Rapid and Broad Detection of H5 Hemagglutinin by an Immunochromatographic Kit Using Novel Monoclonal Antibody against Highly Pathogenic Avian Influenza Virus Belonging to the Genetic Clade 2.3.4.4

Masatoshi Okamatsu

Abstract

Citation: Masatoshi Okamatsu Rapid and Broad Detection of H5 Hemagglutinin by an Immunochromatographic Kit Using Novel Monoclonal Antibody against Highly Pathogenic Avian Influenza Virus Belonging to the Genetic Clade 2.3.4.4. **protocols.io**

dx.doi.org/10.17504/protocols.io.icmcau6

Published: 07 Jul 2017

Protocol

Development of the IC kit to detect H5 HA antigen

Step 1.

Two MAbs, A64/1 and A32/2, were used in the New Linjudge Flu A/H5. The MAb, A64/1, was previously produced using a hybridoma cell line against an H5 low pathogenic avian influenza virus (LPAIV), A/duck/Pennsylvania/10218/1984 (H5N2) [22], and a novel MAb, A32/2, was prepared in a similar method against a clade 2.3.4.4 H5 HPAIV, A/chicken/Kumamoto/1-7/2014 (H5N8) [23]. The improved H5 IC kit was manufactured as described previously [10]. Briefly, the mixture of the anti-H5 HA MAbs, A64/1 and A32/2, were conjugated with colloidal gold with a proper ratio. Anti-mouse immunoglobulin antibodies and the cocktail of A64/1 and A32/2 were then immobilized onto a nitrocellulose membrane to capture antibodies in the control and test judgment regions, respectively.

Viruses

Step 2.

A total of 28 strains of influenza viruses including 26 strains of IAVs, 17 strains of non-H5 viruses, 9 strains of H5 viruses, and 2 strains of influenza B viruses were used (Table 1 and 2). These viruses were propagated in the allantoic cavity of 10-day-old embryonated chicken eggs for 30–48 h at 35°C. The infectious allantoic fluid was harvested and stored at -80°C until use.

Virus titration

Step 3.

Virus titration was performed based on the 50% tissue culture infectious dose ($TCID_{50}$) value by using Madin–Darby canine kidney (MDCK) cells maintained in minimum essential medium supplemented with 0.3 mg/mL L-glutamine, 100 U/mL penicillin G, 0.1 mg/mL streptomycin, 8 mg/mL gentamicin and 10% calf serum. Ten-fold dilutions of viruses in serum-free minimal essential medium were inoculated onto confluent monolayers of cells and incubated at 35°C for 1 h. After 72 h of incubation

at 35°C, the cytopathic effects of the cells were observed. $TCID_{50}$ titers were calculated by the method of Read and Muench (1938). In addition to $TCID_{50}$, the virus infectivity of H5 avian influenza viruses was measured as the 50% egg infectious dose (EID_{50}) by using 10-day chicken embryos. Dilution and titer calculation were performed as described in the $TCID_{50}$ method. The virus titration by EID_{50} and $TCID_{50}$ was performed using the same original working aliquot of each virus.

Evaluation of the specificity and sensitivity of the New Linjudge Flu A/H5, Linjudge Flu A/H5 and ImunoAce Flu (NP)

Step 4.

The detection efficacy of the present kit was compared with a human influenza commercial diagnosis kit, the ImunoAce Flu (NP antigen detection) (TAUNS Laboratories, Inc. Shizuoka, Japan), and the Linjudge Flu A/H5 kit [10]. The test procedure was performed as previously described [10]. In short, 10 µL sample solution was suspended in 90 µL of test solution (TAUNS Laboratories, Inc. Shizuoka, Japan) and the 100 µL suspension was applied to the sample port of each kit. Serial two-fold dilutions of each virus were tested. Results of the kit detection were recorded after 15 min of incubation at room temperature. A single colored line in the control judgment region (C) indicated the absence of H5 HA antigen. The concurrent presence of colored lines in both control and test judgment lines (T) indicated a positive test for H5 HA antigen in the samples. The results of antigen detection were indicated by +/-. The intensity of the positive test line was further recorded on a scale from + to 6+ (S1 Fig.). To standardize the visual judgment of the test line, the optical absorbance value was measured by the fluorescent immunochromato reader DiaScan 10-T (Otsuka Electronics Co., LTD., Osaka, Japan). The detection limit showing the lowest virus titer detectable by each kit was calculated by the equivalent proportion of the original virus titers to the last dilution that was able to yield positive detection. The detection limit was expressed as $\log_{10}$ EID_{50}/test and $\log_{10}$ $TCID_{50}$/test as previously described.

In addition, oropharyngeal and cloalcal swabs collected from 25 healthy chickens in commercial poultry farms were tested to examine cross reactivity of the New Linjudge Flu A/H5 with the field specimens. These samples were also confirmed to be negative with IAV by virus isolation using embryonated chicken eggs as previously described (S1 Table).

Swabs and tissue homogenates of naturally infected birds and experimentally infected chickens with H5N6 HPAIVs

Step 5.

A dead black swan and a dead whooper swan suspected of having natural infections with H5 HPAIVs were transferred to our laboratory for diagnosis. Two H5N6 HPAIVs were isolated from these birds and named A/black swan/Akita/1/2016 (H5N6) and A/whooper swan/Hokkaido/X12/2017 (H5N6), respectively [24]. Simultaneously, tissue homogenates of these birds were prepared for evaluation with the IC kits. Ten percent tissue homogenates were prepared in transport medium (minimal essential medium containing 10 000 U/mL Penicillin G, 10 mg/mL Streptomycin, 0.3 mg/mL Gentamicin, 250 U/mL Nystatin and 0.5% bovine serum albumin fraction V) as test samples and titration of infectivity. Swab samples from these naturally infected birds were not tested since multiple swabbings were formerly performed and used for emergency diagnosis and virus isolation, the subsequent swabs collected in the necropsy might not give appropriate results for kit evaluation. In addition to the natural cases, experimental infection of chickens was performed. Briefly, 12-week-old white-leghorn chickens hatched and raised in our laboratory were used in this study. Three chickens were intranasally infected with $10^{8.4}$ EID_{50} of A/black swan/Akita/1/2016 (H5N6). Each chicken

was housed in a self-contained isolator unit at the BSL3 facility in our laboratory. All chickens were monitored every 24 hours after the inoculation according to the standard protocol [25]. After 2 days post inoculation, swabs and organs of dead chickens were collected for kit evaluation as described above. The test samples were diluted five-fold with the test solution and tested as previously described. The viral infectivity titers in the swabs and tissue homogenates were measured and expressed as $\log_{10}$ $TCID_{50}$/test.

Ethics statements

Step 6.

All the animal experiments were authorized by the Hokkaido University Animal Care and Use Committee (approval numbers: 13-0138) and all experiments were performed per the guidelines of the committee. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.