VALIDATION STUDIES ABSTRACT

Name of the diagnostic kit: *Salmonella* Abortusovis Test
Manufacturer: DIATHEVA s.r.l.
Disease: Salmonellosis in sheep
Pathogen Agent: *Salmonella* Abortusovis
Type of Assay: Enzyme-linked immunosorbent assay ELISA

Purpose of the Assay:
The *Salmonella* Abortusovis test is an Enzyme-linked immunosorbent assay (ELISA) intended for the detection of IgG anti-*Salmonella* Abortusovis in sheep serum samples. The test is designed to be used for the diagnosis of abortive salmonellosis infection and evaluation of antibody response to vaccination, as an ancillary test in conjunction with other methods for serological prevalence survey, or diagnosis and management of *S*. Abortusovis infection within herds, for the following purposes:

1. Demonstrate freedom from infection in a defined population (country/zone/compartment/herd) - Historical freedom
2. To confirm, but not negate, diagnosis of suspect or clinical cases, including confirmation of positive screening tests in individual animals and in herds with infection prevalence ranging from very low to high, based on detection of antibodies in sheep serum.
3. To determine immune status in individual animals or populations (post vaccination)

The test does not distinguish between vaccinated and infected sheep.

Species and Specimens:
The test has been validated for testing serum samples from sheep.

1. **Information on the kit**
   General information on the kit can be found on the DIATHEVA website at www.diatheva.com.

2. **Summary of validation studies**
   Analytical characteristics
   Repeatability: Intra-assay. A CV ≤15% was obtained for positive control and positive samples (low, medium, high concentration) except for negative sample and negative control for with a CV ≤20% was obtained. Higher CV values are considered acceptable and admitted for this kind of samples.
   Inter-assay. A coefficient variation ≤15% was obtained for the negative control, positive control and positive samples (low, medium, high), while a value ≤19% was obtained for negative sample.

   Analytical specificity
   The kit was tested on a panel of serum samples positive for other bacterial species (*Chlamydophila psittaci var. ovis*, *Brucella melitensis* Biot.3, *Listeria monocytogenes*, *Clostridium perfringens*, *Streptococcus ovis*, *Staphylococcus aureus* and *Mycoplasma agalactiae)* that reveal a similar LPS O antigen or species identified as responsible for abortion in sheep. The obtained results indicated that the kit is very specific. No cross-reactions were observed.

Analytical sensitivity

The kit demonstrated an end point titre (Reciprocal of the last dilution of a titration giving a measurable effect) corresponding to 6400 ($p<0,05$).

All the positive serum samples were found positive up to dilution 1:6400

Diagnostic Characteristics

Threshold Determination:

The cut-off point was calculated for each purpose.
Purpose 1: The value 0.516 was selected cut-off for the *Salmonella* Abortusovis.
Purpose 2: The value 0.520 was selected cut-off for the *Salmonella* Abortusovis.
Purpose 3: The value 0.250 was selected cut-off for the *Salmonella* Abortusovis.

Diagnostic sensitivity (DSe) and specificity (DSp) estimates:

Diagnostic parameters of *Salmonella* Abortusovis Test were determined based on the two-way 2 x 2 method. To support fitness for purposes claims DSe and DSp were calculated separately as follows:

Population 1 = 100 naïve sheep (confirmed by SAT)
Population 2 = 100 naïve sheep before vaccination (confirmed by SAT)
Population 3 = 7 + 88 naturally infected sheep (95) (confirmed by culture, PCR and SAT)
Population 4 = 93 vaccinated sheep

Purpose 1: Historical freedom, e.g. contribute to demonstration of freedom from infection in a defined population

The sample panels 1, 2 and 3 were used. The DSp and DSe were reported here below:

		Number of reference samples	
		Known Positive	Known Negative
Test Results	Positive	93	3
	Negative	2	197
		Diagnostic sensitivity **97.89%** (92.60% - 99.74%)	Diagnostic specificity **98.50 % (**95.68% - 99.69%)

Purpose 2: Confirmatory diagnosis of suspect of clinical cases

The sample panels used were 1 and 3. The DSp and DSe were reported here below:

		Number of reference samples	
		Known Positive	Known Negative
Test Results	Positive	94	3
	Negative	1	97
		Diagnostic sensitivity **98.95%** (94.27% - 99.97%)	Diagnostic specificity **97.00%** (91.448% - 99.38%)

Purpose 3: Determination of immune status in individuals or populations post vaccination

The sample panels used were 2 and 4. The DSp and DSe were reported here below:

		Number of reference samples	
		Known Positive	Known Negative
Test Results	Positive	93	0
	Negative	0	100
		Diagnostic sensitivity **100.00%** (96.11% - 100.00%)	Diagnostic specificity **100.00%** (96.38% -100.00%)

Comparative performance
***Salmonella* Abortusovis Test compared to SAT**

Calculated using sample panel 1 and 3

		Number of reference samples	
		Known Positive	Known Negative
***Salmonella* Abortusovis Test**	Positive	95	4
	Negative	0	96
		Diagnostic sensitivity **100.00%** (90.26% - 100.00%)	Diagnostic specificity **96.00 %** (90.07% - 98.90%)

Agreement and discrepancies:

The agreement between SAT (reference method) and *Salmonella* Abortusovis Test have been calculated by the Cohen's Kappa
Kappa= 0.960
SE of kappa = 0.020
95% confidence interval: From 0.921 to 0.999
The strength of agreement is very good.

Reproducibility:

A panel of characterized samples (n=20) and distinct lots of the kits were provided to 3 external laboratories plus 1 internal laboratory of Diatheva facility in order to determine the reproducibility at the end user level. Each laboratory tested the panel, in triplicate, unsupervised, on each of the 2 kit lots provided.

There was 100% agreement between kit lots at each of the sites (including Diatheva).
Obtained results in term of % Cv are summarized in the table below

Sample	**CV Intra-assay**	**CV Inter-assay**
Positive ctr	0.2-3%	≤15%
Negative ctr	0-11%	≤18%
Negative	1.5-21%	≤21%
Low-Medium positive	1-6%	≤21%
High positive	1-15%	≤21%

The β-distribution of the S/P values obtained from the 4 laboratories was calculated showing an excellent concordance.

Application

The test has not yet been incorporated into routine diagnostic regimens.

References

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