Influenza virus type A antibody test kit

Multispecies ELISA

Product code: TRM-575 (2 plates)

- ✓ **Detect antibodies against a broad range of influenza A subtypes**
- ✓ **Can be used with swine and poultry serum**
- ✓ **A shelf life of 24 months**
- ✓ **Approved by the Canadian Food Inspection Agency**

INTRODUCTION

Influenza A is an infectious disease caused by viruses of the family Orthomyxoviridae in the genus influenza virus (which includes also types B, C, and D viruses). Many species of birds and mammals (including swine and human) have been shown to be susceptible to infection with influenza A viruses. These viruses are classified into subtypes based on their haemagglutinin (H) and neuraminidase (N) antigens. At present, 16 H subtypes (H1–H16) and 9 N subtypes (N1–N9) are recognised.

Aquatic birds are a major reservoir of influenza A viruses. The majority of these viruses are of low virulence for commercial birds such as chickens and turkeys. In these species morbidity and mortality may greatly vary depending on various factors such as the species of bird and virulence of the viral strain. Clinical signs may include sudden death, apathy, reduction in feed and water intake, ocular and nasal discharges, coughing, dyspnea, incoordination, torticollis, diarrhea, drop in egg production. To date, highly pathogenic isolates have been associated only with the H5 and H7 subtypes. Such strains are subject to official control.

Infections with influenza viruses A are very common in swine. Clinical signs consist in apathy, reduction in feed and water intake, fever, sneezing and coughing. The morbidity rates may greatly vary while mortality rates are generally low. One relatively stable subtype, H1N1, has been predominant in the swine populations until the mid-1990s. Since that time, various subtypes and variants have been identified worldwide, many of which resulting from reassortments between swine, avian, and/or human viruses. The most common subtypes of swine influenza viruses are H1N1, H1N2, H3N2, and H3N1.

Diagnosis of influenza A virus infections are based on the detection of the virus by isolation (VI) or by reverse transcription-polymerase chain reaction (RT-PCR). Infections can also be diagnosed by detecting antibodies using serological assays such as the Hemagglutination Inhibition (HI) assay or Enzyme-linked immunosorbent assays (ELISA). Serology is greatly hampered by the antigenic diversity among influenza A viruses. This issue can be bypassed by using the virus nucleoprotein (NP) as antigen as it is is very well conserved among the various influenza A viruses.

INTENDED USE

This kit is a competitive ELISA developed by Biovet which allows the detection of antibodies against a broad range of influenza A subtypes.

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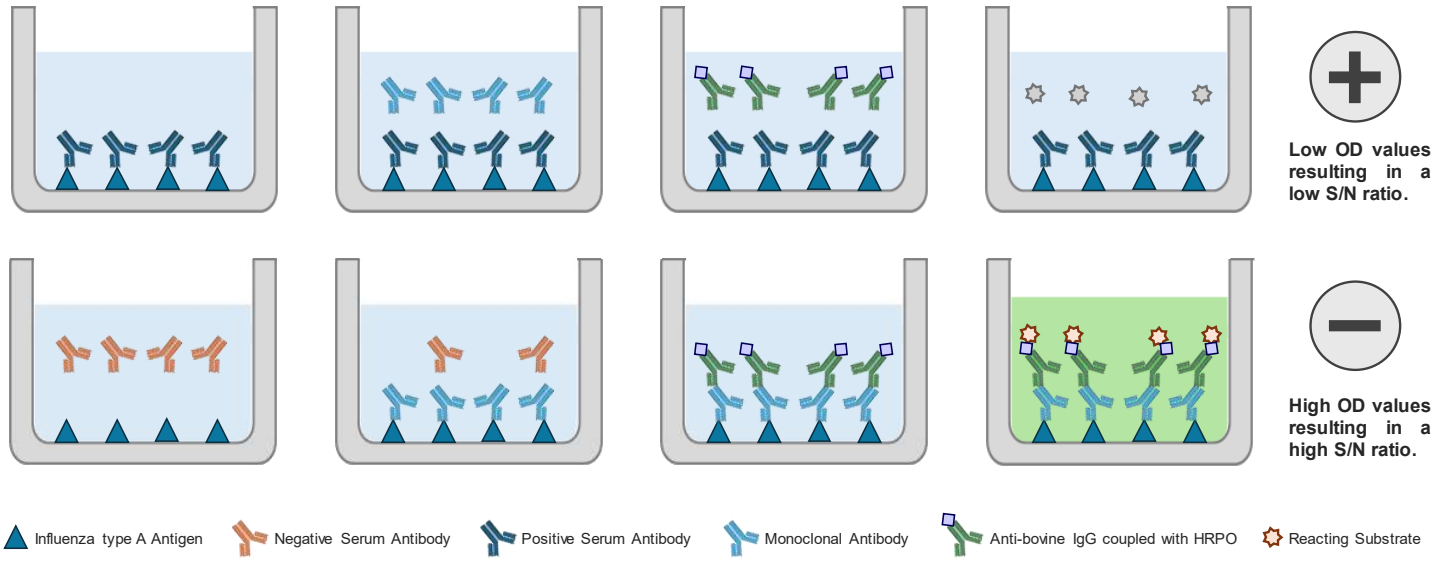
PRINCIPLE OF THE TEST

