

# Serologic Methods Manual

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# Section I

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# Section I

## I. Introduction

### A. Overview

Laboratory animals adventitiously (i.e., accidentally) infected with pathogenic bacteria, viruses, and parasites may not be suitable for research because these adventitious infections may lead to clinical disease and pathological changes, especially in perinatal and immunodeficient animals. Although infections in post-weaning, immunocompetent animals are often subclinical, they can contaminate biological materials and produce other changes that cloud the interpretation of experimental results. Furthermore, some agents indigenous to laboratory animals are zoonotic and while causing asymptomatic infections of their natural hosts, such agents may cause disease in people.

Charles River Research Models and Services (CR-RMS) has pioneered large-scale production of rodents free from adventitious infection using caesarean derivation and barrier maintenance. To prevent infections in transit, these "viral, antibody-free" (VAF) rodents are shipped in filtered crates. Investigators can maintain the VAF status by strict biosecurity that may include the use of barrier rooms, isolators or microisolation units. However, despite the use of rigorous procedures, adventitious infections continue to occur at both breeder and customer facilities. It is therefore essential to perform routine comprehensive health monitoring employing a variety of diagnostic methodologies.

Although barrier rooms have successfully excluded most pathogenic bacteria and parasites, viral contaminations continue to be prevalent. This is because viruses are small, shed in large amounts and highly infectious. Routine monitoring for virus exposure is accomplished by serologic testing for virus-specific antibodies formed as part of the immune response to infection. Serology is also commonly used to test mice and rats for antibodies to viruses, bacteria and other non-viral agents (Table 1) because antibodies are persistent, and assays for their detection are rapid, sensitive and specific when carefully controlled.

Table 1

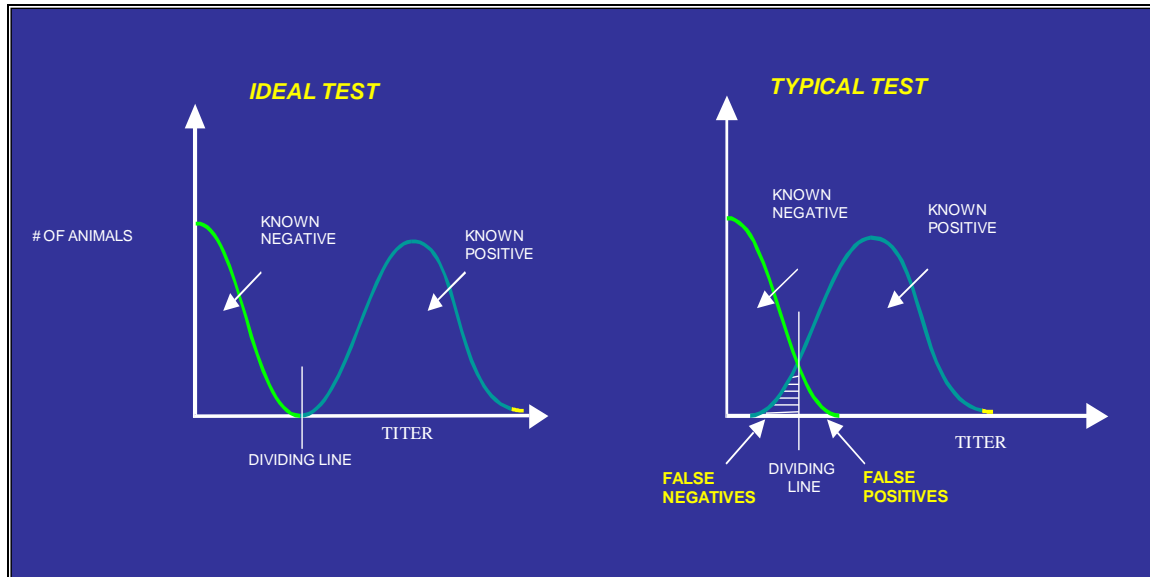
Agent	Abbreviation	Host Species*
Cilia-Associated Respiratory bacteria	CARB	M, R
<i>Clostridium piliforme</i>	CPIL	M, R
Ectromelia virus	ECTRO	M
<i>Encephalitozoon cuniculi</i>	ECUN	M, R, GP, H
Epizootic diarrhea of infant mice virus	EDIM	M
Guinea pig adenovirus	GAV	GP
Theiler's murine encephalomyelitis virus	GDVII	M
Toolan's H-1 virus	H-1	R
Hantaan virus	HANT	M, R
K-virus	K	M
Kilham rat virus	KRV	R
Lymphocytic choriomeningitis virus	LCMV	M, R, GP, H
Mouse adenovirus type-1	MAV-1	M, R
Mouse adenovirus type-2	MAV-2	M, R
Mouse cytomegalovirus	MCMV	M
Murine norovirus	MNV	M
Mouse hepatitis virus	MHV	M
Mouse parvovirus type-1	MPV-1	M
Mouse parvovirus type-2	MPV-2	M
Mouse minute virus	MVM	M
<i>Mycoplasma pulmonis</i>	MPUL	M, R
Mouse thymic virus	MTLV	M
Parvovirus NS-1	NS-1	M, R
Prospect Hill virus	PHV	M
Polyoma virus	POLY	M
Pneumonia virus of mice	PVM	M, R, GP, H
Reovirus	REO	M, R, GP, H
Rat minute virus	RMV	R
Rat parvovirus	RPV	R
Rat Theiler's virus	RTV	R
Rotavirus group B	ROTA-B	R
Sendai virus	SEND	M, R, GP, H
Sialodacryoadenitis (Rat coronavirus)	SDAV	R
Simian virus 5	SV-5	GP, H

\*M = mouse, R = rat, GP = guinea pig, H = hamster, Rb = rabbit

# Section I

While serologic tests are designed to be sensitive and specific, false positive and negative results do occur (Figure 1). Some of the reasons for inaccurate results are described in the CR Technical Bulletin (*Serological Testing to Monitor Rodents for Viral and Mycoplasma Infection - Fall 1990*) on interpretation of serologic results. We strongly recommend that you confirm new positive findings by alternative diagnostic methods and by testing additional animals.

Figure 1



The enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody test (IFA) are extremely sensitive. In the case of the ELISA, the test procedure and reading of results are amenable to automation.

The purpose of this manual is to outline the ELISA and IFA procedures used by the Serology Department at CR-RADS. The following topics will be covered for each assay:

- Methodology Overview
- Material, including equipment and supplies
- Reagent Preparation
- Sample Preparation
- Step-by-step Assay Procedure
- Results Interpretation
- Trouble-shooting

The information provided is intended to help you develop a rodent serologic testing program. We encourage you to contact us with any comments or questions regarding this manual.

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# Section II

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## II. Sample Processing and Results Reporting

### A. Overview

The actual performance of assays is only one part of a successful testing process. Before testing, specimens are collected and submitted with the appropriate sample information. Once received, sample groups and individual specimens are uniquely identified and the tests to be performed on them recorded. They are arranged in an orderly manner on each ELISA test plate or IFA slide, with their specific locations identified on the appropriate forms. Once assays are completed, results are recorded and reported. It is important to have a system for distributing and filing reports such as a Laboratory Information Management System (ILIMS). Finally, specimens are archived for a specific, finite time in case additional or confirmatory testing is requested or required. The current preferred testing method at Charles River is the Multiplexed Fluorometric ImmunoAssay (MFIA) as indicated on the Submission form at the end of this document. The ELISA is used only as a secondary test.

