Validation report

ID Screen® Capripox Double Antigen Multi-species

Multi-species Double Antigen ELISA for detection of antibodies against capripoxviruses including Lumpy skin disease virus (LSDV), sheeppox virus (SPPV) and goatpox virus (GTPV) in serum or plasma from cattle, sheep, goats or other susceptible species

- **First commercially-available ELISA which allows for the detection of Lumpy Skin Disease antibodies**
- **Very high specificity** in CPV-free regions (>99.7%). Should not cross-react with parapox viruses.
- **Detects antibodies against LSDV either in vaccinated or infected animals.**
  At least equivalent sensitivity compared to IPMA and improved sensitivity compared to VNT.
  (Detection of antibodies as of 20 dpv up until at least 7 months post-vaccination.)
- **Easy to handle, with ready-to-use reagents, and allows for high throughput screening without requiring high level containment facilities.**

Introduction

Capripoxvirus is a genus of viruses in the subfamily **Chordopoxvirinae** and the family **Poxviridae.** Capripoxviruses are among the most serious of all animal poxviruses. Sheep, goat, and cattle are known to be natural hosts.

These viruses cause negative economic consequences by damaging hides, wool and compel the establishment to restrict their trade in response to an outbreak.

The genus consists of three species: sheeppox virus (SPPV), goatpox virus (GTPV), and lumpy skin disease virus (LSDV).

The **ID Screen® Capripox Double Antigen Multi-species** ELISA is designed to detect antibodies against the lumpy skin disease (LSDV) and should also cross react with other capripox viruses (CPV): sheeppox virus (SPPV) and goatpox virus (GTPV).

This report summarizes validation data obtained for this test.

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Test principle

Wells are coated with CPV purified antigen. Samples to be tested and controls are added to the microwells. Anti-CPV antibodies, if present, form an antibody-antigen complex. Plates are washed and the conjugate which is a CPV purified antigen labeled with peroxidase (HRP), is added to the microwells. It fixes to the free Fab of the bound serum anti-CPV antibodies. 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 presence of antibodies, a blue solution appears which becomes yellow after addition of the Stop Solution.
- In the absence of antibodies, no coloration appears.

The microplate is read at 450 nm.

Result interpretation: For each sample, the S/P % is calculated:

$$\frac{S}{P}\% = 100\,x(OD_{sample} - OD_{NC}) / (OD_{PC} - OD_{NC})$$

Result	Status
S/P % < 30 %	NEGATIVE
S/P % ≥ 30 %	POSITIVE

Specificity

IDvet internal validation study:

1381 samples from a disease free and non-vaccinated region (France) were tested as follows:

- 867 cattle sera
- 254 sheep sera
- 260 goat sera.

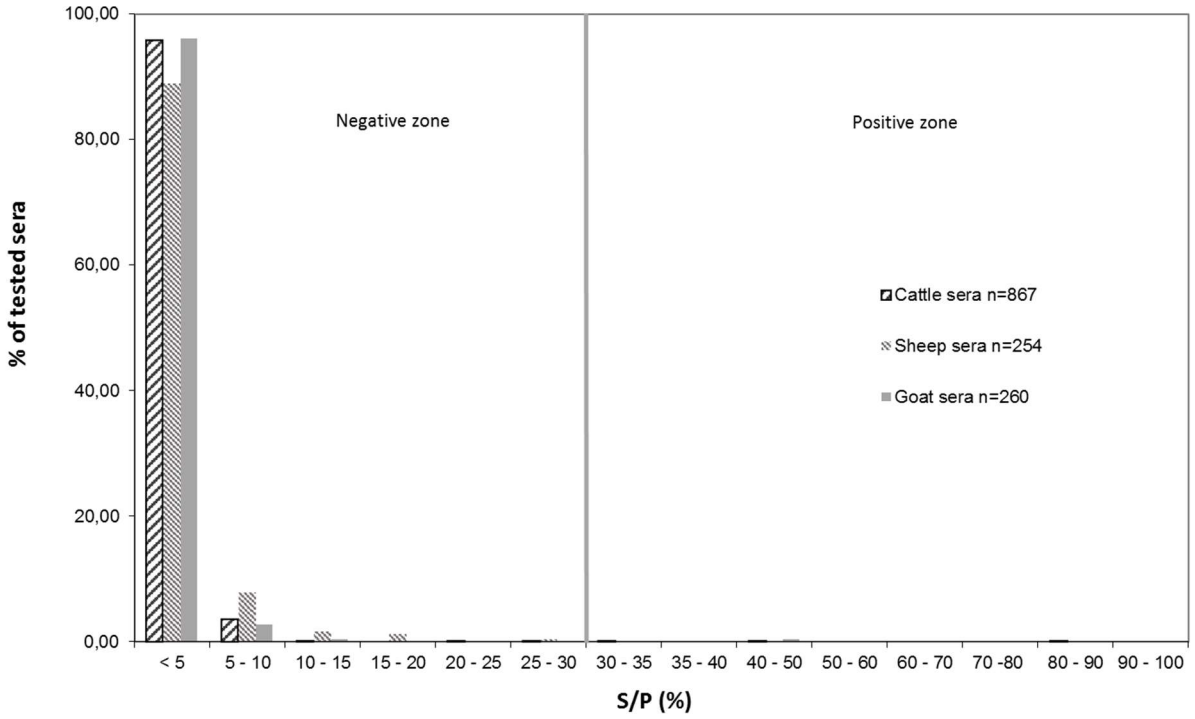

Figure 1: S/P% distribution for negative sera, n=1381.

