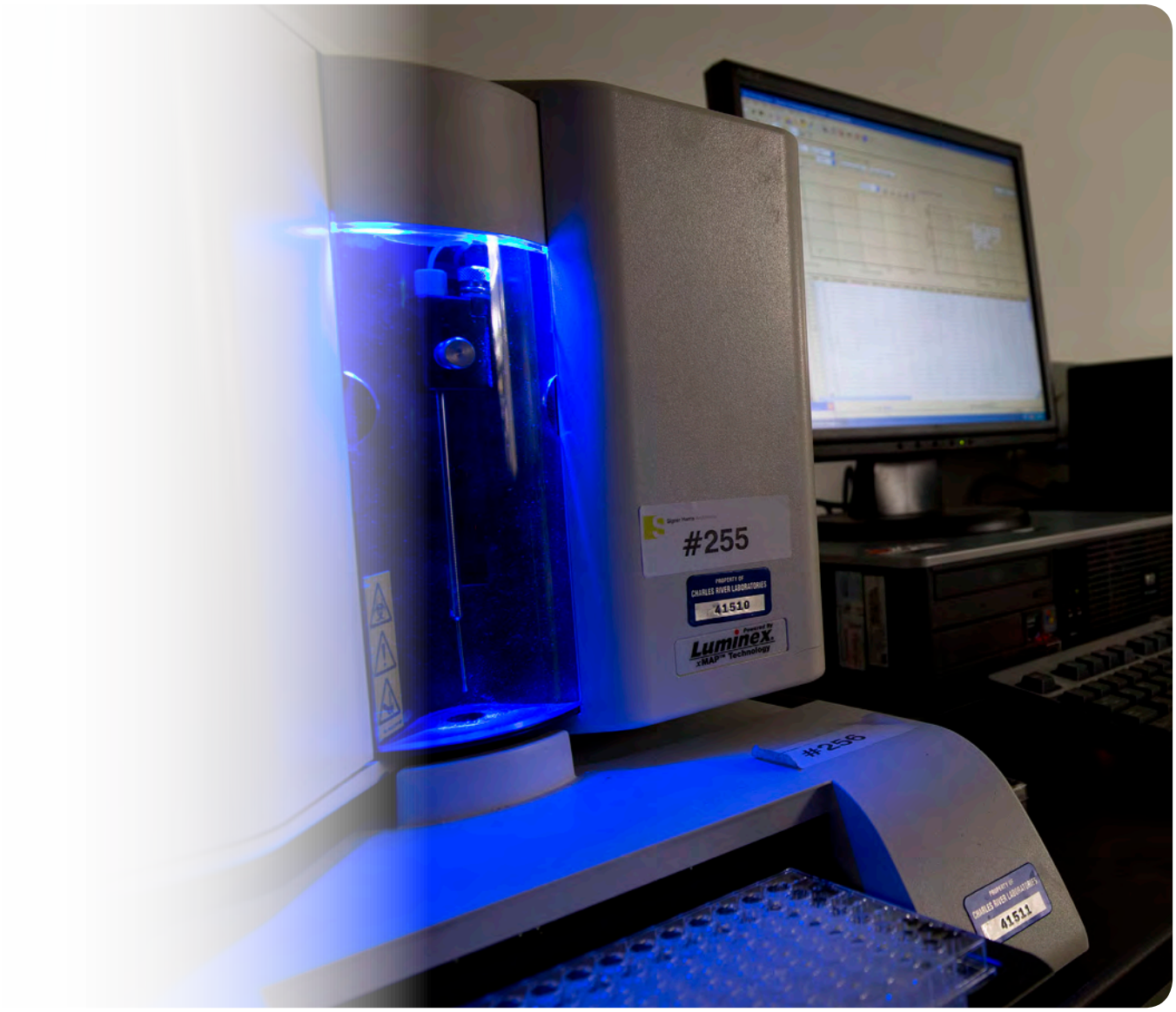

Serologic Methods Manual:

Multiplexed Fluorometric ImmunoAssay® (MFIA®)

charles river

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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.

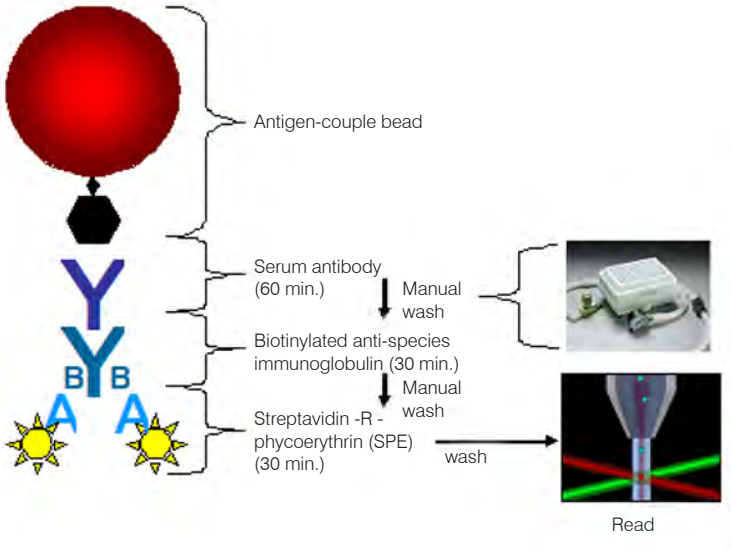
Over the past two decades, the indirect enzyme-linked immunosorbent assay (ELISA) has been the main screening method for serosurveillance due to its sensitivity and ease of automation, while the indirect immunofluorescence assay (IFA) has been the technique of choice for corroborating findings. Both 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, 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 are covalently linked to suspended 5.6 micron, two dye color-coded polystyrene microspheres, i.e., beads (Figure 1A). Since the beads come in 100 distinct color combinations, 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, corresponding to a particular analyte; the other excites the phycoerythrin reporter dye captured during the assay. A minimum number of beads (25 at Charles River Diagnostic Services) are read per assay and the intensity of phycoerythrin fluorescence is reported as a Mean Fluorescence Index (MFI) ranging from 0 to approximately 33,000 (the MFI is discussed more fully in Section H).

