Validation report

ID Screen® Swine Vesicular Disease Competition

5B7 Mab Competitive ELISA for the detection of antibodies directed against the SVD virus in porcine serum or plasma

- **High sensitivity and specificity**, with clear separation of positive and negative results.
- **Calibrated to detect the Primary Reference Serum EU-RS4**, provided by the OIE Reference Laboratory, Pirbright, UK.
- **Convenient and rapid**, with ready-to-use components and results within 2 hours.

Introduction

Swine vesicular disease (SVD) is a contagious viral disease of pigs characterized by fever and vesicles in the mouth and on the snout, feet and teats. The illness may be subclinical, mild or severe, but rarely fatal. The importance of this disease stems from the fact that it cannot be clinically distinguished from foot-and-mouth disease (FMD). Outbreaks of SVD are therefore assumed to be FMD until laboratory tests prove otherwise.

Serology is used for SVD surveillance and export certification. While virus neutralization is the prescribed test for international trade, it is time-consuming, as 2-3 days are required to obtain results. In contrast, results may be obtained by ELISA in only a few hours.

IDvet has developped the ID Screen® Swine Vesicular Disease Competition ELISA to detect antibodies directed against the SVD Virus in pig samples (serum or plasma). The method used by this kit is described in the OIE Manual for Terrestrial Animals, Chapter 2.1.3. It is based on the monoclonal antibody 5b7 developed by Brocchi et al[1].

This report summarizes the validation data for this assay.

Digitally signed by Cucu Vasile
Date: 2025.03.20 13:01:00 EET
Reason: MoldSign Signature
Location: Moldova
MOLDOVA EUROPEANĂ

Test principle

Samples to be tested and controls are added to microwells coated with SVDV antigen.

Anti-SVDV antibodies, if present, form an antibody-antigen complex which masks the SVDV epitopes.

The conjugate (Mab-5B7-HRP) is added to the microwells. It fixes to the remaining free SVDV epitopes, forming an antigen-conjugate-HRP 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 specimen to be tested. In the absence of antibodies, a blue solution appears which becomes yellow after addition of the stop solution. In the presence of antibodies, no coloration appears.

The microplate is read at 450 nm.

Result interpretation:

For each sample, the competition percentage is calculated: $(OD_{sample}) / (OD_{NC}) \times 100$.

Samples with a S/N%:

- less than or equal to 45% are considered positve.
- greater than 45% and less than or equal to 50% are considered doubtful.
- greater than 50% are considered negative.

Analytical sensitivity

Primary and Secondary Reference sera

Analytical sensitivity was evaluated on:

- the Primary Reference Serum EU-RS4.
- the Secondary Reference Serum diluted 1:4000 produced by the OIE Reference Laboratory (Istituto Zooprofilattico Sperimentale, Brescia, Italy).

Serum	Dilution	d.p.i	S/N%	Result
EU-RS4	pure	21	37	Positive
Secondary reference serum	1:4000	21	9	Positive

Table 1: Results for the primary and secondary reference sera.

Results (Table 1):

- The EU-RS4 and the Secondary Reference Serum were found positive diluted 1:4000.

Sera RS1-6, IAH Pirbright

Sera from an experimental infection (sera RS1-6) from IAH, Pirbright were also tested.

Serum	Dilution	d.p.i	S/N%	Result
RS1	pure	0	80	Negative
RS5	pure	5	39	Positive
RS6	pure	6	40	Positive
RS3	pure	15	24	Positive
EU-RS4	Pure	21	37	Positive
RS2	pure	28	6	Positive

Table 2: Results for experimentally-infected sera.

Results (Table 2):

- SVDV antibodies were detected as of 5 days post infection.

Specificity

837 sera from disease-free regions (kindly provided by the *Laboratoire Départemental 22* and Acsediate) were tested.

