Development of a Multiplexed Fluorescent Microsphere Immunoassay for Detection of Antibodies Against Avian Influenza Virus(NP,H5 and H7) and Newcastle Disease Virus (NP)

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Rationale for Development

Wild birds are considered primary hosts of Influenza A in nature. H5 and H7 subtypes are of concern for domestic poultry due to their ability to evolve from low to high pathogenicity

Outbreaks of NDV (velogenic) and AIV H5 and H7 are reportable to OIE. Outbreaks by these viruses in domestic poultry can have devastating economic consequences as discussed in several earlier presentations

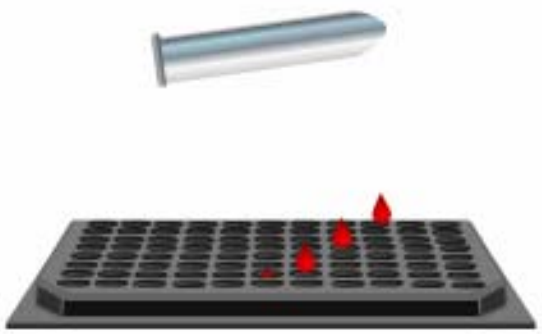
For routine surveillance to maintain export markets, rapid and sensitive assays are needed to detect antibodies to AI proteins

Current serological methods have some limitations

Multiplexing allows maximum information with minimum sample volume and the short turn-around time allows for faster action in the field

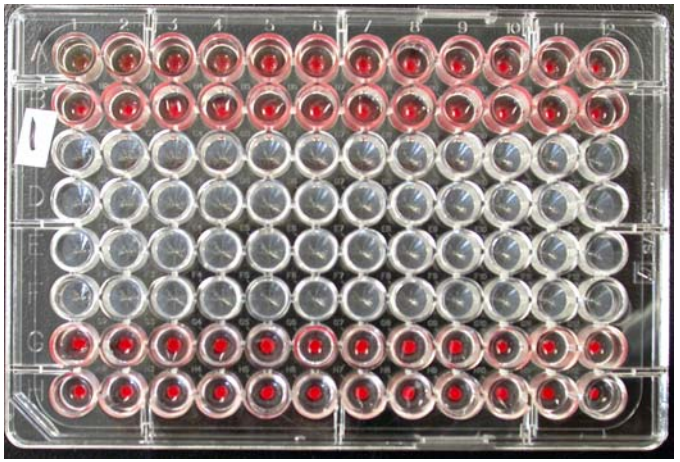
GOAL: an assay that allows detection and subtyping of Influenza A as well as differential diagnosis from Newcastle Disease

Traditional ELISA



1 assay per well

HA/HI assay



1 assay per row

AGID test (“Gold” Standard)

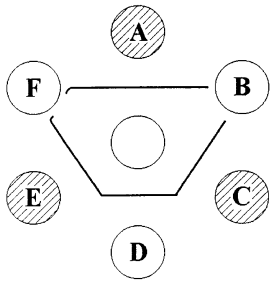
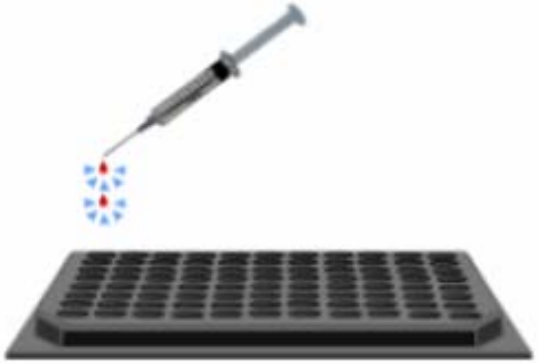


FIGURE 3.—*Immunodiffusion test that has AI AGID antigen in the center well; AI-positive control serum in wells A, C, and E; AI-negative test serum in well B; AI-positive test serum in well D; and weak positive test serum in well F.*

xMAP technology



up to 100 assays per well

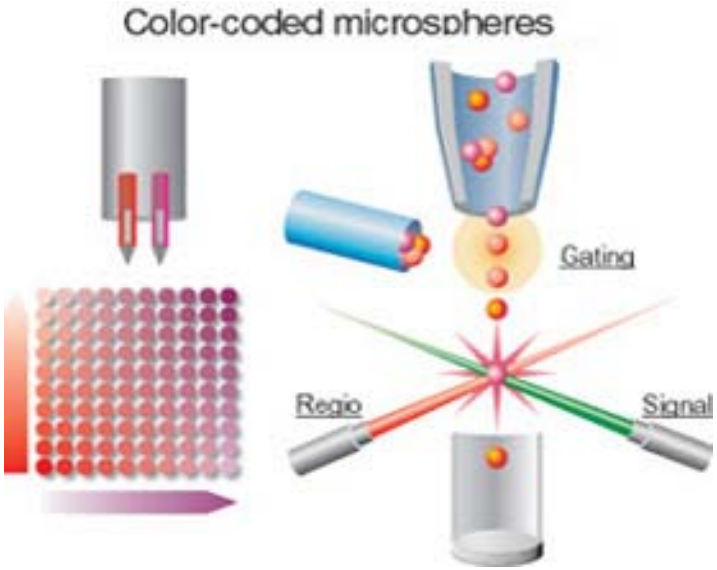
Luminex xMAP Technology

Luminex technology, developed about 15 years ago

Successfully used in wide range of applications in drug discovery, basic research and diagnostics

