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MUMPS VIRUS

ANTIGEN SUBSTRATE SLIDE

CE

NOTE: Changes highlighted

PRODUCT AVAILABILITY

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

Antigen Substrate Slide	Code No. REF
Mumps Virus	MU-8012
Σ Number of Tests	12-Well

INTENDED USE

The Bion MUMPS 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 Mumps Virus IgG or IgM antibodies in human serum. Bion MUMPS 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 Mumps Virus.

SUMMARY AND EXPLANATION

Mumps is an acute, generally self-limiting, contagious disease with moderate fever of short duration. Bilateral or unilateral parotitis is the most common clinical feature. Secondary involvement concerns the testes, ovaries, central nervous system and, more rarely, the pancreas, peripheral nerves, eye, inner ear and other organs.[1]

The incubation period for Mumps Virus ranges between 18 and 21 days. Infections are spread by droplets via the upper respiratory route. Between 25 and 50 percent of all infections are silent. Immunity after infection appears to be lifelong; however, silent reinfections may occur although it is probably an infrequent event. An attenuated live virus vaccine is available which induce lower levels of measurable antibody than natural infection.[1,2]

Only one distinct antigenic type of Mumps Virus is known. Some antigenic cross-reactivity and anamnestic antibody responses exist with other Paramyxoviruses, particularly Parainfluenza Virus type 1, in some serological tests.[1,2,3] However, cross-reacting antibodies do not appear to be a problem when testing for Mumps Virus antibody by immunofluorescence.[1,2,4,22] Paired serum samples should be used for serological analysis, and the initial specimen should be obtained as early as possible after the onset of symptoms. IgG antibodies appear within the first week, reach high titers, and persist as protective antibody for apparent lifelong immunity. A fourfold or greater rise in antibody between paired samples will allow a serological diagnosis of Mumps Virus infection. IgM antibodies appear 2 to 4 days after onset of clinical symptoms, peak at 1 to 2 weeks, persist for about 3 months, and are rarely detectable as long as 6 months after infection. Therefore, single specimens collected within 10 to 15 days after onset are useful for the identification of virus specific immunoglobulin M (IgM).[1,2,5]

Many tests for the determination of antibodies to Mumps Virus have been described. The traditional assays of viral neutralization, hemagglutination inhibition (HI), and complement fixation (CF) all have the drawbacks of either being too cumbersome for routine serological work, or have shortcomings with regard to sensitivity and reliability. Both CF and HI suffer from a relatively low sensitivity, and cross-reacting antibodies to other paramyxoviruses may pose a problem.[1,2] Both immunofluorescence and ELISA tests have the advantages of being sensitive and capable of allowing the separate identification of IgG and IgM viral antibodies for both determination of immune status and diagnosis of acute infection.[1,2]

PRINCIPLE OF THE IFA PROCEDURE

The Bion MUMPS VIRUS ANTIGEN SUBSTRATE SLIDES may be utilized in the indirect fluorescent antibody assay method first described by Weller and Coons[6] and further developed by Riggs, et al.[7] 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 MUMPS VIRUS ANTIGEN SUBSTRATE SLIDES are individually foil-wrapped twelve well slides with a mixture of Mumps 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 MUMPS 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* diagnostic 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 Mumps 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.[8,9,10]

MATERIALS PROVIDED

Bion MUMPS 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. Mumps Virus Positive Human Control Serum
3. Mumps Virus 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[11] or IgG immunoprecipitation.[12,13]

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:[14]

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 MUMPS 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 MUMPS VIRUS ANTIGEN SUBSTRATE SLIDES, the infected cells should exhibit well defined specific fluorescent staining at an intensity of 3+ or greater. The Mumps Virus fluorescent staining pattern consists of fine and coarse cytoplasmic particles in cells that maintain their individuality of size and shape. 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 Mumps 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 Mumps Virus.

A sample is considered negative for Mumps 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 Mumps 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 Mumps Virus antibodies if well defined specific fluorescent staining is observed in the Mumps Virus infected cells at an intensity of 1+ or greater. The Mumps virus fluorescent staining pattern consists of fine and course cytoplasmic particles with the cells maintaining individuality of size and shape. 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 Mumps 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.[15,16] 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 Mumps Virus antibodies indicates a current or previous infection with Mumps. 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. Mumps 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 Mumps Virus should not be used as the only criterion for diagnosis. Paired serum samples (acute and convalescent) and testing for IgM specific Mumps 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 Mumps Virus IFA test. They can be differentiated from Mumps Virus staining in that ANAs stain the nuclei in all cells; whereas, Mumps Virus antibodies exhibit staining only in the 10-50% infected cells.[15]
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.[16] They can be differentiated from the specific antigen staining in that AMA will stain the cytoplasm of all cells; whereas, Mumps 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.[17,18]
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.[19] Therefore, all sera should be treated by ion exchange chromatography[11] or IgG immunoprecipitation[12,13] 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.[19] Therefore, all sera should be treated by ion exchange chromatography[11] or IgG immunoprecipitation[12,13] 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.[22]
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.[21]

