

Serologic Methods Manual: Multiplexed Fluorometric ImmunoAssay™

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TABLE OF CONTENTS

I. INTRODUCTION	3
II. MATERIALS.....	6
A. DISPOSABLES	6
B. EQUIPMENT.....	6
C. REAGENTS NOT SUPPLIED.....	7
D. CHARLES RIVER-SUPPLIED MFIA™ REAGENTS.....	8
III. MFIA™ EQUIPMENT SETUP	9
A. SUSPENSION ARRAY READER SYSTEM.....	9
B. MICROPLATE VACUUM MANIFOLD SYSTEM	11
IV. MFIA™ PROCEDURE.....	12
A. PREPARE SERUM SAMPLES.....	12
B. PREPARE REAGENTS	14
C. PERFORM THE ASSAY	16
D. READ THE TEST PLATES; REVIEW AND EXPORT RESULTS TO EXCEL	18
E. SCORE, INTERPRET, AND REPORT RESULTS.....	19
V. MFIA™ TROUBLESHOOTING GUIDE.....	21
A. SUSPENSION ARRAY READER ERRORS	21
B. CONTROL FAILURES	22
VI. APPENDIX	25
A. AGENT GLOSSARY	25
B. BEAD PROFILES.....	26
C. BEAD-PROFILE CONTROL SERUM SETS.....	28
D. SAMPLE MFIA™ BEAD PROFILE RESEARCH PRODUCT SPECIFICATION SHEET	29
E. CHARLES RIVER MFIA™ RESULTS EXCEL WORKBOOK: RESULTS REPORT.....	30
F. FORMS	31

I. Introduction

At Charles River Laboratories Research Animal Diagnostic Services, ensuring the quality of animal models used in biomedical research is our highest priority. To accomplish this goal, we have developed a number of diagnostic testing strategies and methods to determine if animals have been exposed to adventitious infectious agents. Infections of immunocompetent animals are generally transient, yet serum antibody responses to infection often can be detected within days to weeks and persist throughout the life of the host. As immunoassays for antibodies to etiologic agents are rapid, inexpensive, specific and sensitive, serology is the primary diagnostic methodology by which laboratory animals are monitored for adventitious infections with viruses, mycoplasma, and other fastidious microorganisms.

Over the past two decades, the indirect enzyme-linked immunosorbent assay (ELISA) has been the main screening method for serosurveillance due to its sensitivity and ease of automation, while the indirect immunofluorescence assay (IFA) has been the technique of choice for corroborating findings. Both the ELISA and IFA are performed as *singleplexes*, in which one microbial antibody-antigen reaction is measured per well. The importance of high throughput screening to the biopharmaceutical industry, however, has encouraged development of new technologies that enable many assays to be performed simultaneously in a single well, which is referred to as *multiplexing*.

Using Luminex® Corporation's xMAP® technology, we have developed Multiplexed Fluorometric ImmunoAssay™ (MFIA™) profiles for serosurveillance of various laboratory animal species. The xMAP® technology-based MFIA™ is termed a suspension microarray because antigen, tissue control or immunoglobulin is covalently linked to suspended 5.6 micron polystyrene microspheres, i.e., beads, which are color-coded (Figure 1A). Since the beads come in 100 distinct color sets, as many as 100 different assays can be simultaneously performed in a single microplate well. MFIA™ reactions are analyzed in an xMAP® 96-well-microplate suspension microarray fluorescence reader from Luminex® Corporation (Figure 1B) or one of its partners (e.g., the Bio-Rad Bioplex Suspension Array System). In the reader, beads pass one at a time through a detector where they are exposed to two lasers. One laser excites the internal dyes that identify the bead's color set, which corresponds to an assay; the other excites the phycoerythrin reporter dye captured during the assay. A minimum number of beads (in our case 25) are read per assay and the intensity of phycoerythrin fluorescence is reported as a Median Fluorescence Index (MFI) ranging from 0 to 32,667 (discussed in Section E).

Figure 1

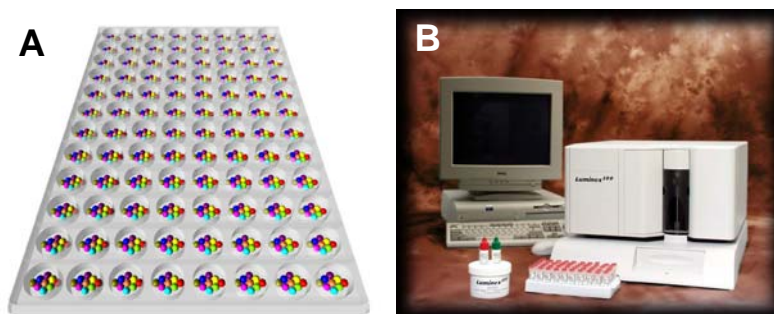


Figure 1: A – Illustration of Bead Color Sets
B – Luminex® xMAP® 100 Instrument

To perform the MFIA™, bead profiles, test sera and other assay reagents are added to wells in filter-bottom 96-well microtiter plates. As is the case for ELISA and IFA, the MFIA™ is performed as a heterogeneous test, meaning that incubations (at 27±2°C on an orbital shaker set to between 400 and 700 RPM) are followed by filter-wash steps to remove unbound serum constituents or labeled reagent. Wash solution added to plate wells is removed by aspiration through well filter-bottoms, which retain the beads. Antigen-antibody complexes formed during the test serum incubation are detected by incubations with biotinylated goat or rabbit antibodies to immunoglobulin of the species being tested (BAG) followed by R-phycoerythrin-labeled streptavidin (SPE) (Figure 2).

Figure 2: MFIA™ Procedure

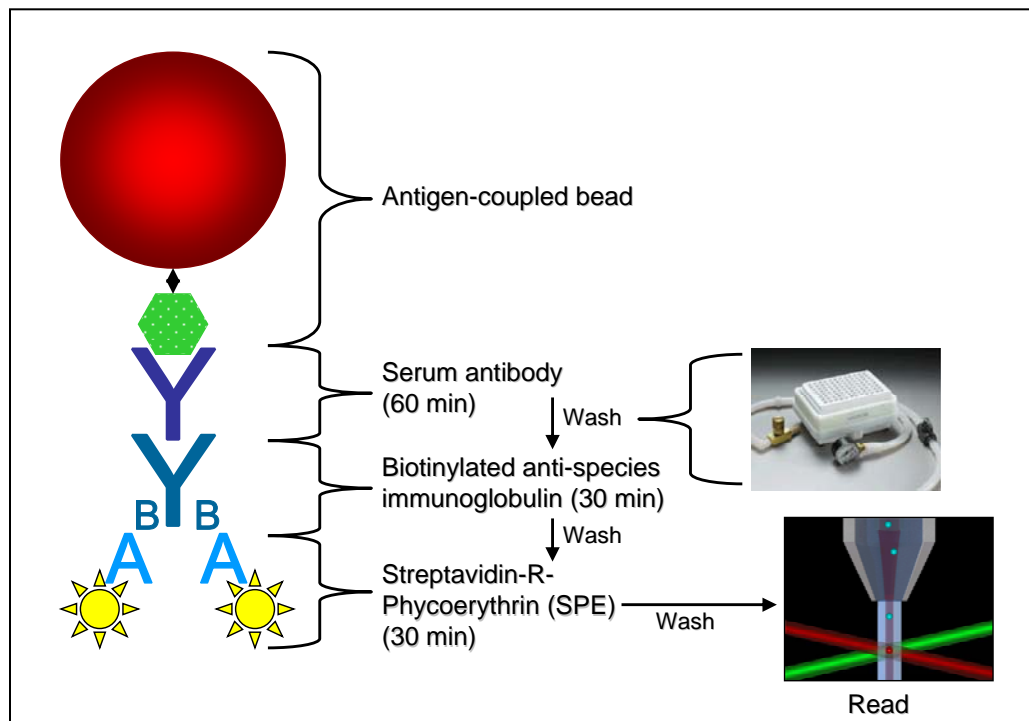


Figure 2: Test Plates are incubated in the dark at 27°C on an orbital shaker set to 400-700 RPM.