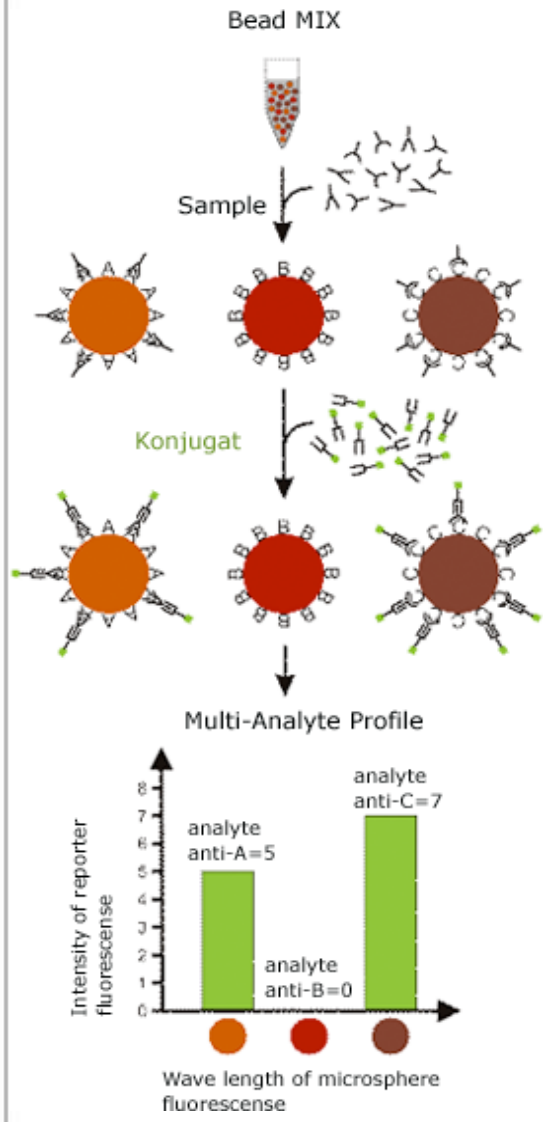
Luminex multiplex assays:

- **Reduce costs, labour and hands-on time**
- **Reduce sample and reagent volume**
- **Front end automation (Deregt et al, 2006)**
- **Highly sensitive due to fluorescence**



Combines flow cytometry, fluorescently-dyed magnetic beads, lasers, and digital signal processing.

Luminex xMAP Technology



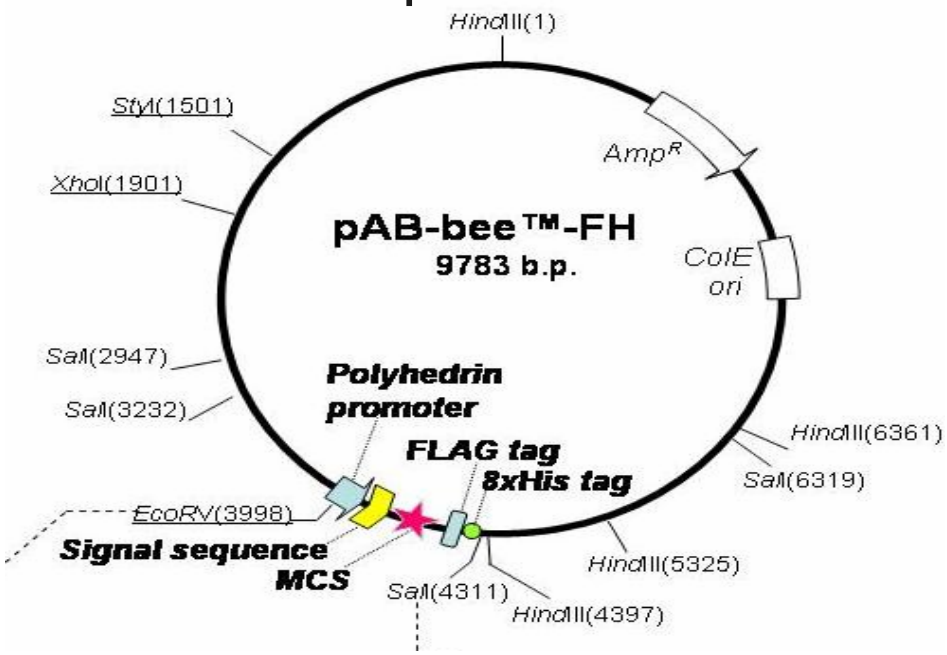
Antigens are coupled to the microspheres- 1 antigen to 1 bead.

