

NOTE: Changes highlighted

SYMBOL DEFINITIONS

Following are the definitions of the symbols found on the various Bion Enterprises, Ltd. (Bion) Product Labels:

 = Consult Directions for Use	 = In Vitro Diagnostic Reagent
 = Store Away From Direct Light	 = Positive Control
 = Storage Temperature	 = Negative Control
 = Expiration Date	 = Endpoint Titer
 = Number of Tests	 = Code Number
 = Amount	 = Lot Number
 = Single Use Only	 = Contains biological material of animal origin
 = Contains human blood or plasma derivatives	

PRODUCT AVAILABILITY

For Bion Product Availability for IFA Test Reagents, see back page.

INTENDED USE

Bion REAGENTS FOR IFA (Indirect Fluorescent Antibody) TESTS may be used in assays for the qualitative and/or semi-quantitative determination of specific IgG or IgM antibodies in human serum. The Bion REAGENTS FOR IFA TESTS are intended for use as an aid in diagnosis of certain autoimmune diseases or for primary or active infection and/or as a determination of immunological experience with specific microorganisms.

SUMMARY AND EXPLANATION

Methods for microbial antibody detection have included indirect fluorescent antibody (IFA) and enzyme immunoassay (EIA).^{1,2} The solid phase immunoassays, such as the IFA and EIA have the advantage of being sensitive, and also able to differentiate between the various antibody classes.

PRINCIPLE OF THE IFA PROCEDURE

The Bion REAGENTS FOR IFA TESTING may be utilized in the indirect fluorescent antibody assay method first described by Weller and Coons³ and further developed by Riggs, et al.⁴ The procedure is carried out in two basic reaction steps:

- Step 1 -** Human serum is reacted with an antigen substrate. Antibodies, if present, will bind to the antigen forming stable antigen-antibody complexes. If no antibodies are present, the complexes will not be formed and serum components will be washed away.
- Step 2 -** Fluorescein labeled antihuman IgG or IgM antibody is added to the reaction site which binds with the complexes formed in step one. This results in a positive reaction of bright apple-green fluorescence when viewed with a properly equipped fluorescence microscope. If no complexes are formed in step one, the fluorescein labeled antibody will be washed away, exhibiting a negative result.

REAGENTS, STORAGE AND STABILITY

ANTIGEN SUBSTRATE SLIDES

Individually foil-wrapped slides of six or twelve wells with tissue culture cells alone, microorganisms alone or antigen infected cells fixed onto each well. The specific cell type or microorganism is identified on the product label. Slides are stable in sealed foil pouches at 8°C or lower until labeled expiration date.

FLUORESCENT ANTIBODY CONJUGATE

Ready to use dropper vial containing 3.5 ml (or 20 mL) fluorescein isothiocyanate labeled goat antihuman immunoglobulins, antihuman IgG (heavy chain specific) or antihuman IgM (heavy chain specific) with Evans Blue counterstain, protein stabilizer, less than 0.1% sodium azide and 0.001% thimerosal. Stable at 2-8°C away from direct light until labeled expiration date.

POSITIVE CONTROL SERUM

Vial containing 0.5 ml positive IgG or positive IgM human control serum with protein stabilizer and 0.005% thimerosal. Stable at 2-8°C until labeled expiration date.

IgM positive controls do not have to be pretreated to remove IgG. When used undiluted, as provided, specific fluorescent intensity of 3+ or greater should be seen. Optionally, the positive control can be titrated to endpoint. If titrated, the control should be serially diluted in PBS. When the control has been tested for the endpoint titer by Bion Enterprises, Ltd., an endpoint titer is printed on the positive control vial. Due to variations within each laboratory (fluorescent microscopy, etc.) each laboratory should establish its own mean titer for each lot of positive control (generally $\pm$ one dilution from stated endpoint).

NEGATIVE CONTROL SERUM

Vial containing 0.5 ml negative human control serum with protein stabilizer and 0.005% thimerosal. Stable at 2-8°C until labeled expiration date.

IgM negative controls do not have to be pretreated to remove IgG. The control is intended to be used undiluted as provided. The staining reaction should exhibit less than 1+ fluorescence.

MOUNTING MEDIUM

Dropper vial containing 3.5 ml (or 20 ml) phosphate buffered glycerol of pH 7.4 $\pm$ 0.2. Stable at 2-8°C until labeled expiration date.

PHOSPHATE BUFFERED SALINE (PBS)

One-liter packet of dry PBS. Stable in sealed packet at 25°C or lower until labeled expiration date.

