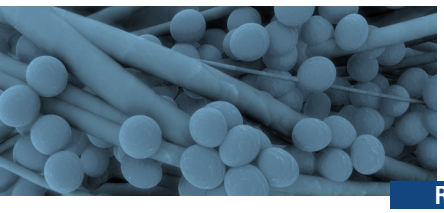
charles river



RESEARCH MODELS AND SERVICES

Mouse and Rat Magnetic Bead MFIA® Qualification

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Purpose and Background:

Animals in breeding colonies are periodically tested by serology and molecular diagnostic methods for routine colony health surveillance (1). Multiplex immunoassays, including the Multiplexed Fluorometric ImmunoAssay (MFIA®) (2–4), based on Luminex® polystyrene (PS) beads have been in use for more than 15 years for routine sero-surveillance of laboratory animals. A new generation MFIA® using magnetic MagPlex® beads (microspheres) was developed for sero-surveillance of mouse and rat colonies.

The MFIA® (1,2) 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 samples 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, identifies the unique color of each bead and simultaneously measures any fluorescent signal. The data compiled and analyzed by computer to calculate a mean fluorescent index (MFI) 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.

Because MagPlex® beads are quick and easy to separate from solution using a magnetic separator, they offer several advantages over PS beads. Pre-filtration of test samples and expensive filter bottom plates which can leak during incubations, are not required when using MagPlex® beads.

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 were compared to PS beads in a validation study assessing limit of detection (diagnostic sensitivity, DSn) and diagnostic specificity (DSp).

Materials and Methods

Purified whole virus lysate and single recombinant viral antigens for common infectious agents in laboratory mice and rats 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, species-specific IgG, and anti-IgG beads, were added to each 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, while 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 or experimentally infected mice or rats. Standard negative assay controls (non-immune) were serum pools collected from historically known antibody negative SPF colonies.

The assay limit of detection (LOD) was evaluated with serially diluted known positive sera (MFIA® high and mid-range immune control sera for each infectious agent) that had been serially diluted two-fold into standard assay diluent (Primary Diluent-1). A total of six dilutions, beginning undiluted and ending at 1/32, were assayed once in duplicate wells using polystyrene beads and magnetic beads. The titration data points represent the average for all MFIA® panel agents with the assay net MFI/1,000 reported. The highest dilution that demonstrated a positive response was selected as the assay LOD.

Additional LOD analysis was performed using sequentially collected monospecific positive sera. Where available, sequentially collected monospecific positive rat and mouse serum samples were assayed once in duplicate wells using only magnetic beads. The time point that demonstrated a positive response was selected as the assay LOD (lowest dilution that gave positive scores) compared to historic polystyrene bead data.

Diagnostic assay sensitivity (DSn) was assessed using 16 known positive sera from naturally or experimentally infected rodents (mice and rats) for one or more of the specific pathogen free (SPF) designated infectious agents (Tables 1 and 2). A set of 16 known negative sera for each species were used from historically known SPF colonies to assess the diagnostic assay specificity (DSp). All samples were tested by two different technicians on three different days, for a total of six runs.

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 sero-surveillance were pooled to create species-specific PS or MAGPIX bead panels.

Table 1: Mouse and Rat MFIA® Bead Panel Members

Bead Panels	Mouse Assessment Plus		Rat Assessment Plus	
Infectious Agents	MPV	MAV-1 & 2	KRV	IDIR
	MVM	ECTRO	RMV	HANT
	NS-1	LDV	RPV	LCMV
	MHV	K	H-1	CARB
	MNV	POLY	NS-1	PIV-3
	TMEV (GD-VII)	MCMV	RTV	REO-3
	EDIM	HANT	SDAV	CPIL
	SEND	ECUN	P.carninii	ECUN
	PVM	CARB	MAV-1 & 2	
	REO-3	MTLV	SEND	
	MPUL	PHV	PVM	
	LCMV	CPIL	MPUL	
Internal Controls	wBAC	MARTH	wBAC	MARTH
	Mouse IgG		Rat IgG	
	Goat anti-mouse IgG		Goat anti-rat IgG	

Results Summary

Limit of Detection (LOD)

To evaluate the limit of detection of the MagPlex® assays versus polystyrene control, sera were titrated and scores between the two bead types were compared. The graphs for analytical performance in Figures 1a-1b below show MFIA® endpoint titration curves of serially diluted MFIA® positive assay controls for individual species and bead type. Average scores for all assays in each species-specific panel were plotted against serum dilutions.

