



Actinobacillus pleuropneumoniae Antibody Test Kit, ELISA

Swinecheck® APP 5a, 5b

Product code: TRM-515 (2 plates)

- ✓ **Highly sensitive ELISA kit**
- ✓ **A shelf life of 24 months**
- ✓ **Approved by the Canadian Food Inspection Agency**
- ✓ **No cross reaction with other APP serotypes**

INTRODUCTION

Actinobacillus pleuropneumoniae (APP) remains an important swine respiratory pathogen in many countries worldwide. A remarkable feature of this organism is that its virulence greatly varies depending on the isolates. This results in clinical situations varying from subclinical infections to peracute mortalities.

Interestingly the virulence of a given isolate correlates well with the serovar in a given geographical location. Many APP serovars, based on capsular polysaccharides (CPS), have been identified so far. Among them, serovars 1, 5 and 7 in North America and 2, 4 and 9 in Europe are the most virulent, even if other serovars such as 8 and 15 may occasionally cause significant losses.



Due to virulence variability the control of APP mainly focuses on the most virulent serotypes. Serological testing is the most efficient tool to monitor APP infections on a herd basis. Numerous serological assays for APP have been developed. However, they greatly vary in their sensitivity and specificity.

Species specific assays (ex. apxIV ELISA) are valuable only in herds presumed to be free of all APP serotypes. In all the other herds serotype specific assays must be used to allow discriminating serotype antibodies. Several APP serogroup/serotype specific serological assays are available. The most sensitive and specific one is the indirect ELISA using LC-LPS as antigen.

INTENDED USE

Swinecheck APP 5a, 5b is an indirect ELISA using LC-LPS as antigen for specifically detecting the antibodies to APP serovar 5a, 5b in porcine serum samples. It has been approved by the Canadian Food Inspection Agency (CCVB file number 780DR/A1.6/D10) in 2001.

PRINCIPLE OF THE TEST

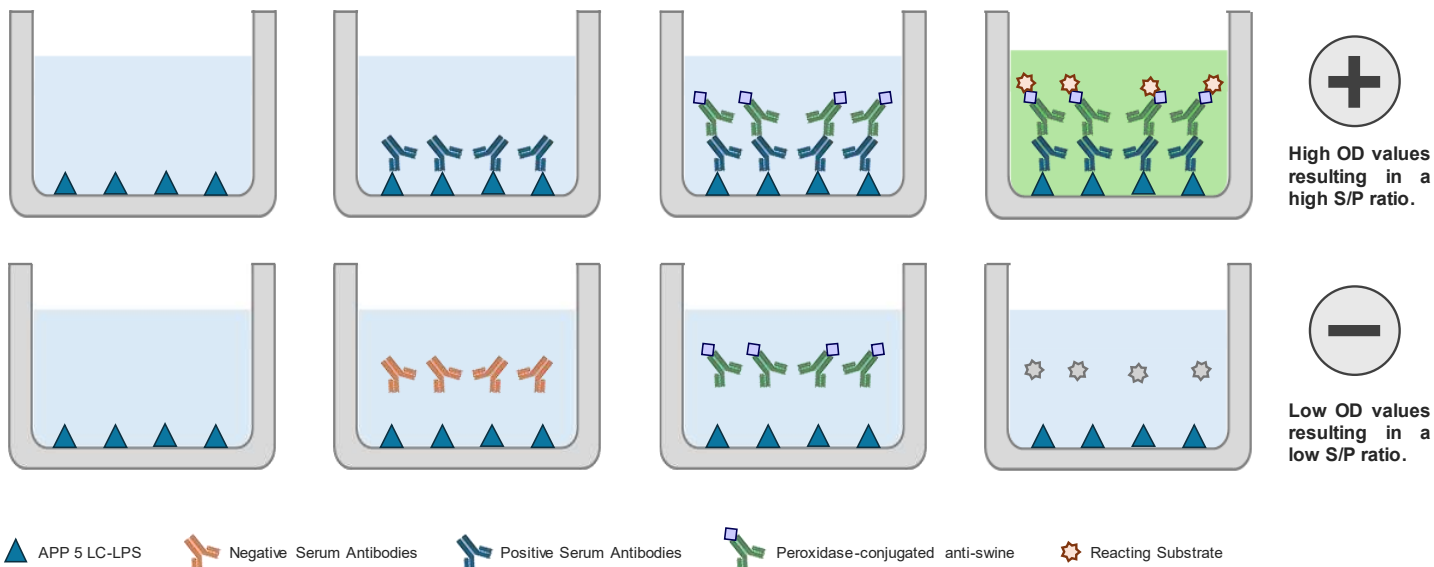
a. Assay description

1. The wells are coated with a purified long chain lipopolysaccharides from the capsule of *A. pleuropneumoniae* serotype 5.

2. Samples are added and incubated for 30 min at 23 ± 2°C. Each well is then washed carefully to remove unbound material.

3. A peroxidase-conjugated anti-swine is added and incubated for 30 min at 23 ± 2°C. Washing steps follow.

4. The substrate is added and incubated for 20 min at 23 ± 2°C. It will react with peroxidase if present. The reaction is stopped, and OD values are measured.



b. Results interpretation

OD values are used to calculate the S/P ratio as follow:

$$\text{S/P ratio} = \frac{\text{OD}_{\text{sample}}}{\text{mean OD}_{\text{pos}}}$$

The status of a test sample is determined with the S/P ratio.