BUFFER PREPARATION

Place contents of a one-liter PBS packet into a one-liter volumetric flask, add *distilled water to the one-liter mark, mix and leave several hours or overnight to dissolve. Reconstituted buffer should have a pH of 7.4 $\pm$ 0.2. Adjust with 1N NaOH or 1N HCL if pH value is outside the stated range. Store in a clean screw capped bottle at 25°C or lower. Stable until labeled expiration date provided no gross contamination is seen. Do not use if pH changes, if the solution turns cloudy, or if a precipitate forms.

* Use deionized water with caution, as pH of this type of water may vary causing the pH of PBS to become unstable upon prolonged storage.

IGG REMOVAL REAGENT

2.5 ml or 20 ml dropper vial containing goat anti-human IgG. Ready to Use. Stable at 2-8°C until labeled expiration date.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use. Thus, only staff trained in methods of in vitro diagnostics may perform the test.
- Substrate slides are for single use only and must not be used more than once.
- Care should be taken when handling substrate slides due to sharp edges.
- The antigenic substrates have been fixed to contain no detectable live microorganisms. However, they should be handled and disposed of as any potentially biohazardous laboratory material.
- Do not remove slides from pouches until ready for testing. Do not use if pouch has been punctured, as indicated by a flat pouch.
- Store all reagents at appropriate temperature. Do not use if stored improperly. All reagents should be brought to room temperature (20-25°C) prior to use.

7. Abnormal test results may be seen if the antigen substrate slides are allowed to dry during the staining procedure.
8. Reagents should not be used beyond stated expiration date.
9. Do not expose conjugate to strong light during storage or use.
10. Avoid microbial contamination of all reagents involved in the testing procedure or incorrect results may occur.
11. Incubation times or temperatures other than those specified may give erroneous results.
12. Reproducibility of endpoint titers relies on accurate pipettors and proper laboratory technique.
13. Reusable glassware must be washed and thoroughly rinsed free of detergents.
14. Care should be taken to avoid splashing or generation of aerosols.
15. Previously frozen specimens after thawing should be thoroughly mixed prior to testing. It is recommended that sera freeze thawed no more than one time. If repeated testing is required, it is suggested that specimen be aliquoted.
16. Sera used to prepare positive and negative controls have been tested by an FDA approved method and found to be negative (were not repeatedly reactive) for the presence of Hepatitis B surface Antigen (HBsAg) and antibodies to Hepatitis C (HepCAb) and HIV 1 & 2. However, because no test method can offer complete assurance of the absence of these or other infectious agents, these reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual "Biosafety in Microbiological and Biomedical Laboratories," 1984 Edition.
17. Patient samples, as well as all materials coming into contact with them, should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual "Biosafety in Microbiological and Biomedical Laboratories", 1984 Edition. Never pipette by mouth. Avoid contact with skin and mucous membranes.
18. The preservatives used in conjugate and controls are toxic if ingested. Azides may react with copper or lead plumbing to form explosive metal azides. When disposing, flush drains with water to minimize build-up of azide and metal compounds.
19. Properly aligned fluorescence microscope with correct filters is imperative.

SPECIMEN COLLECTION

Blood should be collected fasting or at least one hour after meals to avoid lipemic serum, as excess lipids may produce a "film" over the substrate. Aseptically collect 5-8 ml of blood by venipuncture. Allow the blood to clot at room temperature (20-25°C) before separating serum to avoid hemolysis which could interfere with test results. Specimens should be stored refrigerated at 2-8°C and tested within one week of collection. Long term storage should be at -20°C in aliquots to avoid repeated freezing and thawing. Do not store in self-defrosting freezer.

Avoid using contaminated sera as they may contain proteolytic enzymes which will digest the substrate. It is unnecessary to heat inactivate serum specimens prior to testing; however, sera that have been heat inactivated may be used.

When testing paired samples to look for evidence of recent infection, the acute specimen should be obtained as soon as possible after onset of illness and the convalescent specimen obtained 7-14 days later. Acute and convalescent specimens must be tested simultaneously, in the same assay, looking for a significant change in antibody titer between the paired sera. If the first specimen is obtained too late during the course of the infection, a significant rise in the antibody titer may not be detected.