Figure 1. A: Test Plate with Bead Color Sets (multi-colors shown for distinction)
B: Luminex® xMAP® 200 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 (27 ± 2 °C on an orbital shaker) are followed by filter-wash steps to remove unbound serum constituents and labeled reagent. Wash solution added to plate wells is removed by bottom aspiration through well filters, which retain the beads. Antigen-antibody complexes formed during the test serum incubation are detected through successive incubations with biotinylated goat or rabbit antibodies to immunoglobulin (BAG) of the species being tested followed by R-phycoerythrin-labeled streptavidin (SPE), the reporter molecule (Figure 2).

Figure 2. Schematic of the MFIA®; incubations are performed at 27 °C for the time specified, with shaking, to maintain the beads in suspension.



Because of multiplexing, the MFIA® testing process is highly efficient, requiring smaller sample and reagent volumes than traditional singleplex assays. In addition, the breadth of the bead set allows for the incorporation of several internal assay controls to evaluate sample suitability and assay function and to ensure the accuracy of results; these include a tissue control, a species-specific IgG (αIg) and an anti-species immunoglobulin. As in the ELISA and IFA, the tissue control detects non-specific binding of serum immunoglobulin. The αIg control confirms that serum has been added and contains a sufficient immunoglobulin concentration for detection. The anti-species immunoglobulin control bead set demonstrates that the labelled reagents have been properly added and that the Suspension Array Reader is functioning properly.

We have performed comprehensive validation studies of the 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 repeated on different days. You can view a summary of the mouse and rat MFIA® validation study results on the following Charles River Laboratories website:

http://www.criver.com/SiteCollectionDocuments/rm_ld_r_MFIA_summary_report.pdf.

Briefly, the detection limits (i.e., standard immune serum titration endpoints) of the MFIA® were comparable to, and in some cases surpassed, those of the corresponding ELISA. Diagnostic specificity, measured with SPF rodent sera, exceeded 95%; the overall correspondence between ELISA and MFIA® performed on known–positive and known–negative mouse sera was greater than 99%. 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 1-877-274-8371 or askcharlesriver@crl.com to request a copy of the "MFIA® Testing Laboratory: Comprehensive Inventory" and MFIA® reagent order forms.

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. Pipettes
   a. 5-25 mL pipettes with device for aspirating and dispensing fluids (e.g., Pipet-Aid®)
   b. Micropipette tips
3. V-bottom reagent reservoirs (minimizes reagent waste)
4. 0.2 μm bottle-top filter unit and sterile screw-cap bottle
5. Serum filtration plate – **Optional** – Specifications: 96 wells, ≥350 μL capacity per well with 1.0-1.2 μm particle (e.g., glass microfiber) depth-filter bottoms and short drip director (drip director is optional)
6. Microtiter plate – Specifications: 96-well, flat bottom, polystyrene, low protein binding – used for the following:
   a. Stock sample plate
   b. 2X sample plate – unfiltered and filtered
7. Filter-bottom test plate – Specifications: 96 well microplate with lid, 350 μL capacity per well with 1.0-1.2 μm hydrophilic (low-protein-binding) membrane-filter bottom
8. Plate-sealing tape – **Optional** – to seal test plate bottom during the assay procedure (prevents leaks or fluid wicking through plate)
9. Elastic bands – to secure test plate lids
10. Aluminum foil – to block the incubator window and/or to wrap around test plates to achieve darkness during incubations

B. Equipment

1. **96-well microtiter plate vacuum manifold system** set to deliver a vacuum of approximately -1.5 to -2.5 inches of mercury (Hg, equivalent to 38-64 mm)
   a. Components
      i. Microplate vacuum manifold
      ii. Tubing and connectors
      iii. Vacuum gauge
      iv. Vacuum control valve
      v. Vacuum on/off valve
      vi. Laboratory vacuum or vacuum pump
      vii. Waste reservoir
      viii. Trap reservoir or hydrophobic pump-shielding filter
   b. References for setup, calibration and use
      i. Millipore multiscreen separations systems users guide http://www.Millipore.Com/userguides.Nsf/docs/p17479, chapter 2: using the multiscreen vacuum manifold
      ii. Bio-Plex Suspension Array System Hardware Instruction Manual http://www.bio-rad.com/cmc_upload/Literature/39187/4006205D.PDF, section entitled Vacuum Manifold Set Up
2. **Incubator (optional)** – Samples are incubated at room temperature but an incubator can be used to maintain temperature consistency
   a. Specifications:
      i. Incubator temperature: 27 °C. Slight variation (± 2 °C) will not affect the assay.
      ii. Mechanical convection – eliminates cold/warm spots, maintains constant temperature
      iii. Adequate space and power outlet for orbital shaker for bead mixing during incubations
3. **Orbital plate shaker: 4-position shaker** – Test plates are incubated with orbital shaking between 400 and 700 rpm, displacement of approximately 1 cm.
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, version 6.0 or higher.
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	

8. **Plate auto-washer (optional):** For labs with a high throughput, we recommend the use of an automated plate washer such as the Bio-Tek model ELX50/FMW. This unit has a bottom aspiration capability as well as a dispenser to efficiently wash test plates.