Results (Figure 1):

- Out of the 1381 samples tested, 1376 were found negative.
  Measured specificity = 99.6% ($CI_{95\%}$: 99.1 – 99.9%), n=1381.

Species	**Specificity** (%) (number of samples)	$CI_{95\%}$
Cattle	**99.7** (n=867)	99.0 – 99.9 %
Goat	**99.2** (n=260)	97.2- 99.8 %
Sheep	**100.0** (n=254)	98.5 - 100 %

Table 1. Measure of specificity for sera from a CPV disease-free and non-vaccinated region

FLI (Germany) study:

1) 92 cattle sera from a disease free and non-vaccinated region (Germany) were tested at the FLI (Friedrich-Loeffler-Institut), Riems, Germany (Figure 2):

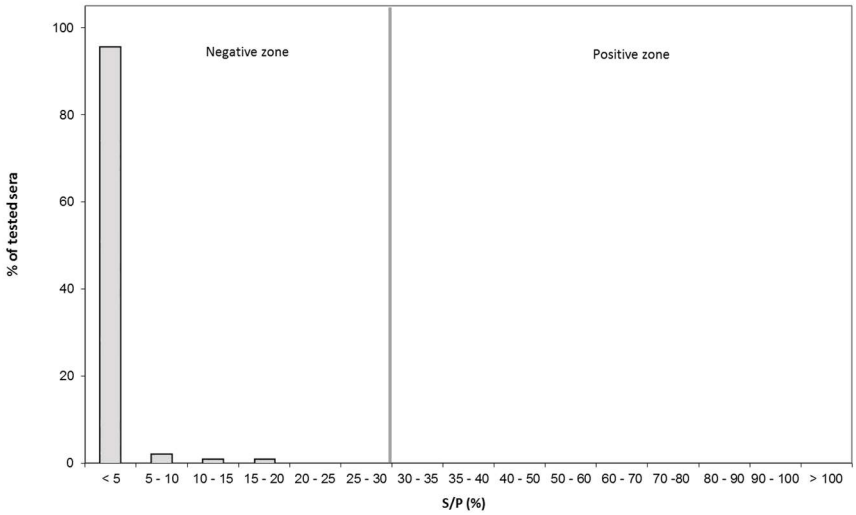

Figure 2: S/P% distribution for negative sera from Germany, n=92.

Results (Figure 2):

- 92 /92 sera were found negative.

  Measured specificity = 100.0 % ($CI_{95\%}$: 96.0- 100.0%), n=92

2) 48 sheep sera and 44 goat sera from a disease free and non-vaccinated (Germany) were tested at the FLI (Friedrich-Loeffler-Institut), Riems, Germany (Figure 3):

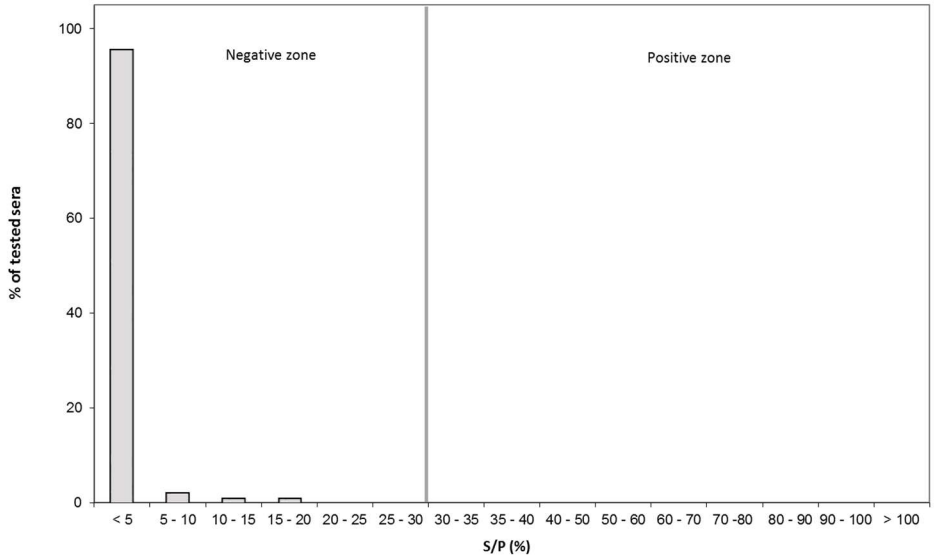

Figure 3: S/P % distribution for negative sera from Germany, n=92.

Results (Figure 3):

- 48/48 sheep sera and 44/44 goat sera were found negative.

  Measured specificity = 100.0 % ($CI_{95\%}$: 96.0- 100.0%), n=92.

CODA-CERVA (Belgium) study:

91 cattle samples from a disease free and non-vaccinated region (Belgium) were tested at CODA-CERVA, Uccle, Belgium (Figure 4).

