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Need for Rapid Detection of Avian Influenza Virus in Antarctic Wildlife

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Summary

Recent global outbreaks of highly pathogenic avian influenza (HPAI) and its potential impact on Antarctic wildlife call for proactive monitoring and rapid diagnosis. Birds and seals in the Antarctic region are at risk, with some species being susceptible to high mortality rates. Rapid diagnosis tool can be used to detect the virus in accordance with established protocols by WHO and WOA. Prompt response and control measures are crucial to prevent further transmission, requiring collaboration among the Parties, particularly between those operating in the Antarctic Peninsula region.

Background

The Committee for Environmental Protection and the Consultative Parties have been continuing their efforts to prevent the introduction of non-natives species including viruses and micro-organisms in accordance with Annex II (Art. 4, Appendix C) and Annex III (Art. 2). Discussions on the concerns of infectious disease outbreaks in Antarctic wildlife had been actively held among the Committee, SCAR, COMNAP, and relevant organizations (ATCM XXII-CEP I-IP4, ATCM XXIII-CEP II-WP32, SATCM XII-CEP III-WP20, ATCM XXIV-CEP IV-WP10, 11, ATCM XXVIII-CEP VIII-WP28, IP63).

The Antarctic Environments Portal has provided a comprehensive information summary on Antarctic wildlife diseases and pointed out the need to establish a structured Antarctic wildlife health surveillance program, as well as the limited or lack of diagnostic investigations in the majority of mortality cases (<https://environments.aq/publications/antarctic-wildlife-diseases-2>).

Since 2022, thousands of highly pathogenic avian influenza (HPAI) outbreaks have been recorded worldwide, and these outbreaks of HPAI (H5N1) have raised concern for wildlife conservation. Avian influenza virus (AIV) can infect birds and mammals, and it is transmitted through feces, contaminated water and soil, direct contact and respiratory droplets. Massive mortality associated with a HPAI in sea lions were reported in Peru (Gamarrá-Toledo et al. 2023) and the outbreak of HPAI (H5N1) in non-poultry has also been detected in Chile and Ecuador (WOAH 2023). The recent outbreaks in the countries of South America suggest high risk of introduction to the sub-Antarctic and Antarctic regions. These regions host a diverse range of bird species and seals which are exposed to the risk of HPAI. In this context, the Scientific Committee on Antarctic Research (SCAR) Expert Group of Birds and Marine Mammals (EG-BAMM)'s Antarctic Wildlife Health Working Group (AWHWG) has released a practical guide for operators who will interact with Antarctic wildlife in August 2022 (Dewar et al. 2022). Given the worldwide reports of outbreaks, particularly in the Antarctic gateway countries, and SCAR's high alertness, a proactive and collaborative approach is required to monitor and reduce the risk of the HPAI.

Rapid Detection of Avian Influenza Virus and Response

Aquatic birds, Anseriformes (e.g. ducks and geese) and Charadriiformes (e.g. gulls and terns), are natural reservoir for avian influenza virus. The HPAI virus can be sufficiently spread by asymptomatic aquatic birds (Khomeenko et al. 2018). However, in the case of African penguins, they are more susceptible to the HPAI infection, resulting in high mortality (Mollini et al. 2020). Antarctic seabirds are taxonomically close to Southern Ocean birds; therefore, monitoring and rapid diagnosis of AIV infection in unsusceptible as well as susceptible species are important.

For rapid AIV detection, commercial diagnostic kits can be used for seabirds and seals in Antarctica, sub-Antarctic regions and neighbouring areas in accordance with the official diagnostic protocols established by the World Health Organization (WHO) and the World Organization for Animal Health (WOAH). Monitoring efforts for early detection of AIV can involve using antigen kits such as AIV Ag Rapid Kit, which can detect AIV antigen in the fecal matter from the cloaca and in scattered feces. The diagnostic reagent in the test kit can detect AIV antigens at 15 minutes after injection of samples. User manual provided by the manufacturer is attached to this information paper. The rapid kits will be distributed free of charge upon request to the monitoring team at the Korea Polar Research Institute (KOPRI), the lead agency for the Korean Antarctic Program (please refer to contact information below for details).