C. Charles River-Supplied MFIA® Reagents

MFIA® reagents are accompanied by research product specification sheets. For shipping and storage conditions, refer to Table 1.

1. **Stock (20X) bead-profile concentrates:** For a list of standard MFIA® profiles go to http://www.criver.com/files/pdfs/research-models/rm_ld_m_mfia_reagents_item_list.aspx. MFIA® profiles are provided as 20X concentrates in 250-300 µL aliquots (sufficient for one full 96-well test plate; 88 samples with 8 controls) suspended in phosphate-buffered saline (PBS) containing Tween 20, sodium azide and 1% BSA. Store stock bead profiles **refrigerated at 2-8 ºC and in darkness as the beads are light sensitive.** DO NOT FREEZE.
2. **Control serum sets:** For a list of available control serum sets go to http://www.criver.com/files/pdfs/research-models/rm_ld_m_mfia_reagents_item_list.aspx. Control sets consist of high- and low-range immune sera, and non-immune sera for verification of assay (analytical) sensitivity and specificity. Volume supplied is sufficient for five test plates. **CONTROL SERUM SETS ARE BEAD-PROFILE-SPECIFIC.** For example, the control set for an assessment profile is different from the set for the tracking profile. Store the sera in a freezer at ≤ -60 ºC. Avoid repeated freeze-thaw cycles and refrigeration for more than two (2) days, as deterioration of signal may occur.
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 five test plates. The recommended working dilution for a reagent, which varies by lot, is indicated in the enclosed research product specification sheet for each lot. Store stock BAG at -20 ºC. SPE is light-sensitive; stock SPE should be stored in the **DARK** at 2-8 ºC. Store working dilutions at 2-8 ºC.

   NOTE: Reagents may be purchased directly from commercial suppliers. However, we strongly encourage you to use the BAG and SPE from Charles River, as we perform stringent quality control testing on each lot to determine optimal working dilutions and to assure suitability for the Charles River MFIA®.
4. **Primary diluent-1 and -2:** Used to dilute test sera and 20X bead profile suspensions. Primary diluent-1 is used for testing of all species except simian, which requires primary diluent-2. The diluent contains proprietary blocking agents that inhibit nonspecific reactivity and therefore is essential for obtaining accurate assay results.

Table 1: Charles River-Supplied MFIA® Reagents – Shipping and Storage Conditions

MFIA® Reagent	Concentration Provided	Temperature Shipped	Temperature/Storage Condition	
			Stock Reagent	Working Dilution
Stock bead-profile	20X	2-8 °C	2-8 °C/Dark	2-8 °C/Dark
Control serum set	2X	Dry Ice	-60 °C	2 days @ 2-8 °C
BAG	Undilute*	2-8 °C	-20 °C to 30 °C	30 days @ 2-8 °C
SPE	Undilute*	2-8 °C	2-8 °C/Dark	30 days @ 2-8 °C/Dark
Primary diluent-1 and -2	N/A	2-8 °C	2-8 °C	N/A

*Refer to the lot-specific research product specifications sheet for working dilution information.

D. Reagents Not Supplied

1. **PBS:** 0.01 M phosphate-buffered saline (PBS), pH 7.4 (for diluting serum)
2. **MFIA® assay buffer (PBS-BSA):** PBS, with 1% (w/v) bovine serum albumin (BSA), pH 7.4 and 0.05% ProClin®: The PBS-BSA reagent can be purchased from an outside vendor. The buffer is used to dilute the BAG, SPE, and as the assay wash buffer.
3. **ProClin® 300 (Supelco®):** An anti-microbial preservative added to PBS and MFIA® assay buffer (0.5 mL ProClin® per liter).
4. Deionized (DI) water

   NOTE: See "MFIA® Testing Laboratory: Comprehensive Inventory" from Charles River Technical Services for specific vendor information.

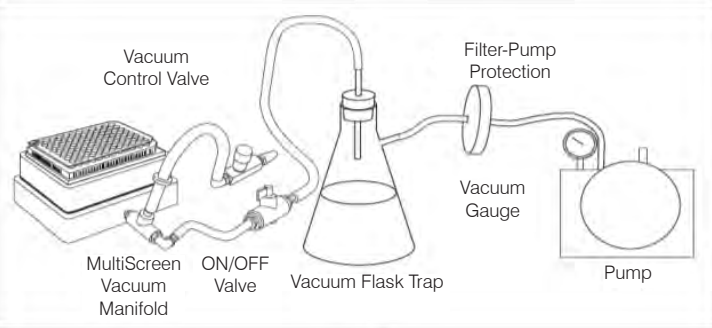
III. MFIA® Equipment Setup

A. 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 inches Hg (-0.7 to -1.3 psi)**. 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 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 adjusted, PBS-BSA-ProClin® should be evacuated from a filter-bottom test plate (e.g., Millipore Multiscreen HTS) in about 5-10 seconds.