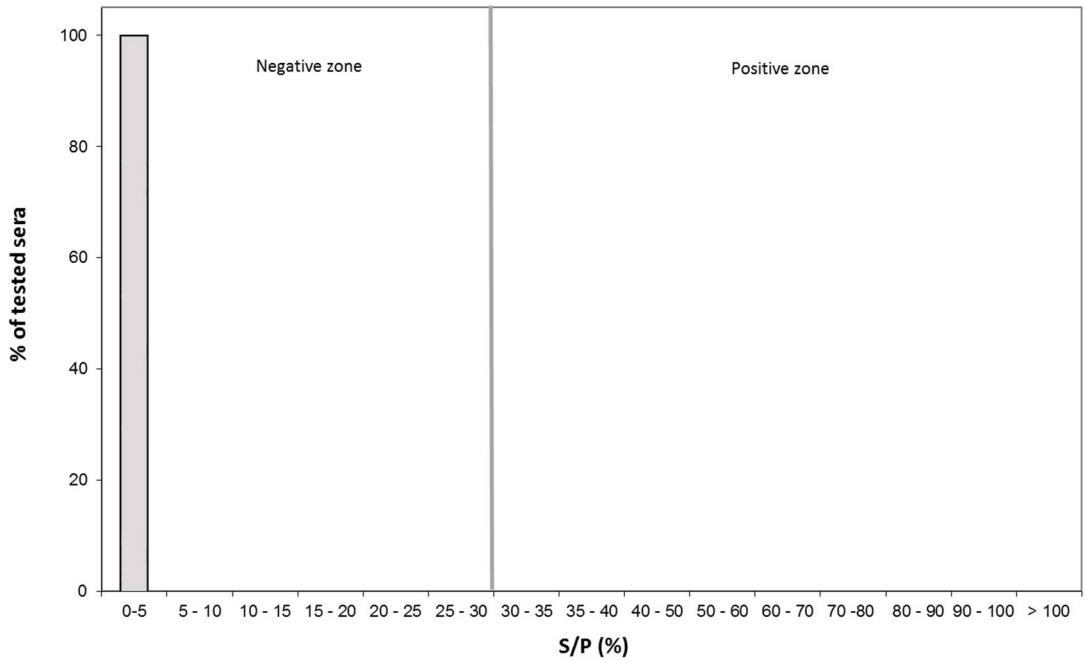

Figure 4: S/P % distribution for negative sera from Belgium, n=91.

Results (Figure 4):

- 91 /91 sera were found negative.

  Measured specificity = 100.0 % ($CI_{95\%}$: 96.0- 100.0%), n=91.

Global specificity calculation:

Species	**Specificity** (%) (number of samples)	$CI_{95\%}$
Cattle	**99.7** (n=1050)	99.2 – 99.9 %
Goat	**99.3** (n=304)	97.6- 99.8 %
Sheep	**100.0** (n=302)	98.8 - 100 %
TOTAL	**99.7** (n=1656)	99.3 – 99.9 %

Conclusion for all studies :

- The **measured specificity** for the **ID Screen® Capripox Double Antigen Multi-species** ELISA is **99.7%** ($CI_{95\%}$: 99.3- 99.9%), n=1656.
- Results from all studies show **an excellent specificty** for the **ID Screen® Capripox Double Antigen Multi-species** ELISA.

Sensitivity

Vaccinated field samples

1) 11 field samples from cattle vaccinated with a commercial live attenuated Neethling strain LSD vaccine, sampled **five months post-vaccination,** were tested with the ID Screen® Capripox Double Antigen Multi-species ELISA ,the IPMA (Immunoperoxydase monolayer assay) test and VNT (Virus Neutralization Test; OIE, 2016). The IPMA and VNT tests were performed at CODA-CERVA (Belgium).

	ID Screen® ELISA	IPMA	VNT
	Cut-off : 30%		
Sample	S/P%	Status	Status	Status
1	303	Pos	Neg	Neg
2	303	Pos	Neg	Pos (Borderline)
3	12	Neg	Neg	Neg
4	224	Pos	Neg	Neg
5	123	Pos	Neg	Neg
6	291	Pos	Neg	Pos (Borderline)
7	95	Pos	Neg	Neg
8	10	Neg	Neg	Neg
9	69	Pos	Neg	Neg
10	270	Pos	Neg	Neg
11	1	Neg	Neg	Neg

Table 2. 11 field samples from vaccinated cattle tested using ID Screen® ELISA, IPMA and VNT

Results (Table 2):

- Out of these 11 field samples from cattle vaccinated against LSD, **8 samples were found positive** with the **ID Screen® Capripox Double Antigen Multi-species** ELISA**, 2 samples** were found **positive by VNT and all were negative** with the **IPMA.**

2) **75 field samples from cattle vaccinated with a commercial** live attenuated **Neethling strain vaccine against LSD (OBP)**, sampled about **one month post-vaccination,** were tested using the ELISA and the IPMA tests. Data (ELISA and IPMA) were obtained at CODA-CERVA, Belgium.

		IPMA		
		Positive	Negative	Total
ID Screen® ELISA	Positive	31	13	**44**
	Negative	9	22	**31**
	Total	**40**	**35**	**75**

Table 3. 75 field samples from vaccinated cattle tested using ID Screen® ELISA and IPMA

Results (Table 3):

- Out of 75 samples from vaccinated animals : the ELISA detected 44 samples as positive.
- The IPMA detected 40 samples as positive (difficulties were encountered in interpreting hemolyzed field samples with the IPMA).

