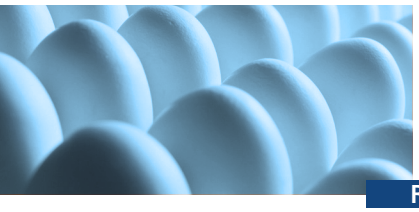
charles river



RESEARCH MODELS

Poultry Magnetic Bead MFIA® Qualification

Purpose and Background

Poultry flocks are tested weekly by serology and molecular diagnostic methods for routine flock health surveillance. Multiplex immunoassays, including the Multiplexed Fluorometric ImmunoAssay (MFIA®) based on Luminex® polystyrene (PS) beads, have been in use for more than 15 years for routine serosurveillance of laboratory animals[1-5]. A new generation MFIA, using magnetic MagPlex® beads (microspheres), was developed for serosurveillance of poultry flocks.

The MFIA is a serologic assay in which an array of uniquely colored microbeads are coated with specific antigens and act as the solid phase of the immunoassay[1-3]. Each unique color can be used to covalently bind a unique antigen, thus multiple antibody determinations can be achieved using a single serum or plasma sample in a single test well. Test sample and antigen-coated microbeads are added to the test well. If antibodies specific to any of the coupled antigens are present in the sample, they will bind to the antigen on the microbeads. Following this primary incubation, the beads are washed to remove non-specific, unbound proteins. A biotinylated anti-species immunoglobulin (conjugate) is added per test well and binds to antibodies bound to the antigen-coupled beads. A second wash is performed to remove unbound conjugate. A fluorescent reporter molecule, streptavidin phycoerythrin, is added to the test well. This molecule exploits biotin-avidin binding, resulting in a detectable fluorescent signal on beads where antibody-bound conjugate is present. The test plate is analyzed by a fluorometer, which then analyzes each bead, identifying the unique color of each bead and simultaneously measures any fluorescent signal. These data are compiled and analyzed by computer and a Mean Fluorescent Intensity (MFI) is calculated for each antigen on each serum sample. An MFI cutoff value is established and samples are classified as either negative, borderline, or positive based on the MFI.

MagPlex beads are quick and easy to separate from a solution using a magnetic separator. Magnetic beads have several advantages over PS beads, including no requirements for pre-filtration of test samples and expensive filter bottom plates, which can leak during incubations.

The purpose of this qualification was to perform the MFIA using infectious agent antibody-positive and -negative sera, and to demonstrate that the MagPlex bead platform is equally sensitive and specific as the PS-based MFIA. Efficacy of these next generation MagPlex beads was compared to PS beads in a validation study using 16 known positive sera from infected birds for one or more of the specific pathogen free (SPF)-designated infectious agents (Table 1). A set of 16 known negative sera were used from historically known SPF poultry flocks. All samples were tested by two different technicians on three different days, for a total of six runs.

Materials and Methods

Purified whole virus lysate and single recombinant viral antigens for common infectious agents found in poultry flocks were individually coupled to PS and MagPlex beads. In addition, several system and sample suitability controls, including tissue control beads to determine sample-related nonspecific antibody binding, chicken IgY, and rabbit anti-chicken IgY beads, were added to the test panel to validate individual runs of the MFIA.

Prepared by:
Charles River Research
Animal Diagnostic Services
251 Ballardvale Street
Wilmington, MA 01887
USA
Date: 13 November 2019

This procedure has been described previously[4], and was performed following the online MFIA® method manual. For each individual bead assay, the net median fluorescence intensity signal (MFI) was calculated by subtracting the tissue control (TC) signal from the antigen (AG) signal. In the following tables and graphs, the results are presented as net MFI/1,000 (or Net MFI in thousands). In this study, values of < 1.5 and ≥ 3 were classified as negative and positive, respectively. Net signals between these cutoffs were called equivocal/indeterminate.

Standard positive assay controls (immune sera) consisted of antiserum or antiserum pools that were collected from naturally infected or intentionally infected birds. Standard negative assay controls (non-immune [NI]) were serum pools collected from historically known antibody-negative SPF birds.

Briefly, analytical sensitivity (limit of detection [LOD]), diagnostic sensitivity (DSn), and diagnostic specificity (DSp) of the MagPlex beads were evaluated. The LOD comparison between PS and MagPlex beads evaluated high and mid-range positive assay controls that were serially diluted twofold into standard assay diluent (Primary Diluent-1). A total of six dilutions were analyzed, beginning undiluted and ending at 1/32. The titration data points represent the average for all MFIA panel agents, with the assay net MFI/1,000 reported.

Diagnostic assay sensitivity (DSn) and reproducibility were measured by analyzing 16 known positive test samples by two technicians (Tech 1 and Tech 2) performing three separate MFIA runs on different days, for a total of six runs. In addition, DSp was measured by analyzing 16 known negative test samples in the same manner.

List of Infectious Agents

Agent-specific antigens and internal controls were coupled individually to PS or MagPix beads. Bead sets for agents used for routine serosurveillance were pooled to create PS or MagPix bead panels.

