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MEASLES - M ANTIBODY TEST SYSTEM

CE

NOTE: Changes highlighted

SYMBOL DEFINITIONS

= Consult Directions for Use
= Store Away From Direct Light
= Storage Temperature
= Expiration Date
= Number of Tests
= Amount
= Single Use Only
= Contains human blood or plasma derivatives

IVD = *In Vitro* Diagnostic Reagent
CONTROL + = Positive Control
CONTROL − = Negative Control
TITER = Endpoint Titer
REF = Code Number
LOT = Lot Number
= Contains biological material of animal origin

Bion MEASLES-M KITS AND REAGENTS

KITS and KIT COMPONENTS	CODE NO.
Measles-M (Measles IgM Antibody) 120 Test Kit	MEM-120
Measles Substrate Slide, twelve wells	ME-6012
Measles IgM Positive Control Serum, 0.5 ml	MEM-6030
Measles Negative Control Serum, 0.5 ml	MEN-6010
Conjugate, IgM with Counterstain, 3.5 ml	CCM-9974
Mounting Medium, 3.5 ml	MM-9985
PBS Packet, One Liter	PBS-9990

INTENDED USE

The Bion MEASLES-M ANTIBODY TEST SYSTEM is an indirect fluorescent antibody assay for the qualitative and/or semi-quantitative determination of Measles (Rubeola) IgM antibodies in human serum.The Bion MEASLES-M ANTIBODY TEST SYSTEM is intended for use as an aid in the diagnosis of recent infection with Measles Virus.

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]

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.[1,2,12]

PRINCIPLE OF THE IFA PROCEDURE

The Bion MEASLES-M ANTIBODY TEST SYSTEM utilizes the indirect fluorescent antibody assay method first described by Weller and Coons[8] and further developed by Riggs, et al.[9] The procedure is carried out in two basic reaction steps:

Step 1 - Human serum (IgM fraction) 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 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

MEASLES VIRUS ANTIGEN SUBSTRATE SLIDES

Ten individually foil-wrapped twelve well slides with a mixture of Measles (CDC Strain) infected and uninfected HEp-2 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 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 titered to endpoint. If titered, 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 ± 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 ± 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 IgM (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 ± 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.
5. Abnormal test results may be seen if the antigen substrate slides are allowed to dry during the staining procedure.
7. Refrigeration (2-8°C) of kit immediately upon arrival will insure stability until labeled expiration date.
8. Reagents should not be used beyond stated expiration date.
9. Substitution of components other than those provided may yield inconsistent results.
10. Care should be taken to avoid splashing or generation of aerosols.
11. Avoid microbial contamination of all reagents involved in the testing procedure or incorrect results may occur.
12. Incubation times or temperatures other than those specified may give erroneous results.
13. Reusable glassware must be washed and thoroughly rinsed free of detergents.
14. Do not expose conjugate to strong light during storage or use.
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. 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 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.

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. IgM separation system
7. Calibrated pipettes to deliver 50 µl, 100 µl and 200 µl, with disposable pipette tips
8. Pasteur pipettes and bulbs
9. Moist chambers
10. Plastic squeeze wash bottle
11. Coplin jars or staining dishes with slide racks
12. 24 x 60 mm #1 coverslips
13. Felt tip marking pen
14. 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 or 250X. The excitation wavelength of FITC is 490nm and the emission wavelength is 520nm.

TEST PROCEDURE

The greatest source of error in IgM testing is interference by the presence of pathogen specific IgG. This can occur in one of two ways.

a. False Negative Reactions may occur due to high levels of specific IgG blocking the IgM in the competition for antigenic sites during the first step of the staining reaction. The IgG, being more avid that the IgM, will react with the antigenic sites first, blocking the IgM from participating in the reaction. The IgM will then be washed away during the first wash, leaving nothing for the anti-IgM conjugate to react with in step two of the staining reaction resulting in a false negative reaction in the presence of pathogen specific IgM.

b. False Positive Reactions may occur when Rheumatoid Factors are present along with pathogen specific IgG antibodies. Some Rheumatoid Factors can be immunoglobulin M anti-IgG. When specific IgG reacts with the antigenic sites during the first step of the staining reaction, the Rheumatoid Factor anti-IgG will then react with the bound IgG. Rheumatoid Factor being an antibody of the IgM class will then react with the anti-IgM conjugate in step two of the staining reaction, resulting in a false positive reaction in the absence of pathogen specific IgM.

It is, therefore, strongly recommended that each patient serum specimen be pre-treated to remove any IgG interference by separating the IgM from the IgG using any of the standard methodologies.[10,11,12] Two such methodologies are ion exchange chromatography[13] and IgG immunoprecipitation.[14,15]

TEST PROCEDURE (continued)

1. **SPECIMEN PREPARATION**

   Separate IgM from IgG by treating patient serum to remove IgG as suggested above.

   Screening:
   It is recommended that screening be carried out at a 1:10 and 1:40 dilution.[16]

   a. Prepare a 1:10 dilution of each patient's serum using one of the above treatments.

   b. Prepare a 1:40 dilution by adding 0.1 ml (100 µl) of the 1:10 dilution to 0.3 ml of PBS.

