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Three Modalities for H5 Influenza Antibody Measurements: Multiplexed Indirect Serology, H5 Bridging Serology, and Inhibition of Sialic Acid Binding

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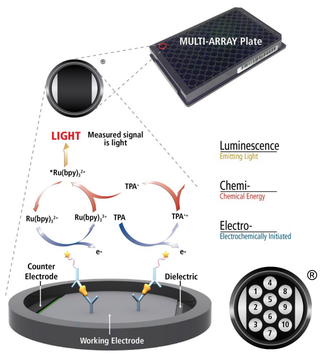
1 Abstract

Influenza causes 31-41 million cases of human infection in a typical year. While most cases are mild, the burden of severe disease falls primarily on children under 5 years of age and adults over 65 years of age. Most cases of human influenza A infection are of the H1 and H3 subtypes. However, there is growing concern that the highly pathogenic avian influenza (HPAI) H5N1 circulating in migratory birds, poultry, and bovines could adapt for human transmission. H5N1 infection in agriculture has led to cow milk recalls, quarantines of herds, and culling of flocks. New high-throughput assays are needed to detect H5N1 from a variety of species and sample types.

To account for multi-species applications, we developed high-throughput plate-based immunoassays to measure antibody responses using three assay formats. All formats use electrochemiluminescence (ECL) technology from Meso Scale Diagnostics, LLC. (MSD) The indirect serology format identifies antibodies to a panel of 10 multiplexed influenza HA antigens (influenza A and influenza B) using a species-specific detection reagent to identify samples with anti-influenza antibodies. The inhibition format identifies potential neutralizing antibodies that inhibit the binding of 2,3' sialic acid to the HA antigen, and can serve as a surrogate for a standard hemagglutination inhibition assay, but with faster turnaround. The bridging serology format identifies antibodies in samples that specifically bind H5 HA and is species-independent. Altogether, these assays rapidly detect anti-influenza antibodies in a variety of species and sample types with high sensitivity.

2 Methods

MSD® electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates.



Electrochemiluminescence Technology

- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.

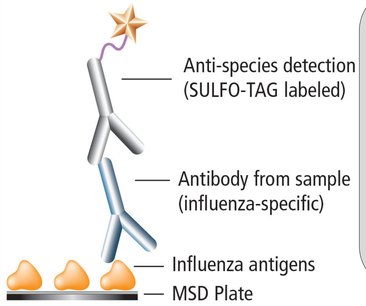
3 Samples

- Normal, healthy human serum was sourced from BioIVT.
- Pooled serum from humans vaccinated against H5N1 was obtained from BEI Resources. (Two samples are from individuals vaccinated against inactivated A/Indonesia/05/2005; and two are from individuals vaccinated against recombinant A/Vietnam/1203/2004).
- Sheep serum samples from vaccinated and control animals were obtained from BEI Resources and Lampire Biological. (Three are from sheep vaccinated against A/Hong Kong/156/1997; and one is from a sheep vaccinated against A/Hong Kong/213/2003 (H5N1).)
- Rabbit serum samples were obtained from BEI Resources and Noble Life Sciences. (Three are from individuals vaccinated against rgA/Vietnam/1203/2004(H5N1); and three are vaccinated against the H5 HA antigen A/Ghana/AVL-763_21VIR750-39/2021, from clade 2.3.4.4b.)
- Goat serum samples were obtained from BEI Resources and SJCRH (One is vaccinated against A/Hong Kong/213/2003 (H5N1)); and one is vaccinated against A/Bald Eagle/FL/W22-134-OP/2022.)
- Ferret sera were obtained from SJCRH where serum was collected 14 days post-challenge with a North American reassortment of H5N1 2.3.4.4b.

Sample Data

For both Indirect Serology and H5 Bridging Serology data sets, samples that quantified at or below the lower limit of detection (LLOD) were set to the LLOD value. Dots represent individual samples, and the solid horizontal line within a sample cluster represents the median concentration for that group. Dotted lines represent the cut-point concentrations, values above which are deemed positive. The cut-points were set to 2 times the LLOD.