- 71 % of samples give similar results with both tests, 17 % were positive only by ELISA and 12% positive only by IPMA.

The S/P % distribution obtained with the ID Screen® Capripox Double Antigen Multi-species ELISA for positive samples is shown in the graph below:

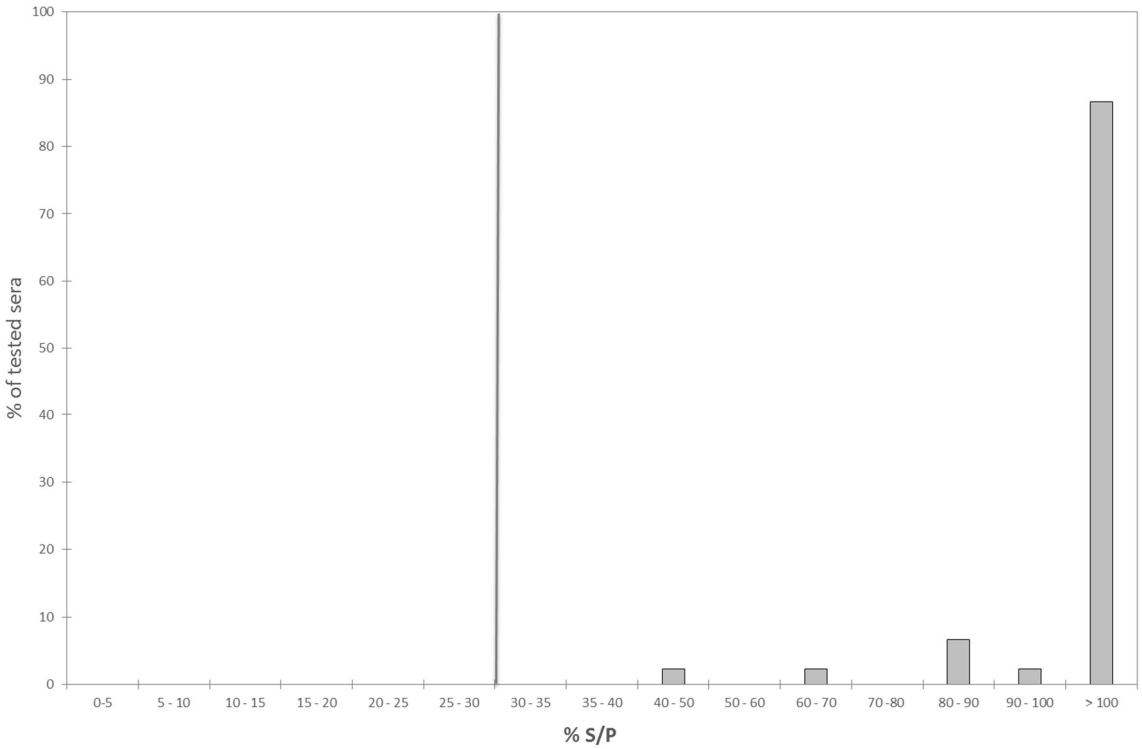

Figure 5: S/P % distribution for ELISA-positive samples, n=44.

Results (Figure 5):

- Only one sample is close to the cut-off, indicating the very good differentiation between positive and negative results by the ID Screen® kit.

3) **48 field samples from cattle vaccinated** with a commercial live attenuated Neethling strain vaccine against LSD (Lumpyvax®, Intervet/MSD) and sampled two months (58 days) post-vaccination were tested using the ELISA. The S/P % distribution obtained with the ID Screen® Capripox Double Antigen Multi-species ELISA is shown in the graph below. Data was obtained by IDvet.

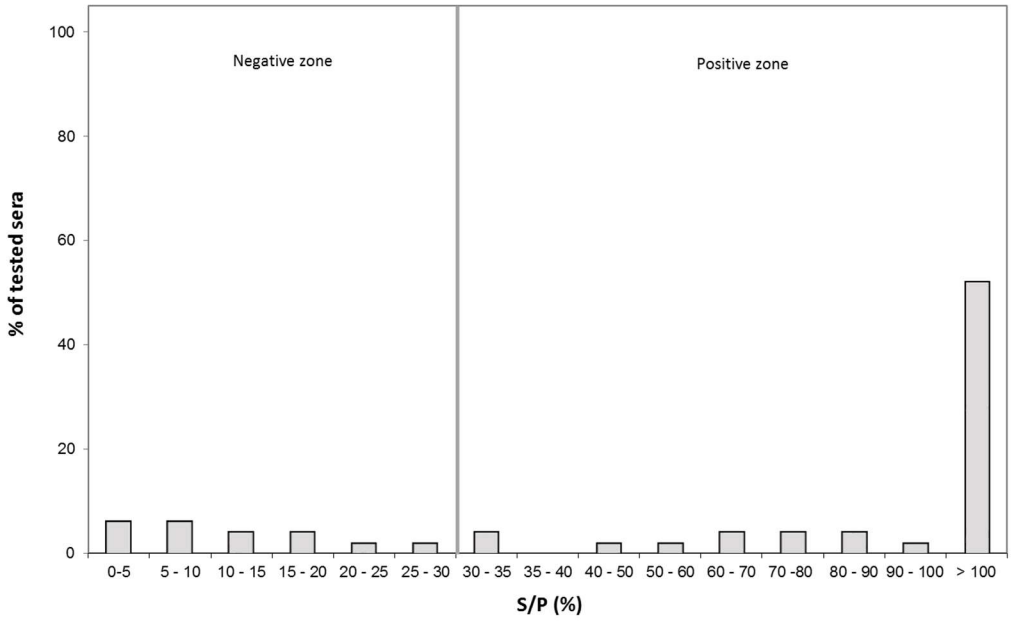

Figure 6: S/P % distribution for 48 cattle field samples at 58 dpv

Results (Figure 6):