   NOTE: It should take approximately 5-10 seconds to completely evacuate the fluid from a test plate. The vacuum should be minimized to attain the evacuation rate described. Too fast an evacuation rate will imbed the beads in the filter matrix and reduce reading efficiency.
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., 10%) bleach or alcohol.

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

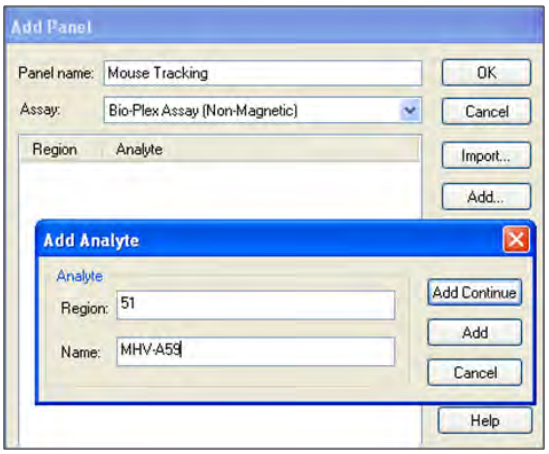


B. Automated Plate Washing - Bio-Tek ELx50 (Optional)

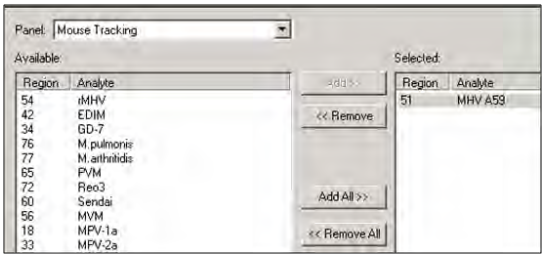
1. This section details the use of the Bio-Tek ELx50 automatic plate washer. It is used following the first and second incubation steps of the MFIA® to remove reagent from a filter-bottom test plate by vacuum, wash the plate and then dispense diluent to each well using an eight-channel dispenser. Additionally, once the incubation steps have been completed, the washer is used to vacuum, wash and dispense diluent prior to reading the test plate in the MFIA® reader.
2. The plate washer is programmed to run a series of small programs linked together to create a complete plate-washing step. Parameters are available to aid in setting up the washer for daily use. At least two linked programs should be established; others may be set up as desired.
   a. Link 1 – for washing the test plate following incubation of primary serum and after the addition and incubation with BAG.
      i. Two cycles of bottom aspiration and washing with 100 µL of MFIA® assay buffer
      ii. One cycle of bottom aspiration and dispensing of 50 µL MFIA® assay buffer
   b. Link 2 – for washing the test plate following incubation with SPE prior to reading the test plate.
      i. Two cycles of bottom aspiration and washing with 100 µL of MFIA® assay buffer
      ii. One cycle of bottom aspiration and dispensing of 125 µL MFIA® assay buffer
3. Prime the washer before use with MFIA® assay buffer.
4. At the end of the day rinse the washer with DI water.
5. Follow manufacturer's maintenance recommendations.

C. Suspension Array Reader System – Bio-Rad Bio-Plex 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:
   a. 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.
   b. 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.
   c. In the MFIA®, the dilution at which sera are tested is low (i.e., 50- or 100-fold). Filtering the serum (optional) should reduce reader issues such as clogs within the fluidics system and slow test plate processing. Serum filtration (optional) 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 (known as a Panel) is created from within a protocol, consisting 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). The following screen shots are from the Bio-Plex Manager software and are intended only as a visual aid.
   a. Define, by name and bead set (i.e., region), all individual assays/analytes to be included in Profile/Panel.



   b. Create a Profile/Panel containing the appropriate bead sets/analytes indicated for specific Charles River MFIA® bead profiles. Creating panels allows new protocols to be quickly populated with a standard set of analytes. Refer to the image below for specific region and analyte information.



   c. For proper presentation of the MFIA® data, create a Profile/Panel in the Bio-Plex Manager corresponding to each Charles River MFIA® bead profile being used.

IV. MFIA® Procedure

A. Prepare Reagents

1. Prepare MFIA® assay buffer for filter-washing the test plates/beads and for diluting BAG and SPE:
   a. Add 1 packet of the PBS-1% BSA powder to 1 liter (1.0 L) of deionized water. Mix on a stir plate until the powder has dissolved completely. Do not agitate the solution.
   b. Add 0.5 mL of ProClin® 300 and mix.
   c. Check the pH, if desired; however when properly reconstituted, the PBS-BSA has a pH of 7.4 ± 0.2.
   d. Filter the solution through a 0.2 μm bottle-top filter unit into sterile, labeled containers (several filters may be needed).
   e. Refrigerate at 2-8 °C.
   f. 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. Since the expiration date is 30 days, the volume prepared can be increased as desired, up to a volume that is sufficient for one month of testing.
   a. Determine the volume of stock BAG, SPE and MFIA® assay buffer you require by performing the following calculations:

      mL MFIA® assay buffer = (6 mL per plate) x (estimated # of plates per week)

      mL stock reagent = (mL MFIA® assay buffer) x (working dil. of BAG or SPE)

      For example:
      Estimated # of test plates per week = 10
      Working dilution of BAG = 1/150

