







MUMPS - G ANTIBODY TEST SYSTEM



NOTE: Changes highlighted

SYMBOL DEFINITIONS

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

SUMMARY AND EXPLANATION

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 induces 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 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.^{1,2,4,14} 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.

Bion MUMPS-G KITS AND REAGENTS

KITS and KIT COMPONENTS

CODE NO.

MUMPS-G (Mumps IgG Antibody) 120 Test Kit	MUG-120
Mumps Substrate Slide, twelve wells	MU-8012
Conjugate, IgG with Counterstain, 3.5 ml	CCG-9972
Mumps IgG Positive Control Serum, 0.5 ml	MUG-8020
Mumps Negative Control Serum, 0.5 ml	MUN-8010
PBS Packet, One Liter	PBS-9990
Mounting Medium, 3.5 ml	MM-9985

CLIA Complexity: High
CDC Analyte Identifier Code: 4007
CDC Test System Identifier Code: 07480

INTENDED USE

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

Even though traditional serologic diagnosis of Mumps requires the identification of a significant (fourfold or greater) rise in IgG 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. Correct interpretation of serologic data depends upon the proper timing of specimen collection in relation to onset of symptoms. Timing is especially important for interpreting negative IgM results. Studies have shown that infection with Mumps Virus results in the detection of IgM antibody within 2 to 4 days following the 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. 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-G ANTIBODY TEST SYSTEM utilizes the indirect fluorescent antibody assay method first described by Weller and Coons⁶ 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 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

MUMPS VIRUS ANTIGEN SUBSTRATE SLIDES

Ten individually foil-wrapped twelve slides with a mixture of Mumps (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 Mumps positive IgG human control serum with protein stabilizer and 0.005% thimerosal. Stable at 2-8°C until labeled expiration date.

When used undiluted as provided, specific fluorescent intensity of 3+ or greater should be seen. Optionally, the positive control can be titrated to endpoint. If titrated, the control should be serially diluted in PBS. When the control has been tested for the endpoint titer by Bion, an endpoint titer is printed on the positive control vial. Due to variations within each laboratory (fluorescent microscope, etc.), each laboratory should establish its own mean titer for each lot of positive control (generally \pm one dilution from stated endpoint).

NEGATIVE CONTROL SERUM

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

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

MOUNTING MEDIUM

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

FLUORESCENT ANTIBODY CONJUGATE

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

PHOSPHATE BUFFERED SALINE (PBS)

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

BUFFER PREPARATION

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

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

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use. Thus, only staff trained in methods of *in vitro* diagnostics may perform the test.
2. Substrate slides are for single use only and must not be used more than once.
3. Care should be taken when handling substrate slides due to sharp edges.
4. The antigenic substrates have been fixed in acetone and contain no detectable live Mumps Virus. However, they should be handled and disposed of as any potentially biohazardous laboratory material.
5. Do not remove slides from pouches until ready for testing. Do not use if pouch has been punctured, as indicated by a flat pouch.
6. All reagents should be brought to room temperature (20-25°C) prior to use.
7. Abnormal test results may be seen if the antigen substrate slides are allowed to dry during the staining procedure.
8. Refrigeration (2-8°C) of kit immediately upon arrival will insure stability until labeled expiration date.
9. Reagents should not be used beyond stated expiration date.
10. Substitution of components other than those provided may yield inconsistent results.
11. 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. Do not expose conjugate to strong light during storage or use.
16. Previously frozen specimens after thawing should be thoroughly mixed prior to testing. It is recommended that sera freeze thawed no more than one time. If repeated testing is required, it is suggested that specimen be aliquoted.
17. Patient samples, as well as all materials coming into contact with them, should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual "Biosafety in Microbiological and Biomedical Laboratories", 1984 Edition. Never pipette by mouth. Avoid contact with skin and mucous membranes.

WARNINGS AND PRECAUTIONS (continued)

16. Sera used to prepare positive and negative controls have been tested by an FDA approved method and found to be negative (were not repeatedly reactive) for the presence of Hepatitis B surface Antigen (HBsAg) and antibodies to Hepatitis C (HepCAb) and HIV 1 & 2. However, because no test method can offer complete assurance of the absence to these or other infectious agents, these reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual "Biosafety in Microbiological and Biomedical Laboratories," 1984 Edition.
17. The preservatives used in conjugates and controls are toxic if ingested. Azides may react with copper or lead plumbing to form explosive metal azides. When disposing, flush drains with water to minimize build-up of azide and metal compounds.

SPECIMEN COLLECTION

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

Avoid using contaminated sera as they may contain proteolytic enzymes which will digest the substrate. It is unnecessary to

heat inactivate serum specimens prior to testing; however, sera that have been heat inactivated may be used.

