



## APPLICATIONS OF IGY ANTIBODIES TO INFLUENZA A VIRUS DETECTION IN INFECTED CELLS BY IMMUNOCYTOCHEMISTRY

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**Introduction and objectives:** Influenza is a disease caused by infection of the host respiratory tract by influenza type A virus that affects a wide variety of species, including human, swine, equine, poultry and sea mammals. The viral RNA is encapsidated by the nucleoprotein (NP), which is a highly conserved protein among influenza A viruses. Detection of influenza virus can be accomplished by assessing specific immune response to the virus, by direct detection of viral RNA or virus antigen in infected tissues. Some diagnostic tests use antibodies produced in mammals to influenza virus detection. However, due to high cost and concerns related to animal welfare, alternative production of antibodies in mammals has been described. The immunoglobulin Y (IgY) is a class of antibodies in poultry that is transferred to the egg yolk. These antibodies are easy to obtain from eggs and their use has been described for immunotherapy, immunodiagnosis and research. The objective of this study was to produce IgY antibodies anti-NP in laying hens immunized with influenza A virus NP, and to evaluate the purified IgY antibodies in influenza virus detection.

**Material and methods:** NP protein was supplied by EMBRAPA. For its production was performed by the cloning of NP gene (from virus isolate A/swine/Brazil/12A/2010, H1N1) in pET23d vector and expression in *E.coli* BL21. Three laying hens of White Leghorn line were immunized with 20 ug of NP via intramuscular. Booster doses were administered on days 14, 28, 42, 84, 126 and 168 of the experiment. Seven days after the seventh dose of the antigen, the produced eggs were collected and IgY antibodies were extracted from yolk by precipitation with ammonium sulfate. Antibody levels were determined by ELISA assay. The specificity of anti-NP IgY was verified by Western blotting. For immunocytochemistry, Madin-Darby Canine Kidney cells (MDCK) were infected with influenza A virus. After 48 hours, the cells were fixed and washed with wash solution. Following, the monolayer cells were incubated with either primary antibody, IgY or monoclonal antibody IgG (1:400). After incubation and washing steps, conjugated antibody anti-IgY or anti-IgG was added to the cells. After the washing step the substrate was added, incubated for 10 minutes and then the plate was washed with water. The cells monolayer was observed under a light microscope.

**Results:** The produced IgY was analyzed by ELISA assay and the results showed that the laying hens immunized with NP protein produced high levels of antibodies when compared to the non-immunized poultry. It was also observed by Western blotting that anti-NP IgY antibodies was specific to NP protein of influenza A virus. Furthermore, MDCK cells infected with influenza A virus showed positive labeling when incubated with either monoclonal antibody IgG or IgY antibody anti-NP in immunocytochemistry test.

**Conclusion:** IgY antibodies specific to NP protein of influenza virus were obtained from egg yolk from laying hens immunized with the NP protein. These antibodies were able to label influenza A virus infected cells, suggesting that anti-NP IgY can be used in the diagnosis of influenza A virus.

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