AESKU.BION
AN AESKU.GROUP COMPANY

VARICELLA ZOSTER
ANTIGEN SUBSTRATE SLIDE

CE

NOTE: Changes highlighted

PRODUCT AVAILABILITY

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

Antigen Substrate Slide	Code No.
Varicella Zoster Virus	VZ-7012
Σ Number of Tests	12-Well
	(REF = Code Number)

INTENDED USE

The Bion VARICELLA ZOSTER VIRUS ANTIGEN SUBSTRATE SLIDES may be used as the antigenic substrate in indirect immunoassay systems for the qualitative and/or semi-quantitative determination of Varicella Zoster Virus (VZV) IgG or IgM antibodies in human serum. Bion VARICELLA ZOSTER VIRUS SUBSTRATE SLIDES are intended for use as an aid in the diagnosis of primary infection, reinfection, or reactivation of the latent virus and as a determination of immunological experience with VZV.

SUMMARY AND EXPLANATION

Varicella (chickenpox) and Zoster (shingles) represent different clinical manifestations of infections with the same virus. Varicella occurs most frequently in children and is characterized by fever and a generalized vesicular exanthem. Zoster generally occurs in adults and consists of a painful, circumscribed eruption of vesicular lesions with accompanying inflammation of associated dorsal root or cranial sensory nerve ganglia.[1] Varicella is the primary infection; whereas, Zoster occurs in individuals with partial immunity resulting from a prior Varicella infection.[1] It is generally considered that Zoster results from reactivation of the virus harbored in a latent state. Episodes of Zoster often occur in individuals undergoing trauma, certain types of drug therapy and in patients receiving immunosuppressive therapy.

Several clinical situations exist where a laboratory diagnosis of Varicella Zoster Virus is crucial. The virus may cause severe or fatal disease in individuals on immunosuppressive therapy or who have genetic defects in their immune system. Progressive, generalized Varicella occurs in as many as 30% of children who acquire chickenpox while on cancer chemotherapy, and mortality has ranged from 7-28%.[1] In older immunodeficient patients, there is an increased risk of disseminated Zoster, and mortality rates range from 3-5% in these infections.[2] Providing quick and reliable specific diagnosis of VZV infection in immunosuppressed patients may guide in the administration of Varicella immune globulin or antiviral agents. Determining the immunity status (presence or absence of antibody) in high risk immunocompromised individuals exposed to VZV infection also guides the physician in the management of these cases.[1] In addition, susceptible hospital staff are a risk to these patients and should be identified. A vaccine is now available for susceptible people.

Care must be taken in all test assays to account for heterotopic antibody titer elevations to VZV found in certain patients with Herpes Simplex Virus infection.[1,3] (See Limitations Section of this insert) The detection of IgM antibody response to VZV does not show this heterotypic reactivity sometimes seen in IgG responses. IgM tests may also be useful in some atypical Zoster infections but it has been found to occur in only approximately 50% of cases.[4]

Methods for microbial antibody detection have included complement fixation (CF), viral neutralization, indirect hemagglutination (IHA), indirect fluorescent antibody (IFA), anticomplement immunofluorescent antibody (ACIF),[5] fluorescent antibody to membrane antigen (FAMA)[6], enzyme immunoassay (EIA) and radioimmunoassay (RIA).[7,8] Of these procedures, the CF test is least sensitive and cannot differentiate between IgG and IgM antibody classes. Neutralization tests are technically complex and time consuming and are generally reserved for seroepidemiologic studies. There is a lack of commercially available reagents for the IHA, RIA, and FAMA tests. Immunoassays, such as the IFA, EIA, and ACIF, have the advantage of being sensitive, specific, and reliable. They are also able to differentiate between the various antibody classes. The potential risk of false positive results must be kept in mind when selecting a test for VZV antibody.

PRINCIPLE OF THE IFA PROCEDURE

The Bion VARICELLA ZOSTER 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 VARICELLA ZOSTER VIRUS ANTIGEN SUBSTRATE SLIDES are individually foil-wrapped twelve well slides with VZV (clinical specimen) infected and uninfected human diploid fibroblast (foreskin) cells fixed onto each well. Each well contains an average of 10-50% infected cells per 200X field.

