Multiplex immunoassays for detection of antibodies against avian influenza

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Avian influenza (AI) is considered an exotic disease in commercial poultry in the United States. However, AI outbreaks in poultry occur occasionally having dramatic economic and health implications. Thus, significant need exists for rapid and sensitive assays for AI detection. Multiplex bead immunoassays hold potential as platform for diagnostic assay development. Whereas traditional immunoassays, such as ELISA, can detect a single analyte per sample, bead immunoassays can potentially detect up to 100 analytes per sample. This could dramatically increase the efficiency of disease surveillance, raising the possibility of a single test capable of detecting influenza-specific antibodies and identifying virus subtype. Other advantages specific to AI include facile profiling of exposure to all influenza subtypes, evaluation of vaccination programs, and differentiation of vaccinated from infected animals (DIVA). The objective of this study was to develop a triplex assay for the detection of antibodies to three AI virus proteins in commercial poultry.

As a preliminary assessment of feasibility, a triplex assay for detection of three AI proteins, NP, M1 and NS1, was developed. Recombinant AI proteins were cloned into baculovirus expression vectors and expressed in insect cells. Immunoassay bead reagents were prepared by covalent coupling of recombinant proteins to fluorescent polystyrene beads. Bead immunoassays were developed using the Luminex platform. Bead couplings were confirmed using chicken polyclonal serum to AI and mouse monoclonal antibodies to NP and NS1 proteins. Reagent preparations were consistent and reproducible, and results were consistent in monoplex and triplex assay formats. A two-dimensional serological optimization was performed to determine ideal assay conditions. The antibody response of chickens infected with AI virus under laboratory conditions was also evaluated. Anti-NP responses were strong and consistent, both over time and between individual birds. On the other hand, anti-NS1 and M1 responses were weaker, highly variable between individual birds, and decreased significantly over time. To assess the specificity of the assay for AI virus, a series of samples known to be positive for other unrelated avian viruses were tested. A high degree of specificity in anti-NP and anti-M1 detection was observed. The multiplex assay was then compared to a commercially available ELISA for detection of anti-NP antibodies in commercial chicken flock samples. The multiplex assay was found to be superior for detection of anti-NP antibodies, with reduction of both false positive and negative samples in a small sample set. The assay was also capable of detecting anti-NP antibodies in commercial turkey flock samples.

This work demonstrates the potential for success of multiplex diagnostic assays for AI and other infectious agents of interest to DHS. These experiments will contribute toward advancing development of antibody detection diagnostics for AI and other infectious agents. They have also laid a clear path for future improvements toward the final goal of a functional end-user assay.