### B. CR-RADS Sample Processing

#### 1. Submission

In order to make initial sample processing simpler and more efficient, we request that customers submit samples diluted 1 part serum to 4 parts buffered saline in standard vials. We require that all submissions are either accompanied by a sample submission form or be preceded by a faxed accession form (Appendix C) on which the customer provides the name and address of the person to be given the results and the following information on each specimen:

- a) Species of origin
- b) A unique identification code
- c) Dilution and any treatment(s), e.g., heat inactivation
- d) Tests to be performed. We refer to panels of assays commonly performed together as profiles. Profiles make it easier for customers to specify the tests they want done.

#### 2. Receipt

- a) We record when a specimen group was received on the submission form and in our Laboratory Information Management System (ILIMS).
- b) The ILIMS assigns an accession order number to each group, e.g., 2000-001234.
- c) Information from the submission form is transferred to ILIMS and a serology log book.
- d) Individual specimens within an accession are given sequential ID numbers generally starting with one.
- e) The information received and recorded is summarized in a setup sheet report generated from the ILIMS.

#### 3. Organization of Samples for Testing

- a) ELISA

ELISAs are performed in 96-well, polystyrene, flat-bottom microtiter plates. Serum specimens are assigned to sample plates based on the profile of tests to be performed. They are then arranged in racks and transferred to the appropriate sample plate. Specimen locations are recorded on a sample plate organization form and entered into our ELISA computer program. Each sample is then transferred into a pair of wells containing diluent. Diluted samples are then transferred into corresponding wells in antigen-coated test plates to perform the ELISA (See Section 3).

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b) IFA

The IFA is performed on Teflon®-coated 18-well, glass microscope slides to which infected and non-infected cells have been attached either combined or in separate wells. The wells to which sera specimens are to be added are recorded on the IFA results form and the samples are then applied to the appropriate slide wells for testing (see Section 4).

#### 4. Recording Results

a) ELISA

Reactions are generally read spectrophotometrically with an ELISA plate reader. At Charles River Research Animal Diagnostic services (CR-RADS) our reader is connected to a PC equipped with specific ELISA software. However, there are a number of excellent commercial programs that can be used for this purpose. Absorbance values from the reader are automatically transferred to the PC where they are compiled into reports by accession.

b) IFA

IFA slides are read visually with a fluorescence microscope and reactions scored on a result form.

#### 5. Reporting and Filing Results

At CR-RADS we report results by group (i.e., accession number). Also indicated on the report are the following:

- a) Accession Number
- b) Sponsor and contact person
- c) Sample information (source, sex, and strain)
- d) Report Distribution

All raw data files are maintained by the CR-RADS Serology Department. Final reports for internal quality control are filed by facility, room and species. Commercial reports are filed by customer.

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## Section III

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### III. Enzyme-Linked Immunosorbent Assay (ELISA)

#### A. Methodology Overview

The indirect ELISA method is the assay most often used for detection of antibodies. Typically, microbial-specific antigen is immobilized on the surface of wells in microtiter plates made of specially-prepared polystyrene. The specimens are incubated in the well to which antibodies may bind. Unbound antibodies are removed by washing. Antibodies that have bound to the attached antigen are demonstrated by incubating first with an enzyme-conjugated anti-immunoglobulin, and then, following a wash to remove unbound conjugate, with a chromogenic enzyme substrate. A colored product develops at a rate proportional to the amount of antigen-specific antibody in the specimen. Color intensity can be assessed visually or spectrophotometrically (in absorbance units) with an ELISA reader.

Ideally, bound antibodies are only to the specified antigen. In practice, however, they may bind non-specifically. In addition to the antigen-coated well, we incubate each sample in an adjacent tissue control well to detect non-specific binding. The tissue control does not contain any material from an infectious agent but is usually prepared from the host system in which the infectious agent is propagated. For example, we propagate MHV virus in mammalian NCTC cells. The tissue control for MHV is a cellular lysate of uninfected NCTC cells. In the case of *M. pulmonis*, however, the tissue control is another cross-reacting rodent mycoplasma, *M. arthritidis*. Wild-type baculovirus infected insect cell lysate is used as the tissue control for recombinant antigens (i.e., NS-1, Hantaan).

A positive result is recorded for a sample if color develops in the antigen well, but not in the tissue control well. Little or no color development in either the antigen or control well for the sample is recorded as a negative result. When a color reaction occurs in the tissue control well in addition to the antigen well, the result is recorded as a tissue-control reaction, TC. A TC result is considered non-specific and does not indicate whether a sample is antibody positive or negative. In the CR-RADS Serology Department we usually retest the sample by an alternative method and, if necessary, we test additional samples from the same source. A more detailed explanation of score calculation and interpretation is in Section 3.E.

#### B. Materials

##### 1. Equipment

###### a) Incubator at 35-40°C

For best sensitivity and reproducibility, test plates should reach the incubation temperature as quickly as possible. To accomplish this, we recommend that you use a mechanically-circulated hot air incubator rather than a convection incubator. In addition, use an incubator with adequate shelf space to avoid stacking plates more than two high. In addition, we recommend covering the plates with sealing tape and incubate in a humidified chamber to prevent evaporation; we use a covered plastic tub with water dampened paper towels.

###### b) Plate Washer (Optional but recommended)

Washing to remove unbound antibody is a crucial step in any solid-phase immunoassay. Although washing can be adequately performed without specialized equipment, a programmable 96-well plate washer offers several important advantages. These include consistency, speed and containment of potentially contaminated fluids aspirated from test plate wells.

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### c) Reader (Optional but recommended)

While you can read test results visually, we recommend that you use an ELISA plate reader equipped with a light source and filter appropriate to the color produced by your substrate. We strongly recommend the use of Kirkegaard Perry Laboratories (KPL) 1-component substrate ABTS-H<sub>2</sub>O<sub>2</sub> that requires a 405nm filter. Most ELISA plate readers have at least one RS-232 serial port, which allows them to send results to a PC for analysis. We have found that using a computer to receive absorbance values and compile reports saves time and effort, and prevents errors.

### d) Pipettors

Reagent and sample preparation, and sample transfers require various pipetting devices that accurately dispense volumes of 10 to 1000 µl. For most purposes that following pipettes are adequate:

Table 2

TYPE	VOLUME (µl)
Single Channel, Adjustable Volume	2 - 10
	10 - 100
	100 - 1000
8 or 12 Channel, Adjustable Volume	5 - 50
	50 - 300
Repeating Pipette with 8 or 12 channel	50

## 2. Description of Key Reagents

### a) Antigen- and Tissue Control-Coated Test Plates

The pattern of how our ELISA plates are coated is shown in Figure 2. Wells in rows A, C, E, and G contain attached test plate specific antigen. The remaining wells (rows B, D, F, and H) are coated with the corresponding tissue control. We routinely add the ELISA plate serum controls into lane 12.