STORAGE AND STABILITY

The Bion VARICELLA ZOSTER 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 VZV. 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 or 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]

MATERIAL PROVIDED

Bion VARICELLA ZOSTER 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. Varicella Zoster Virus Positive Human Control Serum
3. Varicella Zoster 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 the 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 the 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.

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 that 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 the 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 VARICELLA ZOSTER VIRUS SUBSTRATE SLIDES, the infected cells should exhibit less than 1+ fluorescence and appear reddish-orange due to the counterstain.

NOTE: VZV infection of *in vitro* cell cultures can induce Fc-IgG receptors in the cytoplasm of infected cells. IgG antibody from the patient attaches to these Fc receptor sites which then react with the antihuman IgG conjugate and appear as fluorescent perinuclear cytoplasmic inclusions in these cells.[18] This type of staining should be interpreted as negative for VZV antibodies.

Positive Control

Using a positive control serum on Bion VARICELLA ZOSTER VIRUS SUBSTRATE SLIDES, the infected cells should exhibit well defined specific fluorescent staining at an intensity of 3+ or greater. The Varicella Zoster Virus staining pattern is intranuclear inclusions and web-like, membranous cytoplasmic staining. Cells tend to lose individual definition and merge together to form patches of positive staining material. Approximately 10-50% of the cells should exhibit the 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 the 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 to be negative for VZV 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 VZV.

A sample is considered negative for VZV antibodies if the cells exhibit 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 VZV.

... 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).

NOTE: VZV infection of *in vitro* cell cultures can induce Fc-IgG receptors in the cytoplasm of infected cells which inexperienced personnel may interpret falsely as positive readings when doing IgG assays. IgG antibody from the patient attaches to the Fc receptor sites which then react with the antihuman IgG conjugate and appear as fluorescent perinuclear inclusions just outside the nuclear membrane of these cells. This can be differentiated from the specific VZV fluorescent staining of intranuclear inclusions and web-like, membranous cytoplasmic staining.[18] This type of staining should be interpreted as negative for VZV antibodies.

POSITIVE

A serum dilution is considered positive for VZV antibodies if well defined specific staining is observed in the VZV infected cells at an intensity of 1+ or greater. The VZV fluorescent staining pattern is intranuclear inclusions and web-like, membranous cytoplasmic staining with the cells losing individual definition and merging together to form patches of positive staining material. The number of cells exhibiting a positive staining reaction and the type of fluorescent staining should closely approximate that seen with the positive control.

A sample is considered positive for VZV antibodies if it exhibits the characteristic VZV 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.[19,20] 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

Detection of the presence of Varicella Zoster Virus antibodies indicates a current or previous infection with VZV. 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. VZV 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 VZV should not be used as the only criterion for diagnosis. Paired serum samples (acute and convalescent) or testing for IgM specific VZV 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 of 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 VZV IFA test. They can be differentiated from VZV staining in that ANAs stain the nucleus of all cells; whereas, VZV antibodies exhibit staining only in the 10-50% infected cells.[19]
9. Cytoplasmic fluorescence present in the majority of the cells may be due to the presence of antimitochondrial antibodies (AMA) often seen in primary biliary cirrhosis.[20] They can be differentiated from VZV staining in that AMA will stain the cytoplasm of all cells; whereas, VZV will 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 VZV 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.[21] 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.[22]
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.[27] 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. 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.[35]