Because of multiplexing, the MFIA™ testing process is highly efficient, requiring less equipment and smaller sample and reagent volumes than traditional singleplex tests. In addition, we are able to incorporate several internal control assays to verify sample and system suitability and thereby assure the accuracy of results. These include tissue control and IgG anti-test serum species immunoglobulin (α Ig) coated bead sets to evaluate sample suitability. As in the ELISA and IFA, the tissue control detects non-specific binding of serum immunoglobulin. The α Ig control confirms that serum has been added and contains a sufficient immunoglobulin concentration. Another control bead set, coated with serum species immunoglobulin, demonstrates that the labelled reagents and Luminex® reader are functioning properly.

We have performed comprehensive validation studies of MFIA™ to demonstrate diagnostic accuracy, reproducibility, and ruggedness by testing large numbers of known positive and negative serum samples by ELISA, IFA, and MFIA™. Replicate tests of the serum profiles were run by several technicians and on different days. You can view a summary of the mouse and rat MFIA™ validation study results on the Charles River Laboratories website (www.criver.com/info/mfia_summary_report). Briefly, the detection limits (i.e., standard immune serum titration endpoints) of MFIA™ were comparable to, and in some cases surpassed, those of corresponding ELISA. Diagnostic specificity, measured with SPF rodent sera, exceeded 99%; the overall correspondence between ELISA and MFIA™ performed on known–positive and known–negative sera was greater than 95%. Thus, the diagnostic performance of the MFIA™ meets our rigorous acceptance criteria and the high level of quality you have come to expect from us.

Charles River Laboratories has compiled a list of equipment and reagents required for performing MFIA™. Please contact Charles River’s Technical Services at 1-800-338-9680 or comments@crl.com and request a copy of the “MFIA™ Testing Laboratory: Comprehensive Inventory” for specific information.

This method manual includes appendices of infectious agent abbreviations (Appendix A) and a list of reagents (bead profiles and control serum sets) for running MFIA™ tests (Appendices B and C). For future information, or to obtain details for testing other species by MFIA™, please contact Technical Services.

II. Materials

A. Disposables

1. **Serum Vials**
2. **Micropipette Tips**
3. **Pipettes**
4. **5-25 mL Pipettes** with device for aspirating and dispensing fluids
5. **V-Bottom Reagent Reservoirs**
6. **0.2-0.45 μm Bottle-Top Filter Unit and Sterile Screw-Cap Bottle**
7. **Serum Filtration Plate** – Specifications: 96 wells, $\geq 350 \mu\text{L}$ per well with 1.2 μm particle (e.g., glass microfiber) depth-filter bottoms and short drip director
8. **Microtiter Plate** – Specifications: flat bottom, polystyrene, 96-well – used for the following:
 - a) Stock Sample Plate
 - b) 2X Sample Plate - Unfiltered and Filtered
9. **Filter-Bottom Test Plate** – Specifications: 96 well microplate with lid, 350 μL volume per well with 1.2 μm hydrophilic (low-protein-binding) membrane-filter bottom
10. **Plate Sealing Tape** – to seal plate bottom before reading
11. **Elastic Bands** – to secure test plate lids
12. **Aluminum Foil** – to block the incubator window and/or to wrap around test plates to achieve darkness during incubation

B. Equipment

1. **96-Well Microtiter Plate Vacuum Manifold System** calibrated to approximately -1.5 to -2.5 inches of mercury (“Hg”).
 - a) Components
 - (1) Microplate Vacuum Manifold with the following
 - (a) Tubing and connectors
 - (b) Vacuum gauge
 - (c) Vacuum control valve
 - (d) Vacuum ON/OFF Valve
 - (2) Laboratory vacuum or vacuum pump
 - (3) Waste reservoir
 - (4) Trap reservoir or hydrophobic pump-shielding filter
 - b) References for Setup, Calibration, and Use
 - (1) Bio-Plex Suspension Array System Hardware Instruction Manual (http://www.bio-rad.com/cmcs_upload/Literature/39187/4006205D.PDF), section entitled Vacuum Manifold Set Up
 - (2) Millipore MultiScreen Separations Systems Users Guide (<http://www.millipore.com/userguides.nsf/docs/p17479>), Chapter 2: Using the MultiScreen Vacuum Manifold

Serologic Methods Manual: MFIA™

2. **Incubator (optional)** – Samples can be incubated at room temperature but it is suggested to use an incubator for temperature consistency
 - a) Set to the incubation temperature of 27±2°C
 - b) Equipped with mechanical convection to achieve the incubation temperature quickly
 - c) Equipped with a power outlet and room for orbital shaker for bead mixing during incubation
3. **Orbital Plate Shaker:** 4-position shaker – Test Plates are incubated with orbital shaking between 400 and 700 RPM, which roughly corresponds to a variable speed setting of 4 on the shaker
4. **Vortex Mixer:** For mixing bead-profile suspensions
5. **Sonicator Bath:** To disperse bead aggregates
6. **96-Well-Microplate xMAP® Suspension Array Fluorescence Reader System:** This can be purchased directly from Luminex® Corporation or a partner. In addition to the reader, all systems include a computer with software to control the reader and manage test results. We use **Bio-Rad’s Bio-Plex Suspension Array System with Bio-Plex Manager software.**
7. **Single and 8 or 12 Channel Micropipettes:** Reagent and sample preparation and transfers require various micropipettes that can accurately dispense 2 to 1,000 µL. Repeating multi-channel pipettes are best for dispensing reagents that are added to all wells, e.g., bead suspensions, wash solution, and labeled reagents, and for transferring serum samples from one microplate to corresponding wells in another. The following types of micropipettes are recommended:

Pipettor Type	Microliters (µL)	
	Min	Max
Single Channel, Adjustable Volume	2	10
	10	100
	100	1000
8 or 12 Channel, Adjustable Volume	5	50
	50	300
8 or 12 Channel, Repeating	50	

C. Reagents Not Supplied

1. **PBS:** 0.01M Phosphate-Buffered Saline (PBS), pH 7.4
2. **MFIA™ Buffer (PBS-BSA):** PBS, with 1% (w/v) bovine serum albumin (BSA), pH 7.4: The buffer is used to dilute the BAG and SPE and as an assay buffer after the addition of ProClin®.
3. **ProClin® 300 from Supelco®):** An anti-microbial preservative added to PBS and MFIA™ Buffer (0.5 mL ProClin per Liter of PBS-BSA).

NOTE: For specific vendor information, contact Charles River Laboratories Technical Services to receive the “MFIA™ Testing Laboratory: Comprehensive Inventory.”

D. Charles River-Supplied MFIA™ Reagents

MFIA™ reagents are accompanied by a Research Product Specification sheet like the one shown in Appendix D.

1. **Stock (20X) Bead-Profile Concentrates:** The standard rodent bead profiles, shown in Appendix B, are provided as 20X concentrates in 250-300 µl aliquots suspended in phosphate-buffered saline (PBS) containing Tween 20, sodium azide, and 1% BSA. Store Stock Bead Profiles in a refrigerator at 2-8°C and in darkness as the **beads are light sensitive. DO NOT FREEZE.**
2. **Control Serum Sets:** Control sets, shown in Appendix C, consist of high- and low-range immune sera and nonimmune sera for verification of assay (analytical) sensitivity and specificity. **CONTROL SERUM SETS ARE BEAD-PROFILE-SPECIFIC.** For example, the control set for an Assessment profile is different from the Tracking set. Store the sera in a freezer at ≤ -60°C. Avoid repeated freeze-thaw cycles and refrigeration for more than 2 days.
3. **Stock Solutions of Biotinylated Anti-Immunoglobulin (BAG) and Streptavidin-R-Phycoerythrin (SPE):** Stock solutions of these labeled reagents are provided in aliquots sufficient for approximately 5 Test Plates. The recommended working dilution for a reagent, which varies by lot, is indicated in each lot's Research Product Specification sheet. Store working dilutions at 2-8°C.

Note: You have the option of purchasing these reagents directly from commercial suppliers. We strongly encourage you to use the BAG and SPE from Charles River, however, because we perform stringent quality control testing on each lot to assure suitability for the MFIA™ and to select an optimal working dilution.

4. **Primary Diluent:** It is used to dilute control and test sera and 20x bead profile suspensions. It contains proprietary blocking agents that inhibit nonspecific reactivity and therefore is essential for obtaining accurate assay results.