PROCEDURE

Detailed descriptions of indirect immunofluorescence techniques may be found in the references listed in the bibliography.^{5,6,7}

MATERIALS AVAILABLE FROM Bion

1. Antigen Substrate Slides
2. Fluorescent Antibody Conjugates
3. Positive Human Control Serums
4. Negative Human Control Serums
5. Phosphate Buffered Saline (PBS)
6. Mounting Medium
7. IgG Removal Reagent

MATERIALS REQUIRED BUT NOT PROVIDED

1. Disposable test tubes (12 x 75 mm or comparable) and rack
2. Disposable serological pipettes
3. Calibrated pipettes to deliver 50 µl, 100 µl and 200 µl with disposable pipette tips
4. Pasteur pipettes and bulbs
5. Moist chambers
6. Plastic squeeze wash bottle
7. Coplin jars or staining dishes with slide racks
8. 24 x 60 mm #1 coverslips
9. Felt tip marking pen
10. Fluorescence microscope equipped with mercury or tungsten-halogen light source, a 390-490nm excitation filter and 515-520nm barrier filter, and optics to give a total magnification of 200X, 250X or 400X. The excitation wavelength of FITC is 490nm and the emission wavelength is 520nm.

TEST PROCEDURE

The procedure listed below is a general procedure which can be used for most antigen substrate slides. However, the procedure for some of the antigen substrate slides varies from what is outlined below. Therefore it is recommended that the user review the specific Product Insert for the individual antigen substrate being tested, particularly regarding the dilution scheme and materials required.

1. SPECIMEN PREPARATION

Screening:

Each laboratory should establish its own protocol for the preparation of serum screening dilutions. Most indirect fluorescent antibody staining procedures utilize a 1:10 dilution of each patient's serum which can be prepared by adding 0.05 ml (50 µl) of the patient's serum to 0.45 ml of PBS.

NOTE:

If testing for IgM specific antibodies using an IgM specific fluorochrome conjugate, each patient serum specimen must be pre-treated to remove any IgG interference by separating the IgM from the IgG, and then running the screening test on the IgM eluate. Suggested methodologies are ion exchange chromatography⁹ or IgG immunoprecipitation.^{9,10}

Semi-quantitation:

Serum dilutions are utilized to measure antibody titer. Each laboratory should establish its own titrating protocol. The selection of either twofold or fourfold dilution procedures depends upon the experience level and training of the individual(s) reading the fluorescent antibody assay.

The following fourfold serial titration is suggested for IgG testing:

- a. Prepare a 1:10 dilution of each patient's serum by adding 0.05 ml (50 µl) of patient's serum to 0.45 ml of PBS in tube #1.
- b. Add 0.3 ml PBS to tubes #2, #3, #4, and #5.
- c. Using a 100 µl pipette, transfer 0.1 ml (100 µl) from tube #1 to tube #2. Mix. Using a new tip for each dilution, transfer 0.1 ml (100 µl) from the second tube to the third, from the third tube to the fourth, and from the fourth tube to the fifth, mixing after each transfer.

The following twofold titration is suggested for IgM testing:

- a. Prepare a 1:10 dilution of each patient's serum using one of the treatment methodologies mentioned in the "Screening NOTE" above. This will be designated as tube #1.
- b. Add 0.2 ml PBS to tubes #2, #3, #4, and #5.
- c. Using a 200 µl pipette, transfer 0.2 ml (200 µl) from tube #1 to tube #2. Mix. Using a new tip for each dilution, transfer 0.2 ml (200 µl) from the second tube to the third, from the third tube to the fourth, and from the fourth tube to the fifth, mixing after each transfer.

These titrations will have the following dilutions:

Fourfold	Twofold
Tube #1 = 1:10	Tube #1 = 1:10
Tube #2 = 1:40	Tube #2 = 1:20
Tube #3 = 1:160	Tube #3 = 1:40
Tube #4 = 1:640	Tube #4 = 1:80
Tube #5 = 1:2560	Tube #5 = 1:160

2. SLIDE PREPARATION

Remove reagents and as many slides as are required from the refrigerator or freezer and allow to equilibrate to room temperature (20-25°C) for at least five minutes. Remove slides from sealed foil pouches being careful not to touch the antigen surface. Identify each slide using a felt tip marking pen.

3. SPECIMEN APPLICATION

Using separate Pasteur pipettes, apply one drop (20-30 µl) of the positive control, one drop (20-30 µl) of the negative control and one drop (20-30 µl) of each patient serum dilution to individual wells of the slide. Do not touch the antigen surface with the pipette while dropping. Do not allow drops to mix, as cross contamination of samples between wells could cause erroneous results.

4. INCUBATION 1

Incubate in a moist chamber at room temperature (20-25°C) for 30 minutes. THE ANTIGEN MUST NOT BE ALLOWED TO DRY DURING ANY OF THE FOLLOWING STEPS. Nonspecific binding may occur if the reagent is allowed to dry on the slide.

