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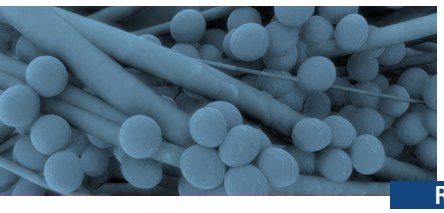

RESEARCH MODELS AND SERVICES

Non-Human Primate Magnetic Bead MFIA® Qualification

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Date: 18 October 2018

Purpose and Background:

Non-human primates (NHPs) in breeding colonies are periodically tested by serology and molecular diagnostic methods for routine colony health surveillance. Multiplex immunoassays, including the multiplexed fluorometric immunoassay (MFIA®) (1–4), based on Luminex® polystyrene (PS) beads have been in use for more than 15 years for routine serosurveillance of laboratory animals. A next-generation MFIA® using the magnetic MagPlex beads (microspheres) was developed for serosurveillance of NHP colonies.

The MFIA® (1–4) 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. 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. This device 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 index (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 solution using a magnetic separator. Magnetic beads have several advantages over PS beads, including not requiring 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 NHPs or humans 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 macaque (rhesus and cynomolgus) colonies. 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 in laboratory NHPs 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, human IgG, and goat anti-human IgG beads, were added to the test panel to validate individual runs of the MFIA®.

The MFIA® procedure has been described previously (4) and performed following the online 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 NHPs or humans. Standard negative assay controls (non-immune) were serum pools collected from historically known antibody-negative SPF macaque (rhesus or cynomolgus) colonies.

Briefly, analytical sensitivity (limit of detection, LOD), diagnostic sensitivity (DSn), and 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 two-fold 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, diagnostic assay specificity (DSp) was measured by analyzing 16 known negative test samples in the same manner.

Results Summary

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: NHP MFIA® Bead Panel Members

Bead Panels	NHP Assessment			
Infectious Agents	SIV mac	HTLV-1/2	rMeasles	rMRV
	SIV gp120	STLV p21	SFV	rLCV
	SRV-2	HVP-2	SV-40	SVV
	SRV gp-20	rBV glycoB	RhCMV	Chagas
Internal Controls	wBAC	Human IgG	Goat anti-human IgG	

Limit of Detection (LOD)

To assess the analytical sensitivity of the MagPlex assays, control sera were titrated and PS and MagPlex bead scores compared. The graph for analytical performance in Figure 1 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.

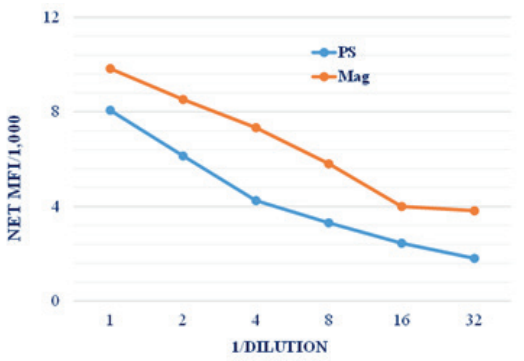

Figure 1. NHP LOD Titrations of MFIA® Control Sera

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 MagPlex assay sensitivity (DSn), data from 16 known positive antiserum samples were analyzed using a Bio-Plex instrument. These samples consisted of NHP and human monospecific and polyspecific antisera.

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 and 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

Bead	Average Net Scores by Control Type			
	High	Low	NI	Blank
Polystyrene	16.3	8.8	0.0	0.1
Magnetic	15.4	9.7	0.0	0.4

Net scores are average of all NHP assays are displayed by control type.

Diagnostic sensitivity is displayed by species and Technician (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)

Tech	Magnetic			
	# Tested	# Pos	% Pos	Score
Tech 1	624	617	98.9%	14.2
Tech 2	624	615	98.6%	14.1
Total	**1248**	**1232**	**98.7%**	**14.2**
Tech	**Polystyrene**			
	# Tested	# Pos	% Pos	Score
Tech 1	624	607	97.3%	14.4
Tech 2	624	587	94.1%	12.5
Total	**1248**	**1194**	**95.7%**	**13.5**

16 known positive immune sera were tested in three separate runs by each of two technicians. Sample Net MF1/1,000 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.

Specificity (DSn) 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 NHP colonies for the tested agents. Data was analyzed from a Bio-Plex instrument (Table 4).

Table 4: Diagnostic specificity (DSp)

Tech	Magnetic			
	# Tested	# Pos	Specificity	Score
Tech 1	858	1	99.9%	0.0
Tech 2	858	2	99.8%	0.0
Total	**1716**	**3**	**99.8%**	**0.0**
Tech	**Polystyrene**			
	# Tested	# Pos	Specificity	Score
Tech 1	858	0	100.0%	0.0
Tech 2	858	0	100.0%	0.0
Total	**1716**	**0**	**100.0%**	**0.0**

16 known negative sera from SPF historically antibody negative sources were tested in three separate runs by each of two technicians. Sample Net MF1/1,000 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.

Conclusion

A total of more than 2,900 assays were performed and the analytical performance of the MagPlex MFIA®, including selectivity and limit of detection, was found to be comparable to or better than those obtained by PS MFIA for NHPs. Overall diagnostic sensitivity of the MagPlex MFIA was 98.7% compared to 95.7% for PS MFIA. Diagnostic specificity of both MagPlex and PS MFIA were $> 99\%$, suggesting that MagPlex MFIA is an acceptable alternative assay for serodiagnosis of adventitious infectious agents of laboratory NHPs.

References

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