- Out of 48 samples from vaccinated cattle, 75% were found positive with the ID Screen® kit (36/48).

These 48 field samples were also tested by the IPMA. The IPMA was performed at CODA-CERVA (Belgium):

		IPMA		
		Positive	Negative	**Total**
ID Screen® ELISA	Positive	12	24	**36**
	Negative	0	12	**12**
	Total	**12**	**36**	**48**

Table 4. 48 field samples from vaccinated cattle tested using the ID Screen® ELISA and IPMA

Results (Table 4):

- Out of 48 samples vaccinated: the ELISA detected 36 samples as positive, and the IPMA detected 12 samples.

- On this panel, **the ID Screen®** kit detects **more efficiently** the **vaccinated samples** compared with IPMA.

4) **48 field samples from cattle vaccinated** with a commercial live attenuated Neethling strain vaccine against LSD (Lumpyvax®, Intervet/MSD), sampled three months (87 days) post-vaccination, were tested using the ID Screen® Capripox Double Antigen Multi-species ELISA: (Data obtained at IDvet)

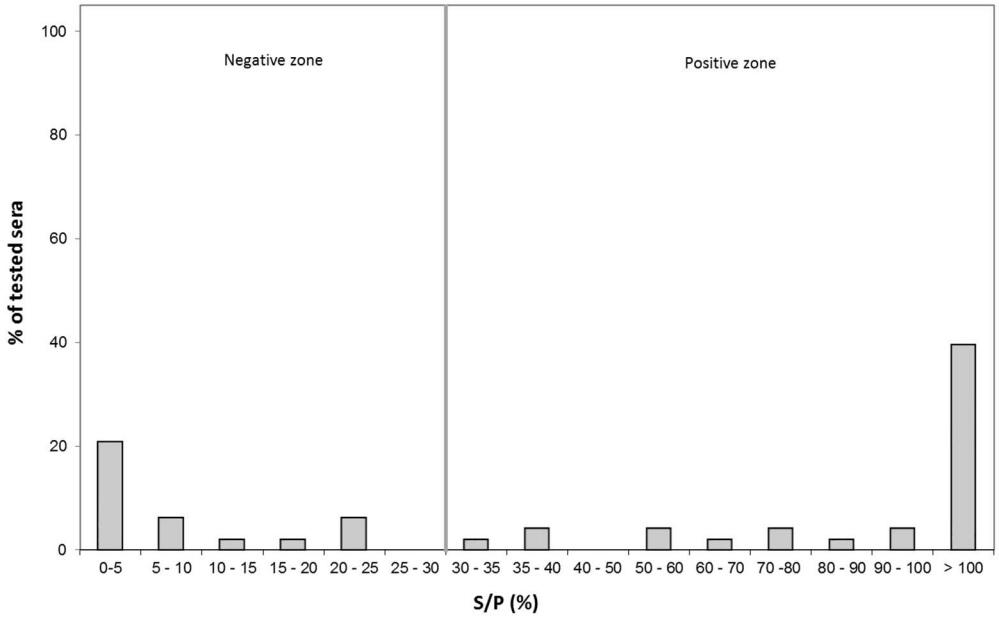

Figure 7: S/P% distribution for 48 cattle field samples at 87 dpv

Results (Figure 7):

- Out of 48 samples from vaccinated cattle, 62.5% were found positive with the ID Screen® kit (30/48).

These 48 field samples were also tested by the IPMA. The IPMA could not give results for 3 samples because they were hemolyzed; 45 samples only were analyzed by IPMA, performed at CODA-CERVA, Belgium (results in table 5):

		IPMA		
		Positive	Negative	**Total**
ID Screen® ELISA	Positive	19	8	**27**
	Negative	2	14	**16**
	Total	**21**	**22**	**45**

Table 5. 45 field samples from vaccinated cattle tested using ID Screen® ELISA and IPMA

Results (Table 5):

- Out of 45 samples vaccinated: the ELISA detected 27 samples as positive, and the IPMA detected 21samples. 19 were found positive by both techniques.

- On this panel, **the ID Screen® kit** detects **more efficiently** the **vaccinated samples** compared with IPMA.

5) **31 cows** from Serbia vaccinated with the commercial LSDV Neethling vaccine (OBP) and sampled between six and seven months post-vaccination, were tested at the FLI (Friedrich-Loeffler-Institut), Riems, Germany using the ID Screen® Capripox Double Antigen Multi-species ELISA (Figure 8).