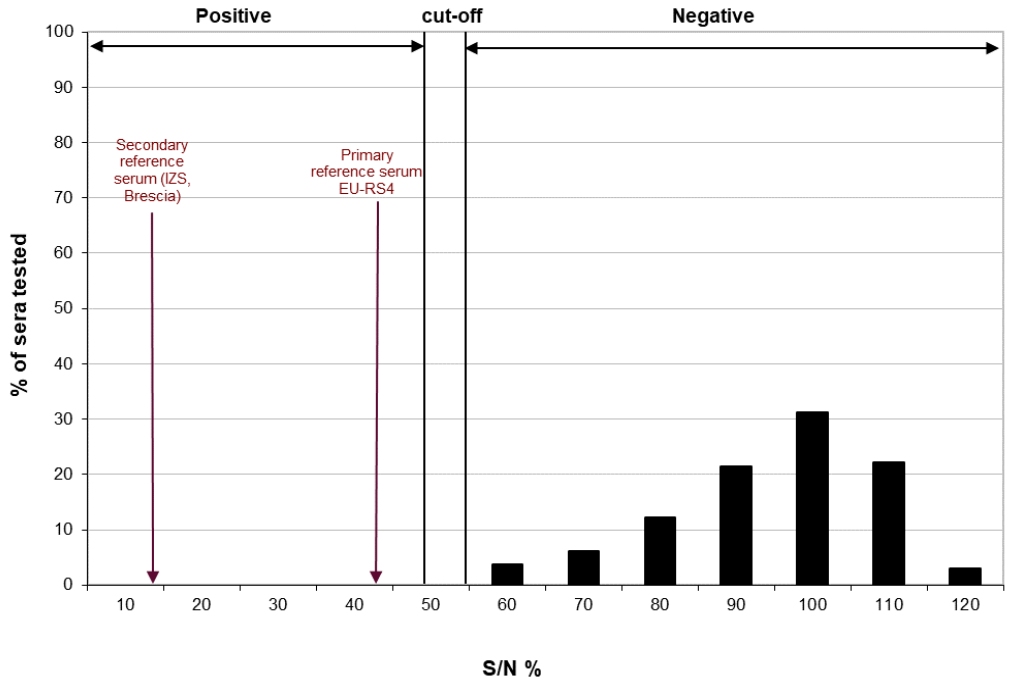

Figure 1: S/N% distribution for disease-free sera.

Results (Figure 1):

- All sera were found negative by the ID Screen® ELISA.
- **Measured specificity = 100% ($CI_{95\%}$: 99.54%-100%), n=837.**

Sensitivity

Correlation with another ELISA

40 sera from naturally-infected Spanish herds were analyzed by the ID Screen® ELISA and another commercially available ELISA (kit A).

	ID Screen® ELISA (cut-off: 45-50%)		Kit A (cut-off: >50%)	
Sample ID	S/N%	Status	S/N%	Results
2	93	-	35	-
5	7	+	98	+
6	3	+	101	+
10	7	+	96	+
16	74	-	22	-
17	3	+	99	+
23	33	+	98	+
24	5	+	92	+
40	16	+	84	+
41	6	+	94	+
47	6	+	91	+
48	4	+	100	+
60	3	+	102	+
68	5	+	101	+
77	3	+	101	+
79	37	+	43	-
80	8	+	94	+
85	6	+	94	+
92	78	-	30	-
94	3	+	101	+
95	4	+	100	+
99	50	-	2	-
100	12	+	92	+
101	4	+	100	+
102	3	+	90	+
107	48	+ / -	27	-
117	17	+	101	+
118	3	+	99	+
122	4	+	4	-
125	3	+	101	+
128	8	+	100	+
129	4	+	100	+
130	5	+	83	+
133	4	+	96	+
136	6	+	98	+
137	6	+	94	+
140	7	+	94	+
143	5	+	80	+
149	5	+	97	+
150	5	+	95	+

Table 3: Results for 40 sera from an infected Spanish herd in two different competitive ELISA tests.

Results (Table 3):

- 37/40 sera gave the same results in both tests.
- 2 sera which were positive, and one which was doubtful with the ID Screen® ELISA, were negative with the other commercial test. These differences could be due to slightly different analytical sensitivities between tests (serum n° 107), or to the use of monoclonal antibodies directed against different epitopes of the SVDV (sera n° 79 and n° 122).

Naturally-infected animals

87 sera from naturally-infected animals were tested (35 sera from Italy and 52 sera from Spain). These sera were from infected herds and were found positive for SVD by VNT.

The same sera were also tested by the OIE reference laboratory (IZS Brescia) using an in-house ELISA (Data not shown).