a. Assay description

1. The wells are coated with a recombinant nucleo-protein (NP) of a type A avian influenza virus. Samples are added and incubated for 30 min at 23 ± 2°C.
2. Each well is washed to remove unbound material. A murine mAb is added and incubated for 45 min at 23 ± 2°C. They compete with the serum for a specific epitope on the recombinant protein
3. After another wash, an anti-mouse conjugated with HRPO is added and incubated for 15 min at 23 ± 2°C. Washing steps follow.
4. The substrate is added and incubated for 10 min at 23 ± 2°C. It will react with HRPO if present. The reaction is stopped, and OD values are measured.



b. Results interpretation

OD values are used to calculate sample / negative (S/N) ratio as follow: **S/N ratio = OD_{sample} / mean $OD_{neg.ctrl}$**

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

Result	S/N ratio
Negative	≥ 0.60
Positive	< 0.60

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

- OD of the Negative Control must be higher than 0.700.
- OD of the Positive Control must be less than 0.400.

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TECHNICAL DATA

a. Sensitivity & Specificity

A panel of porcine serum samples (n = 200) was used for testing assay efficacy. Samples originated from multiple Canadian herds. A hundred samples originated from Canadian herds that were considered free from swine influenza virus. The other half came from herds infected with swine influenza virus. A panel of serum samples from birds free of avian influenza viruses or infected with various subtypes was also used for testing assay efficacy (n = 35). These samples were supplied by the National Centre for Foreign Animal Diseases in Winnipeg. The efficacy was evaluated by calculating the relative sensitivity and the relative specificity (with 95% confidence limits) using Idexx Influenza A Ab Test kit as reference test.

Porcine	Reference test		
	+	-	Total
Biovet +	93	1	**94**
Biovet -	7	99	**106**
Total	**100**	**100**	**200**

Statistic	Value	95% CI
Sensitivity	**93.00%**	**86.11% to 97.14%**
Specificity	**99.00%**	**94.55% to 99.97%**

Avian	Reference test		
	+	-	Total
Biovet +	24	0	**24**
Biovet -	1	10	**11**
Total	**25**	**10**	**35**

Statistic	Value	95% CI
Sensitivity	**96.00%**	**79.65% to 99.90%**
Specificity	**100.00%**	**69.15% to 100.00%**

The agreement with the reference test is considered as "almost perfect" with both species (Kappa: 0,92 for porcine and 0,93 for avian).

b. Stability

The stability of the kit was evaluated with a panel of 9 samples, including 3 negative and 6 positive samples. Samples were tested with 3 serials of the Influenza virus type A antibody test kit that had been stored at 4-8°C for up to 25 months. Results presented are the S/N ratios from the 3 serials.

Sample ID	Serial 1				Serial 2				Serial 3			
	0 mo.	13 mo.	19 mo.	25 mo.	0 mo.	13 mo.	19 mo.	25 mo.	0 mo.	13 mo.	19 mo.	25 mo.
Neg Ctrl	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
Pos Ctrl	0,05	0,05	0,06	0,05	0,06	0,08	0,07	0,09	0,06	0,07	0,08	0,07
Buffer	1,19	1,05	1,07	1,05	1,11	1,00	1,07	0,99	1,13	1,18	1,12	1,12
USDA# 91	0,22	0,19	0,29	0,15	0,20	0,17	0,18	0,21	0,20	0,18	0,17	0,22
USDA # 97	0,35	0,20	0,25	0,19	0,29	0,21	0,20	0,24	0,35	0,20	0,27	0,23
USDA # 99	0,19	0,18	0,19	0,18	0,20	0,20	0,20	0,22	0,22	0,19	0,28	0,25
USDA # 105	0,06	0,05	0,07	0,04	0,07	0,07	0,07	0,11	0,09	0,07	0,07	0,07
USDA # 108	0,25	0,19	0,26	0,15	0,25	0,20	0,19	0,23	0,26	0,19	0,20	0,17
USDA # 112	0,04	0,04	0,04	0,03	0,05	0,06	0,05	0,08	0,04	0,05	0,05	0,04
USDA # 113	0,97	0,90	0,97	0,93	1,00	1,05	1,01	1,02	1,10	1,18	1,07	0,95
USDA # 115	0,95	0,86	0,99	0,90	1,04	0,99	1,00	1,01	1,14	1,16	1,07	0,98
USDA # 118	0,89	0,86	0,90	0,86	0,95	0,95	0,94	0,96	1,02	1,02	0,94	0,89

The ratios remained stable throughout the study, and no changes in result outcomes were observed.

