

Development of a universally applicable ELISA based on recombinant HA and NA antigens for the detection and differentiation of influenzavirus antibodies in animal and human sera

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Key words

Avian influenza, recombinant haemagglutinin, neuraminidase, serology, ELISA, subtype differentiation

Aim of the study

Develop an ELISA assay for differentiation of antibodies against various avian influenza virus subtypes.

Material and methods

Avian influenza virus (AIV) strains were isolated from clinical samples, collected from wild birds in Switzerland. Additional virus isolates were received from cooperating laboratories. Hemagglutinin (HA) and neuraminidase (NA) genes were amplified by PCR and used for protein expression in insect cells. The proteins were purified by FPLC and used as ELISA antigens and for immunisation of chickens, rabbits and mice to produce poly- and monoclonal antibodies. With these antibodies, different ELISA layouts were tested aiming at establishing the differential ELISA system.

The potential of synthetic, AIV subtype-specific peptides as ELISA antigens was evaluated as well. PepSpot-membranes containing complete HA proteins in the form of overlapping, 15 amino acids long peptides were probed with HA-specific sera from rabbits and chickens to identify subtype-specific epitopes.

Results and significance

Recombinant H1, H2, H3, H4, H5, H6, H7, H9, H10, H11, and H12 HA and N1, N2, N3, N5, N6, N8, and N9 NA proteins were expressed. PepSpot analysis with sera against H4, H5 and H12 revealed that linear subtype-specific epitopes do exist and can function as differentiating antigens. However, if additional subtypes were to be differentiated in one test, the cross-reactivity of test sera made a subtype-differentiation impossible. On the other hand, when full-length recombinant HA were used as ELISA-antigens, the cross-reactivity of test sera to intersubtype-conserved epitopes could be reduced in a competitive blocking analysis protocol.

Publications, posters and presentations

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