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

PROCEDURE

MATERIALS PROVIDED

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

MATERIALS REQUIRED BUT NOT PROVIDED

1. One liter volumetric flask or one liter graduated cylinder
2. Distilled water - CAP Type one or equivalent
3. One-liter screw capped container
4. Disposable test tubes (12 x 75 mm or comparable) and rack
5. Disposable serological pipettes
6. Calibrated pipettes to deliver 50 µl, 100 µl and 200 µl, with disposable pipette tips
7. Pasteur pipettes and bulbs
8. Moist chambers
9. Plastic squeeze wash bottle
10. Coplin jars or staining dishes with slide racks
11. 24 x 60 mm #1 coverslips
12. Felt tip marking pen
13. Fluorescence microscope equipped with a mercury or tungsten-halogen light source, a 390-490nm excitation filter and 515-520nm barrier filter, and optics to give a total magnification of 200X or 250X. The excitation wavelength of FITC is 490nm and the emission wavelength is 520nm.

TEST PROCEDURE

1. SPECIMEN PREPARATION

Screening:

Prepare a 1:10 dilution of each patient's serum by adding 0.05 ml (50 µl) of patient's serum to 0.45 ml of PBS.

Semi-quantitation:

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

- a. Prepare a 1:10 dilution of each patient's serum by adding 0.05 ml (50 µl) of patient's serum to 0.45 ml of PBS in tube #1.
- b. Add 0.3 ml PBS to tubes #2, #3, #4 and #5.

- c. Using a 100 µl pipette, transfer 0.1 ml (100 µl) from tube #1 to tube #2. Mix. Using a new tip for each dilution, transfer 0.1 ml (100 µl) from the second tube to the third, from the third tube to the fourth, and from the fourth tube to the fifth, mixing after each transfer. This will give a fourfold titration with the following dilutions:

Tube #1 = 1:10
Tube #2 = 1:40
Tube #3 = 1:160
Tube #4 = 1:640
Tube #5 = 1:2560

TEST PROCEDURE (continued)

2. SLIDE PREPARATION

Remove reagents and as many slides as are required from the refrigerator or freezer and allow to equilibrate to room temperature (20-25°C) for at least 5 minutes. Remove slides from sealed foil pouches being careful not to touch the antigen surface. Identify each slide using a felt tip marking pen.

3. SPECIMEN APPLICATION

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

4. INCUBATION 1

Incubate in a moist chamber at room temperature (20-25°C) for 30 minutes.

NOTE: THE ANTIGEN MUST NOT BE ALLOWED TO DRY DURING ANY OF THE FOLLOWING STEPS. Nonspecific binding may occur if the reagent is allowed to dry on the slide.

5. RINSE 1

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

6. WASH 1

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

7. CONJUGATE APPLICATION

Remove slides from the wash one at a time, shake off excess PBS, dry around outside edges if necessary and return each slide to the moist chamber. Apply one drop of conjugate to each well of each slide, making sure each well is completely covered.

8. INCUBATION 2

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

9. RINSE 2

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

10. WASH 2

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

11. COVERSIP

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

12. READ

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

FLUORESCENT INTENSITY GRADING

Fluorescent intensity may be semi-quantitated by following the guidelines established by the Centers for Disease Control, Atlanta, Georgia:⁸

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

Positive Control

Using the Bion MUMPS POSITIVE IgG CONTROL SERUM as provided with the Bion MUMPS-G ANTIBODY TEST SYSTEM, Mumps 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 titrated control included with each run tests substrate sensitivity, as well as, checks technique, conjugate quality and the microscope optical system. The endpoint titer of each lot of Bion MUMPS POSITIVE IgG CONTROL SERUM must be determined. There must not be more than a twofold difference (+/-) in titer from the stated endpoint. Each run should include the endpoint dilution, one fourfold dilution above and one fourfold dilution below the endpoint dilution. The more concentrated dilution should be positive and the less concentrated dilution negative. If the control does not behave as described, the test results are invalid and the tests should be repeated. If the control again fails to show the proper reaction upon repeat testing, do not report the test results.

READING OF TEST RESULTS

NEGATIVE

A serum dilution is considered negative for Mumps IgG antibodies if the cells exhibit less than 1+ fluorescence and appear reddish-orange due to the counterstain, or if the fluorescence observed is not the specific staining pattern of Mumps Virus.

A sample is considered negative for Mumps IgG antibodies if it exhibits less than 1+ fluorescence at a serum dilution of 1:10 and all greater dilutions, or if the fluorescence observed is not the specific staining pattern of 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 IgG antibodies if, at an intensity of 1+ or greater, there is well defined specific fluorescent staining in the Mumps Virus infected cells. The Mumps Virus fluorescent staining pattern consists of fine and coarse cytoplasmic particles in cells that maintain their individuality of size and shape. 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 Mumps IgG antibodies if it exhibits the characteristic Mumps 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.^{9,10} It is recommended that such samples be diluted beyond the interference for better interpretation. It is possible that autoantibody staining may mask specific staining such that an interpretation cannot be made. Should this occur, test results should be reported as "Unable to interpret due to the presence of interfering antibodies."