      Therefore:
      mL MFIA® buffer = 6 x 10 = 60 mL
      mL stock reagent = 60 mL x 1/150 = 0.4 mL

   b. Add the calculated mL of MFIA® assay buffer and either calculated volume of BAG or SPE stock to a disposable plastic container.
   c. Label the container with the reagent name (BAG/SPE), concentration, ("working dilution") and preparation date. Note: SPE should be stored in a light-block (Amber) container or wrapped in foil.
   d. Store the working dilution reagents in the refrigerator at 2-8 °C.
   e. If you are preparing bulk lots of BAG and SPE, we suggest that you perform quality control on the new lots of reagents once, to qualify the reagents for use. The diluted BAG and SPE are good for one month with proper storage.
3. Prepare working dilution bead suspensions.
   a. **LIMIT THE EXPOSURE OF BEADS TO LIGHT; DO NOT FREEZE.**
   b. Calculate the following quantities by multiplying the **volume per test plate by the # of test plates.**

Reagent	Volume/Test Plate
Primary diluent-1 or -2	5.7 mL
Stock (20X) bead suspension	300 μL (1 vial)

   c. Pipette the calculated mL of primary diluent-1 or -2 (depending on species being tested) into an appropriately-sized disposable tube or bottle. Label the container appropriately; include at least, the profile designation (i.e., Mouse Tracking), suspension concentration (i.e., "working dilution") and the preparation date.
   d. Remove the calculated number of vials from refrigerated storage.
   e. Vortex each vial for 5-15 seconds to fully suspend the beads.
   f. Place the vials in a sonicator bath for approximately 15-30 seconds to break up bead aggregates.
   g. Pipette the calculated volume of 20X bead suspension into the working dilution container to which you added primary diluent.
   h. Store the working dilution beads in a refrigerator at 2-8 °C, in the dark. We have found that working dilution bead-profile suspensions are stable for two weeks when kept at 2-8 °C.

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

B. Prepare Serum Samples for Testing

1. 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.**
2. Dilute sera by adding 1 part serum to 4 parts PBS (1:4 or 5-fold). Alternatively, the same dilution of serum is produced by adding 1 part **whole** blood to 2 parts PBS prior to centrifugation.
3. Keep samples refrigerated for short-term storage (2-3 days) or frozen at -20 °C or below if they need to be stored for more than several days before being tested.
4. Assemble the following materials and reagents. Refer to the Materials section for proper reagent preparation.
   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

C. Prepare EZ-Spot® Samples for Mouse and Rat Testing

1. Collect whole blood from the animal using your method of choice. Note that each dried blood spot (DBS) requires a minimum volume of 25 µL of blood.
2. Blood can be spotted directly from the animal onto the EZ-Spot® card.
   a. To making spotting directly from live animals easier, the EZ-Spot® card can be cut between the sample circles to create 'fingers'. Take care to not cut through the entire card.
   b. **Alternatively,** you can collect blood into EDTA microtainer tubes. Immediately mix the tube well and spot the whole blood onto an EZ-Spot® card.
3. Label the spot with sample ID on the outer flap directly above the spot.
4. Dry the EZ-Spot® card, flap open, for 55-65 minutes at room temperature in a well-ventilated area.
5. Close the flap on the cards containing the DBS and transfer them to a plastic, zip-top bag containing a desiccant packet (10 cards per 1 desiccant packet).
6. Store the cards in plastic bags with a desiccant packet at 2-8 °C. There is no expiration if the cards are stored properly.
7. For EZ-Spot® sample elution reagents and procedure, please contact askcharlesriver@crl.com.

D. MFIA® Test Plate Organization

1. Assign samples to specific wells in test plates designated by sample host species and MFIA® profile.
   a. Record this information, as we typically do, on an MFIA® test plate map or similar form. A blank plate map form is provided in Appendix B. A filled version of this form is shown in Figure 4.

Figure 4: Example of the MFIA® Test Plate Map

Charles River MFIA® Test Plate MAP

Lab: Your Lab

Plate ID: Your Plate 1 Species: Mouse
Profile: Mouse Tracking Lot #: 031811
Control: Mouse Tracking Lot #: 022811

Role	Technician	Date
Setup	JPC	15-Oct-14
Test		

Row/Column:	1	2	3	4	5	6	7	8	9	10	11	12
A	11-01234-1	11-01111-1	5	13	6	8	8	16	8			Mouse-1 High
B	2	2	6	14	C57BL/6-1	K84 - 1	9	CD-1 11-5555 - 1	9			Mouse-1 Low
C	3	3	7	15	2	2	10	2	10			Mouse Mid
D	4	4	8	B23-06/06/11-1	3	3	11	3				NMS
E	5	S-1267-1	9	2	4	4	12	4				NRS
F	6	2	10	3	5	5	13	5				Diluent
G	7	3	11	4	6	6	14	6				
H	8	4	12	5	7	7	15	7				

Remarks:

   b. Test plate locations for standard serum and diluent controls must be reserved. We typically reserve wells in column 12 for these controls. The number of controls varies from 6-8, depending on the test profile chosen.
2. Arrange sample vials in racks by test plate according to the test plate map. This will make it easier for you to transfer samples from vials to their assigned plate wells.

