

MEASLES - G ANTIBODY TEST SYSTEM



NOTE: Changes highlighted

SYMBOL DEFINITIONS

	= 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		

SUMMARY AND EXPLANATION

Measles (Rubeola) is a highly contagious viral disease of childhood characterized by a clinically distinct prodrome of fever, coryza, cough, conjunctivitis and enanthem followed by a generalized maculopapular eruption.¹ The most frequent complication of Measles involves infections of the lower respiratory tract.² A serious complication of Measles is postinfectious encephalitis. A late complication of Measles infection is subacute sclerosing panencephalitis (SSPE), occurring 4 to 17 years after the patient has recovered from Measles.² Measles in pregnancy can have a harmful effect on the fetus, but the effect is less striking and less specific than that seen in Rubella.³ Measles has proven to be a serious problem in the immunosuppressed patient in that it produces a life-threatening illness.⁴ Susceptible persons intimately exposed to a Measles patient have a 99% chance of acquiring the disease.² Measles can occur throughout the year, but epidemics occur primarily in the late winter and early spring.¹

Antibody to Measles Virus appears within 1 to 2 days after onset of rash and peaks 7 days to 2 weeks later. IgM antibodies appear first and persist 3 to 6 weeks. IgG antibodies appear later, peak in 2 to 6 weeks, and gradually decline to low, but persistent levels for life. Infection with Measles Virus confers lifelong immunity to symptomatic reinfection.^{5,6,7} Subclinical reinfection can occur with either vaccine-induced or natural immunity. During such reinfection a boost in titer of IgG antibody occurs, but IgM antibody does not reappear.⁵

Bion MEASLES-G KITS AND REAGENTS

KITS and KIT COMPONENTS

CODE NO.

MEASLES-G (Measles IgG Antibody) 120 Test Kit	MEG-120
Measles Substrate Slide, twelve wells	ME-6012
Measles IgG Positive Control Serum, 0.5 ml	MEG-6020
Measles Negative Control Serum, 0.5 ml	MEN-6010
Conjugate, IgG with Counterstain, 3.5 ml	CCG-9972
Mounting Medium, 3.5 ml	MM-9985
PBS Packet, One Liter	PBS-9990

INTENDED USE

The Bion MEASLES-G ANTIBODY TEST SYSTEM is an indirect fluorescent antibody assay for the qualitative and/or semi-quantitative determination of Measles (Rubeola) IgG antibodies in human serum. The Bion MEASLES-G ANTIBODY TEST SYSTEM is intended for use as an aid in the diagnosis of primary infection and as a determination of immune status.

Antibody to Measles Virus can be detected 11 to 14 days following administration of live, attenuated Measles vaccine.⁵ Atypical Measles can occur in children previously vaccinated with killed Measles Virus vaccines when they become infected with wild Measles. The rash produced is different from that of typical Measles, and may be confused with Rocky Mountain Spotted Fever.²

Serology can be used both to diagnose Measles, and to ascertain the immune status of previously vaccinated individuals. Even though traditional serologic diagnosis of Measles requires the identification of a significant rise in antibody titer between acute and convalescent phase sera, a diagnosis can also be made by the demonstration of the presence of IgM antibody in a single specimen. However, correct interpretation of serologic data depends upon the proper timing of the specimen collection in relation to the onset of the patient's skin rash. Timing is especially important for interpreting negative IgM results.⁷ In addition, Measles serology can be useful in the identification of SSPE in that elevated IgM antibodies to Measles are found in serum and cerebrospinal fluid of SSPE patients.⁵

Methods for Measles antibody detection include Enzyme Linked Immunosorbent Assays (ELISA) and Immunofluorescence Assays (IFA). The IFA and ELISA tests are sensitive and commercially available as well as having the ability to measure separate IgG and IgM antibody classes for both the determination of immune status and diagnosis of acute infection.^{1,2,8}

PRINCIPLE OF THE IFA PROCEDURE

The Bion MEASLES-G ANTIBODY TEST SYSTEM utilizes the indirect fluorescent antibody assay method first described by Weller and Coons⁹ and further developed by Riggs, et al.¹⁰ The procedure is carried out in two basic reaction steps:

Step 1 - Human serum is reacted with the 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 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

MEASLES VIRUS ANTIGEN SUBSTRATE SLIDES

Ten individually foil-wrapped twelve well slides with a mixture of Measles (CDC Strain) infected and uninfected HEp-2 culture cells fixed onto each well. Each well contains an average of 10-50% infected cells per 200X field. Stable in sealed foil pouch at 8°C, or lower, until labeled expiration date.