TITRATION

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

EXAMPLE OF ENDPOINT EXTRAPOLATION:

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

The extrapolated endpoint is reported as 320.

TROUBLESHOOTING

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

INTERPRETATION OF RESULTS

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

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

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

LIMITATIONS OF THE PROCEDURE

1. Mumps Virus IgG antibody test results should be used in conjunction with information available from clinical evaluation and other diagnostic information.

2. A single serological IgG antibody titer to Mumps should not be used as the only criterion for diagnosis. Paired serum samples (acute and convalescent) and testing for IgM specific Mumps antibodies may provide more meaningful data.

3. A negative test result does not necessarily rule out current or recent infection. The specimen may have been collected too early in the disease before demonstrable antibody is present.

4. Lack of significant rise in titer does not exclude the possibility of recent infection but may indicate an acute phase specimen was obtained too late.

5. Positive test results from cord blood or neonates should be interpreted with caution. The presence of IgG antibody in cord blood is usually the result of passive transfer from mother to the fetus. A negative test, however, may be useful in excluding possible infection.¹¹

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 IFA test. They can be differentiated from Mumps
- staining in that ANAs stain the nuclei in all cells; whereas, Mumps antibodies exhibit staining in only the 10-50% infected cells.⁹

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

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

11. If both the positive and negative control substrate cells are not visible when viewed using the fluorescence microscope, it may be necessary to replace or realign the light source and check the specific filters.

12. Cell culture substrate slides may exhibit nonspecific fluorescence due to contamination of antibodies or PBS rinse-wash solutions with bacteria or fungi. It is very important that personnel reading the staining results have experience in fluorescence microscopy.

13. The CDC and FDA jointly determined that the Bion MUMPS-G ANTIBODY TEST SYSTEM falls in the High Complexity category. Thus laboratories performing this test are subject to specific laboratory standards pertaining to certification by CLIA 1988 regulations.

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.¹² Cases have been reported where individuals with serologic evidence of immunity have subsequently developed mumps.¹³ 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 of cross-reactions.^{1,2,4,14}

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}

A study of 38 normal sera from the Midwestern United States using the Bion MUMPS-G ANTIBODY TEST SYSTEM provided information regarding normal adult IgG antibody titers. The titers ranged from less than 1:10 (5%) to 1:320 (8%). The majority of samples evaluated fell between 1:20 and 1:80 (76%).¹⁴

SPECIFIC PERFORMANCE CHARACTERISTICS

Relative specificity and sensitivity evaluations of the Bion MUMPS-G ANTIBODY TEST SYSTEM were conducted on a broad range of patient serum samples. These serum specimens were tested in comparison with another commercially available Mumps IgG Antibody IFA test system for the presence of IgG antibodies to Mumps Virus.

As summarized in TABLE 1, of the one hundred twenty-one serum specimens compared qualitatively, one hundred two were positive and fifteen were negative with both systems. Of the four specimens which could not be interpreted with either system, three were contaminated digesting off the antigen and one had interfering nonspecific staining. There was complete agreement between the two systems with no sera vacillating between a positive or negative result.¹⁴

TABLE 1 - SUMMARY OF RELATIVE COMPARISON TESTING

		BION KIT		Relative Sensitivity	Relative Specificity
		Positive	Negative		
OTHER KIT	Positive	102	0	102/102	
	Negative	0	15		15/15

Twenty-four serum specimens ranging in titer from less than 1:10 to 10,240 were compared semi-quantitatively. Eight specimens were negative with both systems and twelve specimens were positive with both systems. The endpoint of one strongly positive

specimen was not determined. The two systems were essentially equivalent although the results indicate an increase in relative sensitivity with the BION System. The comparative results are summarized in TABLE 2. The tables represent results from four independent readers. If the readers differed, two results are given.¹⁴

TABLE 2 - SUMMARY OF RELATIVE SENSITIVITY TESTING

Spec.#	BION	Other	Spec.#	BION	Other
1	40	20	19	<10	<10
2	80/160	80	20	<10	<10
3	<10	<10	21	<10	<10
4	160	80	22	≥10,240	≥640
5	80	40	23	1280	320/640
6	640/1280	160/320	24	20	10/20
7	640/1280	160/320	25	20	10/20
8	80	20	26	20	10
9	160	80	27	20	10
10	<10	<10	28	80	20
11	<10	<10	29	<10	<10
12	<10	<10	30	10/20	10

SPECIFIC PERFORMANCE CHARACTERISTICS (continued)