3. Transfer samples from vials to a microtiter plate labeled with your Plate ID code; dilute samples in primary diluent-1 or -2 to a 2X concentration.
    a. The 2X concentration for rodent samples corresponds to a 1:24 (or 25-fold) dilution.
    b. The 2X concentration for simian and poultry samples corresponds to a 1:49 (or 50-fold) dilution.
    c. Although only 50 µL of diluted sample is required per multiplex assay, prepare an excess volume of 2X sample (e.g., 125 µL) for the following reasons:
        i. To ensure that the volume of stock sample being diluted is large enough to be accurately pipetted
        ii. To compensate for losses, which occur during sample filtration and transfer
        iii. To have a sufficient quantity for repeat testing
    d. Transfer and dilute samples to the 2X concentration by one of the following methods:
        i. Pipette the appropriate volume of primary diluent-1 or -2 shown in Table 2 into a microtiter plate, labeled "2X Sample Plate" or the like. Pipette the appropriate volume of stock sample shown in 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

Species	Initial Stock Serum Dilution	Transfer Vol (µL)	Primary Diluent (µL)	Primary Diluent Type
Rodent*	Neat	5	120	Primary diluent-1
	1:4 (1/5)	25	100	
Simian	Neat	2.5	122.5	Primary diluent-2
	1:4 (1/5)	12.5	112.5	
Poultry	Neat	2.5	122.5	Primary diluent-1
	1:4 (1/5)	12.5	112.5	

*Rodent includes mouse, rat, hamster, guinea pig and rabbit.

4. Alternatively, first transfer samples from vials to a microtiter plate labeled "Stock Sample Plate" and then dilute the samples 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 sample dilution (i.e., 1:4 or Neat) and "2X", respectively.
    b. Transfer approximately 125 µL of neat or 1:4 diluted sample from a vial to a well in the stock sample plate. Repeat for all samples.
    c. Pipette the appropriate volume of primary diluent as shown in Table 2 to all sample wells in the 2X sample plate. DO NOT add diluent to control wells.
    d. Pipette the appropriate volume of sample, as indicated in Table 2, from the stock sample plate to matching wells in the 2X sample plate containing primary diluent. You can complete this transfer most easily using a multichannel pipette. Mix thoroughly. The final volume of sample 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 re-transferring the sera from vials.

5. Filter the 2X samples (optional).

    NOTE: Although optional, we routinely filter samples to reduce the occurrence of reader clogs and longer-than-expected reading times.
    a. Pipette samples from a 2X sample plate into matching wells in a sample filtration plate. We recommend using a plate with a depth filter (such as glass fiber) rated to remove particles ≥1-1.2 µm, 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 sample 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 inches Hg** until all of the samples 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.

6. Transfer the 2X samples to a test plate as described in Section E below to perform the MFIA®. If testing is not proceeding immediately, **cover and refrigerate the 2X sample plate at 2-8 °C. Perform the MFIA® within 72 hours of plate preparation.** Discard the 2X sample plate at the end of the week.

E. Perform the Assay

1. Make sure the following materials and reagents are readily available (refer to the materials section where necessary).
   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® assay buffer
   g. Microplate vacuum manifold system calibrated to -1.5 to -2.5 inches Hg (-0.7 to -1.3 psi)
   h. Reagent reservoirs
   i. Working dilution stock bead suspensions
   j. Room temperature incubator (optional), set at 27 ± 2 °C, equipped as above
   k. Orbital shaker set to between 400 and 700 rpm
   l. Working dilution; biotinylated anti-species serum immunoglobulin (BAG)
   m. Working dilution; streptavidin-R-phycoerythrin (SPE)
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. **Thoroughly mix the control sera after they are thawed.**
4. Pre-wet test plates as follows:

   NOTE: Failing to pre-wet all wells can cause unequal filtration during the assay.
   a. Dispense 100 µL of MFIA® assay buffer into ALL 96 PLATE WELLS.
   b. Evacuate the MFIA® assay buffer using the microplate vacuum manifold.
5. **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 well of the test plate being used. Refer to the test plate map.
8. Add **50 µL of 2X test sample and control serum set** to their assigned test plate wells. Refer to the test plate map.
   a. Transfer 2X test samples from corresponding wells in the 2X sample plate to the filter-bottom test plate using a multichannel pipette.
   b. Add the control serum set 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 the test plates for 60 minutes** IN DARKNESS with orbital shaking at 400-700 rpm at room temperature. If using an incubator that has a clear glass door, cover the glass or wrap the plate in aluminum foil to limit light exposure.
11. Filter-wash the test plate using the Link 1 program of the automated plate washer or by hand 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® assay buffer** into every test plate well. This step is accomplished most efficiently with a repeating multichannel pipette.

d. Open the ON/OFF valve to evacuate the wells. Close the valve as soon as all wells have been evacuated. **NOTE: Avoid prolonged aspirations** (more than 10 seconds), which can lead to difficulty in re-suspending beads and reduced assay MFI.

e. Repeat steps c and d.

f. Ensure that all liquid 'wicking' has stopped by blotting the test plate on paper towels.

12. Dispense **50 µL of MFIA® assay buffer** into each test well.

13. Dispense **50 µL of working dilution BAG** into each test well. Secure a cover lid to the test plate with an elastic band.

14. **Incubate the test plate for 30 minutes** under the conditions described in the sample incubation step.

15. Filter-wash test plates twice and dispense **50 µL of MFIA® assay buffer** as described above (steps 11-12) and immediately continue to the next step.

16. Dispense **50 µL of working dilution SPE** into each test well. Secure a cover lid to the test plate with an elastic band.