Table 1: Concentration and Temperatures for Charles-River Supplied MFIA™ Reagents

MFIA™ Reagent	Concentration Provided	Temperature Shipped	Temperature / Conditions Stored
Stock Bead-Profile	20X	2-8°C	2-8°C/dark
Control Serum Set	2X	Dry Ice	-60°C
BAG	Stock/Undiluted	Dry Ice	-20°C
SPE	Stock/Undiluted	2-8°C	2-8°C
Primary Diluent	N/A	2-8°C	2-8°C

III. MFIA™ Equipment Setup

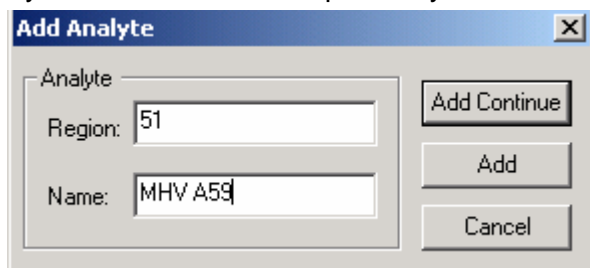
A. Suspension Array Reader System

1. **Maintenance:** The xMAP® suspension array reader is a sophisticated instrument which needs routine care and maintenance including calibration and validation to function properly. We encourage you to purchase a maintenance contract for your reader(s) and to follow manufacturer-recommended maintenance procedures such as the following for the Bio-Plex Suspension Array System that we use:

- Calibrate the instrument daily and validate it monthly. The Bio-Plex System includes a validation kit to evaluate instrument optics alignment, fluidics integrity, report channel performance, and classify efficiency.
- Strictly follow all manufacturer-recommended start-up, shut down, and between-plate wash procedures to prevent clogging of the fluidics system. We find it helpful to run an alcohol flush before shutting down and after a prolonged period of reader inactivity.

In the MFIA™, the dilution at which sera are tested is low (i.e., 50 or 100 fold). Fluidics system clogging and slow reading are prevented by filtering test sera. Serum filtration instructions are presented at the beginning of the MFIA™ Procedure section of this manual.

2. **Creation of MFIA™ Profiles:** In order to perform the MFIA™, you will have to create one or more custom profiles in the suspension array reader management software. In the Bio-Plex Manager software, a custom profile is created from within a protocol, which consists of assays to be run and a plate format indicating which wells are to be read and the sample types they contain (e.g., unknown, control, and standard).
- Assays are defined within a profile by name and bead set (i.e., region) number.



- Part or all of a profile may then be selected for inclusion in a protocol.

Panel: Mouse Tracking

Available:

Region	Analyte
54	rMHV
42	EDIM
34	GD-7
76	M.pulmonis
77	M.arthritis
65	PVM
72	Reo3
60	Sendai
56	MVM
18	MPV-1a
33	MPV-2a

Selected:

Region	Analyte
51	MHV A59

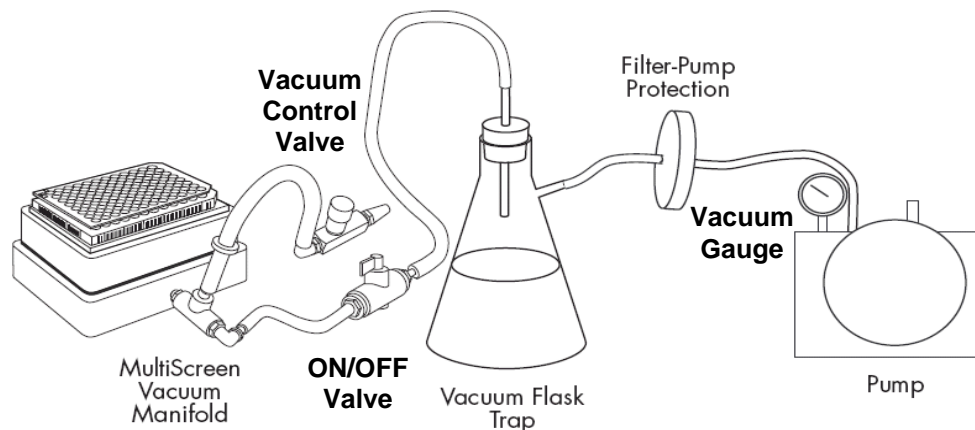
Buttons: Add >>, << Remove, Add All >>, << Remove All

- Thus, you have two options in Bio-Plex Manager for building the Rodent MFIA™ Bead Profiles shown in Appendix B. These include:
 - Adding a Bio-Plex profile for each Charles River MFIA™ Bead-Profile.
 - Creating a single “Rodent MFIA™” profile and separate protocols, with the appropriate assays selected, for each MFIA™ Bead-Profile.

B. Microplate Vacuum Manifold System

1. Configure and assemble the manifold according to the manufacturer's instructions. A diagram of a typical configuration for the MultiScreen Vacuum Manifold from Millipore is presented in Figure 3.
 2. **Proper setup of the vacuum manifold system is critical to obtaining reliable and accurate MFIA™ results.**
 - a) Too much vacuum will reduce the MFI score for positive samples and extend reading times as beads will be difficult to re-suspend properly.
 - b) Too little vacuum will result in extended evacuation times and possibly inadequate washing.
 3. The appropriate vacuum level is **approximately -1.5 to -2.5 "Hg**. Keys to achieving this vacuum level consistently are:
 - a) A constant vacuum source: A dedicated pump is the best way to achieve this.
 - b) A control valve for fine adjustment of the vacuum level: Make sure that the connector leading to the vacuum control valve knob is perpendicular to the manifold to prevent buffer from traveling to the vacuum control valve.
 - c) A gauge that is accurate at the recommended low vacuum level: Since the pump should not be on the bench top with the manifold, the pump vacuum gauge may not be visible. A viewable gauge should be connected to the vacuum line between the manifold and ON/OFF value. A vacuum gauge for this purpose is supplied with the Millipore MultiScreen Vacuum Manifold.
 4. To calibrate and verify the manifold vacuum pressure, we recommend that you follow the procedures provided in the Bio-Plex Suspension Array System Hardware Instruction Manual. If the vacuum pressure is properly calibrated, PBS-BSA-ProClin® should be evacuated from a filter bottom Test Plate (e.g., Millipore Multiscreen HTS) in about 2-4 seconds.
- NOTE:** It should take approximately 5-10 seconds to completely evacuate plates.
5. At the end of each day, when you have finished running tests, rinse the manifold with tap or deionized water to remove buffer salts. You should also occasionally clean and disinfect the manifold by rinsing it with a solution of laboratory detergent followed by dilute (e.g., 5%) bleach or alcohol.

Figure 3: Configuration of Microplate Vacuum Manifold for MFIA™ (from MultiScreen Separations System User Guide)



IV. MFIA™ Procedure

A. Prepare Serum Samples

1. Assemble the following materials and reagents (excluding those required for blood collection).
 - a) Serum Vials and Vial Racks
 - b) MFIA™ Template Form
 - c) Multi- and Single-Channel Micropipettes and Tips
 - d) PBS*
 - e) Primary Diluent*
 - f) Serum Filtration Plates*
 - g) Microtiter Plates*

*See the Materials section
2. Collect blood specimens according to your standard procedure. **Allow the blood specimens to clot fully by holding them at room temperature for at least 30 minutes before centrifugation and serum removal.**
3. Dilute rodent sera by adding 1 part serum to 4 parts PBS (1:4 or 5 fold). Alternatively, produce the same dilution of serum by adding 1 part **whole** blood to 2 parts PBS prior to centrifugation.
4. Keep samples refrigerated for short-term storage or frozen at ≤ -10°C if they need to be stored for more than several days before being tested.
5. Assign sera to specific wells in test plates by serum host species and MFIA™ profile.
 - a) You may record this information, as we typically do, on an MFIA™ Test Plate Map form like the blank one we have provided in Appendix F. An example of this form filled out is shown in Figure 4.