Rapid Diagnosis Procedures

First step

Researchers, experts and trained staffs perform early diagnosis using the pre-provided AIV Ag rapid kit. The sample to be used is cloacal swab, oral swab or feces. The attached manual provides detailed introduction.

Second step

The samples that were diagnosed as positive or suspected to be positive through the test kit should be sent to King Sejong station or KOPRI to identify their genotypes and low or high pathogenicity of AIV. The entire process will take 1 to 2 weeks for the results to be obtained after the sample arrives.

If any of the samples are positive for the HPAI, the findings will be immediately notified to the sample collector and the Parties conducting activities near the sampling site, and will also be reported to the SCAR's EG-BAMM and COMNAP.

Response, Prevention and Control

In the event that HPAI is detected in Antarctic wildlife, SCAR AWHWG Guide should be consulted first. In addition, the following measures could be suggested:

Control measures

To reduce the risk of further transmission of HPAI, control measures should be implemented to restrict the movement of people and to exhaustively disinfect the outer clothing and equipment of researchers who had been in contact with the infected animals, and those who are authorized to be close contact with wild animals.

Communication and coordination

It is crucial to communicate the situation to the Antarctic Treaty parties, coordinate with the relevant stakeholders, and seek expert advice from organizations such as the World Health Organization (WHO) and the World Organization for Animal Health (WOAH).

Research and investigation

Further research should be conducted to better understand the transmission and ecology of HPAI in the Antarctic ecosystem to inform future prevention and control measures.

Monitoring and surveillance

All wildlife populations in the affected area should be closely monitored and tested for HPAI to detect any further cases of the disease and prevent its spread.

Conclusion and Call for Collaboration

Early detection of HPAI in wildlife can help prevent the spread of the virus, reduce its impact on the Antarctic wildlife populations, and protect the health of ecologically related animals and humans that may come into contact with infected animals. In this light, we propose a collaborative effort among the Parties the members of the Committee, and especially the operators who are currently operating in the Antarctic Peninsula region.

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VDRG[®] AIV Ag Rapid kit 2.0

CAT. NO. PP-AIV-12

GENERAL DESCRIPTION

VDRG[®] AIV Ag Rapid kit 2.0 is a lateral flow chromatographic immunoassay for the detection of avian influenza virus (AIV) in an avian cloaca feces or scattered feces.

This is a diagnostic kit to detect AIV antigen by mixing avian cloaca feces with dilution buffer followed by putting them into the sample hole. If there are AIV antigens in the avian cloaca feces, these antigens bind to AIV specific antibody-Cellulose Nano Bead (CNB) conjugates and move on the membrane by capillary forces, and then shows a red line on the test line due to the binding with AIV specific antibodies which are already applied on the membrane. This test kit, the diagnostic reagent can detect AIV antigens quickly and simply at 15 minutes after injection of samples.

KIT COMPONENTS

Components	30 Tests/Kit
① AIV Ag Rapid device	30 tests
② Sample dilution buffer(1ml)	30 vials
③ Swabs	30ea
④ Dropper cap	30ea
⑤ Instruction Manual	1copy

APPEARANCE

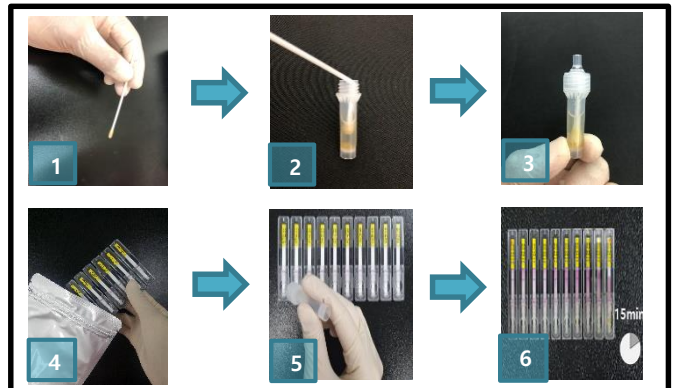
- In a test device : Specimen application round hole (S) is located at lower part of plastic cassette. The location of the test (T) and control (C) lines are marked on the rectangle display. The sample pad, feces separation pad, conjugate pad, nitrocellulose membrane, and absorption pad are attached to the test strip with them overlapped one after another.
- Sample dilution container : There is a transparent and colorless liquid buffer in the plastic container for dilution of a sample.