Interlot and intralot precision of the Bion MUMPS-G ANTIBODY TEST SYSTEM was evaluated by testing twenty-four serum specimens (8 negative and 16 positive over a range of titers). Three different manufacturing lots were tested; and, a single lot was tested in triplicate. In each instance there was no more than a twofold difference (+/-) in titer between any of the comparisons, which is within the confidence limits of this methodology. None of the test sera vacillated between a positive or a negative result.¹⁴

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 suggesting an increased sensitivity of the IFA procedure. The results are summarized in TABLE 3.¹⁵

TABLE 3 - Summary of Comparison of Mumps Antibody Detection by IFA and CF

		Immunofluorescence	
		Positive	Negative
Complement Fixation	Positive	23	0
	Negative	5	4

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 4 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.¹⁵

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 antigen in the Bion MUMPS-G ANTIBODY TEST SYSTEM. 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 antigen substrate. Therefore, false positive reactions for Mumps Virus antibody will most likely not be obtained due to exposure to the Parainfluenza Viruses.¹⁴ The data summary is presented in TABLE 5.

TABLE 4 - Comparison of CF and IFA Mumps Results of Paired Specimens

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

TABLE 5 - Summary of Paramyxovirus Group Specificity Study

Spec.#	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.

BIBLIOGRAPHY

- Norby, E., Mumps Virus, in Manual of Clinical Microbiology, 4th Ed., Lennette, E.H., A. Balows, W.J. Hausler, Jr., H.J. Shadomy., (eds), American Society for Microbiology, Washington, D.C., 774-778, 1985.
- Kleiman, M.B., Mumps Virus Infections, in Laboratory Diagnosis of Viral Infections, Lennette, E.H. (Ed.), Marcel Dekker, Inc., New York, 369-383, 1985.
- Hopps, H.E., and P.D. Parkman, Mumps Virus, in Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections, 5th Ed., Lennette, E.H. and N.H. Schmidt (eds), American Public Health Association, Washington, D.C., 633-653, 1979.
- Rossier, E., H.R. Miller, P.H. Phipps, Mumps Virus Antibody, in Rapid Viral Diagnosis by Immunofluorescence, An Atlas and Practical Guide, Univ. of Ottawa Press, Ottawa, 139-140, 1989.
- Ukkonen, P., M.L. Granstrom, J. Rasanen, M.E. Salonen, and K. Penttinen, Mumps Specific Immunoglobulin M and G Antibodies in Natural Mumps Infection as Measured by Enzyme-linked Immunosorbent Assay, J. Med. Virol 8:131-142, 1981.
- Weller, T.H., A.H. Coons, Fluorescent Antibody Studies with Agents of Varicella and Herpes Zoster Propagated In Vitro, Proc. Soc. Exp. Biol. Med., 86:789-794, 1954.
- Riggs, J.L., R.J. Seiwald, J.H. Burckhalter, C.M. Downs, T.G. Metcalf, Isothiocyanate Compounds as Fluorescent Labeling Agents for Immune Serum, Am. J. Pathol., 34:1081-1097, 1958.
- Lyerla, H.C., F.T. Forrester, The Immunofluorescence (IF) Test, in Immunofluorescence Methods in Virology, USDHHS, Georgia, 71-81, 1979.
- Holborow, E.J., D.M. Weir, G.D. Johnson, A Serum Factor in Lupus Erythematosus With Affinity for Tissue Nuclei, Br. Med. J., 11:732-734, 1957.
- Berg, P.A., I. Roitt, D. Doniach, H.M. Cooper, Mitochondrial Antibodies in Primary Biliary Cirrhosis, Immunol., 17:281-293, 1969.
- Chernesky, M.A., C.G. Ray, T.F. Smith, Laboratory Diagnosis of Viral Infections, Cumitech 15, ASM, Washington, D.C., March 1982.
- Black, F.L., and L.L. Berman, Measles and Mumps, in Manual of Clinical Laboratory Immunology, 3rd Ed., Rose, N.R., H. Freidman, and J.H. Fahey, (eds), American Society for Microbiology, Washington, D.C., 532-535, 1986.
- Zaia, J.A., N.N. Oxman, Antibody to Varicella-Zoster Virus Induced Membrane Antigen: Immunofluorescence Assay Using Monodispersed Glutaraldehyde-Fixed Target Cells, J. Inf. Diseases, 136:519-530, 1977.
- Data on file, Bion, Des Plaines, Illinois.
- Personal Communications, Smith, T., Director, Department of Clinical Microbiology, Virology Laboratory, Mayo Clinic.

AESKU.BION
AN AESKU.GROUP COMPANY



Bion Enterprises, Ltd.
455 State Street, Suite 100
Des Plaines, IL 60016 USA
Phone: +1-847-544-5044
Fax: +1-847-544-5051

EC REP

Qarad, Cipalstraat 3,
B-2440 Geel, Belgium