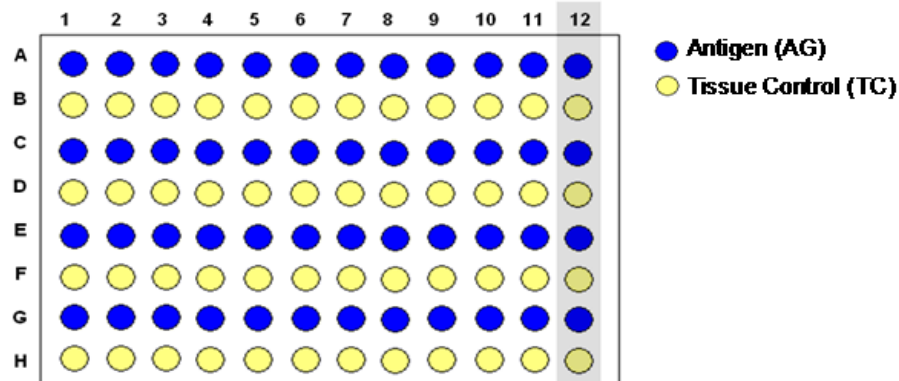


Figure 2

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Our test plates (INTENDED FOR RESEARCH USE ONLY) are available in a 96-well format with removable 8-well strips. Plates with removable strips offer you the flexibility of doing less than 48 tests at a time. As indicated in the Product Specification sheet (Figure 3), CR-RADS antigen-coated plates should be stored in a freezer at -10°C or below. Some ELISA test plates (i.e., LCMV, SV-5, HANT, MPV and NS-1) must be stored at -60°C or below (Table 3). *See Appendix A for a list of equipment manufacturers.*

**Table 3**

ELISA plate	Storage Temperature (±10°C)	
	-20°C	-70°C
CARB	•	
CPIL	•	
ECTRO	•	
ECUN	•	
EDIM	•	
GDVII	•	
MPUL	•	
MAV	•	
MCMV	•	
MHV	•	
PIV-3	•	
PVM	•	
Reovirus	•	
RTV	•	
SDAV	•	
Sendai virus	•	
SV-5	•	
H-1		•
HANT		•
K		•
KRV		•
LCMV		•
MNV		•
MPV		•
MVM		•
NS-1		•
Polyoma virus		•
RMV		•
RPV		•

# Section III

Figure 3



## Charles River Laboratories Research Animal Diagnostic Services

251 Ballardvale Street, Wilmington, MA 01887  
Telephone: 877-274-8371/781-222-6000; Fax: 978-988-9093  
Email: [comments@crl.com](mailto:comments@crl.com); Homepage: [www.criver.com](http://www.criver.com)

### RESEARCH PRODUCT SPECIFICATIONS

**Item #:** PL-041  
**Name:** RTV ANTIGEN-COATED 96-WELL MICROTITER PLATES  
**Lot (Batch):** \_\_\_\_\_  
**Date of Manufacture:** \_\_\_\_\_  
**Date of Expiration:** \_\_\_\_\_

**Description:** Partially-purified antigen and tissue control (an extract of the cell or tissue type in which the agent was propagated) were adsorbed to microtiter plate wells according to the conditions listed on the attached Antigen Purity Quality Control Results sheet. All wells in rows A, C, E, and G are coated with antigen; wells in rows B, D, F, and H are coated with tissue control.

**Form/Storage:** Ready to use. Store at  $-20^{\circ}\text{C}$  or below. Expiration date is 12 months from production date. Storage at  $\leq -60^{\circ}\text{C}$  will increase shelf life.

**Quality Control:** A plate from this research batch was evaluated with a panel of standard mouse and rat control sera by the indirect method of the enzyme-linked immunosorbent assay (ELISA) (Voller et al., Lab. Res. methods Biol. Med. 5:59-82, 1982). Briefly, 50 microliters of standard serum, diluted no less than 1/50 in phosphate-buffered saline (PBS) with 5% bovine serum or in BLOTTO (5% non-fat dry milk in PBS, Johnson et al., Gene. Anal. Techn. 1:3-8, 1984), were added to each of the appropriate antigen wells and adjacent, tissue control wells; the plate was covered and incubated for 40 minutes at  $35-41^{\circ}\text{C}$ . Following several washes with 0.9% saline containing 0.05% Tween 20, 50 microliters of horseradish peroxidase-conjugated, affinity-purified goat anti-rodent IgG were added to each well. After a 40 minute incubation at  $37^{\circ}\text{C}$ , the plate was again washed. One hundred microliters of 0.4mM ABTS-2.0mM  $\text{H}_2\text{O}_2$  chromogenic substrate were added to each well and the plate was incubated at room temperature for 40 minutes. The reaction intensities at 405nm were determined with an ELISA reader. The net absorbance values (antigen-tissue control) were converted to scores by dividing by 0.13. A net score of 3 or above is considered positive. As the results of the attached Antigen Purity quality control report show, positive results occurred only with appropriate immune sera.

**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 and on the attached quality control sheet 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.

ATT-312-1

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### b) High and Low Positive Immune and Nonimmune Control Sera

It is essential that you test standard positive and negative control sera along with your samples to verify assay sensitivity and specificity. Results for control sera are also helpful when troubleshooting. **NOTE:** Our immune control sera are supplied at 2X and therefore must be diluted with an equal volume of serum diluent when used. Non-immune control sera are pre-diluted 5-fold in PBS. You should not repeatedly freeze and thaw control sera or leave them at room or refrigeration temperature for more than 24 hours. We recommend dividing the sera into small volume, single-use aliquots that can be thawed and used at the time the assay is performed.

### c) Enzyme-Conjugated Anti-immunoglobulin

The two enzymes most often used in the ELISA are alkaline phosphatase and horseradish peroxidase (HRP). The quality and concentration of the enzyme-conjugate profoundly affect the accuracy of results. Therefore, the use of an affinity-purified conjugate, optimized for your ELISA system, is crucial. We provide pre-titrated, affinity-purified HRP-conjugated species-specific IgG with recommended working dilutions for use with our test plates.

### d) Chromogenic Substrate

There are a variety of substrates that can be purchased ready-to-use. We have optimized our assays using KPL 1-component substrate ABTS-H<sub>2</sub>O<sub>2</sub> (catalog# 50-66-06) and recommend that you use it in your assays.

### e) Stop Solution

The two types of stop solutions that we have used are 0.12% hydrofluoric acid (HF) and 1% sodium dodecyl sulfate (SDS). As HF is caustic and corrosive, we recommend using SDS.

## 3. Preparation of Buffers, Diluents and Solutions

### a) 1.0M Tris-HCl Stock Solution

COMPONENTS	AMOUNT
Tris - base	12 g
DI H <sub>2</sub> O	qs to 100mL
HCl	various

#### (1) Mixing Instructions

Dissolve 12 grams of Tris base in 60 mL of DI H<sub>2</sub>O. Adjust the pH to 8.7 with concentrated HCl and bring the total volume to 100 mL with deionized water (DI). Filter sterilize (0.2-micron) and store in refrigerator for up to 6 months.

#### (2) Storage

Store at 2°-8° C for up to 6 months.

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- b) **BLOTTO Serum Diluent** – Bovine Lacto Transfer Technique Optimizer (Johnson et al, Gen. Anal. Techn. 1:3-8, 1984). The following recipe is for preparing 100 mL of BLOTTO.

COMPONENTS	AMOUNT
Non-fat Dry Milk	5g
1M Tris Buffer pH 8	5mL
Proclin	50 $\mu$ L
Anti-foam A	33 $\mu$ L
NaCl	0.9g
DI water	95mL

(1) **Mixing Instructions**

Make 50 mM Tris Buffered saline mixing DI H<sub>2</sub>O, 1M Tris Buffer and NaCl. Add Proclin, Anti-foam A and the milk powder. Mix with a magnetic stir bar until the milk is dissolved. Remove large undissolved milk particles by coarse filtration through a screen. We use a 60-micron nylon screen from Sefar America, Depew, NY. Rinse nylon screen between filtrations.

(2) **Addition of Fetal Bovine Serum (FBS)**

Add 20 mL FBS to 80 mL BLOTTO. FBS is added to block false positive reactions caused by antibodies to fetal bovine constituents that may be in the antigen preparation. It is important to 0.2-micron filter the FBS prior to use.

(3) **Storage**

This solution is NON-STERILE. Store at 4°C and use within two weeks.

- c) **Conjugate Diluent** – 15% FBS and 0.9% NaCl in 0.01M Tris-HCl, pH 7.2-7.4. The following recipe is for preparing 100 mL. Note: FBS should be 0.2-micron filtered prior to use.