The fluorescence of the reporter (Streptavidin-R-Phycoerythrin) bound to a biotinylated second antibody determines antigen concentration (green laser).

The fluorescence of the colour-coded bead identifies antigen type (red laser).

Production of Antigens by Baculovirus

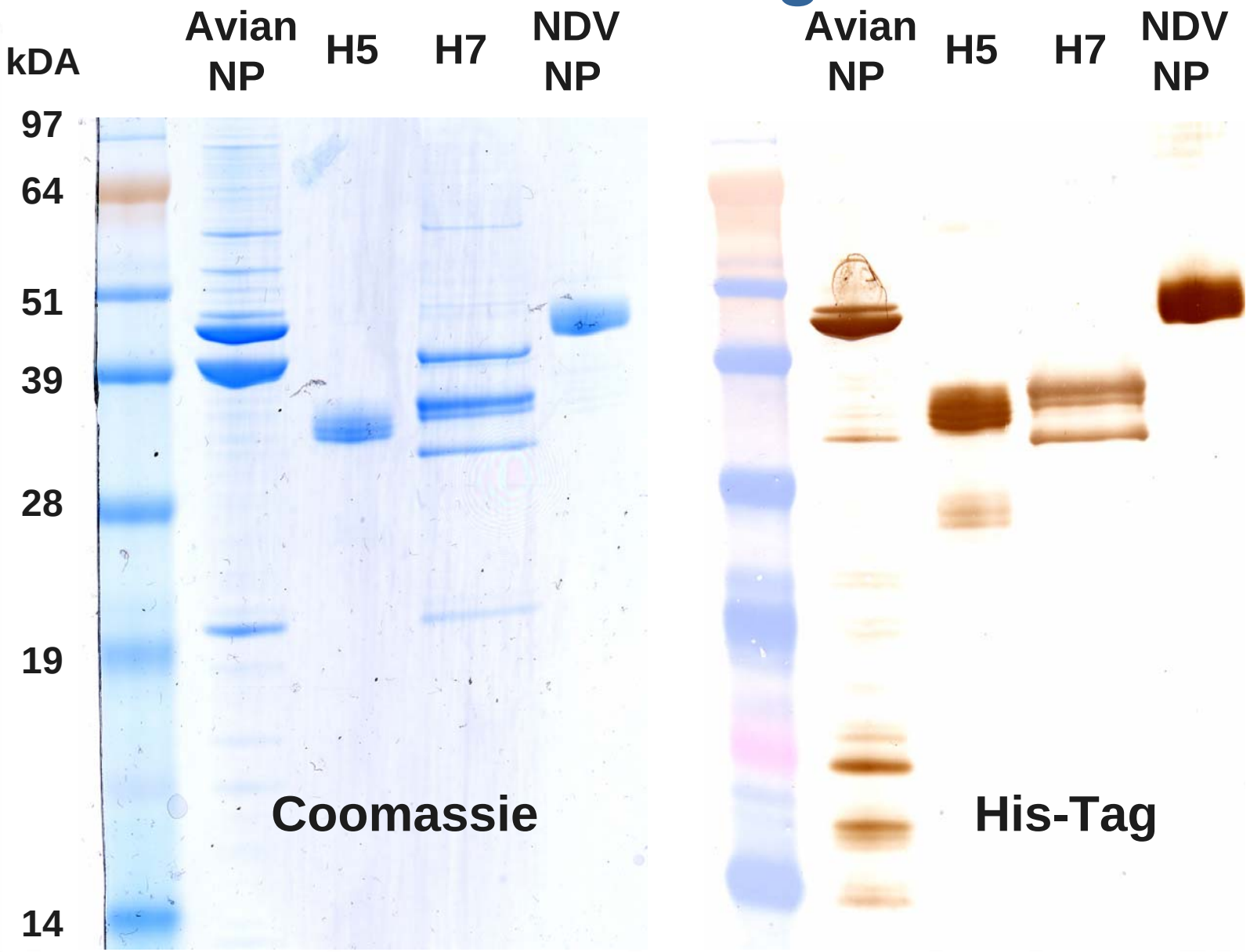
Full length segments or gene fragments are cloned into a transfer vector such as pAB-bee-FH:



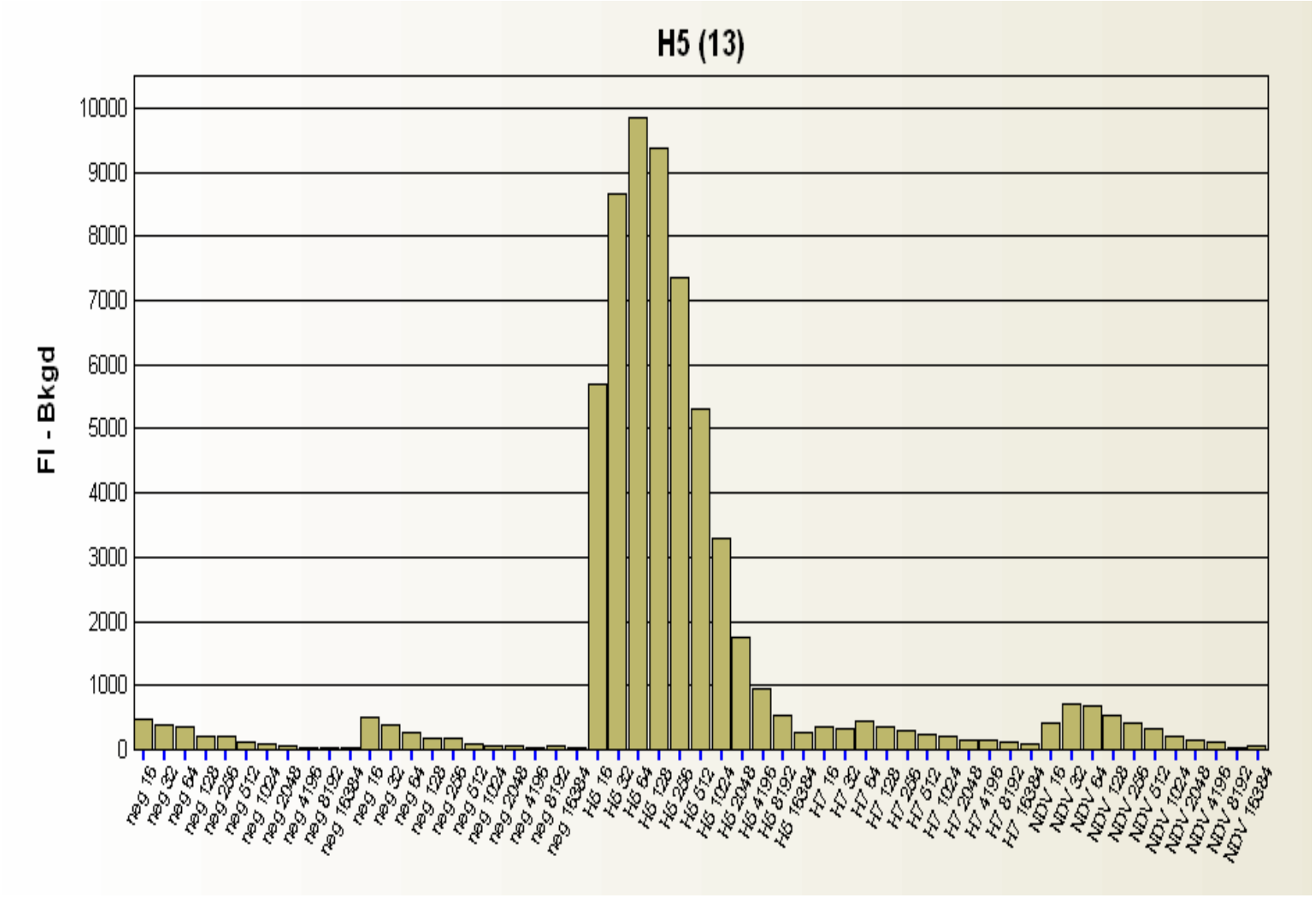
Advantages: C-terminus his-tag for purification of protein, proteins should be secreted into culture media