Figure 4: Example of the MFIA™ Test Plate Map

<u>Charles River MFIA™ Test Plate MAP</u>						Lab: Your Lab					
Plate ID:	Your Plate 1		Species:	Mouse		Role	Technician		Date		
Profile:	Mouse Tracking		Lot #:	031807		Setup	WRS		1/1/2006		
Control:	Mouse Tracking		Lot #:	112206		Test					

Row/Column:	1	2	3	4	5	6	7	8	9	10	11	12
A	06-2345-1	A1-12/10/05-1	YY1	3	363-BSV-110	118	5	13	4	53	61	Poly-High
B	2	2	2	4	111	119	6	14	5	54	62	Poly-Low
C	3	3	3	5	112	120	7	15	6	55	63	Parvo-High
D	4	4	4	6	113	121	8	16	7	56		Parvo-Low
E	5	B23-06/06/06-1	5	23B-DES	114	B22-02/23/05-1	9	17	8	57		NS1-Low
F	6	2	900234-1	24B-DES-1	115	2	10	2005-012345-1	9	58		NMS
G	7	3	1	2	116	3	11	2	CD1-97732-1051	59		NRS
H	8	4	2	3	117	4	12	3	52	60		Diluent

Remarks: _____

Serologic Methods Manual: MFIA™

- b) Test Plate locations for standard serum and diluent controls must be reserved. We typically reserve wells in column 12 for these controls.
- 6. Arrange serum vials in racks by Test Plate according to the Test Plate Map. This will make it easier for you to transfer sera from vials to their assigned plate wells.
- 7. Transfer sera from vials to a Microtiter Plate labeled with your Plate ID code; dilute sera in Primary Diluent to a 2X concentration.
 - a) The 2X serum concentration corresponds to a 1:24 (or 25 fold) dilution of rodent serum.
 - b) Although only 50 µL of diluted serum is required per multiplex assay, prepare an excess volume of 2X serum (e.g., 125 µL) for the following reasons:
 - (1) To ensure that the volume of Stock Serum being diluted is large enough to be accurately pipetted
 - (2) To compensate for losses, which occur during sample filtration and transfer
 - (3) To have a sufficient quantity of 2X serum for repeat testing
 - c) Transfer and dilute sera to the 2X concentration by one of the following methods:
 - (1) Pipette the appropriate volume of Primary Diluent per Table 2 into a Microtiter Plate, labeled 2X Sample Plate. Pipette the appropriate volume of Stock Serum per Table 2 into the 2X Sample Plate containing primary diluent. Mix thoroughly. The final volume of serum and diluent should be 125 µL.

Table 2

Stock Serum		µL Primary
Dilution	µL Transfer	Diluent
Neat	5	120
1:4	25	100

- (2) Alternatively, first transfer sera from vials to a Microtiter Plate labeled “Stock Sample Plate” and then dilute the sera by transferring them from the Stock Sample Plate to another Microtiter Plate labeled as the “2X Sample Plate,” containing Primary Diluent.
 - (a) Label the Stock and 2X Sample Plates with the starting serum dilution (i.e., 1:4 or Neat) and “2X”, respectively.
 - (b) Transfer approximately 125 µL of neat serum or 125 µL of 1:4 diluted serum from a vial to a well in the Stock Sample Plate.
 - (c) Then pipette the appropriate volume of Primary Diluent per Table 2 into the 2X Sample Plate. DO NOT add diluent to control wells.
 - (d) Pipette the appropriate volume of Stock Serum to matching wells in the 2X Sample Plate as indicated in Table 2 above containing primary diluent. You can complete this transfer most easily with a multi-channel pipette. Mix thoroughly. The final volume of serum and diluent should be 125 µL.

NOTE: The main advantage of this process is that additional 2X Sample Plates can be prepared more rapidly vis-à-vis transferring sera from vials.

Serologic Methods Manual: MFIA™

8. Filter the 2X sera (optional).
NOTE: Although optional, we strongly advise you to filter serum samples to reduce the occurrence of reader clogs and longer-than-expected reading times.
 - a) Pipette sera from a 2X Sample Plate into matching wells in a Serum Filtration Plate. We recommend using a plate with a depth filter (such as glass fiber) rated to remove particles $\geq 1\text{-}1.2\ \mu\text{m}$, as depth filters are less likely to clog than membrane filters.
 - b) Label a Microtiter Plate with the plate ID code and "Filtered 2X Sample Plate"
 - c) Remove the vacuum manifold cover and place the "Filtered 2X Sample Plate" in the manifold.
 - d) Replace the manifold cover and seat the Serum Filtration Plate on the manifold-cover gasket, above the Filtered 2X Sample Plate. Be sure that matching wells of the filtration and receiver plates are properly aligned.
 - e) Apply a **low-level vacuum of approximately -1.5 to -2.5 "Hg** until all of the sera have been drawn into the receiver plate. Avoid applying too strong a vacuum as this may lead to foaming and specimen cross contamination.
 - f) Turn the vacuum off and remove the Filtered 2X Sample Plate from the vacuum manifold.
9. Transfer the 2X sera to a Test Plate and perform the MFIA™ within 24 hours, or **cover and store the 2X Sample Plate in a refrigerator at 2-8°C for no longer than 72 hours before MFIA™ testing**. Discard the 2X plates at the end of the week.

B. Prepare Reagents

1. To prepare MFIA™ Buffer for filter-washing of beads and for diluting BAG and SPE:
 - a) Add 1 packet of the PBS-1% BSA powder to 1 liter of deionized water.
 - b) Add 0.5 mL of ProClin® 300.
 - c) Mix until the powder has dissolved completely.
 - d) Check that the pH is 7.4 ± 0.2 .
 - e) Filter the solution through a 0.2 μm Bottle-Top Filter Unit into sterile, labeled containers.
 - f) Refrigerate at 2° to 8°C.
 - g) The reagent expires six months from the day of preparation or when visible contamination is evident.
2. Make working dilutions of BAG and SPE sufficient for a week of testing.
 - a) Warm to room temperature.

Serologic Methods Manual: MFIA™

b) Determine the volume of stock and MFIA™ buffer you require by performing the following calculations:

(1) MFIA™ Buffer = 6 mL per Plate * Estimated # of Plates for Week

(2) mL Stock Reagent = mL MFIA™ Buffer / Reciprocal of Working Dilution

For example:

If

- Estimated # of Test Plates for Week = 10 and
- Reciprocal of Reagent Working Dilution = 150

Then

- mL MFIA™ Buffer = 60
- mL Stock Reagent = 0.4

c) Add the calculated mL of MFIA™ buffer and reagents to a disposable plastic container.

d) Label the container with the reagent name, concentration (i.e., “working dilution”), and preparation date.

e) Store the working dilution reagents in the refrigerator at 2 to 8°C.

f) We suggest that you QC the labeled reagents once in the beginning of each week by performing an MFIA™ on control sera before assaying test sera.

3. Prepare working dilution bead suspensions.

a) Warm to room temperature

b) **LIMIT THE EXPOSURE OF BEADS TO LIGHT.**

c) Calculate the following quantities by multiplying the **Amount per Test Plate by the # of Test Plates.**

Quantity	Amount / Test Plate
Primary Diluent	5.7 mL
Stock (20X) Bead Suspension	300 µL (1 Vial)

d) Pipette the calculated mL of Primary Diluent into an appropriately-sized disposable tube or bottle. Label the container with the profile designation, analyst initials, suspension concentration (i.e., “working dilution”), and the preparation date.

e) Remove the calculated number of vials from the refrigerator.

f) Vortex each vial for around 5-15 seconds to fully suspend beads.

g) Then, place the vials in a sonicator bath for approximately 15-30 seconds to break-up bead aggregates.

h) Pipette the calculated µL of 20X Bead Suspension into the working dilution container to which you added Primary Diluent.

i) Store the working dilution beads in a refrigerator at 2 to 8°C, in the dark. We have found that working dilution bead-profile suspensions are stable for 2 weeks.

NOTE: Vortex and sonicate working dilution bead suspensions just before use.