COMPONENTS	AMOUNT
1.0M Tris-HCl Stock Solution	1mL
NaCl	0.9g
Gentamicin sulfate (50mg/ml)	0.1mL
FBS	15mL
DI H <sub>2</sub> O	qs to 100mL

(1) **Mixing Instructions**

Prepare 100 mL 1.0 M Tris-HCl Stock Solution by dissolving 12 grams of Tris in 60 mL of DI H<sub>2</sub>O. Adjust pH to 7.2 with concentrated HCl and bring volume to 100 mL. Filter sterilize (0.2-micron). To prepare the diluent, add the indicated amounts of each component in the order shown to 60 mL of DI water. Adjust to the final volume to 100 mL with DI water and stir until the NaCl is dissolved.

(2) **Storage**

Store in a refrigerator for up to six months. Note: PBS, pH 7.4 can be used in place of 0.01M Tris-saline.

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- d) **Stop Solution** – 1% SDS. The following recipe is for preparing 100 mL of 1% SDS.

COMPONENTS	AMOUNT
SDS	1g
DI H <sub>2</sub> O	100mL

- (1) Mixing Instructions

Prepare 1% SDS by dissolving 1 gram of SDS into 100ml of DI H<sub>2</sub>O. Filter sterilize into a sterile container.

- (2) Storage

Store 1% SDS in the refrigerator and use within seven days.

- e) **Wash Solution** – 0.9% NaCl, 0.05% Tween 20. The following recipe is for preparing 1000 mL.

COMPONENTS	AMOUNT
NaCl	9g
10% Tween 20	5mL
DI H <sub>2</sub> O	1000mL

- (1) Mixing Instructions

Prepare 10% Tween-20 by mixing 10 mL of Tween with 90 mL of DI H<sub>2</sub>O. Filter sterilize and store at room temperature in a sterile container. Add the specified amount of NaCl and 10% Tween-20 to the DI H<sub>2</sub>O and mix until the salt is dissolved.

- (2) Storage

Store at room temperature and use within five days.

- f) **Working Dilution of Conjugate**

- (1) Mixing Instructions

Freezing the CR-supplied conjugate stock solution should be avoided, therefore do not store the stock solution below -30°C. Dilute the CR-supplied conjugate stock as indicated on the Product Specification Sheet.

- (2) Storage

Refrigerate the diluted conjugate and use within 1 month. We recommend making multiple small volume aliquots for use. This decreases the chance of microbial contamination of a large volume single vessel. Conjugate showing visible signs of contamination (turbidity) should not be used.

# Section III

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## C. Sample Preparation

### 1. Collection and Storage

- a) Careful preparation and proper storage of serum samples are essential to obtain meaningful results. Tests on specimens of poor quality often yield results that are difficult to interpret.
- b) Collect blood following your standard protocols and allow it to clot at room temperature for a minimum of 30 minutes.
  - (1) For samples collected in Microtainer® brand serum separator tubes centrifuge the samples at 6000-15000g for a minimum of two minutes to separate the serum.
  - (2) For samples collected in 16 x 100 mm glass tubes or equivalent, centrifuge at 2000g for 10-15 minutes.
- c) Transfer the separated serum to a new vial. If sterile PBS, pH 7.0 to 7.4, is available, dilute the specimen by mixing one part serum with four parts PBS.
- d) Alternatively, the same final serum dilution of ~1/5 may be achieved by adding PBS directly to the blood: Mix one part blood with two parts PBS, allow the blood to clot, and separate the serum from the clot by centrifugation as described above.
- e) It is best to store serum specimens in sturdy, leak-proof plastic vials at -10°C or below. If they cannot be frozen, refrigerate at 4°C. Specimens refrigerated for more than 24 hours should be protected from the growth of bacteria and fungi by adding an antimicrobial agent such as Proclin.

### 2. Heat Inactivation of Samples

We recommend that you do **NOT** heat inactivate serum samples, as we have observed that this contributes to nonspecific background color development.

### 3. Preparing Sample Plate

- a) First arrange your sample vials (containing serum diluted five-fold with PBS) in racks in the order that they will appear on the test plates.
- b) While samples can be diluted directly in the test plate, we recommend that you make dilutions in separate 96-well low-protein binding microtiter plates.
- c) Serum samples are tested at a dilution of 1/60.
  - (1) To prepare enough diluted serum to perform four tests, add 220µL of BLOTTO diluent to all wells except those reserved for the positive control antisera (we usually reserve wells 12A,B and 12C,D for the high and low positive controls, respectively). Next, add 20 µL of sample to each of two adjacent wells corresponding to antigen and tissue control wells on the test plates.
  - (2) Alternatively, you may prefer to dilute serum 1/30 in order to double the number of test plates that can be filled from a single sample plate from four to eight. To do this, add 225 µL of BLOTTO diluent to all wells except those reserved for the positive control antisera. Then add 45 µL of sample to each of two adjacent wells corresponding to antigen and tissue control wells on the test plates.

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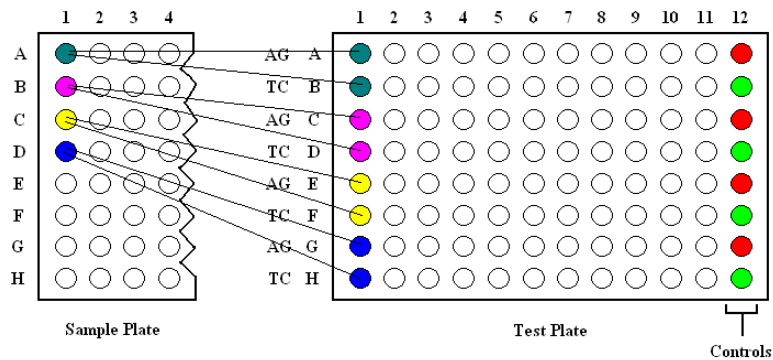
- (3) We typically use wells 12E,F for the non-immune control serum and leave 12G,H as the diluent control. The non-immune control sera should be diluted in the test plate (Step D).
- (4) Cover the sample plates (with another plate) to prevent evaporation.
- (5) Refrigerate and use within seven days.

### D. Testing

#### 1. Transfer Diluted Sera to Test Plates (Figure 4).

- a) Sera can be transferred from a sample plate into test plates with a single channel pipette or multi-channel pipette. However, a 96-well transfer device is most efficient for this purpose.
- b) If the sample plate contains sera diluted 1/60, transfer 50  $\mu\text{L}$  per well.
- c) Alternatively, when the sample plate sera are 2X, i.e., diluted 1/30, first add 25  $\mu\text{L}$  of BLOTTO diluent or PBS to all wells of the test plates. Then transfer 25  $\mu\text{L}$  of sample per well.

Figure 4



#### 2. Add Immune Control Sera to the Reserved Test Plate Wells.

- a) Control sera may be added to any plate location, although we routinely, as noted above, reserve wells in column 12 for this purpose.
- b) Each test plate should include the high and low positive controls. NOTE: The CR positive control sera are supplied at 2X concentration.
- c) For 2X controls, if you haven't already done so, add 25  $\mu\text{L}$  of BLOTTO diluent to each positive control well; then add 25  $\mu\text{L}$  of control serum per well.

#### 3. Incubate the Test Plates at 35-40°C for 40 minutes.

- a) Be sure that all plates are completely covered to minimize evaporation. Incubate in a covered humidified chamber if possible.
- b) Do not stack the plates more than two high.

#### 4. Wash the Test Plates

- a) If you are using an automatic 96-well washer, we recommend 3-5 fill-aspiration cycles. Overfill the well with 350-400  $\mu\text{L}$  if your washer has this capability (aspiration of overflow volume). A soak time between cycles is not necessary.