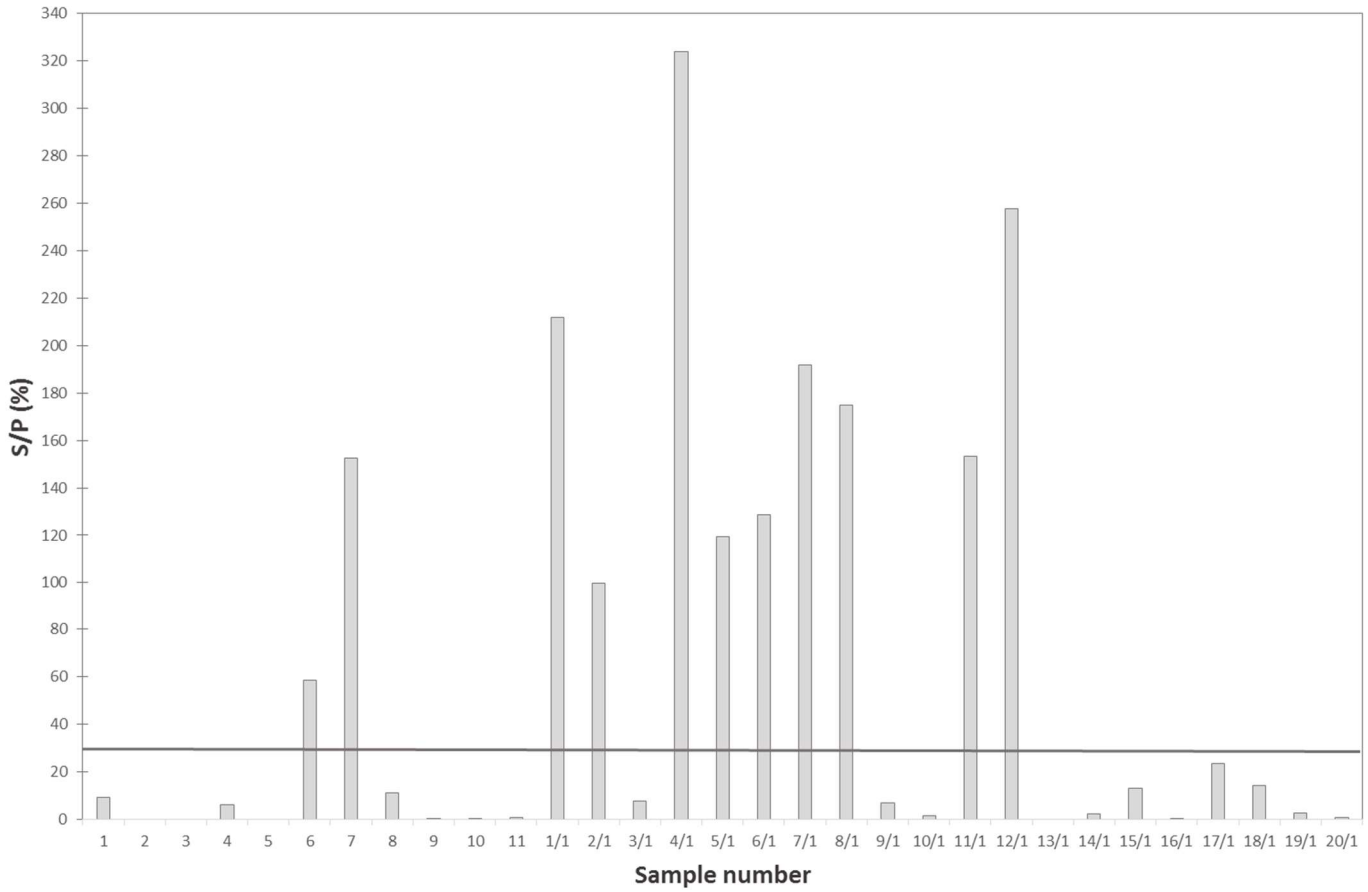

Figure 8: S/P % distribution for 31 cows at 6/7 months post-vaccination

Results (Figure 8):

- Out of 31 samples from vaccinated cattle (6-7 months post vaccination), 35.5% were found positive with the ID Screen® kit (11/31)

Seroconversion:

Data obtained at CODA-CERVA (Belgium):

1) Experimental infection:

Samples from 6 cattle experimentally-infected with LSDV were tested using the ID Screen® Capripox Double Antigen Multi-species ELISA and the IPMA.