Co-transfection with linearized baculovirus DNA

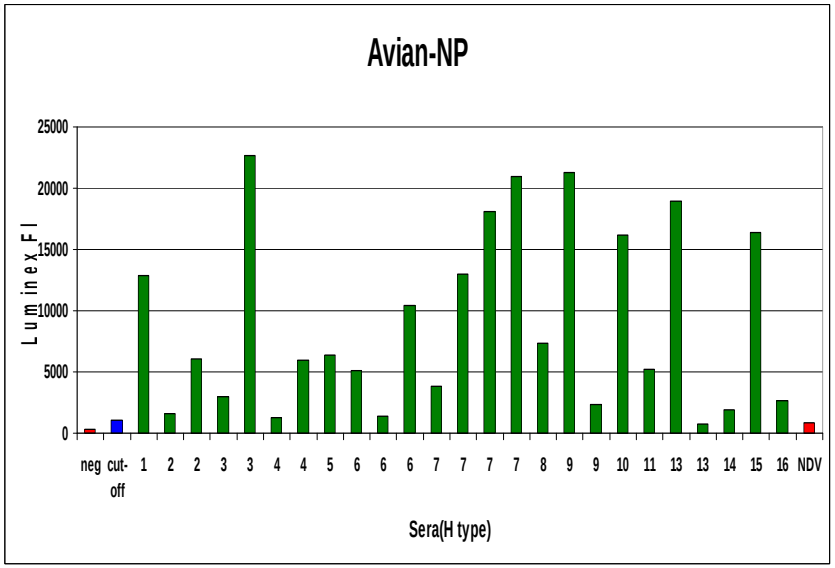
Luminex Antigens

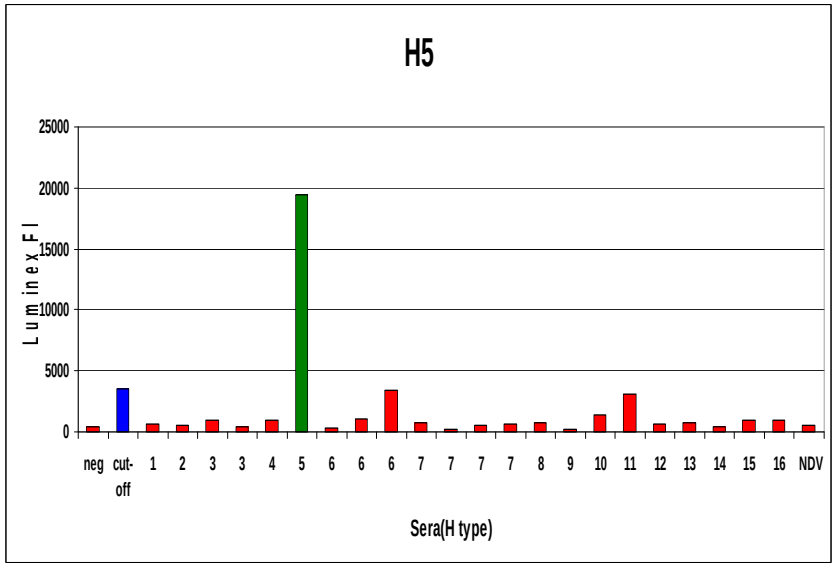


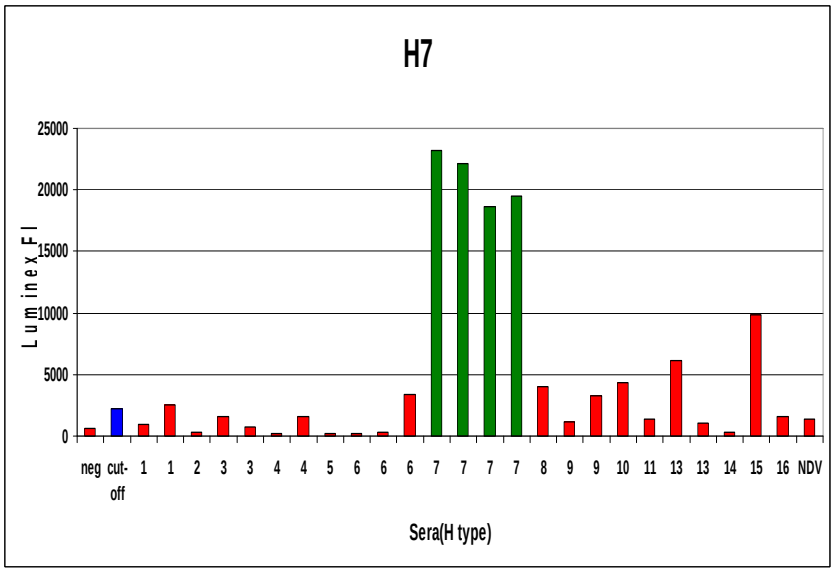
Example: Titration of Antigens

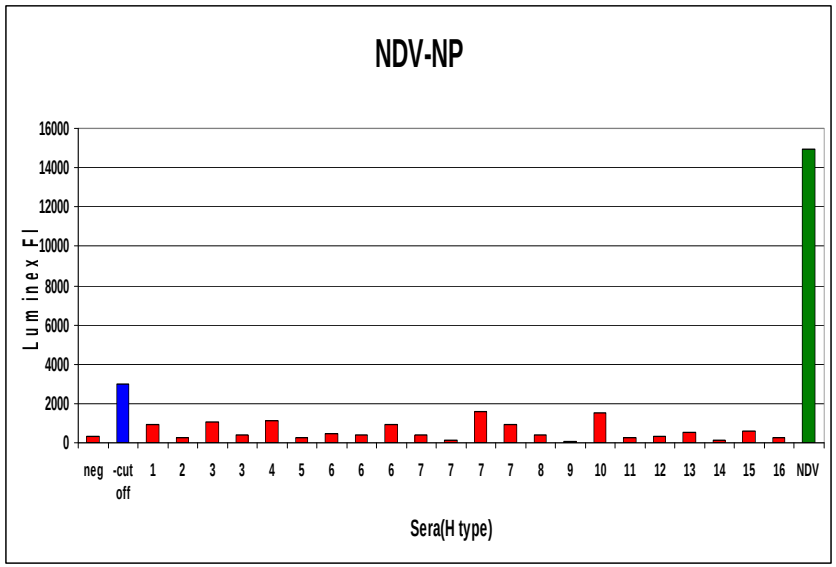


Specificity of Multiplex Assay