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- b) To wash without a plate washer:
  - (1) Prepare a wide waste container with paper towels in the bottom to prevent splattering.
  - (2) To expel the well contents into the waste container, invert and rapidly flick the plate.
  - (3) Using a repeating pipette with a multi-channel manifold, fill all wells with wash solution (~300 uL).
  - (4) Repeat Steps b.2 and b.3 five to six times.
  - (5) Expel the well contents as in Step b.2. Invert and tap the plates against several layers of dry paper towels to remove the last traces of wash fluid.

### 5. Add Diluted Conjugate

- a) Add 50  $\mu$ L of diluted conjugate to all wells and incubate at 35-40°C as above for 40 minutes.

### 6. Wash and Add Substrate and Incubate

- a) Wash the test plates as in Step 4.
- b) Add 100  $\mu$ l of ABTS-H<sub>2</sub>O<sub>2</sub> substrate per well.
- c) Incubate at room temperature for 40 minutes.

### 7. Add SDS-Stop Solution (Optional)

- a) Add 25  $\mu$ l of SDS Stop solution per well.

### 8. Read the Test Plate

- a) The product of the reaction between ABTS-H<sub>2</sub>O<sub>2</sub> and HRP is GREEN. The rate of color development is proportional to the amount of HRP-conjugate bound to the well.
- b) The addition of Stop solution dramatically slows, but does not completely prevent, further color development. It is strongly recommended to read the test plates immediately.
- c) When using an ELISA reader, measure absorbance values at 405 nm. If you are reading at dual wavelengths, set the second filter to 620 nm.

## E. Results Interpretation

### 1. CR-RADS Scoring System

- a) We transmit our optical density (OD) readings from the ELISA reader to a PC where they are converted to scores by dividing by 0.13. In comparison to the 3 decimal place absorbance values, integer scores are easier to read and interpret. This factor converts blanked absorbance values into scores between 0 and ~20.

Value	Formula
Blanked OD	Raw OD – Blank Plate OD Average
Score	Blanked OD/0.13
Net Score	Antigen Score – Tissue Control Score

## Section III

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- b) The Scoring interpretation is given below.

Score <sup>A</sup>			Interpretation
TC	Net	TC + Net	
≥ 2		< 2.5	Negative (-)
		≥ 2.5	Non-specific (TC)
< 2	< 1.5		Negative (-)
	1.5 ≤ Net < 2.5		Borderline, or equivocal (+/-)
	≥ 2.5		Positive (+)

<sup>A</sup> Score: TC = tissue control, Net = Antigen score – TC score.

- c) A result with a high tissue score can still be interpreted as negative provided that the antigen score is less than 2.5.
- d) A result is considered non-specific and recorded as TC when both Score<sub>AG</sub> and Score<sub>TC</sub> are > 2.

## Section III

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### F. Troubleshooting

The following table describes common ELISA issues and their probable cause.

OBSERVATION	POSSIBLE CAUSE	
	COMPONENT	PROBLEM
NO COLOR IN HIGH AND LOW POSITIVE SERUM CONTROLS	CONTROL SERA	<ul style="list-style-type: none"> <li>- Not Added.</li> <li>- Incorrect specificity.</li> <li>- Diluted improperly (ie. too dilute).</li> <li>Inactivated by improper storage or repeated freeze-thaws.</li> </ul>
	CONJUGATE	<ul style="list-style-type: none"> <li>- Incorrect specificity.</li> <li>- Too dilute.</li> <li>- Inactivated by improper storage of repeated freeze-thaws.</li> </ul>
	SUBSTRATE	<ul style="list-style-type: none"> <li>- Alternate ABTS substrate used other than recommend KPL catalog #50-66-06.</li> </ul>
	ANTIGEN	<ul style="list-style-type: none"> <li>- Too dilute or of low potency.</li> <li>- Incorrect agent.</li> <li>- Degraded due to improper storage.</li> </ul>
	READER	<ul style="list-style-type: none"> <li>- At wrong wavelength.</li> <li>- Bulb burned out.</li> <li>- Out of calibration.</li> </ul>
WEAK COLOR DEVELOPMENT IN POSITIVE CONTROL WELLS	COMPONENTS LISTED ABOVE	<ul style="list-style-type: none"> <li>- Plate read in wrong orientation.</li> <li>- Problems listed above.</li> </ul>
	INCUBATION	<ul style="list-style-type: none"> <li>- Temperature too low or exceedingly high.</li> <li>- Time too short.</li> </ul>

## Section III

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### F. Troubleshooting, continued.

OBSERVATION	POSSIBLE CAUSE	
	COMPONENT	PROBLEM
EXCESSIVE BACKGROUND	WASHER	- Too few fill-aspirate cycles.
		- Wash incompletely aspirated after each fill.
		- Fill volume low.
	SERUM SAMPLE	- Sticky due to improper collection and storage or bacterial contamination.
		- Dilution too low or not diluted in BLOTTO serum diluent.
		- From animal immunized or used to grow tumor cells; animal with autoimmune disease.
- Older age animal used.		
CONJUGATE	- Dilution too low.	
	- Poor quality (try another lot or different vendor).	
SUBSTRATE	- Activated non-specifically prior to being added to plate or by contaminants in plate.	
ANTIGEN	- Binds antibody in serum or conjugate non-specifically because 'sticky' or used at too low a dilution.	
INCUBATION	- Time too long.	
	- Temperature too high.	
	- Plates were not completely covered to prevent evaporation of well contents.	
	- No humidity to prevent evaporation of well contents.	

## Section IV

### IV. Indirect Fluorescent Antibody Test (IFA)

#### A. Methodology Overview

The steps of the IFA are very similar to those of the ELISA. Virus-infected cells and uninfected cells are fixed to wells on a glass slide. The fixative is usually cold acetone, which permeabilizes the cell membrane, making the intracellular viral antigens more accessible to antibodies. As with the ELISA, unbound antibodies are removed by washing. Instead of the enzyme conjugate and substrate indicator system used in the ELISA, the binding of primary antibodies to the slide wells in the IFA is demonstrated with a fluorescent dye-conjugated anti-immunoglobulin. After washing to remove unbound conjugate, slides are covered with buffered glycerol and examined with a fluorescence microscope. Epi-illumination is recommended due to the fluorescence being much brighter than transmitted-light darkfield fluorescence resulting in a clearer, crisper image. Fluorescence microscopes have a light source with an exciter filter to exclude all but the appropriate wavelengths and a reflector/barrier filter combination to reflect the light onto the slide, so fluorescence may be observed.

In the IFA, the morphology and location of fluorescence can be evaluated to differentiate specific from non-specific reactions. This is not true for most other serologic tests and is a major advantage of the IFA. Bright, granular fluorescence is typical of a specific antibody-viral antigen reaction. By contrast, diffuse fluorescence suggests a nonspecific reaction. Fluorescence may be mostly nuclear or cytoplasmic depending on the virus (Table 4). Nuclear fluorescence is characteristic of DNA viruses (e.g., the rodent parvoviruses MVM, MPV, RPV, KRV, RMV and H-1). However some minimal cytoplasmic fluorescence may be observed.

**Table 4**

Localization of IFA Fluorescence

AGENT	Fluorescence	
	Nuclear	Cytoplasmic
ECTRO		+
EDIM		+
GDVII		+
HANT		+
H-1	+	+
KRV	+	+
LCMV		+
MAD	+	
MCMV	+	
MHV	+	+
MPV	+	+
MVM	+	+
PHV		+
POLY	+	
PVM		+
REO		+
RMV	+	+
RPV	+	+
SEND		+
SV-55		+
TMEV		+
MPUL <sup>1</sup>		+
CARB <sup>2</sup>	-	-
CPIL <sup>2</sup>	-	-
ECUN <sup>2</sup>	-	-
Toxo. gondii <sup>2</sup>	-	-
MTLV <sup>3</sup>		+

<sup>1</sup>Mycoplasma attaches to the host cell membrane.

