Avian Influenza Virus Antibody ELISA

BIONOTE AIV Ab ELISA

■ Explanation of the Test

Avian influenza (AI) is caused by infection with viruses of the family Orthomyxoviridae placed in the genus *influenzavirus A*. Influenza A viruses are the only orthomyxoviruses known to affect birds. Many species of birds have been shown to be susceptible to infection with influenza A viruses; aquatic birds form a major reservoir of these viruses, but the overwhelming majority of isolates has been of low pathogenicity for chickens and turkeys,and the main birds of economic importance are affected.

The BIONOTE AIV(*Avian Influenza Virus*) Ab ELISA is a Competitive Enzyme Linked Immunosorbent Assay for the qualitative detection of antibody to the most common and prevalent AIV in chicken, duck, turkey, quail, goose, guinea fowl, grey partridge, red partridge, pheasant, swan, horse or pig samples.

The BIONOTE AIV Ab ELISA contains a microplate, which is pre-coated with AIV antigen on the well. For testing, ELISA plates are incubated with an equal mixture of sample and anti AIV antibody-HRP for 30 minutes at 37℃. During first incubation, AIV antibodies present in sample and HRP conjugated anti AIV antibodies competitively bind to the AIV antigen on the well. Following this incubation, all unbounded materials are removed by aspiration and washing. The residual enzyme activity found in the well will be directly inverse proportion to the anti-AIV antibodies in the sample and evidenced by incubating the solid-phase with a substrate solution. The reaction is stopped by adding a stop solution and colorimetric reading will be performed using a spectrophotometer at 450nm and 620nm.

The highly specific selected recombinant AIV antigens on the well enable the BIONOTE AIV Ab ELISA to identify to *Avian Influenza Virus* antibodies in samples, with a high degree of accuracy.

■ Materials Provided

BIONOTE AIV Ab ELISA contains following items to perform the assay.

1	Antigen coated micro-assay plate	96 wells/plate, configured in twelve 1x8 strip
2	Negative Control	SPF chicken serum preserved in phosphate buffer with protein stabilizer. Proclin 300 (0.05%) is added as preservatives.
3	Positive Control	Antibodies to AIV preserved in phosphate buffer with protein stabilizer . Proclin 300 (0.05%) is added as preservatives.
4	Washing solution (10X concentrated)	PBS-Tween 20. Preservative is Proclin 300 (0.05%).
5	Enzyme conjugate	Anti-AIV antibody-HRP, phosphate buffered saline, BSA and stabilizers. Proclin 300 (0.05%) is added as preservatives. Ready to use.
6	Substrate	Tetramethyl-benzidine with citrate-phosphate buffer containing hydro-peroxide (H_2O_2): STORE IN THE DARK. Ready to use.
7	Stopping solution	1N sulfuric acid
8	Adhesive plate sealer	

■ Materials required, but not provided

1) Disposable microplate or test tube for dilution
2) Micro pipette
3) ELISA Washer
4) ELISA Reader
5) Distilled/deionized water

■ Precautions

In order to obtain reproducible results, the following rules must be observed:

1) For in vitro diagnostic use only.
2) Unused materials must be sealed and stored at 2~8℃.
3) Do not mix reagent of different lots.
4) Do not use reagents beyond the stated expiration date marked on the label.
5) Avoid contamination of each reagent with sample or other reagents.
6) Use disposable gloves while handling potentially infectious materials and performing the assay. After assay, wash hands with sanitizers.
7) Substrate and stopping solution should be handled with care. Avoid contact with skin, eyes and mucous membranes. In case of accident, rinse thoroughly with running water.
8) Dispose of containers and residues safely in accordance with national and local regulations.