   Semi-quantitation:
   Serum dilutions are utilized to measure antibody titer. Each laboratory should establish its own titering protocol. The following twofold serial titration is suggested:

   a. Prepare a 1:10 dilution of each patient's serum using one of the treatment methodologies mentioned 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. This will give a twofold titration with the following dilutions:

Tube #1 = 1:10	Tube #4 = 1:80
Tube #2 = 1:20	Tube #5 = 1:160
Tube #3 = 1:40	

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 35-37°C for 90 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 35-37°C for 60 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. **COVERSLIP**
    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 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:[17]

    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-M 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 IgM CONTROL SERUM as provided with the Bion MEASLES-M 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 10-50% 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 titered 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 IgM 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 twofold dilution above and one twofold 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 IgM 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 IgM 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 IgM 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 IgM 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.[18,19] 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.

TROUBLESHOOTING

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

INTERPRETATION OF RESULTS

RESULTS	INTERPRETATION OF SINGLE SAMPLE RESULTS
Less than 10	Negative - Indicates no evidence of recent infection with Measles (Rubeola) Virus. NOTE: 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 - Indicative of a recent primary 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. NOTE: 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.

LIMITATIONS OF THE PROCEDURE

1. Measles Virus IgM antibody test results should be used in conjunction with information available from clinical evaluation and other diagnostic information.
2. 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.[10,11,12] Therefore, all sera should be treated by ion exchange chromatography[13] or IgG immunoprecipitation[14,15] before testing to eliminate possible RF interference.
3. High titers of viral 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.[10,11,12] Therefore, all sera should be treated by ion exchange chromatography[13] or IgG immunoprecipitation[14,15] before testing to avoid this possible problem.
4. 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. Also, only a portion of the IgM antibodies can be recovered.[24]
5. 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; or, the specimen may have been collected too late in the disease after the antibody level is no longer detectable.
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 Virus staining in that ANAs stain the nuclei in all cells; whereas, Measles antibodies exhibit staining only in the 10-50% infected cells.[18]

LIMITATIONS OF THE PROCEDURE (continued)

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.[19] They can be differentiated from Measles staining in that AMA will stain the cytoplasm of all cells; whereas, Measles antibodies exhibit staining in only 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, IgG/IgM separation methods, 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.
13. 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.[20]

SPECIFIC LIMITATIONS OF THE MEASLES VIRUS ASSAY

Measles antibody titers in body fluids may be increased in certain conditions, which have not been correlated with persistent Measles Virus infection.[21]

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.[22]
2. Increased Measles antibody titers have been found in serum and cerebrospinal fluid samples from patients with multiple sclerosis.[23] 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]

SPECIFIC PERFORMANCE CHARACTERISTICS

To investigate the relative sensitivity and specificity of the Bion MEASLES-M ANTIBODY TEST SYSTEM, serum specimens were tested in comparison with results from a certified Immunology Reference Laboratory for the presence of IgM antibodies to Measles Virus. As summarized in TABLE 1, of the twenty specimens compared qualitatively, fourteen were positive and six were negative with both systems. There was 100% overall agreement between both systems with no sera vacillating between a positive or negative result.[24]

TABLE 1 - Summary of Relative Comparison Testing

		BION KIT		Relative Sensitivity	Relative Specificity
		Positive	Negative	100%	100%
Reference Laboratory	Positive	14	0	14/14	
	Negative	0	6		6/6

In addition, the twenty serum specimens ranging in titer from less than 1:10 to 1:320 were compared semi-quantitatively with results from the Reference Laboratory to investigate the relative sensitivity of the Bion MEASLES-M ANTIBODY TEST SYSTEM.
As summarized in TABLE 2, all specimen results agreed with no more than a twofold difference (+/-) in titer between both systems. It appears that the BION system is more sensitive with consistently higher titers. However, the Reference Laboratory uses the criteria that a fluorescent reading intensity of 2+ is the endpoint; whereas, BION uses the criteria of 1+ as the endpoint. The following tables represent results from two independent readers. If the readers differed, both results are given.[24]

TABLE 2 - Summary of Relative Sensitivity Testing

Spec.#	BION	Ref.Lab	Spec.#	BION	Ref.Lab
1	40	40	11	40	20
2	80	40	12	80	40
3	80	40	13	20	20
4	80	40	14	40	20
5	80	40	15	<10	<20
6	80/160	80	16	<10	<20
7	160	80	17	<10	<20
8	80	40	18	<10	<20
9	80/160	80	19	<10	<20
10	40	20	20	<10	<20

Interlot and Intralot precision of the MBL- BION MEASLES-M ANTIBODY TEST SYSTEM was evaluated by testing twenty serum specimens (6 negative and 14 positive over a range of titers). Three different manufacturing lots were tested; and, a single lot was tested in triplicate within two runs. In each instance 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 test sera vacillated between a positive or negative result.[24] This data is summarized in TABLES 3 and 4.

