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MEASLES VIRUS
ANTIGEN SUBSTRATE SLIDE

CE

NOTE: Changes highlighted

PRODUCT AVAILABILITY

The following Measles Antigen Substrate Slides are available individually from Bion Enterprises, Ltd. (Bion):

Antigen Substrate Slide	Code No. REF
Measles Virus	ME-6012
Σ Number of Tests	12-Well

INTENDED USE

The Bion MEASLES (RUBEOLA) VIRUS ANTIGEN SUBSTRATE SLIDES may be used as the antigenic substrate in indirect fluorescent antibody assays for the qualitative and/or semi-quantitative determination of Measles (Rubeola) Virus IgG or IgM antibodies in human serum. Bion MEASLES VIRUS ANTIGEN SUBSTRATE SLIDES are intended for use as an aid in the diagnosis of primary infection and as a determination of immunological experience 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 Hemagglutination Inhibition (HI), Complement Fixation (CF), and more recently Enzyme Linked Immunosorbent Assays (ELISA), Radioimmunoassay (RIA), and Immunofluorescence Assays (IFA). Both CF and HI, which are cumbersome to perform, have shortcomings with regard to sensitivity and reliability. The IFA and ELISA tests have the advantages of having greater sensitivity and being 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 VIRUS ANTIGEN SUBSTRATE SLIDES may be utilized in the indirect fluorescent antibody assay method first described by Weller and Coons[9] 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 (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

Bion MEASLES VIRUS ANTIGEN SUBSTRATE SLIDES are individually foil-wrapped twelve well slides with a mixture of Measles Virus (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.

STORAGE AND STABILITY

The Bion MEASLES VIRUS ANTIGEN SUBSTRATE SLIDES are stable in sealed foil pouches at 8°C or lower until labeled expiration date.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnsotic use. Thus, only staff trained in methods of in vitro diagnsotics may perform the test. IVD
2. Substrate slides are for single use only and must not be used more than once.
3. Use with non-Bion reagents could result in erroneous results.
4. Care should be taken when handling substrate slides due to sharp edges.
5. The antigenic substrates have been fixed and contain no detectable live Measles Virus. However, they should be handled and disposed of as any potentially biohazardous laboratory material.
6. Do not remove slides from pouches until ready for testing. Do not use if pouch has been punctured, as indicated by a flat pouch.
7. Antigen substrate slides should be brought to room temperature (20-25°C) prior to use.
8. Abnormal test results may be seen if the antigen substrate slides are allowed to dry during the staining procedure.
9. Refrigeration (2-8°C) of antigen substrate slides immediately upon arrival will insure stability until labeled expiration date.
10. Antigen substrate slides should not be used beyond stated expiration date.
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. Care should be taken to avoid splashing and generation of aerosols.
15. Previously frozen specimens after thawing should be thoroughly mixed prior to testing. It is recommended that sera is 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.

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.[11,12,13]

MATERIALS PROVIDED

Bion MEASLES VIRUS ANTIGEN SUBSTRATE SLIDES.
Lot Number provided on label. LOT

MATERIALS AVAILABLE FROM Bion

1. Fluorescent Antibody Conjugate with 0.01% Evans Blue counterstain
2. Measles Positive Human Control Serum
3. Measles Negative Human Control Serum
4. Phosphate Buffered Saline (PBS)
5. Mounting Medium

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 a mercury or tungsten-halogen light source, a 390-490 nm excitation filter and 515-520 nm barrier filter, and optics to give a total magnification of 200X or 250X. The excitation wavelength of FITC is 490 nm and the emission wavelength is 520 nm.

TEST PROCEDURE

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 is prepared by adding 0.05 ml (50 µl) of 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 chromatography[14] or IgG immunoprecipitation.[15,16]

Semi-quantitation:

Serum dilutions are utilized to measure antibody titer. Each laboratory should establish its own titering 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 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 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. 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 a negative control serum on Bion MEASLES VIRUS ANTIGEN SUBSTRATE SLIDES, the infected cells should exhibit less than 1+ fluorescence and appear reddish-orange due to the counterstain.

Positive Control

Using a positive control serum on Bion MEASLES VIRUS ANTIGEN SUBSTRATE SLIDES, the 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 in cells that sometimes coalesce to form multinucleated giant cells. Approximately 10-50% of the 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 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 negative for Measles Virus 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 Virus 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 Virus antibodies if well defined specific fluorescent staining is observed in the Measles Virus infected cells at an intensity of 1+ or greater. 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 with the positive control.

A sample is considered positive for Measles Virus antibodies if it exhibits the characteristic 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 the 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 a specific 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 Measles Virus antibodies indicates a current or previous infection with Measles. 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.

LIMITATIONS OF THE PROCEDURE

1. Measles Virus 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 Virus should not be used as the only criterion for diagnosis. Paired serum samples (acute and convalescent) and testing for IgM specific Measles Virus 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 Measles Virus IFA test. They can be differentiated from Measles Virus staining in that ANAs stain the nuclei in all cells; whereas, Measles Virus antibodies exhibit staining only in the 10-50% infected cells.[18]
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 the specific antigen staining in that AMA will stain the cytoplasm of all cells; whereas, Measles Virus 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.[20,21]
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, 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.[22] Therefore, all sera should be treated by ion exchange chromatography[14] or IgG immunoprecipitation[15,16] before testing to eliminate possible RF interference.
15. 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.[22] Therefore, all sera should be treated by ion exchange chromatography[14] or IgG immunoprecipitation[15,16] before testing to avoid this possible problem.
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. Also, only a portion of the IgM antibodies can be recovered.[28]
17. 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 should always be interpreted with caution.[23]

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.[24]

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.[25]
2. Increased Measles antibody titers have been found in serum and cerebrospinal fluid samples from patients with multiple sclerosis.[26] 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%).[27]

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.[28] 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

Bion MEASLES VIRUS ANTIGEN SUBSTRATE SLIDES have been evaluated for the presence of specific Measles Virus using commercial monoclonal antisera. A positive reaction was identified with the infected cell cultures when stained with its corresponding antisera. Also, there was no cross-reactivity with other specific viral or chlamydial monoclonal antiserum and the Measles Virus antigen.[28]

Interlot and intralot precision of the Bion MEASLES SUBSTRATE SLIDES were evaluated by testing for both IgG and IgM antibodies. For IgG, 10 serums specimens (2 negative and 8 positive ranging in titer from 1:20 to 1:1280) were tested on five different lots of slides, and three times on the same lot. For IgM, 20 serum specimens (6 negative and 14 positive ranging in titer from 1:20 to 1:160) were tested on three different lots of slides, and three times on the same lot. 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 sera vacillated between a positive or negative result.[28]

The Immunofluorescent Antibody Assay (IFA) is more sensitive than the traditional Complement Fixation (CF) test in the detection of IgG 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 to 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.[27]

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 1).[28] 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.[27]

TABLE 1 - 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				
	Convalescent	128	>640				

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 2, 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.

TABLE 2 - Cross-reactivity of IgM Antibodies in IFA Tests

Spec.#	Measles	EBV	HSV 1	HSV 2	CMV	VZV	Mumps	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	

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, demons trating that antibodies to these various other viruses exhibit no specificity to the BION Measles antigen substrate.[28]

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.[28]

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