<sup>2</sup>IFA slide wells contain only microorganisms

<sup>3</sup>MTLV-infected thymocytes attach to host cell outer cell membrane.

## Section IV

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### B. Materials

#### 1. Equipment

- a) **2-10 microliter ( $\mu\text{L}$ ) single-channel, adjustable pipette for adding sample**
- b) **2-5 mL repeating pipettes for dispensing FITC-labeled anti-immunoglobulin**
- c) **Humidified Chamber**
- d) **Incubator at 35-40°C**
- e) **Slide Washing Reservoirs**
- f) **Coverslips**
- g) **Fluorescence Microscope**

For fluorescein isothiocyanate (FITC), the microscope should have a mercury or xenon light source with a 495 nm exciting filter and a 520 nm suppression filter. We typically examine slides at magnifications of 100-400X.

#### 2. Description of Key Reagents

##### a) **Antigen-Coated Slides**

For most viruses, we have both virus-infected and uninfected control cells in the same slide well. These slides (INTENDED FOR RESEARCH USE ONLY) are produced by infecting cells grown on the slide with near endpoint dilutions of virus. Slides are incubated for 1-3 days and then are acetone-fixed. As indicated in the Product Specification sheet (**Figure 4**), CR-RADS antigen-coated slides should be stored in a freezer at -10°C or below, preferably at -70°C. Slides stored at -70°C remain antigenic for at least two years.

##### b) **Positive Immune and Nonimmune Control Sera**

It is essential that you test standard positive and negative control sera along with your samples, to verify assay sensitivity and specificity. Results for control sera are also helpful when troubleshooting. NOTE: Our immune control sera are supplied at their working dilutions. Non-immune control sera are pre-diluted 5-fold in PBS. You should not repeatedly freeze and thaw control sera or leave them at room or refrigeration temperature for more than 24 hours. We recommend dividing the sera into small volume, single-use aliquots that can be thawed and used at the time the assay is performed.

##### c) **FITC-Labeled Anti-immunoglobulin**

CR-RADS does supply this component, however, there are many commercial sources of affinity-purified species-specific FITC-labeled IgG. It has been our experience that these conjugates work fine at the dilutions recommended by the manufacturer (generally 1:25-50).

##### d) **Coverslip Mounting Medium** - Tris-Buffered Glycerol, pH 8.7

Mounting medium for FITC is buffered to a basic pH to enhance fluorescence.

##### e) **Wash Solutions:** PBS pH 7.4 and DI H<sub>2</sub>O.

# Section IV

## Figure 4



### Charles River Laboratories Research Animal Diagnostic Services

251 Ballardvale Street, Wilmington, MA 01887

Telephone: 877-274-8371/781-222-6000; Fax: 978-988-9093

Email: [comments@crl.com](mailto:comments@crl.com); Homepage: [www.criver.com](http://www.criver.com)

#### *RESEARCH PRODUCT SPECIFICATIONS*

**Item #:** SL-051  
**Item Name:** PHV ANTIGEN-COATED 18-WELL SLIDES  
**Lot (Batch):** \_\_\_\_\_  
**Date of Manufacture:** \_\_\_\_\_  
**Date of Expiration:** \_\_\_\_\_

**DESCRIPTION:** VERO E-6 Monkey Kidney Cells were infected with PHV and applied to the slide wells. The slides were incubated for a suitable period at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air. They were then dried and acetone-fixed.

**FORM/STORAGE:** Ready to use. Store at -70±10°C or below. At this storage temperature, the slides remain antigenic for at least two years.

**INACTIVATION:** Slides were fixed in acetone for a minimum of 10 minutes. However, they are non-sterile and **may still contain infectious agent**.

**ANTIGEN PURITY:** A slide from this batch was evaluated with a panel of standard sera (both immune and non-immune) by the indirect fluorescent antibody test [(IFA), Lyerla and Forrester, Immunofluorescence Methods in Virology, U.S. Dept of H.E.W., Course No. 8231-C, 1979]. Briefly, 10 microliters of standard serum, diluted no less than 1/10 in phosphate-buffered saline (PBS) or BLOTTO (5% non-fat dry milk in PBS, Johnson et al., Gene. Anal. Techn. 1:3-8,1984) were added to each of the appropriate wells. The slide was incubated at 37°C in a humidified chamber for 30 minutes. It was then gently rinsed in a PBS bath and washed twice by incubating for 5 minutes in each of 2 PBS baths. After a final rinse in deionized water, the slide was dried under a stream of air in a laminar flow hood and then 10 microliters of affinity purified, FITC-conjugated anti-immunoglobulin (generally diluted 1:50) were applied to each well. The slide was incubated for 30 minutes at room temperature and washed as described above. It was then covered with a few drops of 0.1M Tris-buffered glycerol, pH 8.6, and a cover slip. The slide was examined with an epi-illumination fluorescence microscope (with a xenon light source, 450-490 nm filter, 510 nm reflector and 40X Neofluar objective at 400X magnification). A positive reaction was indicated by the observation of granular, yellow-green fluorescence. The intensity of a positive reaction was scored on a scale 1 (weak) to 4 (strong). Minimal diffuse background fluorescence, equivalent to that typically observed with non-immune serum, was recorded as "-". As the results of the attached quality control report show, positive results occurred only with the appropriate immune sera.

**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 and on the attached quality control sheet 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.

ATT-313-1

## Section IV

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### 3. Preparation of Buffers, Diluents and Solutions

- a) **BLOTTO Serum Diluent** – (Refer to ELISA section for preparation)
- b) **Mounting Medium** – 0.1M Tris-Buffered Glycerol, pH 8.7. The following recipe is for preparing 20 mL.

COMPONENTS	AMOUNT
1.0M Tris-HCl Stock Solution, pH 8.7	2 mL
Glycerol	18 mL

(1) **Mixing Instructions**

Add 2 mL of 1.0 M Tris-HCl Stock Solution (refer to ELISA section for preparation) to 18 mL of glycerol mix thoroughly.

(2) **Storage**

Store at room temperature and use within one month.

c) **Diluted Conjugate**

COMPONENTS	AMOUNT
Reconstituted FITC Conjugate Stock	Variable
PBS	Volume

(1) **Mixing Instructions**

Commercial conjugates are generally supplied in the lyophilized form. Follow the manufacturer's instructions to reconstitute the conjugate. Divide into small aliquots and store frozen at -10°C or below. Thaw a vial of FITC conjugate and prepare the recommended working dilution in PBS.

(2) **Storage**

Store at room temperature and use within five days.

d) **Wash Buffer (PBS, pH 7.4):** Sigma (P3813-10 PAK or equivalent)

(1) **Mixing Instructions**

Measure out and dispense one liter of filtered DI H<sub>2</sub>O into a 1L bottle. Open a PBS packet and carefully dispense the entire contents into the bottle. Mix the solution until all components are dissolved.

(2) **Storage**

Store at room temperature and use within six months.

## Section IV

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### C. Sample Preparation

#### 1. Collection and Storage

Collection and storage is the same as for ELISA (Section 3.B). No other sample preparation is necessary.

### D. Testing

#### 1. Prepare Slides

- a) Remove the appropriate type and number of slides from the freezer.
- b) Allow them to warm to room temperature.

#### 2. Organize Test Specimens

- a) Retrieve and arrange your samples.
- b) On an IFA results form, record the well locations of samples and controls used as well as appropriate lot information.