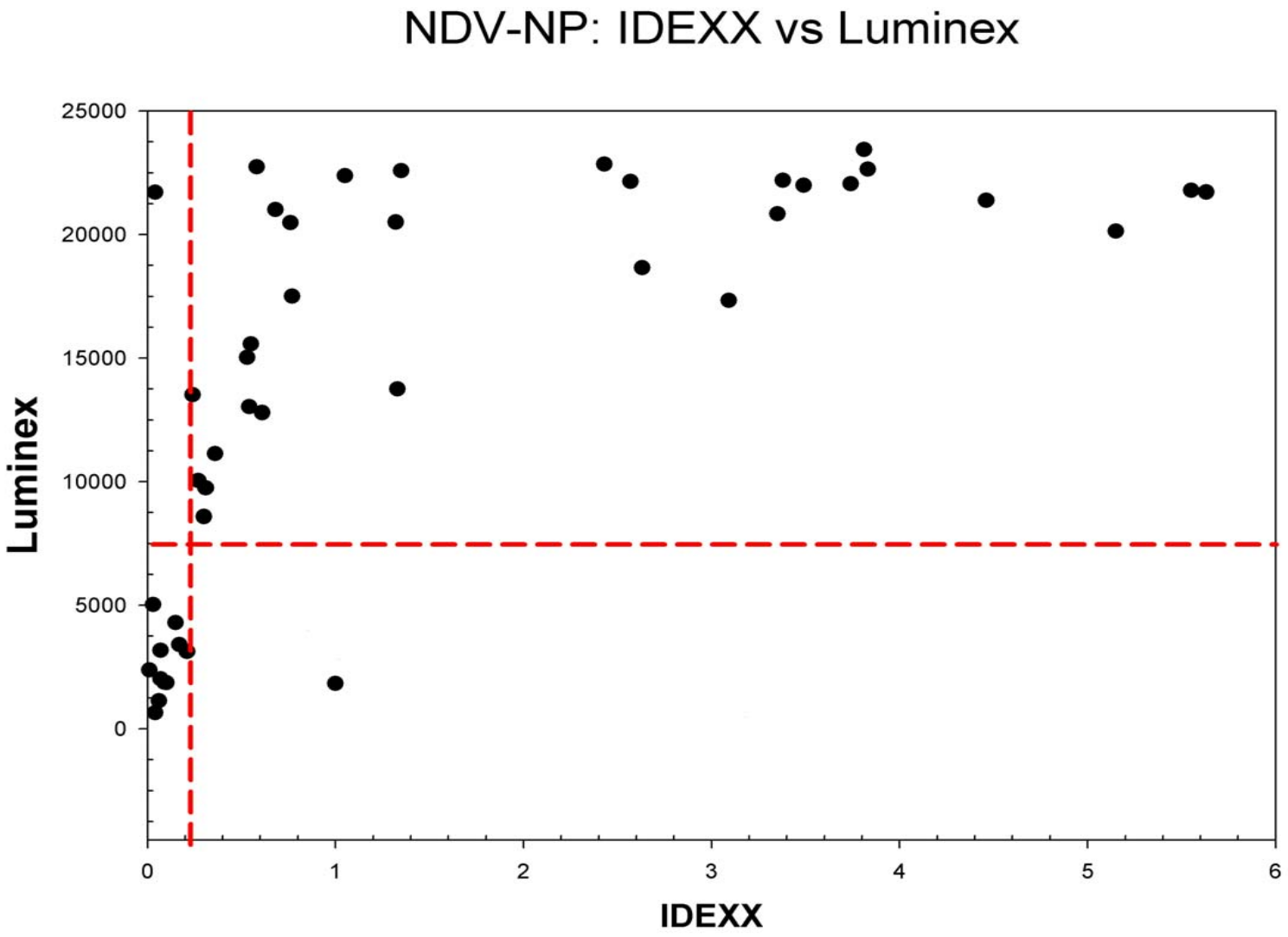




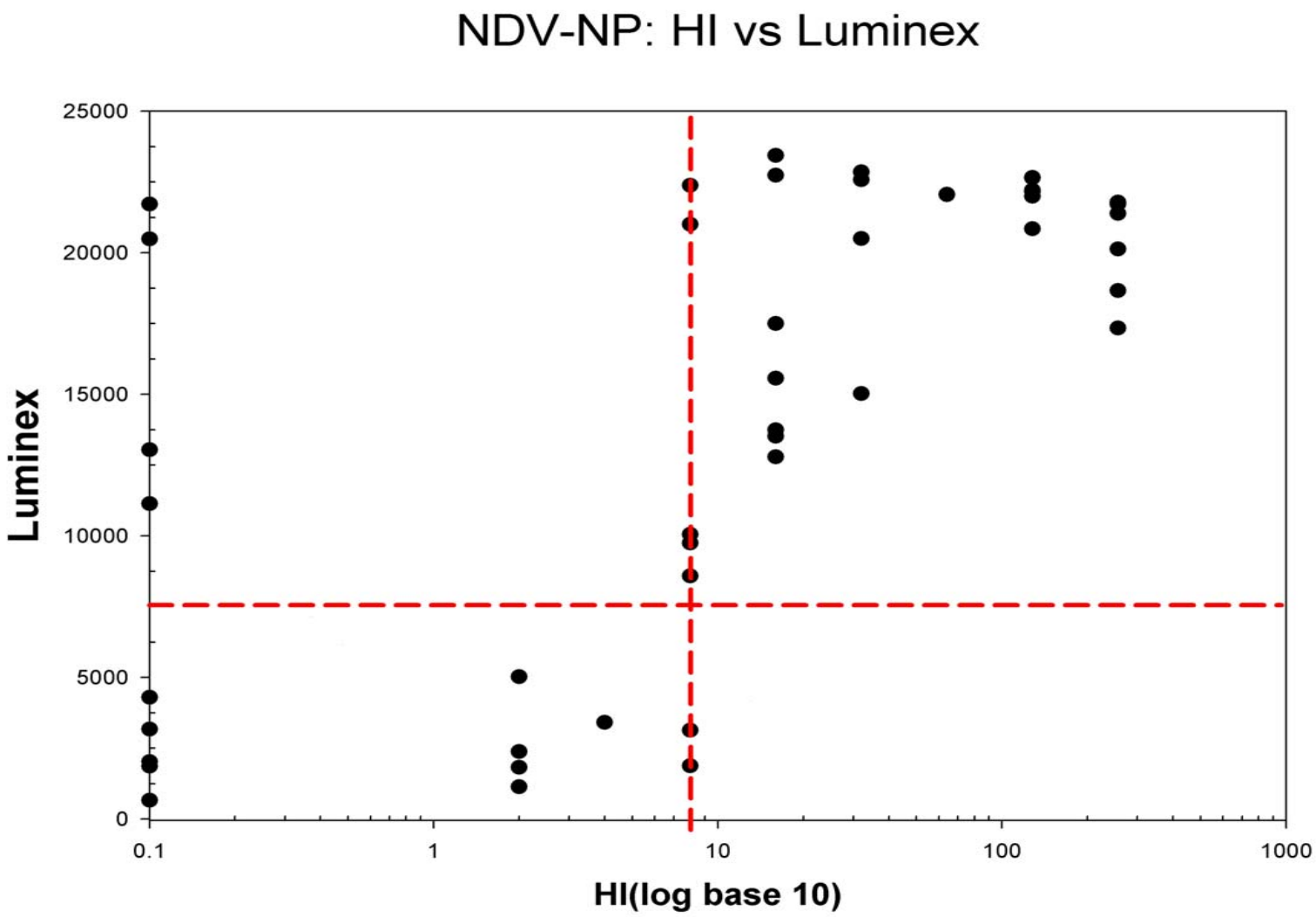




NDV-NP: IDEXX vs Luminex

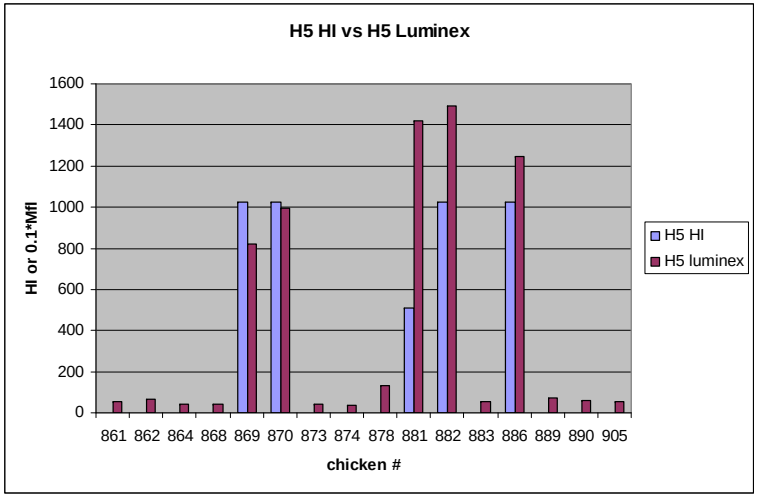


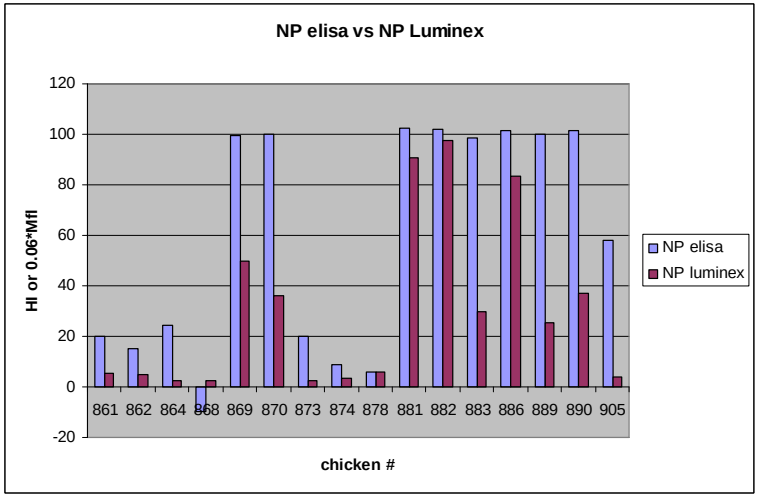
NDV-NP: HI vs Luminex

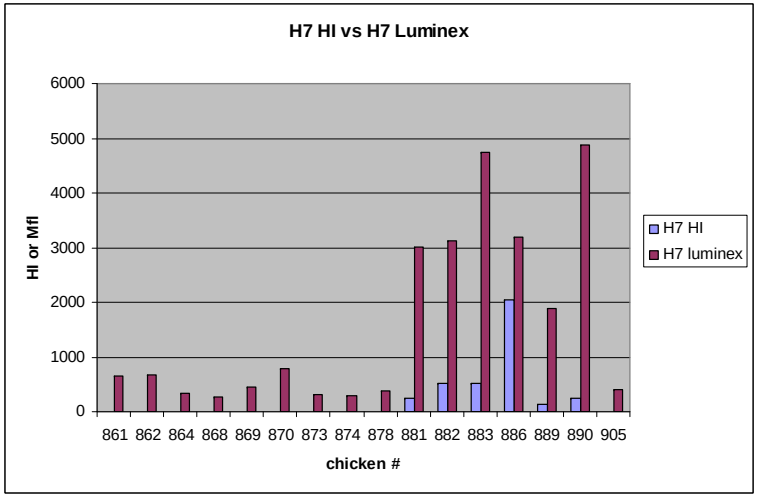


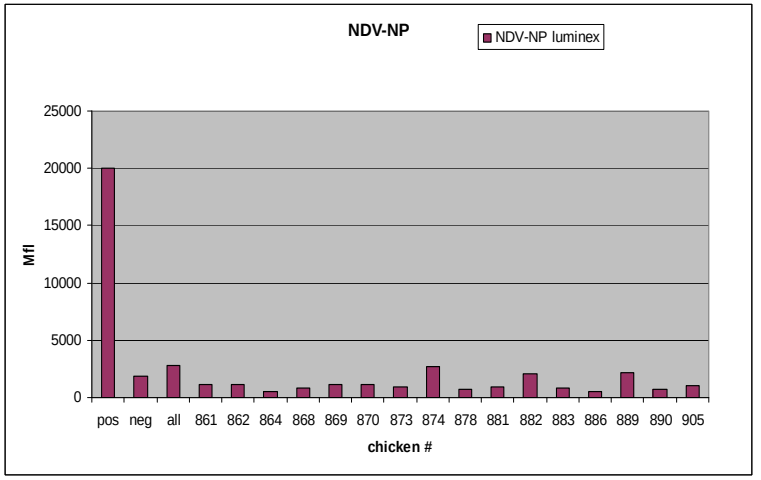
Chicken Vaccine Study Results

A/Chicken/BC/CN-006/2004 - H7N3 (Pre-exposure)
A/Swan/Germany/R65/2006 - H5N1 (Challenge)









CONCLUSIONS

Baculovirus proteins are suitable antigens for the multiplex assay but they may need to be further purified

The multiplex assay shows good correlation with both ELISA and HI assays

This multiplex assay shows good sensitivity and specificity (H5 vs H6 and H7 vs H15 issues – same as in HI assays)

Additional HA and NA recombinant antigens are available for incorporation into the multiplex assay

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