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## Alternative immunoassay techniques for AIV detection: mini review

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### Abstract

In both methods, the detection involved sandwiching of the target AIV between monoclonal antibodies for nucleoproteins and for matrix proteins. In the fluorescent DNA barcode-based immunoassay, fluorophore-tagged oligonucleotides were used as surrogates for signal detection with sensitivity comparable to conventional RT-PCR for allantoic fluid containing AIV. While in the fluorescent bead-based immunoassay, the fluorescent beads were used as the direct detection signal from AIV.

**Keywords:** avian influenza, biobarcode immunoassay, fluorescent beads, immunoassay

### Introduction

Avian flu or bird flu is a highly contagious disease caused by Influenza Virus A type. Influenza viruses belong to *Orthomyxoviridae* family and are RNA viruses having 8-segmented viral genome. AIV causes significant economic losses to the poultry industry worldwide and threatens human life with pandemic. Globally several outbreaks of AIV has occurred with rapid spread of infection with high morbidity and mortality in poultry. Therefore there is an urgent need for rapid diagnostic methods that enable early detection and improve measurements to control the AIV outbreak. Apart from molecular diagnostic methods immunological methods like ELISA <sup>[1]</sup> immunochromatographic strip test <sup>[2]</sup>. Microsphere immunoassay (MIA) is popular because of being rapid, inexpensive, but suffers drawback of low sensitivity. New techniques of Immunoassays based on DNA barcode and fluorescent beads could be an alternative for detection of AIV via immunoreactions between surface antigens (nucleoproteins, matrix proteins) of the AIV and their developed antibodies.

BioBarcode assay was proposed by Nam *et al.* is the only approach that works with sensitivity like of PCR without enzymatic amplification <sup>[4]</sup>. It utilizes two types of particles (a) magnetic micro particle (MMP) functionalized with antibody (primary antibody) which is to capture and isolate the target analyte from the sample solution, and (b) another particle (gold nanoparticle, polystyrene or silica microparticle) anchored with secondary antibodies, which is specific to the same target, and double-stranded ds-DNA <sup>[5]</sup>. One strand of the ds-DNA is immobilized onto the secondary particle probe by covalent bond and the complementary DNA strand released easily by increasing the temperature after completion of the sandwiching immunoreactions. The DNA surrogates for the target of interest and is therefore called a DNA bio-barcode. This DNA bio-barcode can be detected by PCR, DNA microarrays, colorimetric assays, or fluorophore-based assays. Each particle is functionalized with a multitude of DNA strands and thus many DNA barcodes are released for each positive binding event resulting in amplification of the assay. The method has not only a wide dynamic range of detection but also a sensitivity equivalent to that of RT-PCR which outperforms all detection limits of immune-based tests so far. This technique in combination with the shorter detection time and possibility of adapting the method for detection of other viruses or microorganisms, make it a potential tool for surveillance of infectious diseases. Various steps involved are;

1. Preparation of sample
2. Preparation of magnetic microparticle immunoprob
3. Preparation of polystyrene microbead immunoprobe (PMP)
4. Immunoreaction and optical detection.

### Development of a fluorescent bead-based immunoassay for rapid detection of avian influenza virus

Most of the tests for AIV detection are largely based on immunoassays which are assays are

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rapid but have low sensitivity; it is a major concern for mass screening of the AIV. An alternative simple approach lab-on-chip would be to detect the fluorescence signal directly with a simple optical detection system that is suitable for on-field screening tests. In the immunoassay based on fluorescence detection, nano and micro beads conjugation with antibodies or antigen have been used to obtain high sensitive sensing for detection of infectious [6]. These fluorescent beads carry a high amount of fluorophores that generate a photostable and strong fluorescent signal. Moreover, with a high surface-to-volume ratio (SVR), the beads provide better binding sites for the targets. A fluorescent immunoassay (FIA) has been developed for the detection of antibodies against nucleoprotein (NP), matrix and non-structural proteins of [7] and also for multiplex detection of both NP and H5 [8] using spectrophotometer. The procedure requires inactivated virus, Monoclonal antibody (mAb) against the AIV nucleoprotein (NP) and matrix proteins (MP) of AIV. Steps involved in the procedure are

### 1. Antibody conjugation to beads and fluorophore

- a) NP antibody conjugation to magnetic bead
- b) Conjugation of MP antibody on fluorescent carboxyl beads.

### 2. Antibody conjugation confirmation Development of fluorescent immunoassay.

**3. Fluorescent immunoassay:** The two images differentiated by the quantity of fluorescent beads (white spots) confirm the capture of virus by magnetic bead and the formation of sandwich immunoassay. A low background and a low aggregation could be achieved in the developed FIA due to the combined effect of Casein as a blocking buffer and the presence of the Tween20. The replacement of BSA by Casein proved beneficial in reducing the non-specific binding. Lastly Image analysis is done.

### Conclusion

Fluorophore –DNA barcode combined with bead based immunoassay is method which can work as an alternative but is lesser sensitive when compared to PCR. A simple fluorescent bead-based immunoassay (FIA) has been developed for rapid detection of virus in less than 2h. If the FIA is further optimized such as reducing the size of magnetic bead, sensitivity similar of the method could be similar or higher than RT-PCR could be achieved. The developed FIA can be easily integrated with Lab-on-Chip and using portable fluorescent scanner, the system can be used as a sensitive tool for screening of AIV in the field.

### References

1. Sala G, Cordiol P, Moreno-Martinb AM, Tollis E, Brocchi A, Piccirillo, *et al.* Avian Diseases. 2003; 47:1057-1059.
2. Cui S, Chen C, Tong G. Journal of Virology Methods. 2008; 152:102-105.
3. Munch m, Nielsen LP, Handberg KJ, Jorgensen. Archea Virology. 2001; 146:87-97.
4. Nam JM, Stoeva SI, Mirkin BC. Journal of American Chemical Society. 2004; 126:5932-5933.
5. Nam JM, Thaxton CS, Mirkin CA. Science. 2003; 130:1884-1886.
6. Tallury A, Malhotra L, Byrne M, Santra S. Nanobioimaging and sensing of infectious diseases,

Advanced Drug Delivery Reviews. 2010; 62(4-5):424-437.

7. Watson DS, Reddy SM, Brahmakshatriya V, Lupiani B. A multiplexed immunoassay for detection of antibodies against avian influenza virus. Journal of Immunological Methods. 2009; 340(2):123-131.
8. Lupiani B, Mozisek B, Mason PW, Lamichhane C, Reddy SM. Simultaneous Detection of Avian Influenza Virus NP and H5 Antibodies in Chicken Sera Using a Fluorescence Microsphere Immunoassay, Avian Diseases. 2009; 54(1):668-672.