



Rockeby Avian Influenza Virus Antigen Test Kit



RBL-301 (20 Tests)

REVISION DATE: 05/06
LM073-ENG-0



INTENDED USE

The **Rockeby Avian Influenza Virus Antigen Test** kit (AIV Ag) is a rapid one step chromatographic immunoassay for the qualitative detection of avian influenza virus antigen in avian faeces. The kit may be used to test felines.

Note: The test kit is NOT recommended for testing of pigeons.

INTRODUCTION

The avian flu, or the so-called "bird flu", is an infectious disease of birds caused by type A strains of the avian influenza virus (H5N1). All birds are thought to be susceptible to infection with avian influenza, though some species are more resistant to infection than others. Water fowl act as a reservoir of the avian influenza virus by carrying the virus in their intestinal tract and shedding it in their faeces. The virus causes no obvious disease in water fowl but can be highly pathogenic in domestic poultry. The virus is spread to domestic poultry through respiratory secretion and from contact with the faeces of infected birds. Signs of avian influenza are extremely variable, i.e. decreased food consumption, drop in egg count, coughing, sneezing, ruffled feathers, swollen heads, nervous signs like depression and diarrhea. Infection causes a wide spectrum of symptoms ranging from mild illness to a highly contagious and rapidly fatal disease resulting in severe epidemics. This form is characterized by sudden onset, severe illness, and rapid death, with a mortality that can approach 100%.

PRINCIPLE OF ASSAY

The **Rockeby Avian Influenza Virus Antigen Test** kit is a qualitative, one step chromatographic immunoassay to selectively detect the Avian Influenza virus with a high degree of sensitivity. In the test procedure, sample is absorbed through an absorbent membrane and allowed to migrate through the membrane. As the sample proceeds through the membrane, the colored conjugate (colloidal gold conjugate), which was pre-dried on the test strip, migrates with the sample. The sample and the conjugate move through the capture region, precoated with immobilized monoclonal antibody to Avian Influenza virus on the test band region and Protein A on the control band region, and then to the end of the membrane. The bound antibody-antigen complexes are detected by giving a pink-purple color. The control line contains protein A which binds with the dye conjugate. The control band serves as an indication of proper sample addition and migration plus reagent control.

The format provides a clear read out for positive (two lines) and negative (one line) specimens. The appearance of one line for negative specimens gives an added measure of quality control by

demonstrating antibody recognition, assuming that the procedure was done directly and that the reagents are chemically active.

MATERIALS PROVIDED

| | |
|---|------------|
| Rockeby Avian Influenza Virus Antigen test device | 20 devices |
| Test devices packed in individual sealed aluminum pouch with desiccant. | |
| Store at 2°C ~ 30°C. | |
| Specimen tubes containing assay diluent | 20 tubes |
| Plastic tubes with cover, each with 1.0 ml of diluent. | |
| Sample collection swabs | 20 pieces |
| Cotton bud wrapped sample collection sticks. | |
| Disposable droppers | 20 pieces |
| Molded plastic droppers packed with each individual test device. | |
| Instruction Sheet | 1 copy |

PRECAUTIONS



HEALTH AND SAFETY INFORMATION

- In case of an accident or contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Consult a physician immediately in the event that contaminated materials are ingested or come in contact with open lacerations, or other breaks in the skin.
- Wipe any spills of samples promptly with 1% sodium hypochlorite solution.
- Autoclave all used and contaminated materials at 121°C, 15 p.s.i. for 30 minutes before disposal. Alternatively, decontaminate materials in 5% sodium hypochlorite solution for 30 - 60 minutes before disposal in biohazard waste-bags.

ANALYTICAL PRECAUTIONS

- For veterinary *in vitro* diagnostic use only.
- For Professional use only.
- All specimens should be regarded as potentially infectious.
- Gloves must be worn.
- Optimal assay performance requires **STRICT ADHERENCE** to the assay procedure described in this Instruction Sheet. Deviations from the procedure may lead to aberrant results.
- Do not open or remove test device from their individually sealed pouches until ready for use.
- Do not use the test kit if the pouch is damaged or the seal is broken.
- For best results allow all reagents and samples to reach room temperature before use.
- To ensure proper drop delivery, droppers should be held vertically, while gently dispensing one drop at a time, in quick succession.
- Do not reuse test device.
- Do not interchange reagents between kit lots.
- Do not use kit components beyond the expiry date printed on the label(s).

