The **ID Screen® Bluetongue Competition** ELISA allows for the detection of anti-VP7 antibodies in serum and plasma from multiple species.

This document summarizes validation data obtained for this test.



Test Principle

Wells are coated with the VP7 recombinant protein.

The samples to be tested and the controls are added to the microwells. The anti-VP7 antibodies, if present, form an antibody-antigen complex which masks the VP7 epitopes.

An anti-VP7-peroxidase (HRP) conjugate is added to the microwells. It fixes to the remaining free VP7 epitopes, forming an antigen-conjugate-peroxidase complex.

After washing in order to eliminate the excess conjugate, the substrate solution (TMB) is added.

The resulting coloration depends on the quantity of specific antibodies present in the sample to be tested. In the absence of antibodies, a blue coloration appears which becomes yellow after addition of the stop solution. In the presence of antibodies, no coloration appears.

The microplate is read at 450nm.

Result interpretation

For each sample, calculate the competition percentage (S/N%).

Samples presenting a competition percentage:

- greater than or equal to 40 % are considered negative.
- less than 40 % are considered positive.

Analytical sensitivity

As there is no international standard for BTV serology, analytical sensitivity was compared to another commercial ELISA (kit A) through analysis of different dilutions of two positive sera:

- a BTV-4 positive serum, from a Southern Italian slaughterhouse;
- a BTV-8 positive serum, kindly provided by Dr Toussaint and Dr. Clercq, from the CODA CERVA laboratory in Belgium

Results (Table 1):

- Both kits detected the BTV-4 diluted to 1/16.
- The IDvet kit detected an additional dilution of the BTV-8 compared with Kit A.

	BTV-4		BTV-8	
Dilution	IDvet	Kit A	IDvet	Kit A
Pure	+	+	+	+
1/2	+	+	+	+
1/4	+	+	+	+
1/16	+	+	+	+
1/32	-	-	+	-
1/64	-	-	-	-

Table 1: Comparative analytical sensitivity

Sensitivity

Field samples

The following samples from infected herds were tested:

- 300 sera from BTV-8 infected / vaccinated cattle (Germany, Belgium and France, 2007 and 2008).
- 300 sera from BTV-8 infected / vaccinated sheep (Germany, Belgium and France, 2007 and 2008).
- 84 sera from BTV-8 infected / vaccinated goats (Germany, Belgium and France, 2007 and 2008).
- 37 sera from BTV-1 infected cattle (France and Spain, 2008).
- 33 sera from BTV-1 infected sheep (France, 2008).

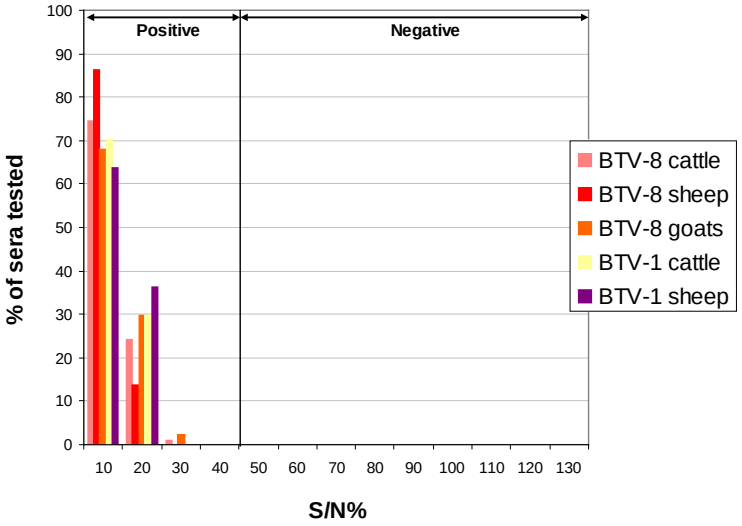


Figure 1: S/N% distribution of positive sera

Results (Figure 1):

- All sera were found positive.
- **Measure sensitivity = 100% ($CI_{95\%}$: 99.49% - 100%), n=754.**

Serotype sensitivity

The following samples were tested using the ID Screen® ELISA:

- antisera to the 26 BTV serotypes;
- serial dilutions of BTV-16 positive samples (4 samples diluted 1/25, 1/50, 1/100 and 1/200);
- 3 BTV-negative sera;
- sera from animals infected by the 5 EHDV (Epizootic Hemorrhagic Disease Virus) serotypes.

Results:

- All 26 serotypes were detected by the ID Screen® ELISA.
- The BTV-16 positive sera were found positive diluted 1/25.
- The BTV-negative samples and EHDV-positive samples were correctly identified as negative.

Correlation with PCR

304 field samples were tested by PCR and the ID Screen® ELISA. These samples came from the 2006 Spring-Summer primo-infection in Belgium.

		BTV-PCR		
		+	-	
ID Screen® ELISA	+	36	1	
	-	1	266	**304**

Table 2: Seroconversion kinetics on time-course sera tested with the ID Screen® ELISA

Results (Table 2):

- Correlation between the PCR and ID Screen® ELISA results were remarkable (99.34%). Such results are due to the fact that the samples came from zone where the disease did not previously exist.
- The sample not detected by ELISA probably corresponds to an animal infected and tested before seroconversion.
- The sample not detected by PCR probably corresponds to an animal after seroconversion and after elimination of the virus.