C. Perform the Assay

1. Assemble the following materials and reagents (excluding those required for blood collection).
 - a) Completed MFIA™ Test Plate Maps
 - b) 2X Sample Plates
 - c) Control Serum Sets*
 - d) Filter-Bottom Test Plates*
 - e) Multi- and Single-Channel Micropipettes and Tips*
 - f) MFIA™ Buffer*
 - g) Microplate Vacuum Manifold System calibrated to -1.5 to -2.5 "Hg*
 - h) Reagent Reservoirs*
 - i) Working dilution Stock Bead Suspensions
 - j) Incubator (optional) at 27±2°C equipped with mechanical convection to achieve the incubation temperature quickly and a power outlet and room for orbital shaker for bead mixing during incubation
 - k) Orbital Shaker set to between 400 and 700 RPM
 - l) Working dilution Biotinylated Anti-Test Serum Species Immunoglobulin (BAG)*
 - m) Working dilution Streptavidin-R-phycoerythrin (SPE)*

*See the Materials section
2. Label each filter-bottom Test Plate with your Plate ID code and the MFIA™ profile.
3. Select Control Serum Sets that correspond to the MFIA™ Profiles being run (Appendix C). **Thoroughly mix the control sera after they are thawed.**
4. **Pre-wet Test Plates** as follows:
 - a) Dispense 100 µL of MFIA™ Buffer per well into ALL 96 WELLS. **NOTE:** Failing to pre-wet all wells can cause unequal filtration during the assay.
 - b) Evacuate the MFIA™ Buffer using the microplate vacuum manifold.
 - c) Observe the vacuum gauge to confirm the pressure is about -1.5 to -2.5 "Hg.
 - d) Approximate the time it takes for the wells to evacuate.
 - e) **DO NOT CONTINUE IF THE MANIFOLD IS MALFUNCTIONING. FOR EXAMPLE, IF:**

The vacuum is too strong (i.e., below -2.5 "Hg) or the evacuation time is too long, that is, more than approximately 10 seconds. You need to recalibrate the vacuum pressure or repair system leaks before continuing.
5. **Warm to room temperature and mix the working dilution bead suspension thoroughly as follows:**
 - a) Vortex for 5-15 seconds
 - b) Sonicate for 15-30 seconds to break up bead aggregates.
6. Pour or pipette the suspension into a V bottom reservoir.
7. Dispense **50 µL of working dilution bead suspension into each Test Plate assay well.**
8. According to the MFIA™ 96-well Test Plate MAP, add **50 µL of 2X test serum, control serum or MFIA™ Buffer (as diluent control) to its assigned Test Plate well.**

Serologic Methods Manual: MFIA™

- a) Transfer 2X test sera from corresponding wells in the 2X Sample Plate to the filter bottom Test Plate using a multi-channel pipette.
- b) Then, add **control** serum and **MFIA buffer as** diluent control to each Test Plate, making sure to use a control set that matches the Test Plate's MFIA™ Profile.
9. Cover each Test Plate with a lid. Secure the lid to the Test Plate with an elastic band so that it does not fall off when shaken during incubation.
10. **Incubate Serum in Test Plates for 60 minutes** in darkness with orbital shaking at 400-700 RPM (corresponding to a shaker variable speed setting of about 4) at room temperature. If using an incubator that has a clear glass door, cover or frost the glass or wrap the plate in aluminum foil to limit light exposure.
11. Filter-wash the beads in each assay well two times as follows:
 - a) Seat the Test Plate securely on the microplate vacuum manifold gasket and evacuate the wells by turning on the vacuum pump and opening the ON/OFF valve.
 - b) When all wells are evacuated, close the ON/OFF valve.
 - c) Dispense 100 µL of MFIA™ buffer into every Test Plate well. This step is accomplished most efficiently with a repeating multi-channel pipette.
 - d) Then open the ON/OFF valve to evacuate the wells. Close the valve as soon as all wells have been evacuated. NOTE: **Avoid prolonged aspirations** (i.e., >10 seconds approximately) which can lead to difficulty in re-suspending beads and reduced assay MFI.
 - e) Repeat steps c and d once.
 - f) **Continue immediately to the next step.**
12. Into each Test Plate assay well, dispense **50 µL of MFIA™ buffer followed by 50 µL of working dilution BAG that has been warmed to room temperature**. Secure a cover lid to the Test Plate with an elastic band.
13. **Incubate BAG in Test Plate wells for 30 minutes** under the conditions described in serum incubation step.
14. Filter-wash Test Plates twice as described above (step 11) and immediately continue to the next step.
15. Into each Test Plate assay well, dispense **50 µL of MFIA™ buffer followed by 50 µL of working dilution SPE that has been warmed to room temperature**. Secure a cover lid to the Test Plate with an elastic band.
16. **Incubate SPE in Test Plate wells for 30 minutes** under the conditions described in the serum incubation step.
17. Filter-wash Test Plates twice as described above (step 11).
18. **Immediately** seal Test Plate bottoms with Plate sealing tape and dispense **125µL** of MFIA™ Buffer into each Test Plate well. Secure a cover lid to the Test Plate with an elastic band.
19. You may then store sealed, covered Test Plates at room temperature in darkness for up to 12 hours before reading.
20. Prior to reading the test plate, re-suspend the beads by shaking for 1 minute.

D. Read the Test Plates; Review and Export Results to Excel

1. Before attempting to read a Test Plate, make sure the reader has been warmed up for 30 minutes or longer and has gone through a start-up wash cycle and calibration.

NOTE: Make sure the instrument is set on LOW to calibrate the CAL 2 Beads.

2. To avoid reader clogs and longer-than-expected reading times, we run a wash cycle in between each Test Plate; an alcohol flush is run at the end of the day and after a prolonged period of reader inactivity.
3. Place each Test Plate on an orbital shaker for at least one minute at 400 to 700 rpm to re-suspend the beads. Read the plate within 10 minutes of re-suspending the beads.
4. Select the appropriate standard protocol and assay profile.
 - a) If necessary, modify the plate format to reflect the number of samples being tested and their locations.
 - b) MFIA™ are NOT quantitative (and hence, we do not create standard curves for interpolation of antibody titers). Therefore, you may define all wells, including those to which we add standard sera, as unknowns.
5. Save the modified protocol under a file name that includes the plate ID code.
6. Run the protocol to read the Test Plate. In the Bio-Plex system, this includes:
 - a) Selecting the protocol Start menu option
 - b) Saving the protocol run as a .rbx (i.e., results) file
 - c) Pressing the Eject button and putting the Test Plate in the plate holder
 - d) Selecting OK, which closes the plate holder and begins the reading process
7. Examine the results report for errors, e.g., inadequate bead counts. Assays with errors should be repeated.
8. Export results to a single Excel worksheet, formatted so that the samples are listed vertically and assays displayed horizontally as shown in the following example:

	A	B	C	D	E	F	G	H	I
1	File Name: L:\NuGenesisArchive\MFIA\Rodent Serology\2006\Results files\042606 63,64 MA+.rbx								
2	Acquisition Date: 26-Apr-2006, 10:47 AM								
3	Reader Serial Number: LX10005136301								
4	Plate ID: Test Plate								
5	RP1 PMT (Volts): 557.02								
6	RP1 Target: 3690								
7									
8				K virus (55)	MHV A59 (51)	rMHV (54)	EDIM (42)	GD-7 (34)	M.pulmonis (76)
9	Type	Well	Description	FI	FI	FI	FI	FI	FI
10	X1	A1	Sample-1	25	24	66	28	39	101
11	X2	B1	Sample-2	31	23	72	35	65	125
12	X3	C1	Sample-3	43	33	92	36	448	111
13	X4	D1	Sample-4	28.5	21	75	22	202	82
14	X5	A2	Sample-5	30	21	40.5	26	34	84
15	X6	B2	Sample-6	39	37	58	37	41	122
16	X7	C2	Sample-7	746	35	63	31	76	101
17	X8	D2	Sample-8	58	44	87.5	50.5	61.5	135
18	X9	A3	Sample-9	80	36	65	46	56	111.5
19	X10	B3	Sample-10	29	45	188	40	32	101
20	X11	C3	Sample-11	84	57	85	55	69	172.5

9. Save the worksheet or transfer it to a workbook setup for MFIA™ results analysis, e.g., the **Charles River MFIA™ Results Excel Workbook**.

E. Score, Interpret, and Report Results

1. Result scores and classification reports can be generated from the **Charles River MFIA™ Results Excel Workbook**. Appendix E shows an example of a scored results report. Other useful reports that can be printed from the workbook include Assay List, Sample List, and Serum Control Results. The MFIA™ Results Excel Workbook is available to you **at no charge**.
2. MFIA™ Scoring
 - a) **Each antibody assay is assigned:**
 - (1) **A Tissue Control (TC) Test:** For most rodent MFIA™ assays, the tissue control is an extract of wild-type baculovirus-infected insect cells. A notable exception is the *Mycoplasma pulmonis* (MPUL) assay, for which a bead set coated with antigen prepared from a different rodent mycoplasma species, *M. arthritidis*, is the tissue control.
 - (2) **An Assay Cutoff:** This value is adjusted for each assay to maximize diagnostic accuracy. For most assays, it is 3000.
 - b) Net Median Fluorescence Index (MFI) are calculated for antibody assays (but not for tissue and other control tests).