- Handle reagent(s) carefully to avoid spills on work surface. Clean up spills with absorbent paper and water.
- Reagents supplied should not present a hazard to health if used in accordance to the instructions stated.

STORAGE AND STABILITY

- Keep the kit at room temperature or refrigerated (2°C~30°C) when not in use. The test device must remain in the sealed pouch until use.
- The test strip is stable through the expiry date printed on the package label.
- DO NOT FREEZE.**
- Do not store the test kit in direct sunlight.

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Tube stand

SPECIMEN COLLECTION AND PREPARATION

- Avian faeces samples (preferably fresh) should be used with this test.
- If faeces specimens are not immediately tested, they should be refrigerated 2°C~8°C. For storage not less than 48 hours, freeze the specimen at -20°C or below.

NOTE: The test kit is NOT recommended for testing of pigeons. Pigeon faeces may give false positive results.

Felines: To test a cat with the AIV Ag test kit, sample collection by a swab from a cat is not easy. So, the following cat sample collection method(s) is recommended.

Method A

- Tracheal washing after anesthesia.
- Swab the exudates.

Method B

- Inject a small amount of normal saline to the nose for sneezing.
- Swab a fit of sneezing.

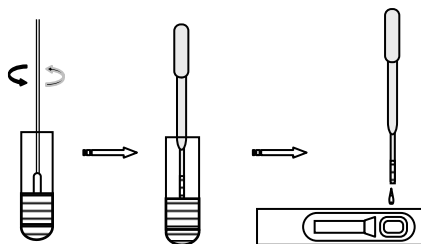
ASSAY PROCEDURE

IMPORTANT: Strict adherence to the assay procedure will ensure optimal assay performance. Deviations from the procedure may lead to aberrant results.

- Take a portion of faeces from a stool sample or from the anal area with the sample collection swab.
- Insert the swab into the specimen tube containing assay diluent.
- Mix the swab until the sample has been dissolved into the assay diluent.
- Leave the test tube until the large particles have settled down to the bottom of the tube.

- Remove the test device from the foil pouch, and place it on a flat and dry surface.
- Using the disposable dropper provided, take the supernatant from extracted sample in the tube.
- Add **eight drops** into the sample hole with the disposable dropper provided (as in figure 1).
- Interpret test results at **10 minutes**.

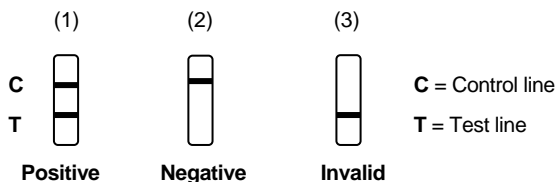
Figure 1.



QUALITY CONTROL

- Positive and negative controls are not included and are optional.
- No line is visible before running the assay. If the control line at position "C" does not become visible after the assay, the test is considered invalid. Positive samples will have an additional colored line at position "T".

INTERPRETATION OF RESULTS



- Positive** for Avian influenza if colored bands appear at the Test line (T) and Control line (C) within the viewing window. Any intensity of line should be considered as a positive.
- Negative** for Avian influenza if only the Control line (C) is visible through the viewing window.
- Invalid** if the Control line (C) is absent. If this occurs, the assay should be repeated using a new device.

LIMITATIONS OF PROCEDURE

Optimal assay performance requires strict adherence to the assay procedure described. Deviation from the procedure may lead to aberrant results. A NEGATIVE result does not exclude the possibility of exposure to or infection with Avian Influenza.

PERFORMANCE

Sensitivity / Specificity:

Table 1 provides the results of a study in Thailand on twenty five healthy and eighteen sick chickens confirmed by HA after egg inoculation. The samples were assayed according to the instruction manual within the kit.