Table 1. Poultry MFIA® Bead Panel Members

Bead Panel Type	Infectious Agent	
	Full Name	Abbreviation
Infectious Agents	Avian encephalomyelitis virus	AE
	Avian influenza virus	AI
	Avian leukiosis virus subgroup A	ALV-A
	Avian leukiosis virus subgroup B	ALV-B
	Avian leukiosis virus subgroup J	ALV-J
	Avian nephritis virus	ANV
	Avian adenovirus	AvAdeno
	Marek's disease virus (conventional antigen)	cMDV
	Egg drop syndrome virus	EDS
	Fowl pox virus	Fowl pox
	Avian hepatitus E virus	HEV
	Infectious bursal disease virus	IBDV
	Infectious bronchitis virus	IBV
	Infectious laryngotracheitis virus	ILTV
	Mycloplasma synoviae	MSYN
	Newcastle disease virus	NDV
	Avian parmyxovirus type 2	PMV-2
	Reticuloendotheliosis virus	REV
	Salmonella species	SALM
Internal Controls	Wild-type baculovirus species	wBAC
	Chicken immunoglobulin Y	Chicken IgY
	Rabbit anti-chicken immunoglubulin Y	Rb α chicken IgY

Results Summary

Limit of Detection (LOD)

To assess the analytical sensitivity of the MagPlex assays, control sera were titrated and PS and MagPlex bead scores were compared. The graph for analytical performance in Figure 1 below shows MFIA endpoint titration curves of serially diluted MFIA positive assay controls. Average scores for all assays in each panel were plotted against serum dilutions.

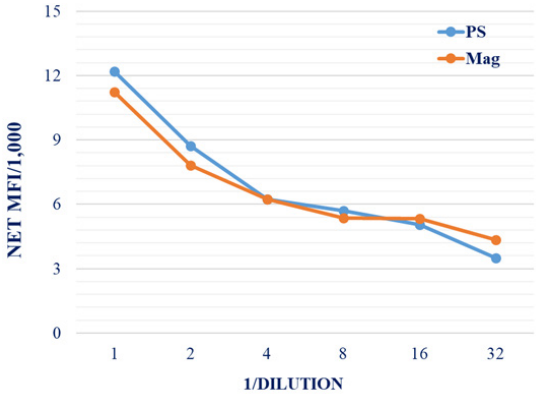


Figure 1. Poultry LOD Titrations of MFIA® Control Sera. Six serial two-fold dilutions of MFIA assay controls were analyzed by PS and MagPlex MFIA. The titration data points represent the average for all bead panel agents with assay net MFI/1,000 reported from a Bio-Plex® 200 instrument.

Diagnostic Sensitivity, Specificity, and Repeatability

To determine DSn, data from 16 known positive antiserum samples were analyzed using a Bio-Plex® instrument. These samples consisted of monospecific and polyspecific poultry antiserum.

Each individual agent (antigen bead) of the test bead panels was considered an individual assay. Not all assays in a panel were expected to score positive for each known positive sample; therefore, only antigen-specific reactions were considered for calculations of sensitivity and specificity. A summary of net scores averaging all assays for each control type is summarized in Table 2.

Table 2. Control Scores: Net scores are average of all poultry MFIA assays are displayed by control type.

Bead	Average Net Scores by Control Type		
	Positive	Non-Immune	Blank
Polystrene	11.0	0.0	0.0
Magnetic	9.3	0.0	0.0

DSn is displayed by species and technician in Table 3. Calculations compared the total number of expected positive reactions (assays) for Tech 1 and Tech 2 for each of their three test runs.

Table 3. Diagnostic sensitivity (DSn): Sixteen known positive immune sera were tested in three separate runs by each of two technicians. Sample net MFI/1,000 of < 1.5 and ≥ 3 were classified as negative and positive, respectively. The values shown represent the average for all panel assays with net MFI/1,000 reported.

Table 3. Diagnostic sensitivity (DSn)

Tech	Magnetic				Polystrene			
	# Tested	# Pos	% Pos	Score	# Tested	# Pos	% Pos	Score
Tech 1	111	110	99.1	11.8	111	111	100.0%	12.2
Tech 2	111	110	99.1	11.2	111	111	100.0%	11.1
Total	**222**	**220**	**99.1**	**11.5**	**222**	**222**	**100.0%**	**11.6**

DSp was calculated comparing the total number of expected negative reactions for the 16 known negative samples to those of both Tech 1 and Tech 2 for each of their three test runs. The known negative samples were collected from historically known SPF poultry flocks for the tested agents. Data was analyzed from a Bio-Plex instrument and summarized in Table 4.

Table 4. Diagnostic specificity (DSp): Sixteen known negative sera from historically antibody-negative sources were tested in three separate runs by each of two technicians. Sample net MFI/1,000 of < 1.5 and ≥ 3 were classified as negative and positive, respectively. The values shown represent the average for all panel assays with net MFI/1,000 reported.

Table 4. Diagnostic specificity (DSp)

Tech	Magnetic				Polystrene			
	# Tested	# Pos	Specificity	Score	# Tested	# Pos	Specificity	Score
Tech 1	1899	0	100.0%	0.0	1899	0	100.0%	0.0
Tech 2	1899	5	99.7%	0.0	1899	2	99.9%	0.0
Total	**3798**	**5**	**99.9%**	**0.0**	**3798**	**2**	**99.9%**	**0.0**

Conclusion

A total of more than 4,000 assays were performed and the analytical performance of the MagPlex MFIA®, including sensitivity, specificity, and limit of detection, was found to be comparable to, or better than, those obtained by PS MFIA for poultry sera. Overall diagnostic sensitivity of the MagPlex MFIA was very similar; 99.1% compared to 100% for PS MFIA. Diagnostic specificity of both MagPlex and PS MFIA were 99.9%, suggesting that MagPlex MFIA is an acceptable alternative assay for serodiagnosis of adventitious infectious agents of poultry flocks.

References

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