SPECIFIC LIMITATIONS OF THE VZV ASSAY

1. A negative test result should be interpreted as indeterminate with respect to the determination of lack of prior infection with VZV and immune status. Some studies have been reported which seem to indicate that immunofluorescent antibody tests may not be as sensitive as fluorescent antibody against membrane antigen (FAMA) in VZV infected cells.[2,6] However, comparative evaluations demonstrated both the standard IF test and the anticomplement immunofluorescent (ACIF) test to be comparable to FAMA in specificity and sensitivity, and offered an alternative to FAMA for the detection of antibody to VZV.[5,23]
2. A significant rise in titer should be used to confirm a clinical diagnosis of atypical Varicella or Zoster infection only if a patient is tested concurrently for IgG antibodies specific for Herpes Simplex Virus (HSV) and does not demonstrate a concurrent significant rise in titer to HSV IgG antibody. A high proportion (up to one-third) of individuals with primary HSV infections who have experienced prior VZV infection show a heterotopic antibody response (by the CF test) to the VZV antigen, making a differential diagnosis between VZV and HSV infection difficult in the absence of clear-cut clinical findings.[1,5,23,24,25]
3. A definitive diagnosis for patients demonstrating significant rises in titer for both VZV and HSV must be made by isolation and/or direct identification of virus or viral antigen from a lesion. The virus causing the infection may not always demonstrate the greater rise in titer. Frequently a differential diagnosis can be made on the basis of the fact that the antibody to the infecting viral type is absent or at a very low titer in the acute phase specimen, whereas antibody to the viral heterotype is already present.[1,26]

EXPECTED VALUES

Most cases of chickenpox occur before the age of 10 years. The incidence of subclinical varicella is estimated to be 5% . About 5 to 10% of adults in the United States are susceptible to varicella, but most adults who believe themselves to be susceptible are actually immune. A history of previous clinical varicella is a good indication that the individual is immune to the infection.[33]

Zoster is contagious to others only in that varicella-susceptible individuals may develop chickenpox following exposure to zoster. Zoster is less infectious to others than varicella. Zoster does not show seasonal distribution as does varicella, and it is more likely to occur in older individuals.[33]

Laboratory diagnosis of varicella zoster infection is crucial in certain situations, such as in patients receiving immunosuppressive therapy for cancer, or those who have abnormal cell-mediated immune responses. Progressive generalized varicella occurs in as many as 30% of children who acquire chickenpox while receiving chemotherapy and radiotherapy, with mortality ranges from 7 to 28%.[34]

SPECIFIC PERFORMANCE CHARACTERISTICS

Bion VARICELLA ZOSTER VIRUS ANTIGEN SUBSTRATE SLIDES have been evaluated for the presence of specific Varicella Zoster Virus using commercial monoclonal antisera. A positive reaction was identified with the infected cell cultures when stained with its corresponding antisera.[35] Also, there was no cross-reactivity with other specific viral or chlamydial monoclonal antisera and the Varicella Zoster Virus Antigen.[35]

As Varicella Zoster Virus (VZV) is a member of the Herpesvirus Group, a study was performed to insure that no cross-reactions occurred between antibodies specific for the other members of this group and the VZV antigen on the BION VZV Substrate Slides. Ten serum specimens with IgG antibodies to Cytomegalovirus, Herpes Simplex Virus (Types 1 and 2) or Epstein-Barr Virus were tested using the Bion VARICELLA ZOSTER VIRUS ANTIGEN SUBSTRATE SLIDES. All ten specimens were negative on the BION VZV Substrate. Thus there was no apparent cross-reactivity as the BION VZV antigen exhibited no specificity for antibodies to other members of the Herpesvirus Group using the IFA test system.[35] Summary of this data is presented in TABLE 1.

In addition, a cross-reactivity study of IgM tests done by IFA was conducted to insure that IgM antibodies to one virus would exhibit no specificity for other antigen substrates. A total of 40 serum specimens were treated to separate IgG and IgM and then tested for IgM. Ten specimens had IgM antibodies to EBV; ten specimens had IgM antibodies to HSV; ten specimens had IgM antibodies to CMV, and ten specimens had IgM antibodies to VZV. These 40 specimens were then tested against all five antigen substrates.

As shown in TABLE 2, there did not appear to be any specific pattern of cross-reactions between antibodies of any one virus against the other four antigens except between HSV types 1 and 2. There was some other multiple positive results. Some with comparatively low titers (*) may be of little or questionable significance while others had higher titers (**) which may be of importance.[35]

TABLE 1 -Summary of Herpesvirus Group Specificity Study

Spec. No.	VZV	CMV	EBV	HSV1	HSV2
1	<10	640	80	<10	<10
2	<10	160	<10	<10	<10
3	<10	<10	<10	80	40
4	<10	<10	<10	80	20
5	<10	40	<10	<10	80
6	<10	20	<10	<10	<10
7	<10	10	<10	40	40
8	<10	20	160	<10	<10
9	<10	<10	<10	80	20
10	<10	160	<10	<10	<10