NOTE: For IgM testing, incubate the substrate slides in a moist chamber at 35-37°C for 90 minutes.

5. RINSE 1

Remove slides from moist chamber and rinse GENTLY with PBS using a squeeze wash bottle. Do not focus the PBS stream directly onto the wells. To prevent cross contamination tilt slide first toward wells 1-6 and, running a PBS stream along the midline of the slide, allow the PBS to run off the top edge of the slide. Then, tilt the slide toward wells 7-12 and repeat this procedure, allowing the PBS to run off the bottom edge of the slide. For six well slides, tilt slide down and run the PBS stream across the slide above the wells, allowing the PBS to run off the bottom edge of the slide.

6. WASH 1

Place slides in Coplin jars or staining dishes and wash in two changes of PBS for not less than five minutes or more than ten minutes each, agitating gently at entry and prior to removal.

7. CONJUGATE APPLICATION

Remove slides from the wash one at a time, shake off excess PBS, dry around outside edges if necessary and return each slide to the moist chamber. Apply one drop of an appropriate fluorescent antibody (IgG or IgM) conjugate with counterstain (diluted to its predetermined proper working dilution) to each well of each slide, making sure that each well is completely covered.

8. INCUBATION 2

Incubate in a moist chamber at room temperature (20-25°C) for 30 minutes. Protect slides from excessive light.

NOTE: For IgM testing, incubate in a moist chamber at 35-37°C for 60 minutes.

9. RINSE 2

Remove slides from moist chamber and rinse GENTLY with PBS using a squeeze wash bottle. As suggested in step 5., do not focus PBS stream directly onto the wells.

10. WASH 2

Place slides in Coplin jars or staining dishes and wash in two changes of PBS for not less than five minutes or more than ten minutes each, agitating gently at entry and prior to removal.

11. COVERSIP

Remove slides one at a time from the last PBS wash, shake off excess PBS and immediately add two to four drops of mounting medium across the slide. Tilt slide and rest the edge of the coverslip against the bottom of the slide allowing the mounting medium to form a continuous bead between the coverslip and slide. Gently lower the coverslip from the bottom of the slide to the top, being careful to avoid air bubbles. Drain excess mounting medium by holding the edge of the slide against absorbent paper. Wipe off back of slide.

12. READ

Examine stained slides as soon as possible using a properly equipped fluorescence microscope. It is recommended that slides be examined on the same day they are stained. If any delay is anticipated, store slides in the refrigerator (2-8°C) away from direct light and read the following day. Do not allow mounting medium to dry between slide and coverslip. If drying should occur, add additional mounting medium or recoverslip slide.

FLUORESCENT INTENSITY GRADING

Fluorescent intensity may be semi-quantitated by following the guidelines established by the Centers for Disease Control, Atlanta, Georgia:¹¹

- 4+ = Maximal fluorescence; brilliant yellow-green.
- 3+ = Less brilliant yellow-green fluorescence.
- 2+ = Definite but dull yellow-green fluorescence.
- 1+ = Very dim subdued fluorescence.

The degree of fluorescent intensity is not clinically relevant and has only limited value as an indicator of titer. Differences in fluorescence microscope optics, filters and light sources may result in differences of 1+ or more fluorescent intensity when observing the same slide using different microscopes.

QUALITY CONTROL

SPECIFICITY CONTROL

Both a positive and negative antibody control must be included with each run. These controls must be examined prior to reading test samples and should demonstrate the following results:

Negative Control

Using the Bion NEGATIVE CONTROL SERUM as provided, the microorganism, infected cells or ANA HEp-2 cells should exhibit less than 1+ fluorescence and appear reddish-orange due to the counterstain.

Positive Control

Using the Bion POSITIVE IgG or IgM CONTROL SERUM as provided, the microorganism, infected cells or ANA HEp-2 cells should exhibit well defined specific fluorescent staining at an intensity of 3+ or greater.

See Table "FLUORESCENT STAINING PATTERNS".

Each control must demonstrate the expected reaction in order to validate the test. If the controls fail to appear as described above, the test results should not be reported and the test should be repeated. If upon repeat testing the controls still fail to show the proper reaction, do not report test results.