17. **Incubate SPE in test plate wells for 30 minutes** under the conditions described in the sample incubation step.

18. Filter-wash the test plate by hand as above (step 11) or by using the Link 2 program of the automated plate washer.

19. Dispense **125 µL of MFIA® assay buffer** into each test well.

20. **Optionally,** seal test plate bottoms with plate-sealing tape.

    a. If 'wicking' or drips are noted on the test plate, sealing the bottom of the plate is an efficient method for preventing further fluid loss.

    b. If there has been significant loss of fluid from the wells prior to reading, re-wash the plate as in steps 18-19 above.

21. Secure a cover lid to the test plate with an elastic band.

    a. Prior to reading the test plate, re-suspend the beads by shaking for 1 minute.

    b. You may then store sealed, covered test plates at room temperature in darkness for up to 12 hours before reading.

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

Before attempting to read a test plate, make sure the Bio-Rad Bio-Plex reader has been warmed up at least 30 minutes and that the start-up wash cycle and calibration procedures have been performed.

NOTE: Follow the manufacturer's instructions when performing all start-up procedures (i.e., make sure the instrument is set on LOW to calibrate the CAL 2 beads).

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.

1. 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.

2. Select the appropriate standard protocol and assay profile previously established (section III.C).

    a. Modify the plate format to reflect the number of samples being tested and their locations.

    b. The MFIA® is NOT quantitative. We do not create standard curves for interpolation of antibody titers. Therefore, you may define all wells, including those to which we add control sera, as unknowns, designated with "X" in the Bio-Plex Manager.

3. Run the protocol to read the test plate. For the Bio-Plex system:

    a. Select the protocol Start menu option.

    b. Save the protocol run as a *.rbx (i.e., results) file; name appropriately.

    c. Press the Eject button and place the test plate in the plate holder.

    d. Select OK; the plate holder will close and test plate will be read.

4. Observe the first sample to verify that the selected bead panel matches the bead profile for the test wells; during the read, all bead regions of the selected profile should be populated for each sample.

    a. If there are beads falling outside their designated bead region on the protocol display, an incorrect profile selection has been made.

        i. For Bio-Plex Manager software version 6.0 and above, allow the reader to finish the plate, then reselect the proper profile and recreate the report file. The newer software versions record data for all analytes present.

ii. For older Bio-Plex Manager software versions, stop the plate read, reselect the proper profile and then reread the plate.

b. If there are regions that are not filling in, then agents have been selected that are not in the profile selected. Excessive read times will be evident. Stop the read, reselect the proper profile or eliminate the incorrect agents and reread the plate.

5. Examine the results report for errors, such as inadequate bead counts or failing IgG scores. Assays with errors should be repeated. Refer to Section V, MFIA® Troubleshooting Guide if necessary.

G. Exporting MFIA® Plate Data

To analyze the results, the Bio-Plex raw plate data (*.rbx file) is exported as a Microsoft® Excel® spreadsheet. Charles River has developed the MFIA® Results Excel Workbook, where test plate results are imported as individual sheets. These results are then analyzed based on MFI, background signal and tissue reactions to generate a final report of scored results, sample classification and interpretation. Detailed use of the Charles River MFIA® Results Excel Workbook is provided in a separate document.

1. Export the test plate data to a single Excel worksheet, formatted so that the samples are listed vertically and the assays are 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	86	55	69	172.5

2. These results can be saved as individual workbooks or transferred (imported) directly to the **Charles River MFIA® Results Excel Workbook,** or similar workbook that has been established to analyze MFIA® test plate data.

a. Open the **Charles River MFIA® Results Excel Workbook,** or similar workbook.

b. Use the "Move or Copy Worksheet" (right-click on the sheet name) command to insert the exported test plate worksheet into the results workbook.

c. Name the worksheet following the plate data results naming conventions established at the time the results workbook was created.

d. The workbook automatically analyzes the results for reporting. Refer to the MFIA® Results Excel Workbook (version March 2011): Instructions for Use for information on printing reports. Appendix A shows an example of a scored results report.

H. Scoring MFIA® Results

The following section briefly describes the MFIA® test plate data analysis. Additional documentation and a detailed description of scoring and interpretation are available in the MFIA® Results Excel Workbook (version March 2011): Instructions for Use or by contacting Charles River's Technical Services.

1. The assay mean fluorescence index (MFI) reflects the reactivity of the sample to the specific antigen.

2. Each antibody assay is assigned a tissue control test and a cut-off value.

a. **Tissue control (TC) test:** For most 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. All other tissue controls are extracts from uninfected microbial host cells or cell lines.

b. **Assay cutoff:** This value is the minimum fluorescence expected for a positive sample and is adjusted for each assay to maximize diagnostic accuracy. For most assays, the fluorescence cutoff is 3000.

3. A net MFI is calculated for antibody assays by the following formula.

$$\text{Net MFI} = \text{MFI}_{\text{Antibody Assay}} - \text{MFI}_{\text{Tissue Control}}$$

4. Net MFI and TC MFI are converted to scores by comparison to the specific assay cutoff. These formulae were developed to enhance the diagnostic sensitivity to equivocal and tissue-reactive samples.

a. For samples where the net MFI is elevated with the TC MFI low or 0, the analysis is simply:

Score = (Net MFI/Cutoff MFI)*3

b. For samples where the net MFI is elevated with the TC MFI equal to or greater than the assay cutoff, the following equation is used. Samples are scored accordingly with any TC score shown in brackets:

Score =[(Net MFI - Cutoff MFI)/1000] + 3

Example: Raw MFI = 8200, TC = 4000, Cutoff = 3000; Net MFI = 4200; Score "4(4)"

5. The scores range, in general, from 0 to 30, with conditional calculations used in borderline and atypical values to give meaningful scores. Scoring results in this manner facilitates interpretation and visual inspection of the results report.