Net MFI = MFI_{Antibody Assay} – MFI_{Tissue Control}
 - c) Net MFI and TC MFI are converted to scores by comparison to the Assay Cutoff as follows:
 - (1) **Net or TC MFI < Assay Cutoff: Score = (Net MFI/Cutoff MFI)*3**
 - (2) **Net or TC MFI ≥ Assay Cutoff: Score = ((Net MFI–Cutoff MFI)/1000) +3**

By using these formulas, a Net or TC MFI equal to the Assay Cutoff MFI is always given a score of 3, irrespective of the Cutoff. Scoring results in this manner facilitates visual inspection and results interpretation.

When the Assay Cutoff MFI = 3000, Score = Net or TC MFI/1000. Since the cutoff for the vast majority of assays is equal or close to 3000, you could elect to use this simple formula for scoring your results without a substantial loss of diagnostic accuracy.
 - d) **Ig or αIg Score = Raw MFI/1000.** These controls are assigned a Pass/Fail Cutoff MFI, typically ≥ 8000. Scores below the Cutoff MFI/1000 fail.
3. MFIA™ Results Interpretation
 - a) Assay results should only be interpreted if its system-suitability control results meet the following acceptance criteria:

Assay System Suitability Control Acceptance Criteria		
Control	Acceptable Result	
	Score	Classification
High Range Immune Serum	≥ 4.5	Positive
Low Range Immune Serum	≥ 1.5	≥ Borderline
Nonimmune Serum	< 2.5	≤ Borderline
Diluent	< 2.5	≤ Borderline
Ig Bead Set*	≥ Cutoff/1000	Pass

*Bead set coated with serum species immunoglobulin

Serologic Methods Manual: MFIA™

- b) If system suitability control results are satisfactory, assay scores are classified as shown in the following table:

MFIA™ Score Classification					
Score				Classification	
α Serum Ig*	TC	Net	TC + Net		
< Cutoff MFI/1000				Fail (F)	
≥ Cutoff MFI/1000	≥ 2		< 2.5	Negative (-)	
			≥ 2.5	TC Reaction (T)	
	< 2		< 1.5		-
			≥ 1.5 & < 2.5		Borderline (B)
		≥ 2.5		Positive (+)	

*Bead set coated with IgG anti-test serum species immunoglobulin (Ig): A failing score for this sample suitability control could result from the addition of insufficient sample, too high a sample dilution, incorrect species of testing serum from an immunodeficient host.

V. MFIA™ Troubleshooting Guide

Charles River Laboratories Technical Services and professional staff are available to assist you in troubleshooting your MFIA™ tests. Please note that Charles River Laboratories recommends that you contact the supplier for any equipment-related problems.

A. Suspension Array Reader Errors

Symptom	Cause	Solution
Very High Test Scores	CAL 2 Beads calibrated on HIGH	➤ Make sure CAL 2 Beads are calibrated on LOW.
Bead count low	Wrong bead profile or protocol selected	➤ In the bead grid window, verify that all white ovals (denoting the regions of bead sets selected for the protocol) contain black dots (representing beads). Holding the cursor over a specific oval will cause the assigned bead set and antigen to appear. ➤ Select the correct profile and reanalyze the results.
	Profile too dilute	➤ Check calculations and pipette calibration.
	Beads aggregated	➤ Vortex and sonicate 20X bead concentrate. ➤ Vortex and sonicate working bead suspension before use.
	Beads lost during assay procedure	➤ Vacuum setting too high or left on too long; recalibrate vacuum and try not to evacuate plate for more than 10 seconds. ➤ Check whether filtered microplate is of the correct type.
	Did not shake plate sufficiently before reading	➤ Check orbital shaker setting. ➤ Mix plate on orbital shaker for at least 1 minute and no more than 10 minutes before reading plate.
	Plate wells leaked	➤ Seal bottom of plate with plate sealing tape before adding diluent and reading.
	Reader clogged	➤ Refer to suspension array reader manual for instructions on clearing clogs. ➤ Filter test sera and run wash cycle between plates if you are not doing so.
	Beads are not re-suspended in 125µl of diluent	➤ Vacuum filter the plate and re-suspend the beads in 125 µl of diluent. Mix plate on orbital shaker for at least 1 minute and no more than 10 minutes before reading plate.
Beads falling outside of oval range	Beads exposed to too much light	➤ Avoid prolonged, direct exposure to light. ➤ Perform all incubations in darkness.
	Bubbles may be present in the sample lines	➤ Use the “Remove Bubbles” option on the Bio-Plex reader to remove bubbles.
Bead clusters not surrounded by oval	Incorrect bead profile or protocol chosen	➤ Modify the results file assays selections and reanalyze the results.

B. Control Failures

Symptom	Cause	Solution
Positive-range control scores low	Wrong serum control set	➤ Make sure that the serum control set matches the bead profile.
	BAG or SPE incorrectly diluted	➤ Check calculations and micropipette calibration.
	BAG or SPE not stored properly or expired	➤ Store Bag Stock at <20°C, SPE stock at 2-8°C, and discard when expired.
	Incorrect BAG	➤ Check that BAG is specific to test serum IgG
	Insufficient quantity of control serum added	➤ Confirm that the micropipette used to dispense control serum was set to 50 µL. ➤ After thawing, mix the control serum thoroughly.
	Sera not stored properly or expired	➤ Store frozen at -60°C or below. ➤ Once thawed, refrigerate at 2-8°C for no longer than a week. ➤ Avoid repeated freeze-thaw cycles.
	Vacuum set too high or left on too long	➤ Recalibrate vacuum and try not to evacuate plate for more than 10 seconds.
	Incubation inadequate	➤ Make sure incubator is set to 27±2°C. ➤ Use a mechanical convection incubator to bring Test Plates to temperature quickly. ➤ Incubate on orbital shaker running at 400-700 RPM. ➤ Adhere to incubation times.
Nonimmune serum/diluent are positive	Positive serum added to assay wells	➤ Verify that control sera were added in the correct wells, for instance, that they were not added in reverse order.
	Well leaked and results are "Ghost" beads from previous well	➤ We have observed that when a well leaks and is not read, results similar to those for the prior well may be reported. ➤ To prevent the wells from leaking, seal plate bottoms with Plate sealing tape before re-suspending the beads for reading.

Symptom	Cause	Solution
Control sera are TC Reactive	Beads were not diluted in Primary Diluent	<ul style="list-style-type: none"> ➤ The Primary Diluent, which can be purchased from Charles River, contains blocking agents that are essential for MFIA™ specificity.
	Beads were not adequately washed	<ul style="list-style-type: none"> ➤ Confirm that all wells are emptying during filter-washing. ➤ If the vacuum is too weak, increase it to the recommended -1.5 to -2.5 "Hg. Replace the vacuum manifold gasket if it is worn and seal system leaks. ➤ Be sure to fill all Test Plate wells when washing.
	BAG or SPE working dilution too concentrated	<ul style="list-style-type: none"> ➤ Check calculations and micropipette calibration. ➤ Make sure that you are using the working dilutions recommended in the Research Product Specification sheets for the current reagent lots.
A test serum is TC Reactive	Serum was not sufficiently dilute	<ul style="list-style-type: none"> ➤ Verify that stock rodent serum is diluted 1:4. ➤ Confirm that 2X rodent serum is diluted 1:24.
	Serum was not diluted in Primary Diluent	<ul style="list-style-type: none"> ➤ Always dilute sera in Primary Diluent from Charles River.
	Serum was from an inoculated or autoimmune host	<ul style="list-style-type: none"> ➤ Serum from a host with autoimmune disease or one that has been parenterally inoculated with a biological material may contain antibodies that react with host cell and culture medium constituents used in the propagation of a microorganism. ➤ Test serum from immunocompetent animals that have not been parenterally inoculated with biological material.