SENSITIVITY CONTROL

A titrated control included with each run tests substrate sensitivity, as well as, checks technique, conjugate quality and the microscope optical system. The endpoint titer of this control must be determined and there must not be more than a twofold difference (+/-) in titer from this determined endpoint. Each run should include the endpoint dilution, one twofold or fourfold dilution above and one twofold or fourfold dilution below the endpoint dilution. The more concentrated dilution should be positive and the less concentrated dilution negative. If the control does not behave as described, the test results are invalid and the tests should be repeated. If the control again fails to show the proper reaction upon repeat testing, do not report the test results.

READING OF TEST RESULTS

NEGATIVE

A serum dilution is considered to be negative for the specific antibodies being analyzed if the microorganism, infected cells or ANA HEp-2 cells exhibit less than 1+ fluorescence, or if the fluorescence observed is not the specific staining pattern described in the Table "FLUORESCENT STAINING PATTERNS".

A sample is considered negative for the specific antibodies being analyzed if it exhibits less than 1+ fluorescence at the screening serum dilution and all greater dilutions, or if the fluorescence observed is not the specific staining pattern described in the Table "FLUORESCENT STAINING PATTERNS".

...Negative samples may exhibit fluorescent staining of the microorganism, infected cells or ANA HEp-2 cells slightly greater than the negative control, but less than 1+.

...Nonspecific staining of all cells observed in some sera at low dilutions is most likely due to the presence of autoantibodies against cellular components in either the nucleus or cytoplasm.

...Staining of areas other than the specific staining pattern described in the Table "FLUORESCENT STAINING PATTERNS" should be interpreted as negative and attention should be directed to specific steps in the staining method (e.g., RINSE and WASH steps).

NOTE: Infection of in vitro culture cells by Herpesviruses (CMV, EBV, HSV1, HSV2, or VZV) can induce an Fc-IgG receptor in the cytoplasm of infected cells which may result in false positive readings when doing IgG assays. This most often occurs with CMV and HSV1. IgG antibody from the patient attaches to these Fc receptor sites which then react with antihuman IgG conjugate and appear as fluorescent perinuclear cytoplasmic inclusions just outside the nuclear membrane of these cells. This can be differentiated from the specific viral fluorescent staining which is generally nuclear inclusions or homogeneous staining of both the nucleus and cytoplasm.^{12,13} The perinuclear Fc-IgG receptor site staining should be interpreted as negative.

POSITIVE

A serum dilution is considered positive for the specific antibodies being analyzed if well defined specific fluorescent staining is observed in the microorganism, infected cells or ANA HEp-2 cells at an intensity of 1+ or greater. See Table "FLUORESCENT STAINING PATTERNS".

The number of cells or microorganisms exhibiting a positive staining reaction and the type of fluorescent staining pattern should closely approximate that seen in the positive control.

A sample is considered positive for the specific antibodies being analyzed if it exhibits the characteristic staining pattern with a fluorescent intensity of 1+ or greater at the screening serum dilution.

NOTE: When using an infected cell antigen substrate, each field should contain cells that exhibit no apple-green fluorescence. Should most of the cells in the patient test wells fluoresce apple-green in the nucleus and/or cytoplasm, an autoimmune staining reaction due to the presence of autoantibodies should be considered.^{14,15} It is recommended that such samples be diluted beyond the interference for better interpretation. It is possible that autoantibody staining may mask specific staining such that an interpretation cannot be made. Should this occur, test results should be reported as "Unable to interpret due to the presence of interfering antibodies."

TITRATION

If a semi-quantitative titration is performed, the result should be reported as the reciprocal of the last dilution in which 1+ apple-green fluorescent intensity of the specific staining pattern is detected. When reading fourfold serial dilutions, endpoints can be extrapolated where necessary.

EXAMPLE OF ENDPOINT EXTRAPOLATION:

1:10 = 4+
1:40 = 3+
1:160 = 2+
1:640 = +/-

The extrapolated endpoint is reported as 320.

TROUBLESHOOTING

Possible solutions to problems that may occur in immunofluorescent assays are discussed in an accompanying brochure entitled "TROUBLESHOOTING IN IMMUNOFLUORESCENCE".

INTERPRETATION OF RESULTS

Detection of the presence of specific microbial antibodies indicates a current or previous experience with the organism. A significant (fourfold or greater) increase in titer between acute and convalescent serum samples and/or a positive test for IgM specific antibodies usually indicates evidence of a recent or active infection. See ANA product insert for ANA interpretation.