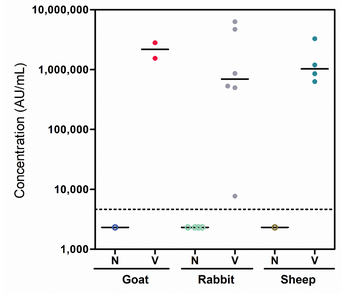
4 Indirect Serology



Protocol

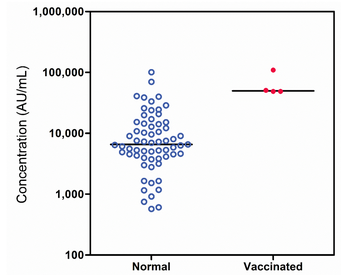
1. Block pre-coated plates for 30 minutes at room temperature (RT).
2. Wash and add calibrator, control, or diluted sample (50 µL per well). Incubate 2 hours at RT.
3. Wash and add species-specific detection antibody solution (50 µL per well). Incubate 1 hour at RT.
4. Wash and add MSD read buffer (150 µL per well). Analyze with MSD instrument.

H5-vaccinated Animals Generate Antibodies that Bind H5 A/Ghana



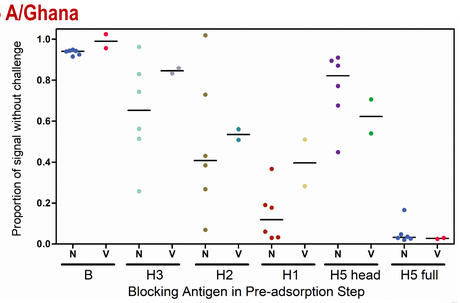
Normal (N) and vaccinated (V) animal serum samples were diluted 1:10⁶ and tested for binding to H5 A/Ghana antigen. A secondary antibody that is specific to the species IgG was used for detection. Samples were calibrated to arbitrary units using human polyclonal serum.

H5-vaccinated and Unvaccinated Human Serum Contains Antibodies that Bind H5 A/Ghana



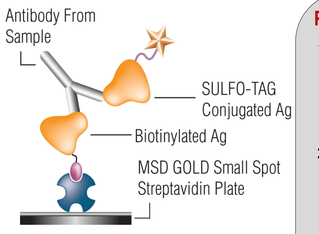
Normal and vaccinated human serum samples were diluted 1:5,000 and tested for binding to H5 A/Ghana antigen. Samples were calibrated to arbitrary units using human polyclonal serum.

Human Serum Contains Anti-HA Antibodies that are Cross-reactive with H5 A/Ghana



Normal (N) and vaccinated (V) human sera were diluted 1:5,000 in assay diluent containing 20 µg/mL of an influenza HA antigen to allow any reactive antibodies to adsorb to the antigen. The diluted samples were assessed for binding to the H5 A/Ghana antigen using the MSD assay. Data are reported as the percentage of signal, compared to the no-antigen control. The results demonstrate that both the normal and vaccinated samples have antibodies from prior influenza A exposures that cross-react with H5 A/Ghana.

5 H5 Bridging Serology Assay

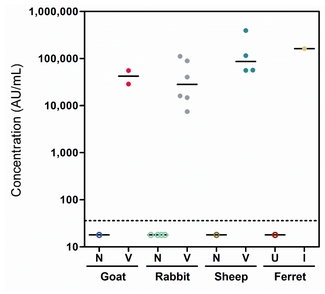


The antigens in the H5 Bridging Serology Assay are recombinant constructs with the HA head domain (amino acids 48-326) of the clade 2.3.4.4b H5N1 strain A/Ghana/AVL-763_21VIR7050-39/21.

Protocol

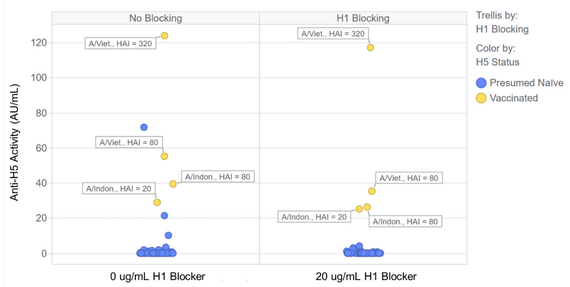
1. In a polypropylene plate, incubate calibrator, controls, and diluted samples with 1:1 molar ratio of biotinylated capture antigen (Ag) and SULFO-TAG conjugated detection antigen for 1 hour at RT.
2. Transfer solution with formed complexes from off-line incubation plate to a pre-blocked MSD streptavidin plate (50 µL per well). Incubate 1 hour at RT.
3. Wash and add MSD read buffer (150 µL per well). Analyze with MSD instrument.