#### 3. Place Specimens and Controls on Slides and Incubate

- a) Add 5  $\mu$ l of BLOTTO diluent to all wells except those reserved for positive control sera.
- b) Using the IFA Form as a guide, add the test specimens and negative controls to the appropriate wells. The volume of specimen or control per well is 5  $\mu$ l (final dilution 1/10). Spread each sample over the whole well being careful not to scrape the surface.
- c) Add 10  $\mu$ l of positive control serum to the appropriate well(s).
- d) Incubate the slides at 35–40°C in a humidified chamber for 30 minutes.

#### 4. Wash

- a) Gently aspirate the serum samples from the individual wells. Carefully rinse the slides in PBS by gentle agitation. This initial quick rinse step helps prevent cross-contamination
- b) Fill three separate wash reservoirs with PBS and a fourth with DI H<sub>2</sub>O.
- c) Incubate the slides in the second PBS-filled reservoir for 2-5 minutes.
- d) Move the slide to the next wash reservoir and incubate for another 2-5 minutes.
- e) Transfer the slides to the DI water wash reservoir and rinse.
- f) Air dry. DO NOT BLOT.

#### 5. Add Conjugate and Incubate

- a) Using a repeating pipettor, add 10  $\mu$ l of diluted FITC conjugate to all wells.
- b) Incubate the slides at 35–40°C in a humidified chamber for 30 minutes.

#### 6. Wash and Cover with Mounting Medium

- a) Wash the slides as in Step 4.
- b) Add a drop of mounting medium and place a coverslip on each slide. Avoid trapping air bubbles. Avoid using too much mounting medium, as the coverslip will float and the excess medium may get on the microscope objective.

## Section IV

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### 7. Read Results

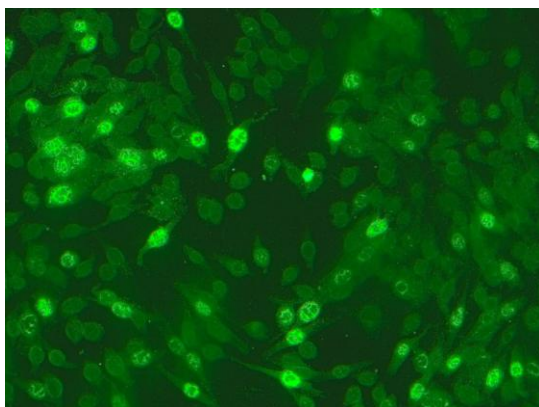
- a) It is recommended to read the slides immediately to verify that the assay worked (positive and negative controls are acceptable). Samples may be read immediately or the slides may be stored at -20°C for up to one week without deterioration of the fluorescence.
- b) Examine the slides with the fluorescence microscope at a magnification of 100-400X. FITC fluorescence is yellow-green in color.
- c) After examination of the positive and negative controls, score the results as follows:

FLUORESCENCE	SCORE
Minimal, comparable to non-immune control	-
Very dim, granular	1
Moderate granular	2
Bright granular	3
Glaring granular	4
Diffuse	TC

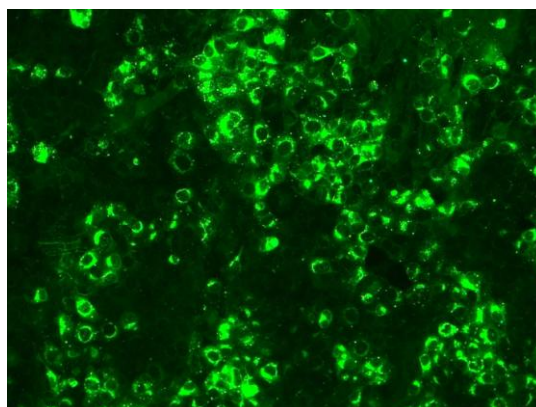
### D. Results Interpretation

Correct reading of IFA reactions takes practice. Bright granular fluorescence is typical of specific antibody-viral antigen reactions, whereas diffuse fluorescence suggests a non-specific tissue reaction (TC). A TC reaction is also probable when the percentage of fluorescing cells or the location of the fluorescence is markedly different from that observed in the positive control well. In the case of certain DNA viruses, such as the rodent parvoviruses MVM, KRV and H-1, strong nuclear fluorescence is characteristic, while other viruses show predominantly cytoplasmic fluorescence (Table 4).

### Examples of IFA Staining



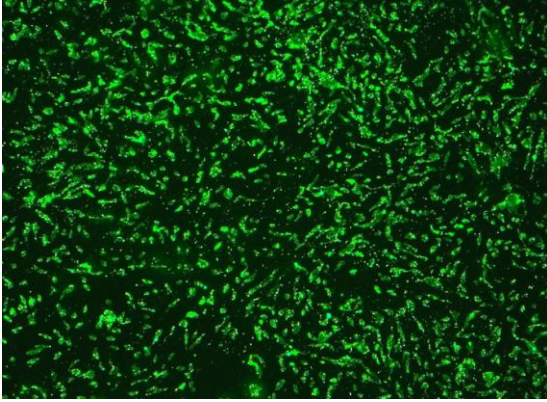
**Nuclear: MVM**



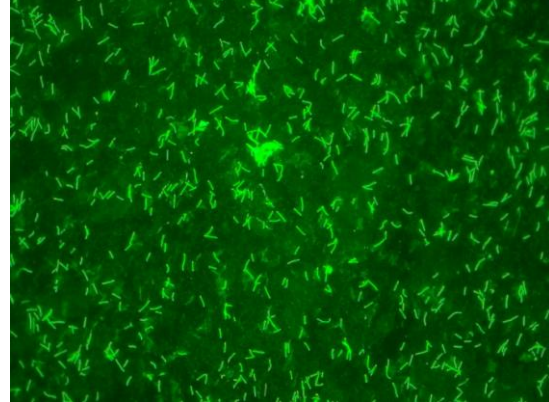
**Cytoplasmic: Hantaan**

## Section IV

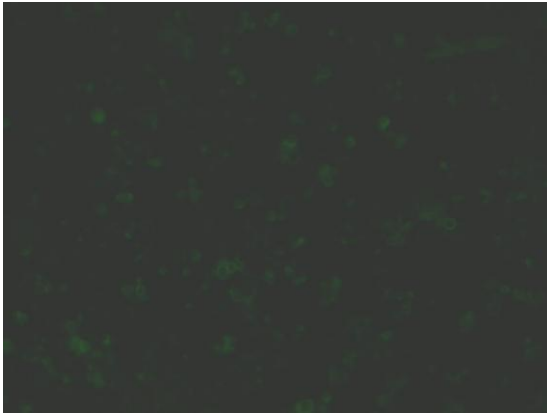
---



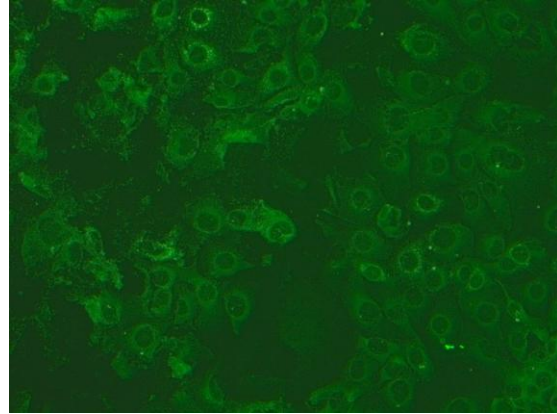
**Mycoplasma**  
*M. pulmonis* attached to host cell membrane



**Bacterial: *C. pil***



**Negative serum (MAD IFA slide)**



**TC/ Non-specific (EDIM IFA slide)**

## Section IV

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### E. Troubleshooting

The following table describes common IFA issues and their probable cause.