LIMITATIONS OF THE PROCEDURE

1. Antibody test results should be used in conjunction with information available from clinical evaluation and other diagnostic information.
2. A single serological IgG antibody titer should not be used as the only criterion for diagnosis. Paired serum samples (acute and convalescent) and testing for IgM specific antibodies may provide more meaningful data.
3. A negative test result does not necessarily rule out current or recent infection. The specimen may have been collected too early in the disease before demonstrable antibody is present.
4. Lack of significant rise in titer does not exclude the possibility of recent infection but may indicate that an acute phase specimen was obtained too late.
5. In some instances, high IgG or IgM antibody levels in the first of paired specimens may prevent the detection of increases in total antibody, resulting in apparently stationary total antibody titer.
6. Test results on specimens from immunosuppressed patients and pregnant women may be difficult to interpret.
7. Positive test results may not be valid in persons who have received blood transfusions or various blood products within the past several months.
8. Antinuclear antibodies (ANA) present in serum may interfere with the IFA test. They can be differentiated from specific antibody staining in that ANAs stain the nuclei in all cells; whereas, specific antibodies exhibit nuclear inclusion staining only in the 10-50% infected cells.¹⁴
9. Cytoplasmic fluorescence in the majority of the cells may be due to the presence of antimitochondrial antibodies (AMA) often seen in primary biliary cirrhosis.¹⁵ They can be differentiated from the specific antigen staining in that AMA will stain the cytoplasm of all cells; whereas, specific antibodies exhibit staining in only the 10-50% infected cells.
10. Positive test results from cord blood or neonates should be interpreted with caution. The presence of IgG antibodies in cord blood is usually the result of passive transfer from mother to the fetus. A negative test, however, may be useful in excluding possible infection. Because of the possibility of contamination of cord blood with maternal IgM, it is prudent to confirm positive viral IgM antibody results on cord blood samples by testing a follow-up specimen from the infant, preferably within the first five days of life.^{16,17}
11. Endpoint reactions may vary between laboratories due to differences in type or condition of fluorescence microscope employed, diluting apparatus, IgG/IgM separation methods, as well as the experience level of personnel performing the assay.
12. If both the positive and negative control substrate cells are not visible when viewed using the fluorescence microscope, it may be necessary to replace or realign the light source and check the specific filters.
13. Cell culture substrate slides may exhibit nonspecific fluorescence due to contamination of antibodies or PBS rinse-wash solutions with bacteria or fungi. It is very important that personnel reading the staining results have experience in fluorescence microscopy.
14. If testing for IgM specific antibodies, high titers of specific IgG when present in the patient serum may compete with the pathogen specific IgM for the antigen sites resulting in a false negative IgM reaction.¹⁸ Therefore, all sera should be treated by ion exchange chromatography⁸ or IgG immunoprecipitation^{9,10} before testing to avoid this possible problem.
15. If testing for IgM specific antibodies, the presence of Rheumatoid Factor (RF) in serum may cause a false positive reaction if pathogen specific IgG is also present. Routine RF tests may not be sensitive enough to detect small amounts of RF which exist within the normal range, but are sufficient to cause a false positive reaction in the more sensitive IFA technique.¹⁸ Therefore, all sera should be treated by ion exchange chromatography⁸ or IgG immunoprecipitation^{9,10} before testing to eliminate possible RF interference.
16. Two methods such as immunoprecipitation and ion exchange chromatography have been commonly used for neutralizing or removing possibly interfering IgG antibodies prior to testing for specific IgM antibodies in IFA tests. Immunoprecipitation neutralizes all classes of IgG while not affecting the IgM levels; however, high levels of IgG may need to be treated with proportionally increased amounts of the precipitating reagent. Ion exchange chromatography will only eliminate IgG subclasses 1, 2 and 3 with subclass 4 (usually less than 5% of the total IgG) remaining in the fraction with the IgM.
17. IgG removal reagents may lower the total amount of IgM present in serum after treatment. The amount removed can be as high as 25% of total IgM present¹⁹. The effects of IgG removal on the sample may vary by assay type performed (IFA, ELISA, etc.). The specific IgM may vary by assay specificity of related organism as well. Inherent cross-reactivity between related viruses, previous infections with related viral species, and bacterial infections is also possible²⁰. It is suggested that paired serums be treated and processed for antibody titers at the same time to minimize processing differences. Serums tested for acute infection should be checked for the patient's clinical history and repeated if indeterminate. Paired samples are recommended for analysis.
18. IgM serology performed by IFA is very attractive since it combines specificity with sensitivity and in most cases only a single serum sample is required. However, the need for careful interpretation of the significance of positive IgM tests in relationship to patient's clinical situations must be emphasized. It is essential to have an awareness of understanding of the many problems associated with IgM testing to avoid the many pitfalls that can trap the most experienced of workers. IgM results must always be interpreted with caution.²¹

SPECIFIC PERFORMANCE CHARACTERISTICS

See specific Product Inserts for additional performance characteristics on individual Antigen Substrates.