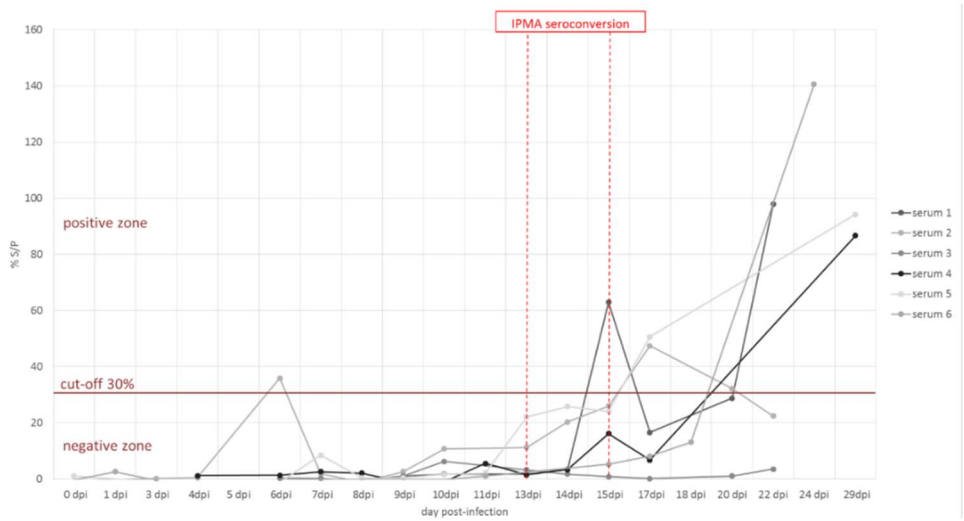

Figure 9: S/P % values for 6 experimentally infected cattle

Results (Figure 9):

- Using the **ID Screen® kit**, seroconversion is observed **between 22 and 29 dpi,** while I**PMA** detects them between **13-15 dpi.**

2) Experimental Vaccination:

Samples from 6 cattle experimentally-vaccinated against LSD were tested using the ID Screen® Capripox Double Antigen Multi-species ELISA and the IPMA:

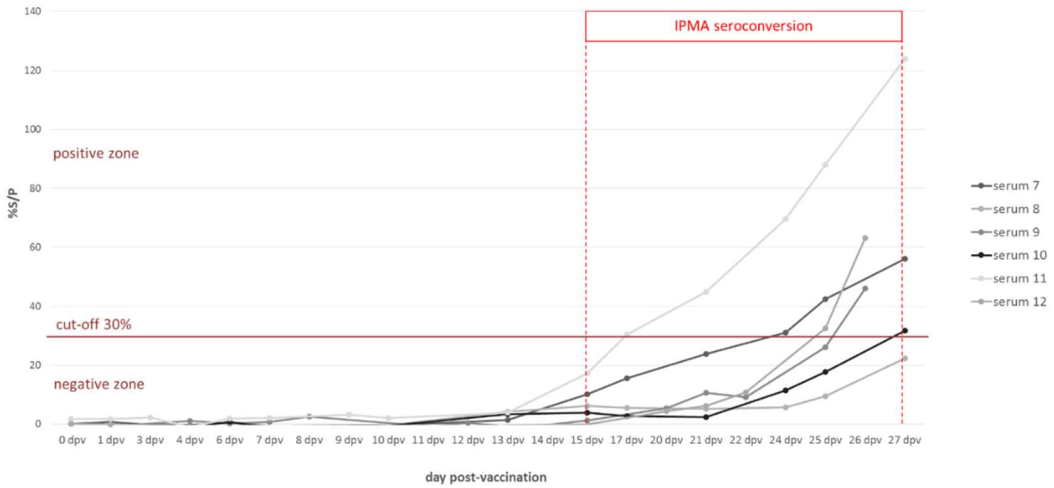

Figure 10: S/P % values for 6 experimentally vaccinated cattle

Results (Figure 10):

- With the **ID Screen® kit** seroconversion is observed between 17 to 29 dpi, while I**PMA** detects them between **15-27 dpi.**

Data obtained at FLI (Germany):

1) **Sera from 6 cattle experimentally infected with LSDV-Neethling vaccine strain** were tested using the ID Screen® Capripox Double Antigen Multi-species ELISA. (Data obtained at FLI, Germany).

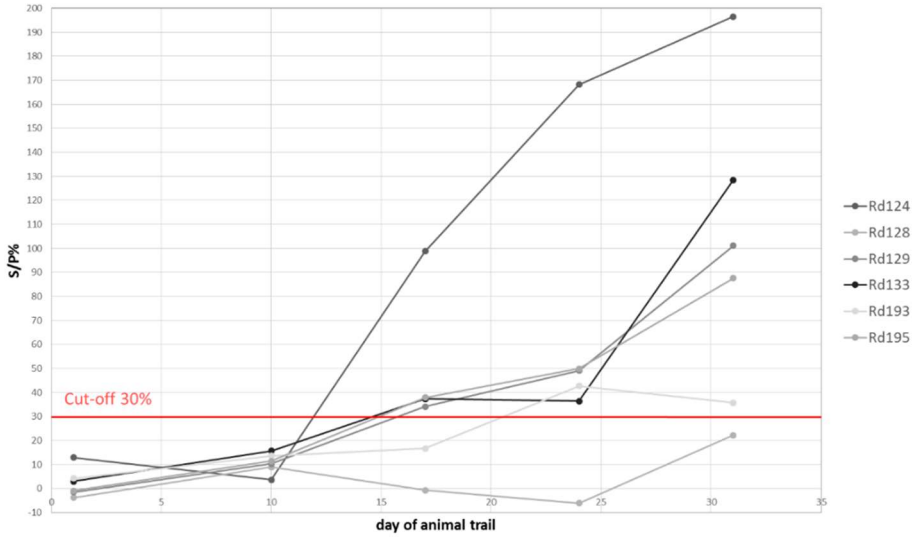

Figure 11: S/P % values for 6 experimentally infected cattle

Results (Figure 11):

- Seroconversion is observed between 12 and 21 dpi for 5/6 infected samples using the **ID Screen® kit.**
- For one sample (Rd128), seroconversion did not occur at 30 dpi, suggesting that this sample might seroconvert after 30 dpi (data not available later than 30 dpi).