POSITIVE CONTROL SERUM

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

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, an endpoint titer is printed on the positive control vial. Due to variations within each laboratory (fluorescent microscope, 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

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

The control is intended to be used undiluted as provided. The staining should exhibit less than 1+ fluorescence.

MOUNTING MEDIUM

One dropper vial containing 3.5 ml phosphate buffered glycerol of pH 7.4 \pm 0.2. Stable at 2-8°C until labeled expiration date.

FLUORESCENT ANTIBODY CONJUGATE

Two ready to use dropper vials, each containing 3.5 ml fluorescein isothiocyanate labeled goat antihuman IgG (heavy chain specific) with 0.01% Evans Blue counterstain, protein stabilizer, less than 0.1% sodium azide and 0.001% thimerosal added. Stable at 2-8°C away from direct light until labeled expiration date.

PHOSPHATE BUFFERED SALINE (PBS)

Two one-liter packets 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.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use. Thus, only staff trained in methods of *in vitro* diagnostics may perform the test.
2. Substrate slides are for single use only and must not be used more than once.
3. Care should be taken when handling substrate slides due to sharp edges.
4. The antigenic substrates have been fixed in acetone and contain no detectable live Measles Virus. However, they should be handled and disposed of as any potentially biohazardous laboratory material.
5. Do not remove slides from pouches until ready for testing. Do not use if pouch has been punctured, as indicated by a flat pouch.
6. 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. Refrigeration (2-8°C) of kit immediately upon arrival will insure stability until labeled expiration date.
9. Reagents should not be used beyond stated expiration date.
10. Substitution of components other than those provided may yield inconsistent results.
11. Do not expose conjugate to strong light during storage or use.
12. Care should be taken to avoid splashing or generation of aerosols.
13. Avoid microbial contamination of all reagents involved in the testing procedure or incorrect results may occur.
14. Incubation times or temperatures other than those specified may give erroneous results.
15. Reusable glassware must be washed and thoroughly rinsed free of detergents.
16. 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.
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.

WARNINGS AND PRECAUTIONS (continued)

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 to 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. The preservatives used in conjugates 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.

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

MATERIALS PROVIDED

1. Measles Virus Antigen Substrate Slides
2. Fluorescent Antibody Conjugate
3. Positive Control Serum
4. Negative Control Serum
5. Phosphate Buffered Saline (PBS)
6. Mounting Medium

MATERIALS REQUIRED BUT NOT PROVIDED

1. One liter volumetric flask or one liter graduated cylinder
2. Distilled water - CAP Type one or equivalent
3. One-liter screw capped container
4. Disposable test tubes (12 x 75 mm or comparable) and rack
5. Disposable serological pipettes
6. Calibrated pipettes to deliver 50 µl, 100 µl and 200 µl, with disposable pipette tips
7. Pasteur pipettes and bulbs
8. Moist chambers
9. Plastic squeeze wash bottle
10. Coplin jars or staining dishes with slide racks
11. 24 x 60 mm #1 coverslips
12. Felt tip marking pen
13. Fluorescence microscope equipped with a 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 and/or 250X. The excitation wavelength of FITC is 490nm and the emission wavelength is 520nm.

TEST PROCEDURE

1. SPECIMEN PREPARATION

Screening:

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.

Semi-quantitation:

Serum dilutions are utilized to measure antibody titer. Each laboratory should establish its own titrating protocol; however, the following fourfold serial titration suggested:

- 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. This will give a fourfold titration with the following dilutions:
 - Tube #1 = 1:10
 - Tube #2 = 1:40
 - Tube #3 = 1:160
 - Tube #4 = 1:640
 - Tube #5 = 1:2560

TEST PROCEDURE (continued)

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 5 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.

NOTE: 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.

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 conjugate to each well of each slide, making sure 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.