H5-vaccinated Animals Generate Antibodies that Bind H5 A/Ghana



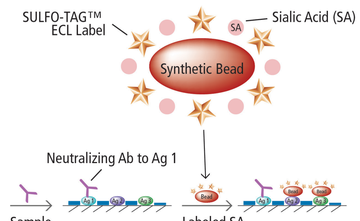
Normal (N), vaccinated (V), uninfected (U), and infected (I) animal serum samples were diluted 1:1,000 and tested for H5-specific antibodies by bridging serology.

Vaccinated Human Serum Contains H5-specific Antibodies



The anti-H5 antibody activity of presumed negative (blue) and positive pooled H5 vaccine study (yellow) samples was measured in the absence (left panel) or presence (right panel) of free H1 HA to remove H1 cross-reactivity. The vaccine study samples are labeled to indicate the vaccine strain (A/Vietnam/1203/04 or A/Indonesia/5/2005) and the HAI titer reported by BEI Resources against the vaccine strain.

6 Sialic Acid Inhibition Assay

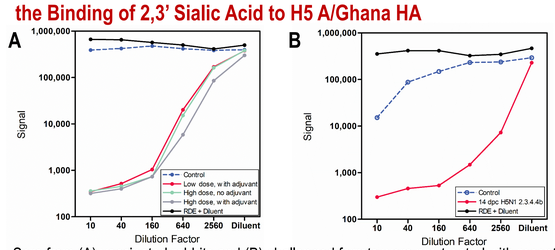


Protocol

1. Block pre-spotted plates for 30 minutes at RT.
2. Wash and add controls and diluted sample (25 µL per well). Incubate 1.5 hours at RT.
3. Add sialic acid-coated detection particles (25 µL per well). Incubate 2 hours at RT.
4. Wash and add MSD read buffer (150 µL per well). Analyze with MSD instrument.

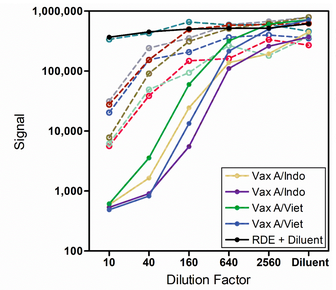
The particles in the Sialic Acid Inhibition Assay are labeled with SULFO-TAG for detection and 2,3'-linked sialic acid for binding to the influenza hemagglutinin antigen.

Antibodies in Vaccinated Rabbits and Challenged Ferrets Inhibit the Binding of 2,3' Sialic Acid to H5 A/Ghana HA



Sera from (A) vaccinated rabbits and (B) challenged ferrets were pre-treated with receptor destroying enzyme (RDE) to cleave any sialic acids in the sample, diluted, and analyzed for the ability to inhibit the binding of detection particles with 2,3' sialic acid moieties.

Antibodies in Human Serum Inhibit the Binding of 2,3' Sialic Acid to H5 A/Ghana HA



Sera from normal (dotted lines) and vaccinated (solid lines) humans were pre-treated with RDE, diluted, and analyzed for the ability to inhibit the binding of detection particles with 2,3' sialic acid moieties.

7 Acknowledgements

We thank Dr. Richard Webby and Jennifer DeBeauchamp from SJCRH for ferret and goat serum samples. We thank BEI Resources for human, sheep, rabbit, and goat serum samples.

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8 Summary and Conclusions

- Here, we demonstrate 3 modalities to evaluate anti-influenza antibodies in animal and human serum.
- Animals demonstrated potent serological responses during vaccination or challenge.
- Human H5 vaccination generated measurable antibody responses to H5 HAs.
- Binding assays for H5 responses should consider the potential for cross-reactivity from pre-existing antibodies to other HA subtypes.
- Vaccinated and challenged animals and humans produce antibodies that inhibit the binding of 2,3' sialic acid with the HA antigen.

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