I. MFIA® Results Interpretation and Classification

1. **Ig or αIg score (Ig bead set assay controls) = Raw MFI/1000.** These controls are typically assigned a pass/fail cutoff MFI value of ≥ 8000 (score ≥ 8), however, the actual values vary depending on the bead lot. Scores below the indicated value result in a failure of the assay (see below).
2. Test plate results should only be interpreted if the system-suitability and serum control results meet the following acceptance criteria:

Acceptance Criteria for Assay Controls		
Control	Acceptable Result	
	Score	Classification
High-range immune serum	≥ 4.5	Positive
Low-range immune serum	≥ 1.5	≥ Borderline
Non-immune serum	< 2.5	≤ Borderline
Diluent	< 2.5	≤ Borderline
Ig bead set*	≥ Cutoff/1000	Pass

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

3. If any of the test plate assay controls fail, the results are not acceptable and must be repeated.

NOTE: If the high-range immune serum control fails but all other controls are acceptable, the results are valid.

4. If test plate assay control results are satisfactory, individual assay scores are classified as shown in the following table:

MFIA® Score Classification			
Score			Classification
TC	Net	TC + Net*	
≥ 2	< 0.5	< 2.5	Negative (–)
	≥ 0.5	≥ 2.5	TC Reaction (T)
< 2	< 1.5		-
	1.5 ≤ X < 2.5		Borderline (B)
	≥ 2.5		Positive (+)

*A classification of negative can still be determined with a non-zero TC score provided the TC + Net score (= Ag score) is negative. The TC + Net determination is not necessary when the TC score is < 2.

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 recommends that you contact the supplier for any equipment-related problems.

A. Suspension Array Reader Errors

Symptom	Possible Cause	Solution
Very high test scores	CAL 2 beads calibrated on HIGH	• Make sure CAL 2 beads are calibrated on LOW.
Bead count low	Incorrect bead profile or protocol selected	• In the bead grid window, verify that all white ovals (denoting the regions of the 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.
	Beads too dilute	• Check calculations and pipette calibration.
	Beads too aggregated	• Vortex and sonicate 20X bead concentrate. • Vortex and sonicate working bead suspension before use. • Manually re-suspend the test plate wells to disperse aggregates and reread the plate.
	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 the 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 after adding diluent and before 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 125 µL 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 (photo-bleached)	• 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 assay selections and reanalyze the results.

B. Control Failures

Symptom	Possible Cause	Solution
Low positive control scores	Incorrect 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, IN DARKNESS 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.
	Plate wells leaked	• Seal bottom of plate with plate-sealing tape after adding diluent and before reading.
Non-immune 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.
Control sera are TC-reactive	Beads were not diluted in primary diluent	• The primary diluent, which can be purchased from Charles River, contains blocking agents that are essential for MFIA® specificity.
	Beads were not adequately washed	• 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 inches of 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	• 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.

Control Failures (continued)

Symptom	Possible Cause	Solution
A test serum is TC-reactive	Serum was not sufficiently dilute	• 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	• Always dilute sera in primary diluent from Charles River.
	Serum was from an inoculated or autoimmune host	• Serum from a host with an autoimmune disorder 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 propogation of a microorganism. • Test serum from immunocompetent animals that have not been parenterally inoculated with biological material.
Ig assay score is below cutoff	BAG or SPE potency inadequate	• 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	• 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.
	Plate wells leaked	• Seal bottom of plate with plate-sealing tape after adding diluent and before reading.
αIg assay score is below cutoff	Volume of serum added was insufficient	• 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 multichannel pipette.
	Serum was too dilute	• 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	• For short-term storage, refrigerate sera at 2-8 °C. For long-term storage, freeze sera at -10 °C or below.
	Serum species incorrect	• Test the serum using the correct profile.
	Serum collected from immunodeficient host	• Immunodeficient hosts are not suitable for serosurveillance serologic testing. Perform serology on immunocompetent adult animals.
	Plate wells leaked	• Seal bottom of plate with plate-sealing tape after adding diluent and before reading.

Laboratory: Your Lab

Plate ID: Sample Plate **Operator:** WSHEK **Reader Serial #:** LX10005136301
Species: Mouse **Assay Date:** 3-May-06 **Acquisition Date:** 26-Apr-2006, 10:47 AM

Assay				Well:	*Net Scores (TC Score >=2)*														
					A12	B12	C12	D12	E1	F1	G1	H1	E2	F2	G2	H2	E3	F3	G3
					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
Bead #	**Antigen**	**Cutoff**	**Type**																
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	**MAV-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	1	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	13	14	13	14	13	12
36	**Rat IgG**	8000	IgG		16	17	16	16	15	15	16	15	15	15	15	14	14	14	13

B. Form

Charles River MFIA® Test Plate MAP

Lab: _____

Plate ID: _____
Species: _____
Panel: _____

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												

Reagent	Lot	Expiration

askcharlesriver@crl.com
www.criver.com