In the months following these analyses, the percentage of PCR-positive samples decreased, while the number of serology-positive samples remained constant.

Experimentally infected animals

12 sheep, experimentally infected with BTV-4, were bled 0, 4, 7, 14, 21, 28, 35 and 42 days post-infection. Serum samples were tested with the ID Screen® Bluetongue Competition ELISA.

Sample	Day 0	Day 4	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
1	-	-	-	+	+	+	+	+
2	-	-	-	-	-	+	+	+
3	-	-	-	-	+	+	+	+
4	-	-	-	+	+	+	+	+
5	-	-	-	+	+	+	+	+
6	-	-	-	+	+	+	+	+
7	-	-	-	+	+	+	+	+
8	-	-	-	+	+	+	+	+
9	-	-	-	+	+	+	+	+
10	-	-	-	+	+	+	+	+
11	-	-	+	+	+	+	+	+
12	-	-	+	+	+	+	+	+

Table 3: Seroconversion kinetics for experimentally infected sheep.

Results (Table 3):

- Seroconversion was detected at least 7 days post-infection.

Vaccinated animals

The following animals were tested with the ID Screen® Bluetongue Competition ELISA:

- 1 sheep, vaccinated with a South African BTV-2 live vaccine, and bled 0, 7, 14, 21, 28, 35, 42 and 49 days post-vaccination (dpv);
- 5 sheep, vaccinated with Merial 2,4 vaccine, and bled 0, 7, 38, 74 and 108 dpv.

Days post vaccination	S/N%
0	**119**
7	**91**
14	**46**
21	**59**
28	**40**
35	**31**
42	**15**
49	**8**

Table 4: Seroconversion kinetics of a BTV-2 vaccinated serum (cut-off: <40%).

Days post vaccination (dpv)	S/N % result				
	Serum 1	Serum 2	Serum 3	Serum 4	Serum5
0	139	125	139	129	125
7	77	47	114	62	58
38	42	53	50	90	62
74	**38**	**7**	**7**	**7**	**9**
108	**7**	**8**	**7**	**8**	**8**

Table 5: Seroconversion kinetics of 5 vaccinated sheep (cut-off: <40%).

Results (Tables 4 & 5):

- For the BTV-2 vaccinated sheep, the kit detected seroconversion as of 35 dpv.
- For sheep vaccinated with the Merial 2,4 vaccine, the kit detected seroconversion between 38 and 74 dpv.

Specificity

1500 ovine sera, 650 bovine sera and 311 caprine sera from disease-free regions in Southern France (Aveyron and Herault) were tested. These samples were collected prior to the 2006 BTV outbreak in France.

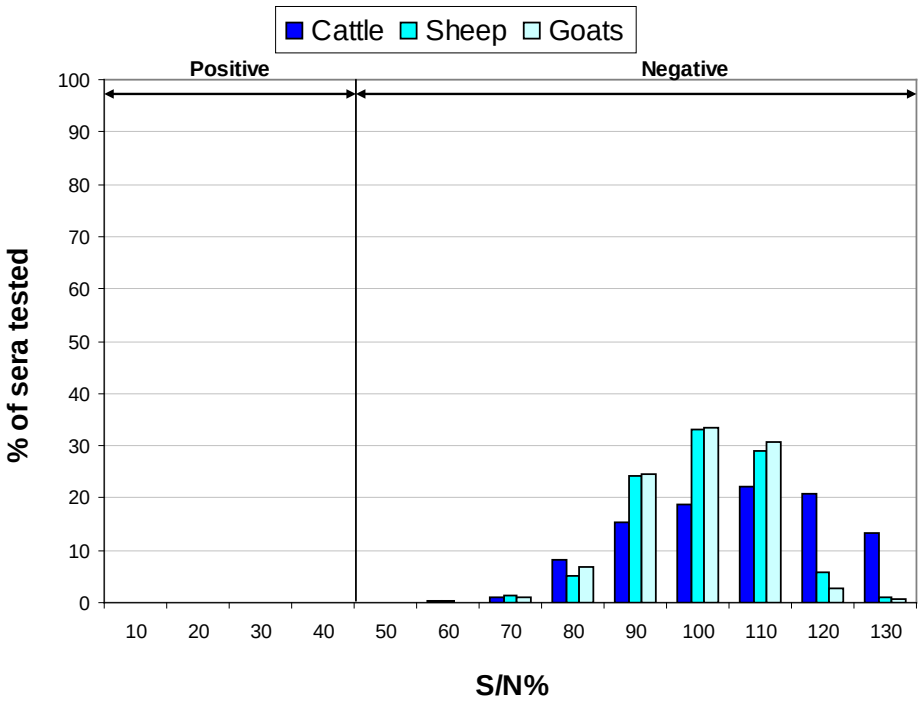


Figure 2: S/N% distribution of negative sera (origin: France).

Results (Figure 2):

- Out of the 1500 sera tested, all bovine and caprine sera were found negative. One ovine serum was found positive.
- **Measured specificity = 100% ($CI_{95\%}$: 99.84% - 100%), n=2461.**

Conclusion

The **ID Screen® Bluetongue Competition** ELISA kit demonstrates excellent specificity and sensitivity and high correlation with PCR and detects seroconversion at least 7 days post infection.

The kit is an easy-to-use and reliable tool for Bluetongue disease surveillance.

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