

Anigen AIV Ag ELISA

1. Explanation of the Test

Avian influenza (AI) is caused by infection with viruses of the family Orthomyxoviridae within 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 are a major reservoir of these viruses, but the overwhelming majority of isolates have been of low pathogenicity for chickens and turkeys, the main birds of economic importance to be affected.

The Anigen AIV (*Avian Influenza Virus*) Ag ELISA is an Enzyme Linked Immunosorbent Assay for the qualitative detection of AIV antigen in samples.

The Anigen AIV Ag ELISA test contains a microplate, which is pre-coated with AIV specific monoclonal antibody in the wells. For testing, all specimens are added to the wells and incubated for 60 minutes. After washing, the conjugate (AIV monoclonal antibodies conjugated with a peroxidase) is added to the wells and it competes with the antigens in the specimens. The higher the titre of antigen in the specimen, the less conjugate will be bound to the antigen in the well. Following this incubation step, all unbound material is removed by aspiration and washing before adding the substrate solution. The residual enzyme activity found in the well will be directly proportional to AIV antigen in specimens and will be detected by incubating the solid-phase with a substrate solution. The reaction is stopped by addition of the stopping solution and colorimetric reading is performed by using a spectrophotometer at 450nm and 620nm.

The specially selected AIV antibodies used as capture material in this test enables the Anigen AIV Ag ELISA to identify *Avian Influenza Virus* antigens in specimens with a high degree of accuracy.

2. Materials Provided (96Tests/Kit)

Anigen AIV Ag ELISA contains the following items:

- 1) Antibody coated microplate: 96 wells, configured in 12 x 8 wells.
- 2) Negative Control: 1vial (0.3ml) of protein-stabilized buffer tested non-reactive for AIV antigen. Sodium azide (0.1%) added as a preservative.
- 3) Positive Control: 1vial (0.3ml) of recombinant AIV poultry antigen. Sodium azide (0.1%) added as a preservative.
- 4) Sample Diluent: 1vial (15ml) of phosphate buffer, Sodium azide (0.1%) added as a preservative
- 5) Washing solution (20X concentrated): 1vial (50ml) of PBS-Tween 20. Preservative: Proclin 300 (0.05%) Note: Before use, take the contents of one vial, and make it up to 1 litre with distilled water. If undissolved crystals are present, re-suspend the solution by warming the vial at 37 degrees C for a few minutes.
- 6) Enzyme Conjugate: (101X concentrated): 1vial (0.2ml) of monoclonal anti-AIV-HRP, BSA and stabilizers. Proclin 300 (0.05%) added as a preservative.
- 7) Conjugate Diluent: 1vial (15ml) of phosphate buffered saline, BSA and stabilizers. Preservative: Proclin 300 (0.05%)
- 8) Substrate A: 1 vial (7.5ml) of tetramethyl-benzidine with citrate-phosphate buffer: STORE IN THE DARK.
- 9) Substrate B: 1 vial (7.5ml) of citrate-phosphate buffer containing H₂O₂

- Note: before use, make 1:1 mix with substrate A and substrate B.
- 10) Stopping solution: 1 vial (15ml) of 1N sulfuric acid. Ready to use.
 - 11) Adhesive plate sealer
 - 12) Instructions for use

3. Precautions

In order to obtain reproducible results, the following instructions must be followed:

- 1) For in vitro diagnostic use only.
- 2) Do not mix the reagents from different lot numbers.
- 3) Use thoroughly clean glassware, free from contamination of metal ions or oxidating substances.
- 4) Use disposable gloves while handling potentially infectious material and performing the assay.
- 5) ?Substrates and stopping solution should be handled with care. Avoid contact with skin, eyes and mucous membranes. In case of an accident rinse thoroughly with running water.

4. Specimen Collection and Storage

[For cloaca, trachea or feces swab]

- 1) Fully swab from cloaca or trachea
- 2) Insert the swab into the 500ul of PBS
- 3) Stir and extract the samples from the swab.
- 4) Centrifuge at 12,000 rpm for 1 minute to settle large particles
- 5) Take 10ul of the supernatant

[For kidney, trachea tissue or feces]

- 1) Make 10% suspension (w/v) with PBS
- 2) Centrifuge at 12,000 rpm for 1 minute to settle large particles
- 3) Take 10ul of the supernatant

If specimens are not immediately tested, they should be refrigerated at 2 - 8 degrees C. For storage periods longer than three days, freeze the specimen at - 20 degrees C or below. Specimens should be brought to room temperature prior to use. Specimens containing precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.