Bion MICROBIAL ANTIGEN SUBSTRATE SLIDES have been evaluated for the presence of specific microbial antigens using commercial monoclonal antisera. In each case, positive reactions were identified with the specific microorganism or antigen infected cell cultures when stained with its corresponding antisera.²¹ Also, there was no cross-reactivity observed between the microorganism or antigen infected cell cultures when they were reacted with monoclonal antisera which were not specific for that particular antigen substrate.²¹

Components have been tested to evaluate their performance requirements for IFA tests and to confirm their lot to lot consistency and product usability. Control serums are tested for specificity to show a positive staining reaction (pattern) with their particular matching substrate. For sensitivity testing, they must give an expected negative reaction (<1+ reaction, undiluted) or positive reaction (3 to 4+ reaction, undiluted) and a titer (1+) at the stated endpoint. Conjugates, Mounting Media and PBS Buffer have been tested for expected results with positive controls with established endpoints (1 to 2+ reaction) appropriate for the various substrates, as well as negative controls (<1+ reaction) and PBS Buffer (negative for nonspecific reactions).

Each laboratory should determine its own performance characteristics using all reagents assembled to perform indirect fluorescent antibody assays.

FLUORESCENT STAINING PATTERNS

MICROBIAL AGENT	FLUORESCENT STAINING PATTERN
Adenovirus	The infected cells exhibit both nuclear and cytoplasmic staining. More apparent intranuclear inclusions are seen at periphery of the nucleus.
Antinuclear Antibodies (HEp-2 Cells)	Various staining patterns are exhibited in cell nuclei; peripheral, homogeneous, speckled and nucleolar. See ANA insert for specific information on staining patterns.
Borrelia burgdorferi (Lyme Disease)	Staining of the complete spirochetal microorganism.
Chlamydia (LGV-1)	The infected cells exhibit intracytoplasmic inclusion bodies found near the nucleus. Also, pinpoint elementary bodies are often observed both on and between the tissue culture cells.
Coxsackie B Virus	The infected cells display a variety of patterns from solid staining of the entire cell, to speckly staining of some cells, to just the rim of the cell staining.
Cytomegalovirus	The infected cells exhibit intranuclear inclusion body staining. Cytoplasmic staining can also be seen with strong positive sera, but perinuclear staining observed in some of the infected cells is nonspecific.
Echovirus	The infected cells display a variety of patterns from solid staining of the entire cell, to speckly staining of some cells, to just the rim of the cell staining.
Epstein-Barr Virus Viral Capsid Antigen (VCA)	The infected cell population displays solid staining of the entire cell.
Herpes Simplex Virus Type 1	The infected cells exhibit both nuclear and cytoplasmic fluorescence. HSV-1 demonstrates a more apparent membrane staining. Nonspecific perinuclear staining is more apparent with weak positive or negative primary sera. Patches of cells tend to merge, losing their individuality.

FLUORESCENT STAINING PATTERNS (continued)

MICROBIAL AGENT	FLUORESCENT STAINING PATTERN
Herpes Simplex Virus Type 2	The infected cells exhibit both nuclear and cytoplasmic fluorescence, but the intranuclear staining is more apparent with HSV-2. Patches of cells tend to merge, losing their individuality.
Influenza A Virus	The infected cells exhibit staining in the nucleus alone, in the cytoplasm alone, or in both the nucleus and cytoplasm.
Influenza B Virus	The infected cells exhibit staining which may be somewhat uneven, but is found in the nucleus alone, the cytoplasm alone, or in both the nucleus and cytoplasm.
Measles (Rubeola) Virus	The infected cells exhibit staining of cytoplasmic granules. The cells may coalesce forming multinucleated giant cells.
Mumps Virus	The infected cells exhibit staining of fine and coarse cytoplasmic particles. Cells tend to maintain individuality of size and shape.
Mycoplasma pneumoniae	The infected cells exhibit staining of small glass rod-like forms, and solid colonies with reddish-orange background cells. Colony staining alone may be seen with low-titered sera. Ring-like subunits around the colony rims may be seen in high-titered IgM sera.
Respiratory Syncytial Virus	The infected cells exhibit staining of fine cytoplasmic particles. Cells coalesce into large syncytial masses with indistinguishable cell walls.
Varicella Zoster Virus	The infected cells exhibit staining of both intranuclear inclusions and web-like, membranous cytoplasmic staining. Cells tend to lose individual definition and merge together forming patches of positive staining material.