TABLE 3 - Summary of Interlot Precision

Spec.#	Lot 1	Lot 2	Lot 3	Spec.#	Lot 1	Lot 2	Lot 3
1	40	40	40	11	20/40	40	40
2	80	80	40	12	80	80	80
3	80	80	80	13	20	20	20
4	80	80	80	14	20/40	40	40
5	80	80	80	15	<10	<10	<10
6	80/160	80/160	80/160	16	<10	<10	<10
7	160/32	320	80/160	17	<10	<10	<10
8	160	80/160	80	18	<10	<10	<10
9	80	160	160	19	<10	<10	<10
10	40	20/40	40	20	<10	<10	<10

TABLE 4 - Summary of Intralot Precision

Spec.#	1st Test	2nd Test	3rd Test	Spec.#	1st Test	2nd Test	3rd Test
1	80	40	40	11	20/40	40	20/40
2	160	80	80	12	80	80	80
3	80	80	80	13	20	20	20
4	80	80	80	14	40	20/40	20/40
5	80	80	80	15	<10	<10	<10
6	160	80/160	80/160	16	<10	<10	<10
7	320	160	160/320	17	<10	<10	<10
8	80/160	80	160	18	<10	<10	<10
9	80/160	80	80	19	<10	<10	<10
10	40/80	40	40	20	<10	<10	<10

SPECIFIC PERFORMANCE CHARACTERISTICS (continued)

In a routine Viral Serology Laboratory, it is not commonly known if ANA, Rheumatoid Factors or any other interfering substances are present; therefore, it is recommended that some kind of treatment to eliminate these interfering substances be done before testing for specific IgM antibodies. As it is important to realize that there may be some limitations in pre-treatments to remove IgG antibody, further studies were done on serum specimens to determine the actual immunoglobulin levels before and after treatment. Nine sera (6 normal and 3 disease state) were pre-treated by the two commonly used methods of immunoprecipitation and ion exchange chromatography. Immunoprecipitation appears to neutralize essentially all of the IgG, none of the IgA and none of the IgM. Whereas, ion exchange chromatography removed only approximately 97% of IgG and all of the IgA. However, it also removed an average of 61% of the IgM from six sera and all of the detectable IgM from three normal sera. It appears from this study, although limited in number, that the immunoprecipitation method is more efficient at eliminating the possibly interfering IgG while leaving the possibly significant IgM available for testing.[16,24]

Several limitations must be kept in mind when interpreting IgM serology tests. This is true even after separation treatment of IgG and IgM eliminates the problems of Rheumatoid Factors and IgG competitive binding. IgM specific antibody responses can occur not only with primary infection but can be attributed to reactivation or reinfection particularly in Herpesvirus infections.[12,25] Also, it is well known that dual infections can occur and have been reported between several members of the Herpesvirus Group.[26,27,28,29] In addition, with some viruses such as CMV and EBV, IgM specific antibodies may continue for many months. Thus, a person may have residual IgM from one infection and become infected with a different agent.[12,25] It is not uncommon for one childhood infection to follow closely after another. Also, an infection with a new agent may cause an anamnestic response in IgM from the recent previous infection.[30] Therefore, requesting a convalescent specimen looking for a change in IgM antibody levels between paired specimens may help clarify the actual current infection from the previous past infection.

A cross-reactivity study was conducted to insure that IgM antibodies to Measles virus would exhibit no specificity for other antigen substrates. Ten serum specimens positive for Measles IgM antibodies were treated to separate IgG and IgM and then tested for IgM antibodies to EBV, HSV1, HSV2, CMV, VZV and Mumps. As summarized in TABLE 5, there did not appear to be any specific pattern of cross-reactions between the IgM antibodies of Measles and the other six antigens. As indicated by *, there were comparatively low titers with EBV and HSV in two specimens which may be of little or questionable significance. In addition, 47 specimens with IgM antibodies to EBV, HSV1, HSV2, CMV, VZ or Mumps were tested on the BION Measles antigen substrate and were found to be less than 1:10, demonstrating that antibodies to these various other viruses exhibit no specificity to the BION Measles antigen substrate.[24]

TABLE 5 - Cross-reactivity of IgM Antibodies in IFA Tests

Spec.#	Measles	EBV	HSV 1	HSV 2	CMV	VZV	Mump	Comment
1	80	<10	<10	<10	<10	<10	<10	
2	80	<10	<10	<10	<10	<10	<10	
3	80	<10	<10	<10	<10	<10	<10	
4	320	10	20	<10	<10	<10	<10	*
5	80	<10	<10	<10	<10	<10	<10	
6	320	<10	<10	<10	<10	<10	<10	
7	80	<10	<10	<10	<10	<10	<10	
8	320	<10	<10	<10	<10	<10	<10	
9	80	<10	10	<10	<10	<10	<10	*
10	80	<10	<10	<10	<10	<10	<10	

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 patients' clinical situations must be emphasized. It is essential to have an awareness or 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.[24]

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