■ Collection and Storage of Sample

[Collection and Storage]

1) For routine serologic flock monitoring, it is suggested that at least 30 or more samples per flock be randomly collected at standard time intervals(i.e. every four weeks).
2) This ELISA was evaluated at serum/plasma from chicken, duck, turkeys, quails, geese, guinea fowls, grey partridges, red partridges, pheasants, swans, horses, and pig and egg yolk from duck and chicken. Other species are not evaluated.
3) Fresh serum, plasma, or egg yolk samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely.
4) If samples are not immediately tested, they should be stored at 2~8℃. For keeping samples more than 3days, freeze the sample at - 20℃ or below. They should be brought to room temperature prior to use.
5) For egg yolk samples, 2ml of egg yolk should be mixed with 2ml of PBS (pH7.2) using vortex agitator. And then the mixed egg yolk should be centrifuged by 3,000rpm for 30 minutes. The supernatant can be used as specimens.

[Precautions]

1) Use fresh sample. Hemolyzed or contaminated sample might cause false result.
2) Remove the blood corpuscle in samples before use. They may cause non-specific reaction.
3) Sodium azide in sample may affect the test result.

■ Preparation of Reagents and Samples

1) Allow all reagents and samples to come to room temperature(18~25℃) for 30minutes before use.
2) Unused microplate wells should be stored sealed in the enclosed plastic bag at 2-8℃. It should be used as soon as possible. But do not reuse micro wells or pour reagents back into their original bottles once dispensed.
3) Mix samples thoroughly by gentle inversion. If necessary, any visible particulate matters in the samples should be removed by low-speed centrifugation.
4) **Washing solution (10X concentrated)**: The wash concentrated must be diluted 1 : 9 with distilled/deionized water before use. i.e. add 50ml of Washing solution to 450ml of distilled water and mix well.
5) **Storage and stability**

Material / Reagent	State	Storage	Stability
Working washing solution	Once prepared	Room temp.(18~25℃)or 2~8℃	1 week

■ Procedure of the Test

1) Prepare the microplate wells for negative control 3 wells, positive control 2 wells and each of the samples to each wells
2) Add 50 ㎕ of positive control(PC), negative control(NC), and samples into each well.
3) Add 50 ㎕ of conjugate solution(Ready to use) into each well containing PC, NC and sample.
   * Note: If number of sample is over 100, add 55 ㎕ of sample and 55 ㎕ of conjugate into the sample dilution plate(not provided). After that, add 100 ㎕ of mixed sample into the microplate(provided).
4) Mix well on vibrating mixer and cover the microplate with adhesive plate sealer.
5) Incubate the wells at 37±1℃ for 30 minutes.
6) Wash the wells at 6 times with 350 ㎕ of diluted washing solution and aspirate all liquid from the wells.
7) Add 100 ㎕ of substrate(Ready to use) to each well.
8) Incubate the wells for 10 minutes at room temperature(18~25℃).
9) Add 100 ㎕ of stopping solution to each well.

10) Read the absorbance of the wells with a bichromatic spectrophotometer at 450nm with reference wavelength at 620nm. Reading must be completed within 30minutes from the end of assay.

Interpretation of the Result

[Test validation]

1) Mean OD_{450} of the negative control(OD_{450}NCx) is more than 1.0.
2) Mean OD_{450} of the positive control(OD_{450}PCx) is more than - 0.005 and less than 0.5000.
3) If either of these values is out of range, test is considered invalid and the samples should be retested.

[Calculation of PI value]

1) Calculate PI (Percent inhibition) value by each sample, using the formula percent inhibition

PI value = [1-(OD_{450} of sample/ OD_{450}NCx)] x 100

For example)

- OD_{450}NCx : 2.040 , OD_{450}PCx : 0.059 ,
- OD_{450}sample : 1.950
- PI value = [1-(1.950/2.040)] x 100 = 4.4 → This sample is considered as negative

* If PI value is minus(-), it's considered as 0.

[Interpretation of the result]

1) Based on the PI value and animal species, the samples are classed as follows:

* Interpretation of egg yolk samples is also the same criteria.