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PRODUCT AVAILABILITY

The following Products are available individually from Bion

	12-well	6-well
<u>Antigen Substrate Slides</u>	<u>Code No.</u>	<u>Code No.</u>
Adenovirus	AD-3112	
Antinuclear Antibodies	AN-1012	AN-1006
Borrelia burgdorferi (Lyme Disease)	BB-6112	
*Chlamydia (LGV-1)	CH-4112	
Coxsackie B1-6 Screen	CB-3312	CB-3306
*Cytomegalovirus	CM-2012	
Echovirus Screen	EC-4412	
Epstein-Barr Virus (EBV) VCA	EB-5012	
Herpes Simplex Virus, Type 1	HS1-3012	
Herpes Simplex Virus, Type 2	HS2-4012	
Influenza A Virus	IA-1112	
Influenza B Virus	IB-2112	
Measles (Rubeola) Virus	ME-6012	
Mumps Virus	MU-8012	
Mycoplasma pneumoniae	MP-1212	MP-1206
Respiratory Syncytial Virus	RS-9012	
Varicella Zoster Virus	VZ-7012	

<u>Control Serums, 0.5 ml per Vial</u>	<u>Neg</u> <u>Code No.</u>	<u>IgG</u> <u>Code No.</u>	<u>IgM</u> <u>Code No.</u>
ANA Positive, Homogeneous		ANP-1040	
ANA Negative	ANN-1010		
ANA Nucleolar		NUC-1042	
ANA Anti-Centromere, (ACA)		ACA-1043	
ANA Ribonucleoprotein, (RNP)		RNP-1044	
ANA Sjogren's Syndrome A, (SS-A)		SSA-1045	
ANA Sjogren's Syndrome B, (SS-B)		SSB-1047	
ANA Scleroderma-70, (Scl-70)		SCL-1046	
Borrelia burgdorferi	BBN-6110	BBG-6120	BBM-6130
*Chlamydia	CHN-4110	CHG-4120	CHM-4130
*Cytomegalovirus	CMN-2010	CMG-2020	CMM-2030
Epstein-Barr Virus (VCA)	EBN-5010	EBG-5020	EBM-5030
Herpes Simplex Virus 1 & 2	HSN-3510	HSG-3520	HSM-3530
Influenza A Virus	IAN-1110	IAG-1120	
Influenza B Virus	IBN-2110	IBG-2120	
Measles (Rubeola) Virus	MEN-6010	MEG-6020	MEM-6030
Mumps Virus	MUN-8010	MUG-8020	MUM-8030
Mycoplasma pneumoniae	MPN-1210	MPG-1220	MPM-1230
Respiratory Syncytial Virus	RSN-9010	RSG-9020	
Varicella Zoster Virus	VZN-7010	VZG-7020	VZM-7030

<u>Conjugates, dropper bottles</u>	<u>Size</u>	<u>Code No.</u>
Anti-human Immunoglobulin, for ANA testing	3.5 ml	CCP-9970
Anti-human Immunoglobulin, for ANA testing	20 ml	CCP-9920
Anti-human Immunoglobulin, for ANA testing	60 ml	CCP-9960
Anti-human IgG, for all IgG testing except EBV	3.5 ml	CCG-9972
Anti-human IgG, for all IgG testing except EBV	20 ml	CCG-9922
Anti-human IgM, for all IgM testing except EBV	3.5 ml	CCM-9974
Anti-human IgM, for all IgM testing except EBV	20 ml	CCM-9924
Anti-human IgG for EBV testing	3.5 ml	CXCG-9972
Anti-human IgG for EBV testing	20 ml	CXCG-9922
Anti-human IgM for EBV testing	3.5 ml	CXCM-9974
Anti-human IgM for EBV testing	20 ml	CXCM-9924
(Customized sizes are also available.)		

<u>Miscellaneous Reagents</u>	<u>Size</u>	<u>Code No.</u>
*Phosphate Buffered Saline (PBS)	1-Liter	PBS-9990
*Mounting Medium	3.5 ml	MM-9985
*Mounting Medium	20 ml	MM-9986
IFADiluent	60 ml	DIL-9993
IFADiluent	125 ml	DIL-9994
IFADiluent	250 ml	DIL-9995
*IgG Removal Reagent (for IgM testing)	2.5 mL	GBR-9983
*IgG Removal Reagent (for IgM testing)	20 mL	GBR-9981

* Not  Marked



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