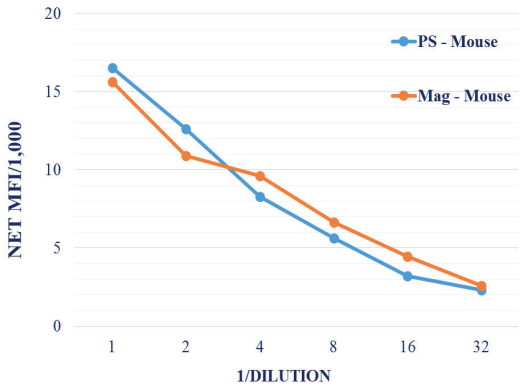

Figure 1a. Mouse

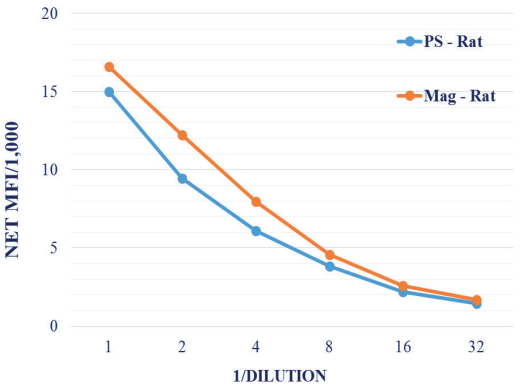

Figure 1b. Rat

Figures 1a-1b. Mouse and Rat 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.

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 mouse and rat monospecific 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 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:

Net scores are average of all assays in the species-specific bead panels and are displayed by control type.

Bead	Species	Average Net Scores by Control Type			
		High	Low	NI	Blank
Polystyrene	Mouse	14.8	7.2	0.0	0.0
	Rat	13.1	5.8	0.0	0.0
	Total	**14.0**	**6.6**	**0.0**	**0.0**
Magnetic	Mouse	15.9	9.3	0.0	0.0
	Rat	15.7	8.8	0.0	0.0
	Total	**15.8**	**9.1**	**0.0**	**0.0**

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):

Sixteen 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 (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 mouse and rat colonies for the tested agents. Data was analyzed from a Bio-Plex instrument (Table 4).

Species	Tech	Magnetic				Polystyrene			
		# Tested	# Pos	% Pos	Score	# Tested	# Pos	% Pos	Score
Mouse	Tech 1	267	264	98.9%	15.4	267	262	98.1%	13.9
	Tech 2	267	252	94.4%	12.8	267	252	94.4%	12.0
Mouse Total		**534**	**516**	**96.6%**	**14.1**	**534**	**514**	**96.3%**	**13.0**
Rat	Tech 1	285	274	96.1%	14.3	285	284	99.6%	12.8
	Tech 2	285	276	96.8%	11.6	285	285	100.0%	13.5
Rat Total		**570**	**550**	**96.5%**	**12.9**	**570**	**569**	**99.8%**	**13.2**
Grand Total		**1104**	**1066**	**96.6%**	**13.5**	**1104**	**1083**	**98.1%**	**13.1**

Table 4: Diagnostic Specificity (DSp):

Sixteen known negative sera from SPF 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.

Species	Tech	Magnetic				Polystyrene			
		# Tested	# Pos	Specificity	Score	# Tested	# Pos	Specificity	Score
Mouse	Tech 1	2415	13	99.5%	0.0	2415	0	100.0%	0.0
	Tech 2	2415	6	99.8%	0.0	2415	0	100.0%	0.0
Mouse Total		**4830**	**19**	**99.6%**	**0.0**	**4830**	**0**	**100.0%**	**0.0**
Rat	Tech 1	1893	20	98.9%	0.0	1893	0	100.0%	0.0
	Tech 2	1893	17	99.1%	0.0	1893	0	100.0%	0.0
Rat Total		**3786**	**37**	**99.0%**	**0.0**	**3786**	**0**	**100.0%**	**0.0**
Grand Total		**8616**	**57**	**99.4%**	**0.0**	**8616**	**0**	**100.0%**	**0.0**

Sequentials

Serum specimens were collected temporally from experimentally infected rodents and tested by PS and MagPlex® beads to compare the earliest time of detectable seroconversion. Briefly, post-weaning CD-1® (ICR) mice or CD (SD) rats from Charles River barrier colonies were inoculated with diluted virus seed intranasally, orally, and/or intraperitoneally. Four animals were infected per time point. Animals were euthanized and blood collected on various days post-inoculation/infection (e.g., days 0, 7, 10, 14, 21, 28, and 35 DPI).