Symptom	Cause	Solution
Ig assay score is below cutoff	BAG or SPE potency inadequate	<ul style="list-style-type: none"> ➤ If BAG or SPE potency is inadequate, the Ig control failure should occur in most or all assay wells in Test Plates receiving the reagents. ➤ Verify that the BAG and SPE were stored and diluted properly and did not expire. Confirm that the BAG was specific for immunoglobulin of the species being tested. ➤ You should consider evaluating the potency of new batches of working dilution BAG and SPE by testing a set of control sera, particularly before processing a large number of sera.
	Well(s) not completely evacuated	<ul style="list-style-type: none"> ➤ When a well is not completely empty prior to the addition of diluent and reagent, the final dilution may be too high. ➤ Ensure that all Test Plate wells have been evacuated before adding diluent and reagent.
αIg assay score is below cutoff	Volume of serum added was insufficient	<ul style="list-style-type: none"> ➤ Confirm that the micropipette used to dispense the serum was calibrated and set to 50 μL. ➤ Make sure that the tips fit the micropipette properly, especially when using a multi-channel pipette.
	Serum was too dilute	<ul style="list-style-type: none"> ➤ Check calculations and micropipette calibration. ➤ If you are pipetting 5 μL or less of Stock Serum, consider preparing a larger volume of 2X serum.
	Serum was degraded	<ul style="list-style-type: none"> ➤ For short term storage, refrigerate sera at 2 to 8°. For long term storage, freeze sera at -10°C or below.
	Serum species incorrect	<ul style="list-style-type: none"> ➤ Test the serum in the correct profile.
	Serum collected from immunodeficient host	<ul style="list-style-type: none"> ➤ Immunodeficient hosts are not suitable for serosurveillance serologic testing. Perform serology on immunocompetent adult animals.

VI. Appendix

A. Agent Glossary

Agent	Abbreviation	Family/Order	Subfam/Genus	Species*
Bovine Rotavirus	BRV	Reoviridae	Rotavirus	M, Rb
Cilia-associated respiratory bacillus	CARB			M, R, Rb
<i>Clostridium piliforme</i>	CPIL	Clostridaceae		M, R, Rb
Mousepox (Ectromelia)	ECTRO	Poxviridae	Orthopoxvirus	M
<i>Encephalitozoon cuniculi</i>	ECUN	Pleistophoridae	Encephalitozoon	M, R, H, GP, Rb
Epizootic diarrhea of infant mice virus	EDIM	Reoviridae	Rotavirus	M
Toolan's H-1 Virus	H-1	Parvoviridae	Parvovirus	R
Hantaan Virus	HANT	Bunyaviridae	Hantavirus	M, R
Mouse Pneumonitis Virus (K Virus)	K	Papovaviridae	Polyomavirus	M
Kilham Rat Virus	KRV	Parvoviridae	Parvovirus	R
Lymphocytic Choriomeningitis Virus	LCMV	Arenaviridae	Arenavirus	M, R, H, GP, Rb
Adenovirus	MAV	Adenoviridae	Mastadenovirus	M, R, GP, Rb
Mouse Cytomegalovirus	MCMV	Herpes	Betaherpesvirus	M
Mouse Hepatitis Virus	MHV	Coronaviridae	Coronavirus	M
Murine Norovirus	MNV	Caliciviridae	Norovirus	M
Mycoplasma pulmonis	MPUL	Mycoplasmataceae	Mycoplasma	M, R
Mouse Parvovirus	MPV	Parvoviridae	Parvovirus	M
Minute Virus of Mice	MVM	Parvoviridae	Parvovirus	M
Parainfluenza virus type 1	PIV-1/SEND [^]	Paramyxoviridae	Paramyxovirus	H, GP, Rb
Parainfluenza virus type 2	PIV-2/SHFV [^]	Paramyxoviridae	Paramyxovirus	H, GP, Rb
Parainfluenza virus type 3	PIV-3/SV-5 [^]	Paramyxoviridae	Paramyxovirus	R, GP
Polyoma Virus	POLY	Papovaviridae	Polyomavirus	M
Pneumonia Virus of Mice	PVM	Paramyxoviridae	Pneumovirus	M, R, H, GP
Rat Coronavirus	RCV	Coronaviridae	Coronavirus	R
Reovirus	REO	Reoviridae	Orthoreovirus	M, R, H, GP, Rb
Rat Minute Virus	RMV	Parvoviridae	Parvovirus	R
Rat Parvovirus	RPV	Parvoviridae	Parvovirus	R
Sialodacryoadentitis Virus	SDAV	Coronaviridae	Coronavirus	R
Sendai Virus	SEND	Paramyxoviridae	Respirovirus	M, R, H, GP, Rb
Shipping Fever Virus	SHFV	Paramyxoviridae	Paramyxovirus	Rb
Simian Virus-5	SV-5	Paramyxoviridae	Paramyxovirus	H, GP, Rb
Theiler's Murine Encephalomyelitis Virus	TMEV/GDVII [^]	Picornaviridae	Cardiovirus	M, R
Vaccinia	VACC	Poxviridae	Orthopoxvirus	M

* M=Mouse, R=Rat, GP=Guinea pig, H=Hamster, Rb=Rabbit; [^] SEND, SHFV, SV-5 and GDVII are the strains of PIV-1, PIV-2, PIV-3 and TMEV propagated for production of antigens.

Serologic Methods Manual: MFIA™

B. Bead Profiles

Note: Profiles may be modified. The exact assays and bead sets that each profile-lot comprises are indicated in the lot's Research Products Specifications sheet.

Species: Profile/Item #:				Mouse					Rat			
				Parvovirus RG-MDB-1	Prevalent RG-MDB-7	Tracking RG-MDB-2	Assessment RG-MDB-3	Assessment Plus RG-MDB-4	Prevalent RG-MDB-8	Tracking RG-MDB-5	Assessment Plus RG-MDB-6	
Assay												
Type	Agent	Coupled Reagent	Bead Set									
Microbial Antibodies	MNV	MNV	21		+	+	+	+				
	SEND	SEND	60			+	+	+		+	+	
	PIV-3	Bovine PIV-3	63								+	
	PVM	PVM	65			+	+	+		+	+	
	MHV/ SDAV	MHV-A59 NP	54		+	+	+	+	+	+	+	+
		MHV-A59	51		+	+	+	+	+	+	+	+
		MHV-S	20		+	+	+	+	+	+	+	+
	MVM	MVM VP2	56	+	+	+	+	+				
	MPV-1	MPV-1 VP2	18	+	+	+	+	+				
	MPV-2	MPV-2 VP2	33	+	+	+	+	+				
	KRV	KRV VP2	46						+	+	+	
	H-1	H-1 VP2	44						+	+	+	
	RPV	RPV VP2	19						+	+	+	
	RMV	RMV VP2	25						+	+	+	
	Parvo	NS-1	62	+	+	+	+	+	+	+	+	
	REO	REO-3	72			+	+	+		+	+	
	GDVII	GDVII	34		+	+	+	+	+		+	
	EDIM	BRV	42		+	+	+	+				
	LCMV	LCMV	45				+	+			+	
	HANT	HANT	43					+			+	
	MAV	MAV-1	47					+	+			+
		MAV-2	66					+	+			+
	POLY	POLY	64					+	+			
	K	K	55					+	+			
	ECTRO	ECTRO	37					+	+			
	MCMV	MCMV	52						+			
	MPUL	MPUL	76			+	+	+		+	+	
	CARB	CARB	35						+		+	
CPIL	CPIL	74						+		+		
ECUN	ECUN	75						+		+		
Internal Control	Tissue	wBAC	53	+	+	+	+	+	+	+	+	
		MARTH	77			+	+	+		+	+	
	Labeled Reagents	Mouse IgG	27	+	+	+	+	+				
		Rat IgG	36						+	+	+	
	Sample Addition	α Mouse IgG	26	+	+	+	+	+				
	α Rat IgG	24						+	+	+		

Note: Please check individual Bead Profile lot specification sheet for exact bead member composition.

B. Bead Profiles (continued)

				Species:		
				Hamster	Rabbit	
				Assessment RG-MDB-300	Tracking RG-MDB-200	Assessment Plus RG-MDB-201
Assay						
Type	Agent	Coupled Reagent	Bead Set			
Microbial Antibodies	SEND	SEND	60	+		+
	PIV-2	SV-5	63	+		+
	PVM	PVM	65	+		
	REO	REO-3	72	+		+
	EDIM	BRV	42			+
	LCMV	LCMV	45	+		+
	MAV	MAV-1	47			+
		MAV-2	66			+
	CARB	CARB	35		+	+
	CPIL	CPIL	74			+
ECUN	ECUN	75	+	+	+	
Internal Control	Tissue	wBAC	53	+	+	+
	Labeled Reagents	Hamster IgG	27	+		
		Rabbit IgG	36		+	+
	Sample Addition	α Hamster IgG	26	+		
α Rabbit IgG		24		+	+	

Note: Please check individual Bead Profile lot specification sheet for exact bead member composition.