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c. Repeatability

The intra lot repeatability (A) was evaluated using 10 samples with a reactivity ranging from negative to strongly positive. Each sample was tested 4 times in a pre-licensing serial of the kit. The inter lot study (B) was conducted the same way with 4 plates of 4 distinct serials and a different panel of samples.

A

Sample ID	Well 1	Well 2	Well 3	Well 4	Mean ± SD	CV (%)
Neg Ctrl.	1,00	1,00	1,00	1,00	1,00 ± 0,00	**0,0%**
Pos Ctrl.	0,06	0,05	0,06	0,06	0,06 ± 0,00	**6,7%**
426908-11	0,05	0,04	0,05	0,04	0,05 ± 0,00	**9,4%**
428961-2	0,19	0,16	0,20	0,19	0,18 ± 0,02	**10,4%**
425763-4	0,16	0,13	0,15	0,14	0,15 ± 0,01	**9,8%**
426908-14	0,45	0,34	0,40	0,40	0,40 ± 0,04	**10,7%**
425529-1	0,64	0,61	0,64	0,66	0,64 ± 0,02	**3,1%**
429641-5	0,74	0,67	0,72	0,73	0,71 ± 0,03	**4,7%**
429879-5	0,92	0,76	0,94	0,85	0,87 ± 0,08	**9,6%**
429641-3	0,94	0,82	0,98	0,92	0,92 ± 0,07	**7,4%**
425527-4	0,83	0,77	0,86	0,89	0,84 ± 0,05	**5,9%**
427247-3	0,95	0,90	1,00	1,02	0,97 ± 0,05	**5,4%**

B

Sample ID	Serial A	Serial B	Serial C	Serial C	Mean ± SD	CV (%)
Neg Ctrl.	1,00	1,00	1,00	1,00	1,00 ± 0,00	**0,0%**
Pos Ctrl.	0,05	0,06	0,06	0,06	0,06 ± 0,00	**8,6%**
USDA# 91	0,22	0,20	0,20	0,20	0,20 ± 0,01	**4,0%**
USDA # 97	0,35	0,29	0,35	0,31	0,33 ± 0,03	**9,1%**
USDA # 99	0,19	0,20	0,22	0,21	0,21 ± 0,01	**6,8%**
USDA # 105	0,06	0,07	0,09	0,08	0,07 ± 0,01	**17,3%**
USDA # 108	0,25	0,25	0,26	0,26	0,25 ± 0,01	**2,8%**
USDA # 112	0,04	0,05	0,04	0,04	0,04 ± 0,01	**17,5%**
USDA # 113	0,97	1,00	1,10	0,97	1,01 ± 0,06	**6,1%**
USDA # 115	0,95	1,04	1,14	0,94	1,02 ± 0,10	**9,3%**
USDA # 118	0,89	0,95	1,02	0,90	0,94 ± 0,06	**6,3%**
USDA # 130	0,05	0,07	0,08	0,06	0,07 ± 0,01	**15,2%**

Data showed are S/N ratios. The results demonstrate the kit's high repeatability. All samples from the intra-lot study had a coefficient of variation under 15%. Three samples from the inter-lot study had a CV slightly over 15%, but each was strongly positive with a S/N ratio under 0.10.

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CONCLUSION

The “Influenza virus type A antibody test kit” demonstrates satisfactory diagnostic performances (sensitivity, specificity, repeatability) and has a shelf life of 24 months at 2-8°C.

KIT COMPOSITION

Components	Quantity
12 strips of 8 wells coated with recombinant type A Influenza Ag	2
Ready-to-use Positive Control	2.5 mL
Ready-to-use Negative Control	2.5 mL
Ready-to-use Sample Dilution Buffer	100 mL
Concentrated Wash Solution (10X)	2 x 100 mL
Ready-to-use anti-NP mAb	25 mL
Concentrated HRPO Conjugate (300X)	300 µL
Ready-to-use Substrate	25 mL
Ready-to-use Stop Solution	25 mL

CFIA-licensed FEO; 780DR/I5.0/D10

For more info, contact us at: 1-888-824-6838, option 3
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