SAMPLE PREPARATION

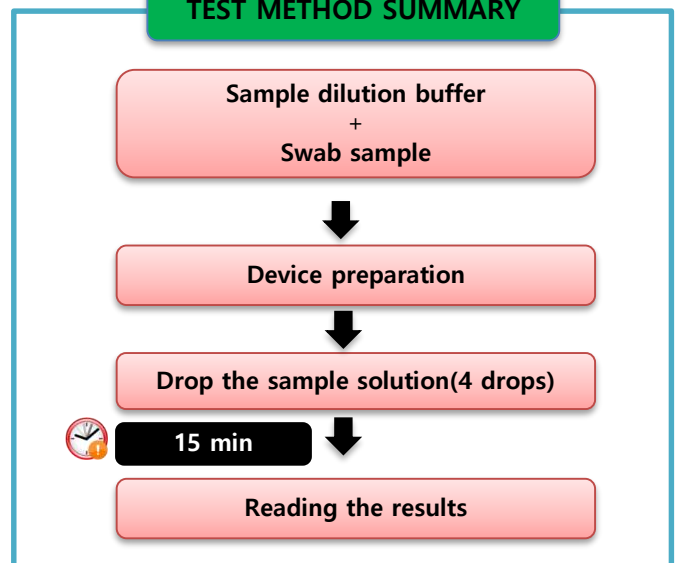
- Use avian feces as samples.
- Take the sample by pricking inside of feces deeply or pulling out directly through avian's anus.
- Then mix together the sample and dilution solution.

TEST PROCEDURE

- Swab the feces from the stool or rectums using the sample collection swab.
- Put the sample swab(③) in a tube containing the sample diluent(②), mix it 10 times, then cut the groove into the swab and cut off the rod, and let the head of the swab fall into the tube.
- Attach the dropper cap (④) to the tube containing the sample dilution solution and the cut swab to close it.
- Place the VDRG[®] AIV Ag Rapid kit 2.0 device(①) on a flat surface.
- Take the supernatant of sample solution using dropper, and then instill 4 drops into the test device.
- Verify the result at 15 minutes.



TEST METHOD SUMMARY

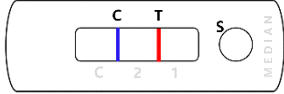


VDRG® AIV Ag Rapid kit 2.0

CAT. NO. PP-AIV-12

INTERPRETATION OF RESULT

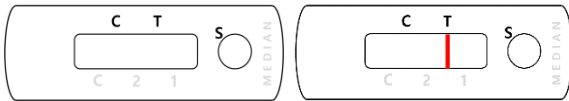
1. Positive : When there are the blue control line and the red test line.



2. Negative : When there are a blue control line but no test line.



3. Re-test : When there is a test line but no control line, or there are no control line and test line.



* Regardless of AIV presence, a control line should always appear. The control line is needed to check whether abnormal reaction occurs or not, so if there is no control line, re-test should be performed.

PRECAUTIONS

1. For in-vitro animal diagnostic use only.
2. Read this instruction manual thoroughly and follow all steps strictly for successful use of the product.
3. Extended exposure of this Rapid Test Device to moisture may decrease test performance. Therefore, open the device right before use (<10 minutes).
4. Make sure to use a separate test tube, dropper, and cotton swab for each sample.
5. Do not touch the membrane in the device. The results may be affected.
6. Do not use test device and reagents after expiration date.
7. Wear personal protective equipment (PPE) such as lab coat, goggle, and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
8. All test samples should be considered potentially infectious and all items contacting the samples should be considered contaminated.
9. After use, all wastes should be sterilized with high-pressure steam at 121 degrees Celsius for ≥ 15 minutes or comparable methods.
10. This Rapid Kit is made for preliminary test only. The result should be confirmed by other laboratory tests for final diagnosis.

STORAGE AND STABILITY

Store all reagents at 2~30°C. Do not freeze. Reagents remain stable until the expiration date when stored as instructed.

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