Result	S/P ratio
Negative	Less than 0.40
Suspicious *	Less than 0.50 but greater or equal to 0.40
Positive	Greater or equal to 0.50

* A second serum sample should be collected 2 weeks later and retested

The following criteria must be met in order to validate the test:

- Negative control ratio must be less than 0.15.
- Mean of positive control ODs must be greater than 0.800.

TECHNICAL DATA

a. Sensitivity & Specificity

A panel of 334 field samples were characterized by the GREMIP of the Faculty of Veterinary Medicine of Université de Montréal. The samples were then tested with the Swinecheck APP 5a, 5b kit, and the results compared. Results are shown with suspect results as negative (A) and suspect results as positive (B).

A	GREMIP +	GREMIP -	Total	Statistic	Value	95% CI
Biovet +	76	31	107	Relative sensitivity	93.83%	86.18% to 97.97%
Biovet -	5	222	227	Relative specificity	87.75%	83.06% to 91.52%
Total	81	253	334	Kappa value	0.74	

B	GREMIP +	GREMIP -	Total	Statistic	Value	95% CI
Biovet +	122	26	148	Relative sensitivity	90.37%	84.10% to 94.77%
Biovet -	13	173	186	Relative specificity	86.93%	81.44% to 91.28%
Total	135	199	334	Kappa value	0.76	

The Swinecheck® APP 5a, 5b showed excellent sensitivity, specificity and a moderate agreement with the reference test.

b. Stability

A panel of 10 samples, 5 negatives and 5 positives, were tested with 3 distinct serials from 0 to 24 months post-production. Results presented S/P ratios.

Samples	Reactivity	Serial A		Serial B		Serial C	
		0 mo.	24 mo.	0 mo.	24 mo.	0 mo.	24 mo.
29	Negative	0,14	0,13	0,09	0,08	0,09	0,11
112	Negative	0,11	0,11	0,07	0,06	0,07	0,08
67	Negative	0,11	0,09	0,06	0,06	0,06	0,08
98	Negative	0,11	0,10	0,07	0,08	0,08	0,10
101	Negative	0,27	0,23	0,21	0,19	0,23	0,22
21	Positive	0,65	0,60	0,52	0,65	0,57	0,52
6	Positive	0,96	0,96	1,03	1,11	0,99	1,06
19	Positive	0,84	0,85	0,99	0,87	0,89	0,94
23	Positive	1,10	1,04	1,17	1,36	1,13	1,24
28	Positive	0,78	0,69	0,77	0,67	0,84	0,70

The S/P ratios for each sample remained stable throughout the study, and no changes in result outcomes were observed.

c. Repeatability

The inter lot repeatability was evaluated using a panel of 10 samples with a reactivity ranging from negative to strong positive. Samples were tested in 6 different series and results presented are S/P ratios. Mean and standard deviation were used to calculate the Coefficient of Variation (CV).

Sample ID	Reactivity	Serial 1	Serial 2	Serial 3	Serial 4	Serial 5	Serial 6	Mean	±	SD	CV (%)
31	Negative	0,09	0,05	0,05	0,04	0,06	0,03	0,05	±	0,02	38,7
38	Negative	0,11	0,07	0,07	0,08	0,08	0,06	0,08	±	0,02	22,0
39	Negative	0,15	0,09	0,10	0,11	0,12	0,10	0,11	±	0,02	19,1
40	Negative	0,20	0,16	0,16	0,16	0,16	0,14	0,16	±	0,02	12,0
41	Negative	0,30	0,24	0,27	0,27	0,26	0,23	0,26	±	0,02	9,5
42	Weak Pos.	0,51	0,47	0,50	0,45	0,43	0,43	0,47	±	0,03	7,4
43	Positive	0,68	0,73	0,78	0,65	0,66	0,67	0,70	±	0,05	7,2
44	Positive	0,84	0,87	0,86	0,76	0,82	0,84	0,83	±	0,04	4,7
45	Positive	0,96	0,97	1,09	0,99	0,97	1,06	1,01	±	0,05	5,4
35	Positive	1,16	1,29	1,30	1,25	1,36	1,35	1,29	±	0,07	5,7

The results show the Swinecheck® APP 5a, 5b kit's high repeatability. No positive sample had a CV over 15%.

d. Cross Reactions

Eventual cross-reactions with other serotypes of APP were evaluated by testing strong positive sera against APP 1, 2, 3, 5, 6, 7, 10, 12 and 13 with a serial of the Swinecheck® APP 5a, 5b kit. Samples were run in duplicate, and results shown are mean S/P ratios.

Sample ID	S/P
Pos ctl	1,00
Neg ctl	0,03
Buffer	0,01
APP 1	0,03
APP 2	0,11
APP 3	0,12
APP 5	0,99
APP 6	0,03
APP 7	0,04
APP 10	0,03
APP 12	0,07
APP 13	0,05

No cross-reactions were observed with other serogroups/serotypes.

CONCLUSION

The Swinecheck APP 5a, 5b antibody ELISA test kit has demonstrated suitable performances in terms of repeatability, stability, sensitivity, specificity and agreement when compared to the GREMIP APP 5 ELISA used as “gold standard”.

KIT COMPOSITION

Components	Quantity
12 x 8 wells strips coated with APP 5 antigens	2
Ready-to-use Positive Control	2.5 mL
Ready-to-use Negative Control	2.5 mL
Concentrated Conjugate	Variable
Concentrated Wash Solution (10X)*	2 x 100 mL
Ready-to-use Substrate	24 mL
Ready-to-use Stop Solution	24 mL



CFIA-licensed FEO; 780DR/A1.6/D10

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