OBSERVATION	POSSIBLE CAUSE	
	COMPONENT	PROBLEM
NO OR WEAK FLUORESCENCE IN POSITIVE CONTROL WELL	CONTROL SERA	<ul style="list-style-type: none"> <li>- Not Added.</li> <li>- Incorrect specificity.</li> <li>- Diluted improperly (ie. too dilute).</li> <li>- Inactivated by improper storage or repeated freeze-thaws.</li> </ul>
	CONJUGATE	<ul style="list-style-type: none"> <li>- Incorrect specificity.</li> <li>- Too dilute.</li> <li>- Inactivated by improper storage or repeated freeze-thaws.</li> </ul>
	SLIDE	<ul style="list-style-type: none"> <li>- Percentage of infected cells too low.</li> <li>- No cells or cells scraped off during procedures.</li> <li>- Wrong virus.</li> </ul>
	FLUORESCENCE MICROSCOPE	<ul style="list-style-type: none"> <li>- Wrong light source.</li> <li>- Incorrect exciting/suppression filter.</li> <li>-Not aligned properly.</li> </ul>
EXCESSIVE BACKGROUND	SERUM SAMPLES	<ul style="list-style-type: none"> <li>- Dilution too low.</li> <li>- BLOTTO blocking diluent not used.</li> <li>- Source animals parentally immunized or have autoimmune disease.</li> </ul>
	CONJUGATE	<ul style="list-style-type: none"> <li>- Dilution too low.</li> <li>- Poor quality , not affinity purified.</li> </ul>
	SLIDE	<ul style="list-style-type: none"> <li>- Cells not spread out. This increases background and reduces specific fluorescence.</li> <li>- Bacterially contaminated during preparation.</li> <li>- No humidity to prevent evaporation of well contents.</li> </ul>

# Section V

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## V. Appendices

### A. Equipment List for Serology Testing

#### 1. Pipettors, Single and Multi-channel

<b>Company</b>	<b>Address</b>
Eppendorf	102 Motor Parkway Hauppauge, NY 11788 (800) 645-3050 <a href="http://www.eppendorf.com">www.eppendorf.com</a>
Rainin	P.O.Box 2160 7500 Edgewater Drive Oakland, CA 94621-0060 <a href="http://www.rainin.com">www.rainin.com</a>
Finnpette (ThermoScientific)	P.O. Box 117 Rockford, IL 61105 (800) 874-3723 <a href="http://www.piercenet.com">www.piercenet.com</a>
Hamilton	4970 Energy Way Reno, NV 89502 (800) 648-5950

#### 2. Plate Washers/Readers

<b>Company</b>	<b>Address</b>
Biotek	P.O. Box 998 Highland Park Winooski, VT 05404 <a href="http://www.biotek.com">www.biotek.com</a>
Dynex Technologies	14340 Sullyfield Circle Chantilly, VA 20151-1621 <a href="http://www.dynextechnologies.com">www.dynextechnologies.com</a>

## Section V

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### B. Reagent Suppliers for Serology Testing

<b>Company</b>	<b>Address</b>
Pierce (ThermoScientific)	P.O. Box 117 Rockford, IL 61105 (800) 874-3723 <a href="http://www.piercenet.com">www.piercenet.com</a>
Kirkegaard & Perry	910 Klopper Road Gaithersburg, MD 20878 (800) 638-3167 <a href="http://www.kpl.com">www.kpl.com</a>
Sigma	P.O. Box 14508 St. Louis, MO 63178 (800) 325-3010 <a href="http://www.sigma-aldrich.com">www.sigma-aldrich.com</a>
Fisher Scientific	2000 Park Lane Drive Pittsburgh, PA 15275 (800) 766-7000 <a href="http://www.fishersci.com">www.fishersci.com</a>

# Section V

## C. Serology Submission Form

Page 1



To be completed at Charles River

Accession No: \_\_\_\_\_

Date Received: \_\_\_\_\_

### Serology Submission Form

**Contact Information**

Institution: \_\_\_\_\_

Address: \_\_\_\_\_

Contact: \_\_\_\_\_

Phone: \_\_\_\_\_

Fax: \_\_\_\_\_

Email: \_\_\_\_\_

**Billing Information**

Institution: \_\_\_\_\_

Address: \_\_\_\_\_

Contact: \_\_\_\_\_

Phone: \_\_\_\_\_

Fax: \_\_\_\_\_

Email: \_\_\_\_\_

PO #: \_\_\_\_\_

**Payment method is required for efficient turn-around of results.**

Credit Card:     VISA     MC     AMEX    Credit card Number: \_\_\_\_\_

Name on Credit Card: \_\_\_\_\_ Exp Date: \_\_\_\_\_ Security Code: \_\_\_\_\_

### Sample Information

To the best of my knowledge these samples and/or specimens do not contain any infectious agent or material which might pose a threat to human health. If this is not the case, please call Charles River before sending samples.

Signed: \_\_\_\_\_ Date: \_\_\_\_\_

Species\* (Check One):     Mouse     Rat     Hamster     Guinea Pig     Other \_\_\_\_\_

\*Please use one form per species.                      Total number of samples submitted: \_\_\_\_\_    Source: \_\_\_\_\_

Sample ID			Profile / Test(s)	Heat Inact	Other Treatment	Strain
Vial #	Customer ID	Dilution				
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Comments: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Please include a copy of this form in the container in which the samples are shipped. See instructions on pages 3.

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Page 1 of 3

# Section V

Submission form, page 2



Sample ID			Profile / Test(s)	Heat Inact	Other Treatment	Strain
Vial #	Customer ID	Dilution				
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						

For Charles River Use Only

**Comments:** \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

# Section V

Submission form, page 3



## Instructions for Completing the Serology Submission Form

### General

Please complete the following information:

- Fill out the Contact and Billing Information as completely as possible.
- Enter the payment method information completely to ensure efficient reporting of results.
- Please sign and date to verify the biohazard status.

### Sample Information

- Circle the species origin of the sample and indicate the date of shipment. Use one form per species.
- Indicate the room or facility of origin as the Source.
- For each sample, record all pertinent information including:
  - Vial number.
  - Indicate any reference identification in the "Customer ID". Designations will appear on the results report as the "Code".
  - Dilution of the sample; as described on the Sample Submission Information Sheet, we request that the sample be diluted one part serum to four parts PBS. Record this information as either 1:4 (i.e. 1 part to 4 parts) or 1/5 (1 part in 5).
  - For each sample, indicate the appropriate Profile/Test Name. See table below for general naming conventions.
- We prefer that you do not heat inactivate samples. If heat-inactivation of samples was performed (56°C for 30 minutes), please check the appropriate box and note this as a comment.
- Please describe any other treatments that have been performed on any sample, e.g. receptor destroying enzyme (RDE) digestion, or kaolin absorption.

MFIA Profile	Abbreviation	Individual Test
Parvovirus screen	PV	MFIA: Test Name
Tracking	T	IFA: Test Name
Prevalent	PR	
Assessment	A	
Assessment Plus	AP	

### Shipping Requirements

- When shipping samples, please follow all International Air Transport Association (IATA) guidelines.
- Serum: At least 200  $\mu$ L of diluted serum is requested. Samples should be frozen ( $\leq -20^{\circ}\text{C}$ ).
- We provide IATA-compliant sample shipping containers (sample collection materials, shipping labels and containers, etc.). Simply contact Technical Services (1-800-338-9680) to request materials for your samples for diagnostic testing. Alternatively, you may order shipping materials 24 hours a day via our website at [www.criver.com/info/quotes](http://www.criver.com/info/quotes). Shipping materials are offered at no charge to you.

Please ship samples to:

**Charles River  
Serology Laboratory  
251 Ballardvale Street  
Wilmington, MA 01887  
Tel: 1-800-338-9680**