Repeatability

Intra-plate repeatability was evaluated by measuring the coefficient of variation (CV %) for 36 repetitions of a strong positive sample, and 60 repetitions of a weakly positive sample.

Results are considered conform if the CV % is less than 15%. OD results are shown in Table 6 below.

0,351	0,378	0,327	0,355	0,360	0,771	0,782	0,349	0,329	0,341	0,343	0,357
0,337	0,333	0,317	0,309	0,359	0,727	0,757	0,325	0,317	0,331	0,317	0,330
0,382	0,330	0,319	0,334	0,358	0,714	0,798	0,322	0,305	0,317	0,316	0,330
0,862	0,825	0,869	0,833	0,867	0,741	0,775	0,731	0,757	0,650	0,622	0,753
0,772	0,773	0,858	0,776	0,827	0,748	0,769	0,761	0,770	0,748	0,728	0,753
0,345	0,333	0,317	0,331	0,329	0,741	0,769	0,331	0,329	0,302	0,323	0,334
0,368	0,331	0,323	0,314	0,336	0,686	0,802	0,319	0,314	0,326	0,305	0,342
0,369	0,359	0,358	0,350	0,377	0,715	0,794	0,322	0,316	0,322	0,337	0,330

	Average OD	Standard deviation	Minimum	Maximum	CV%
Strong positive sample	0,767	0,055	0,622	0,869	7
Weak positive sample	0,355	0,019	0,302	0,382	6

Table 6 : Repatability study for the ID Screen® ELISA (results expressed as OD values)

Results (Table 6):

- The CV % obtained were 6% for the weak positive sample and 7% for the strong positive sample, demonstrating an excellent repeatability.

Stability

The shelf-life of the products is evaluated by the technique of accelerated ageing. The stability of the plates, the positive control (C+) and the conjugate were tested by evaluating the residual activity of individual components after storage at 37°C ± 2°C, with respect to storage at 4°C ± 3°C. The measured residual activity at 37°C ± 2°C should be greater than 75% after one month.

Results are shown in Figure 12.

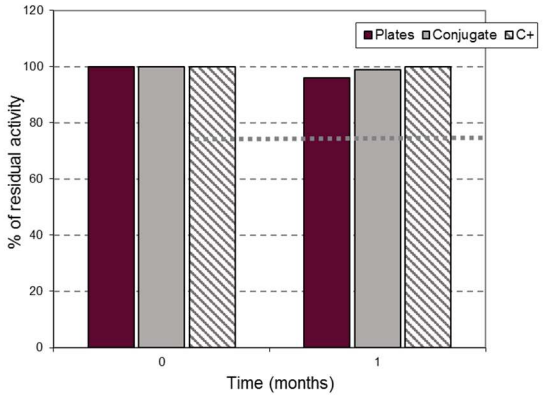

Figure 12: Percentage of residual activity of the plates, positive control and conjugate after stability testing at 37°C.

Results (Figure 12):

- The plates showed a residual activity of 96%, the positive control of 100%, and the conjugate of 99% indicating high component stability.

Conclusion

- The **ID Screen® Capripox Double Antigen Multi-species** ELISA kit demonstrates **high specificity** and good sensitivity.
- The kit detects seroconversion:
  - between 17 and 29 dpv for vaccinated animals
  - between 12 and 29 dpi for infected animals
- The kit is easy-to-use and results are obtained in less than 3 hours.

The **ID Screen® Capripox Double Antigen Multi-species** ELISA is a reliable tool for the detection of antibodies against the Lumpy Skin Disease with high reproducibility, repeatability, and robustness.

Acknowledgements

IDvet would like to thank:

- CODA CERVA, Uccle, Belgium
- FLI, Riems, Germany
- The Pirbright Institute, Pirbright, UK

Related products

Differential diagnosis: LSD should be differentiated clinically from pseudolumpy skin disease "PLSD" (caused by the Bovine Herpesvirus 2, BHV-2), hypoderma bovis infection, besnoitiosis urticaria, photosensitization, bovine lymphangitis, and mycotic dermatitis.

ID Screen® Besnoitiosis indirect 2.0 (product code : BSNTB):
Indirect biwell ELISA for the detection of antibodies against Besnoitia besnoiti in bovine serum or plasma

ID Screen® BHV-2 indirect (product code : BHV2S):
Indirect ELISA for the detection of anti-BHV-2 antibodies in cattle sera or plasma

References

EFSA AHAW Panel, 2015. Scientific Opinion on LSD. EFSA Journal 2015;13(1):3986, 73 pp.

E. Tuppurainen and N. Galon. Control and eradication of LSD in South East Europe, 27th conf. of the OIE Commission for Europe, presented at the SGE LSD2 (2016, Lisbon, Portugal).

OIE (2016). Lumpy skin disease [Chapter 2.4.13]. In Manual of diagnostic tests and vaccines for terrestrial animals 2016. Paris, France: OIE.

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

ID.vet
Innovative Diagnostics

310, rue Louis Pasteur
34790 Grabels – FRANCE

Phone + 33 (0) 4 67 41 49 33
Fax + 33 (0) 4 67 45 36 95

info@id-vet.com
www.id-vet.com