SPECIFIC LIMITATIONS OF THE MUMPS VIRUS ASSAY

Mumps and Parainfluenza Viruses share common antigens, and serologic cross-reactions are possible.[3] Therefore, a positive result may not always mean immunity to the virus that is being evaluated.[20] Cases have been reported where individuals with serologic evidence of immunity have subsequently developed Mumps.[21] Cross-reactions might account for these occasional reported instances of reinfection.[3] However, this serologic evidence is based primarily upon complement fixation assay systems; whereas, testing by immunofluorescence does not appear to have this problem.[1,2,4,22]

EXPECTED VALUES

The medical literature indicates that approximately one-third of Mumps Virus infections are subclinical or silent in clinical presentation. Many adults fail to identify the fact that they have had Mumps even though laboratory studies indicate a previous infection.[1,2] Only 5% of the adult households studied lack detectable Mumps antibody although many more reported a negative medical history. Evaluation of a group of medical students revealed the fact that 88% reporting no prior history of Mumps were found to have antibody to the virus.[2]

Introduction of the live, attenuated Mumps vaccine in 1967 resulted in the detection of measurable antibody in more than 90% of the recipients of the vaccine. However, antibody titers are lower than those observed after a natural Mumps infection.[2,3]

SPECIFIC PERFORMANCE CHARACTERISTICS

Bion MUMPS VIRUS ANTIGEN SUBSTRATE SLIDES have been evaluated for the presence of specific Mumps Virus using commercial monoclonal antisera. A positive reaction was identified with the infected cell cultures when stained with its corresponding antisera.[22] Also, there was no cross-reactivity with other specific viral or chlamydial monoclonal antiserum and the Mumps Virus antigen.[22]

Interlot and intralot precision of the Bion MUMPS SUBSTRATE SLIDES were evaluated by testing for both IgG and IgM antibodies. For IgG, 24 serum specimens (8 negative and 16 positive ranging in titer from 1:20 to greater than 1:1024) were tested on three different lots of slides, and three times on the same lot. For IgM, 35 serum specimens (13 negative and 22 positive ranging in titer from 1:20 to 1:640) 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.[22]

The Immunofluorescent Antibody Assay (IFA) is more sensitive than the traditional Complement Fixation (CF) test in the detection of antibody to Mumps Virus, which allows for the determination of immune status. In a study of 32 serum samples, there was 84% overall agreement between the two procedures with five samples positive by IFA and negative by CF indicating the increased sensitivity of the IFA procedure.[23]

IFA has an additional advantage over CF by its capability of allowing for separate identification of specific IgG and IgM antibodies. This allows for the diagnosis of acute infection with a single specimen (IgM), as well as, by a fourfold rise in titer (IgG and IgM) between acute and convalescent specimens. Performing both the IgG and IgM tests provides the physician with a more useful complete picture of the patient's immunological status.

TABLE 1 summarizes the CF and IFA results on several paired serum specimens. A fourfold, or greater, rise in titer by the CF test for all five sets of paired specimens indicate a serological diagnosis of Mumps Virus infection. With the IFA tests, four of the patients with positive IgM results already on their acute specimens could have been diagnosed immediately without waiting for the convalescent specimen. Diagnosis of the fifth patient required testing both acute and convalescent specimens for a significant rise in titer. With the IgG test alone, three patients could be diagnosed with recent Mumps infection based on a fourfold, or greater, rise in titer between paired samples. However, without the accompanying positive IgM results, two patients with equally high IgG titers on both acute and convalescent specimens showed no clear-cut evidence of recent infection. In these cases, testing for IgM specific Mumps antibodies was necessary to confirm the diagnosis.[23]

TABLE 1 - Comparison of CF and IFA Mumps Results of Paired Specimens

Patient	Specimen	Complement Fixation	Immunofluorescence	
			IgG	IgM
1	Acute Convalescent	<2 512	160 >640	- +
2	Acute	<2 32	>640 >640	+ +
3	Convalescent	<2 128	40 >640	+ +
4	Acute Convalescent	<2 256	10 40	+ +
5	Acute Convalescent	<2 256	160 160	+ +

The Mumps Virus is a member of the Paramyxovirus Group. A study was performed to insure that no cross-reactions occurred between antibodies specific for the Parainfluenza members of this group and the Mumps Virus using Bion MUMPS ANTIGEN SUBSTRATE SLIDES. Eleven of the twelve sera tested had antibodies to Parainfluenza types 1,2 and/or 3. There was one specimen negative for all three antibodies. All sera evaluated were negative when tested on the BION Mumps Virus antigen substrate. Therefore, false positive reactions for Mumps Virus antibody will most likely not be obtained due to exposure to the Parainfluenza Viruses.[22] The data summary is presented in TABLE 2.

TABLE 2 - Summary of Paramyxovirus Group Specificity Study

Spec. No.	Mumps	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3
1	<10	<10	4+ at 1:10*	3+ at 1:10*
2	<10	<10	320	160
3	<10	20/40	640	160
4	<10	1280	160	1280/2560
5	<10	160	80	80/160
6	<10	40	<10	160
7	<10	640	640	320
8	<10	640	320/640	160
9	<10	<10	160	80
10	<10	80/160	40	320
11	<10	160	40	160
12	<10	<10	<10	<10

*Quantity not sufficient to titer out to endpoint.

Each laboratory should determine its own performance characteristics using all reagents assembled to perform the IFA test.

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