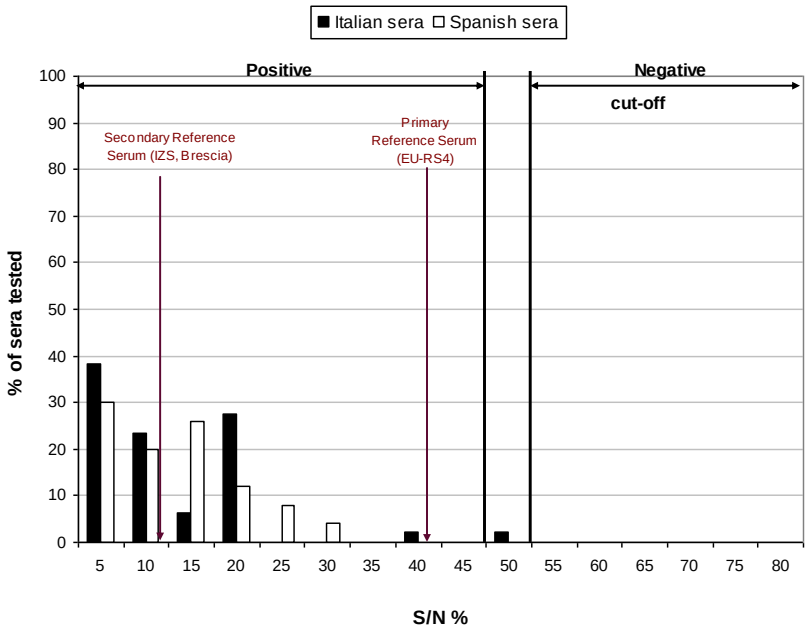

Figure 2: S/N% distribution for sera from infected animals.

Results (Figure 2):

- 86/87 sera were found positive by the ID Screen® ELISA, 1 serum gave a doubtful result.
- **Measured sensitivity = 98.85% ($CI_{95\%}$: 93.77%-99.8%), n=87.**
- Test agreement between the ID Screen® ELISA and IZS Brescia in-house ELISA was 100%.

Robustness

The ID Screen® test robustness was evaluated by testing the maximum and minimum of incubation time as defined in the instructions for use:

- Samples incubation: 60 minutes ± 6 minutes at 37°C (± 3°C);
- Conjugate incubation: 30 minutes ± 3 minutes at 37°C (± 3°C);
- Substrate Solution incubation: 15 minutes ± 2 minutes at 21°C (± 5°C).

For each condition, the test is validated if:

- The mean value of the negative control OD (OD_{NC}) is greater than 0.700 ($OD_{NC} > 0.700$).
- The ratio of the mean values of the Positive and Negative Controls (OD_{PC} and OD_{NC}) is less than 0.3.

Optical densities at 450 nm obtained in each condition for both the negative and positive controls and the S/N% values obtained for 3 dilutions of a positive serum and 2 negative samples are detailed in Table 7.

Incubation time Sample / conjugate / Substrate	54 min / 27min / 13 min	60 min / 30min / 15min	66 min / 33 min / 17 min	
Positive control	0,090	0,161	0,168	DO 450nm
Positive control	0,102	0,137	0,137	
Negative control	1,177	1,360	1,438	
Negative control	1,106	1,366	1,460	
OD_{PC} > 0,7	√	√	√	
OD_{PC} / OD_{NC} < 0,3	√	√	√	
MRI-NC-002 diluted 1:2	38	37	41	S/N%
MRI-NC-002 diluted 1:4	68	60	60	
MRI-NC-002 diluted 1:8	90	86	89	
Negative sample 1	99	104	106	
Negative sample 2	93	88	81	

Table 7: Robustness study for the ID Screen® ELISA (results expressed as OD values at 450nm).

Results (Table 7):

- For each time condition, the test validation criteria for both the positive and negative controls were obtained.
- For each time condition, the S/N% values obtained for each condition were similar, and analytical sensitivity was constant, thereby demonstrating the excellent robustness of the ID Screen® ELISA.

Phone + 33(0)4 67 41 49 33 ● Fax + 33(0)4 67 45 36 95
info@id-vet.com ● www.id-vet.com

Repeatability

Repeatability was evaluated by calculating the coefficient of variation (CV) for 60 repetitions of the primary reference serum (EU-RS4), and 36 repetitions of the negative control of the kit. The CVs obtained were found to be between 3 and 8%.

Conclusion

The ID Screen® Swine Vesicular Disease Competition ELISA correctly identifies the OIE reference sera, and demonstrates excellent specificity and high sensitivity.

The kit is easy-to-use, flexible and reliable serological test, which gives results in less than two hours.

Reference:

(1) *Brocchi, E.; Berlinzani, A..; Gamba, D. & Simone, F. (1995).* ***Development of two novel monoclonal antibody-based ELISAs for the detection of antibodies and the identification of swine isotypes against swine vesicular disease virus****. J. Virol. Metods, 52, 155-67.*

History of revisions

Version	Edit date	Reference	Type of revision	Revision made
1014	07/2018	DOC648	Update : Addition/Edition of validation data	• Version 1014 was created when the stop solution formula was changed. This modification had no impact on the test performances. • Robustness data were added.

Phone + 33(0)4 67 41 49 33 ● Fax + 33(0)4 67 45 36 95
info@id-vet.com ● www.id-vet.com