C. Bead-Profile Control Serum Sets

Species	Profile	Control Set Item #	Polyspecific		Parvovirus			Nonimmune		Diluent
			High	Low	VP High	VP Low	NS1 Low	Mouse	Rat	
Mouse	Parvo	RG-MCL-1			√	√	√	√	√	√
	Prevalent	RG-MCL-7	√	√	√	√	√	√	√	√
	Tracking	RG-MCL-2	√	√	√	√	√	√	√	√
	Assessment	RG-MCL-3	√	√	√	√	√	√	√	√
	Assessment Plus	RG-MCL-4	√	√	√	√	√	√	√	√
Rat	Prevalent	RG-MCL-8	√	√	√	√	√	√	√	√
	Tracking	RG-MCL-5	√	√	√	√	√	√	√	√
	Assessment Plus	RG-MCL-6	√	√	√	√	√	√	√	√

Species	Profile	Control Set Item #	Polyspecific		Rotavirus	Nonimmune		Diluent
			High	Low	Low	Hamster	Rabbit	
Hamster	Assessment	RG-MCL-300	√	√		√		√
Rabbit	Tracking	RG-MCL-200	√	√		√	√	√
	Assessment Plus	RG-MCL-201	√	√	√	√	√	√

D. Sample MFIA™ Bead Profile Research Product Specification Sheet



251 Ballardvale St.
Wilmington, MA 01887
Telephone (978) 658-6000

RESEARCH PRODUCT SPECIFICATIONS

NAME: **MULTIPLEXED FLUOROMETRIC IMMUNOASSAY (MFIA) BEADS**

ITEM NO. :

RG-MBD-1 _____	RG-MBD-5 _____
RG-MBD-2 _____	RG-MBD-6 _____
RG-MBD-3 _____	RG-MBD-7 _____
RG-MBD-4 _____	RG-MBD-8 _____

LOT (BATCH): _____

DESCRIPTION: Partially purified antigen- and tissue control -coupled microbeads, organized into serology testing profiles as indicated in the following table:

ITEM NO.	SPECIES	PROFILE PACKAGE	AGENTS INCLUDED
RG-MBD-1	MOUSE	PARVOVIRUS	MPV-1, MPV-2, MVM, NS-1; plus internal control beads to verify assay performance
RG-MBD-7	MOUSE	PREVALENT	PARVOVIRUS PROFILE and MHV, MNV, TMEV, EDIM
RG-MBD-2	MOUSE	TRACKING	PREVALENT PROFILE and SEND, PVM, REO, MPUL
RG-MBD-3	MOUSE	ASSESSMENT	TRACKING PROFILE and LCMV, MAV, ECTRO, K, POLY
RG-MBD-4	MOUSE	ASSESSMENT PLUS	ASSESSMENT PROFILE and MCMV, HTN, ECUN, CARB, CPIL
RG-MBD-8	RAT	PREVALENT	RPV, H-1, KRV, RMV, NS-1, SDA/RCV, TMEV; plus internal control beads to verify assay performance.
RG-MBD-5	RAT	TRACKING	RPV, H-1, KRV, RMV, NS-1, SDA/RCV, SEND, PVM, REO, MPUL; plus internal control beads to verify assay performance
RG-MBD-6	RAT	ASSESSMENT PLUS	TRACKING PROFILE and TMEV, LCMV, MAV, HTN, ECUN, CARB, CPIL, PI-3

FORM/STORAGE: Beads are provided as a 20X suspension in phosphate buffered saline containing Tween, sodium azide and 1% BSA. Dilute 20-fold in Primary Diluent (Charles River Laboratories item #RG-MDL-1) to yield a suspension sufficient to perform 96 assays. Store refrigerated at 2-8°C in the dark. DO NOT FREEZE. Expiration is 1 year from receipt date if the product is properly stored.

PERFORMANCE: Beads from this batch were evaluated for potency and purity with a panel of standard immune and non-immune rodent sera. The batch was deemed acceptable because only the appropriate immune sera gave positive results; the median fluorescence index (MFI) scores for standard high and low immune positive control sera were as expected.

Note: FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE. Nothing on this sheet is to be construed as a recommendation to use this research product in violation of any patents. The information presented above is believed to be accurate. However, said information and product are offered without warranty or guarantee since the ultimate conditions of use and the variability of how the materials are treated are beyond our control. No claims beyond replacement of unacceptable material or refund of purchase price shall be allowed.

E. Charles River MFIA™ Results Excel Workbook: Results Report

Well:		Net Scores (TC Score >=2)																
		A12	B12	C12	D12	E1	F1	G1	H1	E2	F2	G2	H2	E3	F3	G3		
Assay	Antigen	Cutoff	Type	Sample-45	Sample-46	Sample-47	Sample-48	Sample-49	Sample-50	Sample-51	Sample-52	Sample-53	Sample-54	Sample-55	Sample-56	Sample-57	Sample-58	Sample-59
60	SEND	3000	Antigen	12	3	0	0	0	0	0	0	0	0	0	0	0	0	0
65	PVM	3000	Antigen	11	3	0	0	0	0	0	0	0	0	0	0	0	0	0
54	rMHV-A59	3000	Antigen	15	3	0	0	0	0	0	0	0	0	0	0	0	0	0
51	MHV-A59	3000	Antigen	14	3	0	1	0	0	0	0	0	0	0	0	0	0	0
56	MVM	3000	Antigen	1	0	7	3	0	0	0	0	0	0	0	0	0	0	0
18	MPV-1	3000	Antigen	25	23	19	10	0	0	0	0	0	0	0	0	0	0	0
33	MPV-2	3000	Antigen	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
62	NS-1	3000	Antigen	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0
34	GDVII	3000	Antigen	17	3	0	1	0	0	0	0	0	0	0	0	0	0	0
72	REO	4000	Antigen	14	2	0	0	0	0	0	0	0	0	0	0	0	0	0
42	EDIM	3000	Antigen	10	2	0	0	0	0	0	0	0	0	0	0	0	0	0
45	LCMV	3000	Antigen	19	4	0	1	0	0	0	0	0	0	0	0	0	0	0
43	HANT	3000	Antigen	16	3	0	1	0	0	0	0	0	0	0	0	0	0	0
37	ECTRO	3000	Antigen	13	4	0	0	0	0	0	0	0	0	0	0	0	0	0
47	MAY-1	3000	Antigen	15	3	1	2	0	0	0	0	0	0	0	0	0	0	0
66	MAV-2	3000	Antigen	18	5	0	1	0	0	0	0	0	0	0	0	0	0	0
52	MCMV	3000	Antigen	9	2	0	0	0	0	0	0	0	0	0	0	0	0	0
64	POLY	3000	Antigen	11	4	0	0	0	0	0	0	0	0	0	0	0	0	0
55	K	3000	Antigen	14	7	0	0	0	0	0	0	0	0	0	0	0	0	0
76	MPUL	3000	Antigen	15	4	0	1	0	0	0	0	0	0	0	0	0	0	0
77	MARTH	3000	TC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	CARB	4000	Antigen	11	2	0	0	0	0	0	0	0	0	0	0	0	0	0
74	CPIL	3000	Antigen	14	3	0	0	0	0	0	0	0	0	0	0	0	0	0
75	ECUN	3000	Antigen	18	4	0	1	0	0	0	0	0	0	0	0	0	0	0
53	wBAC	3000	TC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	Mouse IgG	8000	IgG	13	14	13	14	13	13	13	14	14	14	13	14	14	13	12
36	Rat IgG	8000	IgG	16	17	16	16	15	15	16	15	15	15	15	14	14	14	13

F. Forms

Charles River MFIA™ Test Plate MAP

Lab: _____

Plate ID: _____ Species: _____
 Profile: _____ Lot #: _____
 Control: _____ Lot #: _____

Role	Technician	Date
Setup		
Test		

Row/Column:	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Remarks: _____