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 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 as 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 MEASLES NEGATIVE CONTROL SERUM as provided with the Bion MEASLES-G ANTIBODY TEST SYSTEM, the infected cells should exhibit less than 1+ fluorescence and appear reddish-orange due to the counterstain.

Positive Control

Using the Bion MEASLES POSITIVE IgG CONTROL SERUM as provided with the Bion MEASLES-G ANTIBODY TEST SYSTEM, Measles infected cells should exhibit well defined specific fluorescent staining at an intensity of 3+, or greater. The Measles Virus fluorescent staining pattern consists of cytoplasmic granules with the infected cells sometimes coalescing to form multinucleated giant cells. Approximately 0-50% of cells should exhibit this specific staining pattern with the uninfected cells staining reddish-orange due to the counterstain.

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 each lot of Bion MEASLES POSITIVE IgG CONTROL SERUM must be determined. There must not be more than a twofold difference (+/-) in titer from the stated endpoint. Each run should include the endpoint dilution, one fourfold dilution above and one 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 negative for Measles IgG antibodies if the cells exhibit less than 1+ fluorescence and appear reddish-orange due to the counterstain, or if the fluorescence observed is not the specific staining pattern of Measles Virus.

A sample is considered negative for Measles IgG antibodies if it exhibits less than 1+ fluorescence at a serum dilution of 1:10 and all greater dilutions, or if the fluorescence observed is not the specific staining pattern of Measles Virus.

- ... Negative samples may exhibit fluorescent staining of the infected 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 viral infected cells should be interpreted as negative and attention should be directed to specific steps in the staining method (e.g., RINSE and WASH steps).

POSITIVE

A serum dilution is considered positive for Measles IgG antibodies if, at an intensity of 1+ or greater, there is well defined specific fluorescent staining in the Measles Virus infected cells. The Measles Virus fluorescent staining pattern consists of cytoplasmic granules in cells that sometimes coalesce to form multinucleated giant cells. This pattern is exhibited in 10-50% of the cells with the remaining uninfected cells staining reddish-orange due to the counterstain. The number of cells 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 Measles IgG antibodies if it exhibits the characteristic Measles Virus staining pattern with a fluorescent intensity of 1+ or greater at a serum dilution of 1:10 or greater.

NOTE: 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.^{12,13} 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 may 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

RESULT	INTERPRETATION OF SINGLE SAMPLE RESULTS
Less than 10	Negative - Indicates no previous infection with Measles Virus and susceptibility to this agent. <u>NOTE:</u> This may represent a primary infection with the humoral immune response not yet developed to detectable levels. If infection with Measles Virus is still suspected, a second specimen should be obtained 7-14 days later, and the paired specimens tested simultaneously, looking for a seroconversion.
10 or Greater	Positive for Measles Virus antibody. This may represent: 1. a primary infection; 2. a past experience with Measles Virus and usually indicates immunity; 3. a passively acquired antibody from recent blood transfusions, organ transplantation, or transplacental transfer.

NOTE: The titer of a single specimen should not be the only criteria used to aid in the diagnosis of Measles infections. Paired samples (acute and convalescent) must be collected and tested simultaneously in the same assay to look for a seroconversion or a significant rise in titer. When only a single specimen is available, testing for IgM specific Measles Virus antibodies may help to confirm a diagnosis of active Measles Virus infection.

ACUTE RESULT	CONVALESCENT RESULT	INTERPRETATION OF PAIRED SAMPLE RESULTS
Less than 10	Less than 10	Not likely to be an acute Measles Virus infection. <u>NOTE:</u> This may represent a primary infection if time of obtaining the second specimen is too soon after the first. If this condition is suspected, obtain a third specimen 7-14 days after the second specimen and run the three simultaneously, looking for a seroconversion.
Less than 10	10 or Greater	Most likely a primary infection with Measles Virus unless the individual has recently acquired passive antibody.
10 or Greater	10 or Greater but with less than a fourfold difference in titer from the acute specimen	Not significant evidence of current infection. Most likely a previous experience with Measles Virus. This may represent: 1. a primary infection, but the interval between the first and second specimens may not have been long enough for development of a fourfold rise in antibody titer. If this condition is suspected, obtain a third specimen 7-14 days after the second specimen and run the three simultaneously looking for a significant rise in antibody titer; 2. a primary infection, if first specimen is obtained late after onset and antibodies have already reached a plateau; 3. a passively acquired antibody from transplacental transfer, blood transfusion, etc. <u>NOTE:</u> Testing for IgM specific Measles Virus antibodies may help to confirm a diagnosis of active Measles Virus infection when there is less than a fourfold difference in titer between the acute and convalescent specimens.
10 or Greater	10 or Greater with a fourfold or more difference in titer from the acute specimen	Usually indicates an active or recently active Measles Virus infection.