Species	Chicken	Duck	Turkey	Quail	Goose	Guinea Fowl	Grey Partridge	Red Partridge	Pheasant	Swan	Horse	Swine
Positive PI value	≥ 50	≥ 50	≥ 85	≥ 50	≥ 50	≥ 50	≥ 50	≥ 50	≥ 50	≥ 50	≥ 50	≥ 50
Negative PI value	<50	<50	<85	<50	<50	<50	<50	<50	<50	<50	<50	<50

2) Use the following guidelines to establish influenza antibody flock status. Please note that the ELISA is a flock test and flock decisions should not be made on individual samples or very small flock samples (i.e. less than 10 samples/flock).

ELISA result	Presumed Flock Status	Recommended Action
All samples negative	No antibody to AIV	None. Monitor on an ongoing basis (e.g. every 4~6 weeks)
At least 1 sample positive	Antibody to AIV present	Flock AIV status should be confirmed with additional serological tests and virus isolation.

Limitations and Interferences

1) Failure to add specimen in the procedure could result a false negative. Repeat testing should be considered where there is clinical suspicion of infection.
2) Other clinically available tests are required if questionable results are obtained. As other diagnostic tests, a definitive clinical diagnosis should not be based on the result of a single test. It is recommended the diagnosis decision is made by the clinician after all clinical and laboratory findings have been evaluated.

Storage and Stability

1) All reagents should be stored at 2~8℃.
2) Shelf life is 12 months. Do not use after the stated expiry date.

Packaging Unit

	96Test	480Tests
Micro plate	1plate	5plates
Negative Control	0.3ml X 1vial	1.5ml X 1vial
Positive Control	0.3ml X 1vial	1.5ml X 1vial
10X Washing Solution	50ml X 1bottle	250ml X 1bottle
Enzyme conjugate	8ml X 1bottle	40ml X 1bottle
Substrate (Ready to use)	12ml X 1bottle	60ml X 1bottle
Stopping Solution	15ml X 1bottle	80ml X 1bottle
Adhesive plate sealer	2 ea	10 ea
Instructions for use	1 ea	1 ea

Performance characteristics

1) Sensitivity and Specificity

The BIONOTE AIV Ab ELISA has been evaluated with positive and negative clinical samples by Hemagglutination inhibition Test(HI), Agar Gel Immunodiffusion Test(AGID), Single Radial Hemolysis Test(SRH), and other ELISA.

Species	Chicken	Duck	Turkey	Quail	Goose	Guinea Fowl	Grey Partridge	Red Partridge	Pheasant	Swan	Horse	Swine
Tested No.	1,613	178	213	46	25	19	38	5	18	4	63	266
Sensitivity (%)	98.2	97.5	97.1	100	100	87.5	100	not tested	100	100	100	91.1
Specificity (%)	97.3	93.8	100	97	100	100	100	100	62.5	100	not tested	97.4

* This evaluations were carried out at O.I.E. reference laboratories and institutes.

2) Serotype validation

The BIONOTE AIV Ab ELISA has been validated against following antisera.

H1N1	H2N3	H3N2	H3N8	H4N8	H5N1	H5N2	H5N3	H6N2	H7N1	H7N3
H7N7	H8N4	H9N2	H9N7	H10N1	H11N6	H12N5	H13N6	H14N5	H15N6	

3) Antibody detection comparison between serum and egg yolk after experimental vaccination of H5N3 or H9N2

	Serum antibody detection		Egg York antibody detection	
	H5N3 vaccination	H9N2 vaccination	H5N3 vaccination	H9N2 vaccination
*DPI 7	**20/20	18/20	0/10	0/10
DPI 8	-	-	2/10	0/10
DPI 10	-	-	1/10	5/10
DPI 12	-	-	10/10	6/10
DPI 14	21/21	19/20	20/20	13/20
DPI 15	-	-	10/10	10/10

*DPI: Day Post Inoculation

* *Positive samples/Tested samples

Precision

Within-run and between-run precisions have been determined by the testing 10 replicates of three specimens: a negative serum, a low positive serum and a strong positive serum. The C.V (%) of negative, low positive, and strong positive values were within 10% of the time.

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