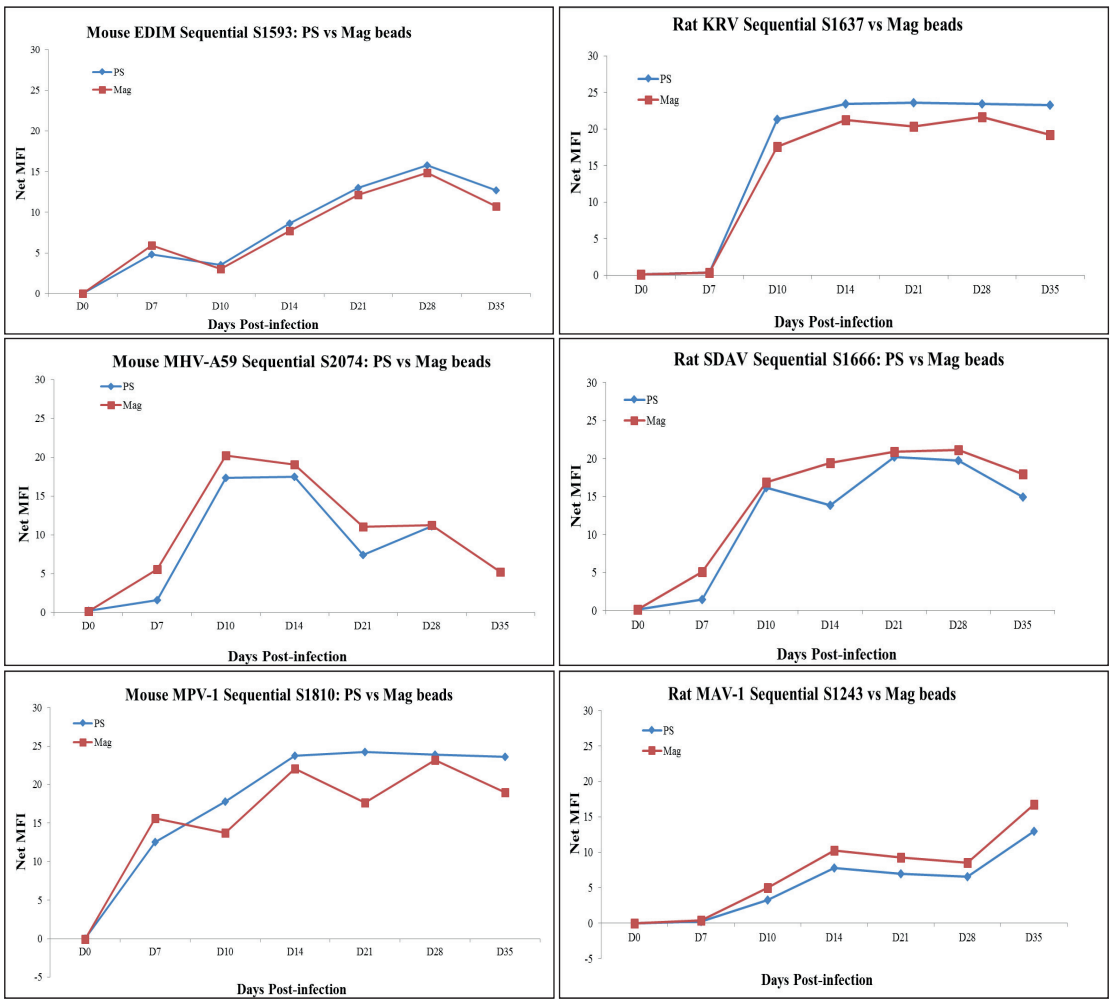

Figure 2a.

Figure 2b.

Figures 2a-2b. Seroconversion antibody profiles for three mouse agents (2a) EDIM, MHV strain A59, MPV-1 strain; and rat agents (2b) KRV, SDAV, MAV-1 strain. PS and MagPlex® bead seroconversion profiles were compared to verify equivalence between the two bead assays.

Conclusion

More than 9,700 assays were performed and analytical performance of the mouse and rat MagPlex® MFIA®, including selectivity and limit of detection, was found to be comparable to, or better than, those obtained by PS MFIA® for mice and rats. Overall diagnostic sensitivity of the MagPlex® MFIA® was approximately 97%, compared to approximately 98% for PS MFIA®. Both PS and MagPlex® beads showed similar seroconversion sensitivity, (i.e., can detect antibodies at the same DPI as well as comparable average scores at each DPI). 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 mice and rats.

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