5. Preparation of Reagent Solutions

- 1) Samples and positive and negative control solution: Do not dilute.
- 2) Enzyme Conjugate (101X concentrated): The enzyme conjugate concentrate must be diluted 1 to 100 with conjugate diluent before use.
- 3) Wash solution (20X concentrated): The concentrated wash solution must be diluted 1 to 19 with distilled/deionized water before use.
- 4) Substrate: Substrate A must be mixed 1 to 1 with substrate B before use.

6. Test Procedure

- 1) Prepare enough test wells for 2 x negative controls, 2 x positive controls and each of the test samples.
- 2) Add 100ul of the sample diluent to all wells. Add 10ul of the positive control and negative control (2 wells each) and 10ul of the test samples to each of the assigned wells.

- 3) Cover the microplate with an adhesive plate sealer and mix well on vibrating mixer. Mixing is very important to get the reproducible results.
- 4) Incubate the plate 37 degrees C for 60 minutes.
- 5) Wash the wells 6 times with 350ul of diluted washing solution. Aspirate all liquid from the wells.
- 6) Add 100ul of MAb-HRP (1:100 dilution in the conjugate diluent) to all wells.
- 7) Incubate the plate at 37 degrees C for 30 minutes.
- 8) Wash the wells 6 times with 350ul of diluted washing solution. Aspirate all liquid from the wells.
- 9) Add 100ul of the substrate to each well.
- 10) Incubate the plate for 10 minutes at room temperature.
- 11) Add 100ul of the stopping solution into each well.
- 12) Read the absorbance of the wells with a bichromatic spectrophotometer at 450nm with reference wavelength at 620nm. Reading must be completed within 1 hour from the end of assay.

7. Interpretation of the Results

1) Test validation

The individual values of the absorbance for the negative and positive control are used to calculate the mean value if

$$-0.010 \leq A(\text{Neg.}) \leq 0.200$$

$$A(\text{Pos.}) \geq 1.00$$

If one of the absorbance values of the negative control is outside the specification, this value can be ignored. Both absorbance values of the positive control must comply with the specification. If these specifications are not met, the test must be repeated.

2) Evaluation

Calculate the mean absorbance of the negative controls, and then calculate the cut-off value by adding 0.100 $N_{cx} + 0.100 = \text{cut-off value (C.O.)}$

Calculate the S/C.O: Divide the absorbance value of the sample by the cut-off value (C.O.). Based on the criteria of the test, the samples are classed as follows:

Test results:

- i. S/C.O. of sample < 1 Negative
- ii. S/C.O. of sample \geq 1 Positive

Samples with a test result which is equal to or greater than the cut-off value, should first be retested in duplicate. If, in the retest, the mean absorbance is again equal to or greater than the cut-off, these samples should always be verified using a confirmatory test.

8. Limitations and Interferences

- 1) The test procedure, precautions and interpretation of results sections of this test kit must be followed closely when testing.
- 2) Common sources for mistake: kits used beyond the expiry date, poor washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
- 3) Please do not use kit beyond the expiry date indicated on the kit box and reagent labels.

- 4) Failure to add specimen in the procedure could result in a falsely negative test. Repeat testing should be considered where there is clinical suspicion of infection.

9. Storage and stability

- 1) Store at 2-8 degrees C. This test kit is stable until the expiration date printed on the packaging and on the label of each component of the kit (before opening).
- 2) Open vial stability of materials / reagents

Material / reagent	State	Storage	Stability
Coated Microplate	Once opened	2 - 8 degrees C, sealed	1 month
Negative Control	Once opened	2 - 8 degrees C	3 month
Positive Control	Once opened	2 - 8 degrees C	3 month
Sample Diluent	Once opened	2 - 8 degrees C	1 month
Enzyme Conjugate	Once opened	2 - 8 degrees C	1 month
Conjugate Diluent	Once opened	2 - 8 degrees C	1 month
Substrate A	Once opened	2 - 8 degrees C	1 month
Substrate B	Once opened	2 - 8 degrees C	1 month
Washing solution	Once opened	2 - 8 degrees C	Expiry date
Working Washing solution	1:20 diluted	2 - 8 degrees C Room temp	3 month 2 weeks
Stopping solution	Once opened	Room temp	Expiry date

10. Packaging Unit: 96 Tests/kit, 480 Tests/kit

11. Precision

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

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