LIMITATIONS OF THE PROCEDURE

1. Measles Virus IgG 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 to Measles should not be used as the only criterion for diagnosis. Paired serum samples (acute and convalescent) and testing for IgM specific Measles 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 an acute phase specimen was obtained too late.

5. Positive test results from cord blood or neonates should be interpreted with caution. The presence of IgG antibody 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.¹⁴

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 Measles IFA test. They can be differentiated from Measles staining in that ANAs stain the nuclei in all cells; whereas, Measles antibodies exhibit staining only in the 10-50% infected cells.¹²

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.¹³ They can be differentiated from the specific antigen staining in that AMA will stain the cytoplasm of all cells; whereas, Measles antibodies exhibit staining only in the 10-50% infected cells.

10. Endpoint reactions may vary between laboratories due to differences in type or condition of fluorescence microscope employed, diluting apparatus, as well as the experience level of personnel performing the assay.

11. 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.

12. 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.

SPECIFIC LIMITATIONS OF THE MEASLES ASSAY

Measles antibody titers in body fluids may be increased in certain conditions, which have not been correlated with persistent Measles Virus infection.¹⁵

1. Increasing antibody titers against Measles Virus and certain other viruses, e.g., Rubella, have been associated with chronic active hepatitis, systemic lupus erythematosus and infectious mononucleosis.¹⁶

2. Increased Measles antibody titers have been found in serum and cerebrospinal fluid samples from patients with multiple sclerosis.¹⁷ Titers of antibodies against other enveloped viruses have also been found to be increased in a considerable portion of patients with multiple sclerosis. The significance is not known.

EXPECTED VALUES

The prevalence and age incidence of Measles are related to population density, economic and environmental factors, and to the usage of Measles vaccine.¹

In the absence of Measles vaccine, 50% of children have antibody by the age of 5, and 90-95% have antibody by the age of 15. Among younger children with Measles, the peak incidence occurs in infants under 2 years of age. With the introduction of the Measles vaccine, the age-specific incidence has shifted upwards as the overall incidence of Measles has declined.⁵

A study was performed on fifty normal blood donors from the Midwestern United States using the BION Measles IFA procedure to determine the normal adult IgG antibody titer to Measles. In this study, the titers ranged from 1:10 (12%) to 1:640 (2%) with the mean titer being 1:40 (56%).¹⁸

At a private suburban hospital in the Midwestern United States, a total of 7,319 Measles IgG tests were performed using the BION Measles IFA procedure between April, 1989 through May, 1990. Eighty-eight percent or 6,418 were positive and twelve percent or 901 were negative.¹⁹ The now recommended schedule of routinely administering second doses before entering high school has lowered the percent of negative results from vaccine failures.

SPECIFIC PERFORMANCE CHARACTERISTICS

Relative specificity and sensitivity evaluations of the Bion MEASLES-G ANTIBODY TEST SYSTEM were conducted on a broad range of patient serum samples. These serum specimens were tested in comparison with the results from an independent, certified Reference Laboratory which also utilizes the Indirect Immunofluorescent Test to detect antibodies to the Measles Virus.