Table 1: Sensitivity and specificity of Rockeby Avian Influenza Type A kit.⁷

| | Positive | Negative | Total |
|----------|----------|----------|-------|
| Positive | 18 | 0 | 18 |
| Negative | 0 | 25 | 25 |
| Total | 18 | 25 | 43 |

Results of another study in Indonesia on 10 cloacal swab and faeces samples from various avian species found that the Rockeby Kit was able to detect all ten positive samples (10/10).

Influenza A Subtype Reactivity⁷

| Test site | Subtype | Results |
|---------------|---------|----------|
| Singapore | H3N8 | Positive |
| | H6N1 | Positive |
| | H7N1 | Positive |
| | H9N2 | Positive |
| | H5N2 | Positive |
| | H5N3 | Positive |
| Thailand | H1N1 | Positive |
| | H5N1 | Positive |
| Indonesia | H5N1 | Positive |
| | H5N2 | Positive |
| | H5N3 | Positive |
| | H7N3 | Positive |
| | H7N7 | Positive |
| WHO Australia | H5N1 | Positive |

Influenza B and non-Influenza Reactivity⁷

| Test site | Other avian virus | Results |
|-----------|------------------------------|----------|
| Singapore | Influenza B | Negative |
| | Paramyxovirus Type 2 | Negative |
| | Newcastle Disease | Negative |
| Indonesia | Newcastle Disease | Negative |
| | Infectious Bronchitis | Negative |
| | Infectious laryngotracheitis | Negative |
| | Egg Drop Syndrome | Negative |
| | Infectious Bursal disease | Negative |

Table 2: Comparative Performance of Rockeby Avian Influenza Type A kit against HA and RT-PCR at different concentrations of virus.⁷

| Virus Level (ELD ₅₀ /ml) | C25SL02P3 | | | C17Dx01P3 | | |
|-------------------------------------|-----------|-----|--------|-----------|-----|--------|
| | Rockeby | HA | RT-PCR | Rockeby | HA | RT-PCR |
| 10 ⁹ | 2+ | 128 | + | 2+ | 128 | * |
| 10 ⁸ | 2+ | 64 | + | 2+ | 16 | * |
| 10 ⁷ | 2+ | 8 | + | 2+ | 1 | + |
| 10 ⁶ | - | <1 | + | - | <1 | + |
| 10 ⁵ | - | <1 | - | - | <1 | + |
| 10 ⁴ | - | <1 | - | - | <1 | + |

* Not done

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- Bird Flu fact sheet, Department of Disease Control, Public Health Ministry Thailand.
- OIE. 2000. Highly pathogenic avian influenza In: Manual of standards for diagnostic tests and vaccines, 4th ed.
- Perkin LEL. and Swayne DE. 2003. Comparative Susceptibility of selected avian and mammalian species to a Hong Kong-origin H5N1 high-pathogenicity avian influenza virus. Avian Disease 47:956-967.
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- Swayne DE. and Halvorson DA. 2003. Influenza In: Diseases of poultry. 11th ed. Iowa State Press, Iowa, USA.
- Webster RG. 1997. Predictions for future human influenza pandemics. Journal of Infectious Disease 176 (supp. 1): 14-19.
- Rockeby biomed confidential data on file.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no expressed warranty other than that the test kit will function as an *in vitro* diagnostic assay within the specifications and limitations described in the Instruction Sheet when used in accordance with the instruction contained herein. The manufacturer disclaims any warranty expressed or implied, including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any other purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product. The manufacturer shall not be liable to the purchaser or third parties for any damage, injury or economic loss howsoever caused by the product in the use or in the application thereof.

TECHNICAL PROBLEMS / COMPLAINTS

Should there be a technical problem / complaint:

- Note the kit(s) lot number(s) and the expiry date.
- Retain the kit(s) and the test device(s).
- Contact Rockeby biomed or your local distributor.

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The following are graphical symbols used in or found on Rocheby biomed products and packaging. These symbols are the most common ones appearing on diagnostic devices and their packaging. They are explained in more detail in the British and European Standard BS EN 980: 2003.



Use by
Synonym for this :
Expiry Date



In vitro diagnostic
medical device



Batch Code
Synonyms for this are:
Lot Number
Batch Number



Catalogue Number



Temperature Limitation



Attention.
See Instruction for
Use



Manufacturer



Do not reuse



Contains sufficient for
<n> tests



Consult instructions
for use