As summarized in TABLE 1, of the one hundred serum specimens compared qualitatively, seventy-five were positive and twenty-five were negative with both systems. There was complete agreement between the two systems with no sera vacillating between a positive or negative result.¹⁹

TABLE 1 - SUMMARY OF RELATIVE SPECIFICITY TESTING

		BION Kit		Relative Sensitivity	Relative Specificity
		Positive	Negative	100%	100%
Reference Laboratory	Positive	75	0	75/75	
	Negative	0	25		25/25

SPECIFIC PERFORMANCE CHARACTERISTICS (continued)

As summarized in TABLE 2, twelve serum specimens ranging in titer from less than 1:10 to 1:1280 were compared semi-quantitatively. Nine specimens were positive and three specimens were negative with both systems with no more than a twofold (+/-) difference in titer between the two systems. The two systems were essentially equivalent although the results indicate a slight increase in relative sensitivity with the BION System. This is most likely due to a difference in reading criteria with the Reference Laboratory using a fluorescent intensity of 2+ for the endpoint titer and BION using a 1+ cutoff.¹⁹

TABLE 2 - SUMMARY OF RELATIVE SENSITIVITY TESTING

Spec.#	BION	Reference Lab	Spec.#	BION	Reference Lab
1	<10	<10	7	160	80
2	<10	<10	8	640	160
3	<10	<10	9	320	160
4	40	10	10	640	320
5	40	20	11	640	640
6	80	40	12	1280	640

Interlot and intralot precision of the Bion MEASLES-G ANTIBODY TEST SYSTEM was evaluated by testing ten serum specimens (2 negative and 8 positive over a range of titers) using five different lots of kits as well as testing them three times on the same kit lot. There was no more than a twofold difference (+/-) in titer between any of the comparison testings, which is within the confidence limits of this methodology. None of the sera vacillated between a positive or negative result. Summaries of this data are presented in TABLES 3 and 4.¹⁹

TABLE 3 - SUMMARY OF INTERLOT PRECISION

Spec.#	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5
1	<10	<10	<10	<10	<10
2	<10	<10	<10	<10	<10
3	<10	<10	<10	<10	<10
4	40	20/40	40	20	40
5	40	40	40	40	40
6	80	80	80	40	80
7	160	80/160	160	80	160
8	320	160	160	160	160
9	640	640	640	320	640
10	1280	640/1280	1280	640	1280

TABLE 4 - SUMMARY OF INTRALOT PRECISION

Spec.#	1st Test	2nd Test	3rd Test
1	<10	<10	<10
2	<10	<10	<10
3	<10	<10	<10
4	40	40	20/40
5	40	40	40
6	80	80	40/80
7	160	160	160
8	320	160	160
9	640	640	640
10	1280	1280	640/1280

The Immunofluorescent Antibody Assay (IFA) is more sensitive than the traditional Complement Fixation (CF) test in the detection of antibody to Measles Virus. In a study of thirty-five serum samples, there was 80% overall agreement between the two procedures with seven samples positive by IFA and negative by CF. The IFA titers were approximately 8-16 fold higher than those of the CF assay indicating the increased sensitivity of the IFA procedure. This additional sensitivity allows for the determination of immune status.¹⁸ The results are summarized in TABLE 5.

TABLE 5 - SUMMARY OF COMPARISON OF MEASLES ANTIBODY DETECTION BY IFA AND CF

IFA	CF	
	Positive	Negative
	Positive	Negative
	19	7
	0	9

As summarized in TABLE 6, five sets of paired serum specimens (acute and convalescent) were found to have fourfold, or greater, rises in titer to Measles by the CF procedure indicating a serological diagnosis of Measles infection. IFA results to Measles for these paired specimens were then determined (TABLE 6). IFA tests also confirmed a Measles diagnosis with fourfold, or greater, rises in titer. However, the acute specimens were already positive by IFA and would suggest the performance of an immunofluorescent IgM test might confirm the diagnosis of acute infection with a single specimen without waiting for a convalescent specimen. This gives immunofluorescence an additional advantage over complement fixation by allowing the separate identification of specific IgG and IgM antibodies.¹⁸

TABLE 6 - COMPARISON OF CF AND IFA MEASLES RESULTS OF PAIRED SPECIMENS

Patient	Specimen	CF	IFA-IgG	Patient	Specimen	CF	IFA-IgG
1	Acute	<2	10	4	Acute	<2	10
	Convalescent	128	>640		Convalescent	128	160
2	Acute	<2	40	5	Acute	<2	10
	Convalescent	1024	>640		Convalescent	128	>640
3	Acute	<2	10		Acute	<2	10
	Convalescent	128	>640		Convalescent	128	>640

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