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

No. of Spec.	EBV	HSV1	HSV2	CMV	VZV
9 1	160-12-80 1280	<10 <10	<10 <10	<10 10*	<10 <10
10	<10	80-320	20-160	<10	<10
9 1	<10 80**	<10 <10	<10 <10	10-160 640	<10 <10
7 1 1 1	<10 >80** <10 20*	<10 20* 20* <10	<10 20* 20* <10	<10 <10 <10 <10	40-320 640 80 80

Several limitations must be kept in mind when interpreting IgM serology tests. This is true even after separation treatment of IgG and IgM eliminates the problems of Rheumatoid Factors and competitive IgG binding. IgM specific antibody responses can occur not only with primary infection but can be attributed to reactivation or reinfection particularly in CMV, HSV, EBV and VZV infections.[22,27] It is also well known that dual infections can occur and have been reported between several members of the Herpesvirus Group.[28,29,30,31] In addition, with some viruses such as CMV and EBV, IgM specific antibodies may continue for many months. Thus, a person may have residual IgM from one infection and become infected with a different agent.[22,27] It is not uncommon for one childhood infection to follow closely after another. Also, an infection with a new agent may cause an anamnestic response in IgM from the recent previous infection.[32] Therefore, requesting a convalescent specimen looking for a change in antibody levels between paired specimens may help to clarify the actual current infection from the previous past infection.

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 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 must always be interpreted with caution.[35]

BIBLIOGRAPHY

1. Schmidt, N.J., Varicella-Zoster Virus, in: Manual of Clinical Microbiology, Fourth Ed., Lennette, E.H., A. Balows, W.J. Hausler, Jr., and H.J. Shadomy, ASM, Washington, D.C., 720-727, 1985.
2. Zaia, J.A., Clinical spectrum of varicella-zoster virus infections, in: The human herpesviruses: an inter-disciplinary perspective, Nahmias, A.J., W.R. Dowdle, and R.F. Schinazi (eds), Elsevier-North Holland, Inc., New York, 10-19, 1981.
3. Schmidt, N.J. Further evidence for common antigens in herpes simplex and varicella-zoster virus, J. Med. Virol 9:27-36, 1982.
4. Schmidt, N.J. and A.M. Arvin, Sensitivity of different assay systems for immunological M responses to varicella-zoster virus in reactivated infections (zoster). J. Clin. Microbiol. 23:978-979. 1986.
5. Gallo, D., and N.J. Schmidt, Comparison of Anticomplement Immunofluorescent and Fluorescent Antibody-to-Membrane Antigen Tests for Determination of Immunity Status to Varicella-Zoster Virus and for Serodifferentiation of Varicella-Zoster and Herpes Simplex Virus Infections, J. Clin. Microbiol. 14:539-543, 1981.
6. Williams, V., A.A. Gershon, and P.A. Brunell, Serological response to varicella-zoster membrane antigens measured by indirect immunofluorescence, J. Infect. Dis. 130:669-672, 1974.
7. Gershon, A.A., Varicella Zoster Infections in Laboratory Diagnosis of Viral Infections, Lennette, E.H. (ed), Dekker, New York, 329-340, 1985.
8. Waner, J.L., and T.H. Weller, Varicella-Zoster Virus, in: Diagnostic Procedures For Viral, Rickettsial And Chlamydial Infections, Sixth Ed., Schmidt, N.J. and R.W. Emmons (Eds.), America Public Health Association, Inc., Washington, D.C., 379-406, 1989.
9. 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.
10. Riggs, J.L., R.J. Siewald, 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.
11. Goldman, M., Fluorescent Antibody Methods. New York, Academic Press, 1968.
12. Kawamura, A. Jr., Fluorescent Antibody Techniques and Their Application. Baltimore, University Park Press, 1969.
13. Nairn, R.C., Fluorescent Protein Tracing, Baltimore, Williams and Wilkins, 3rd Ed., 1969.
14. Johnson, R.B., and R. Libby, Separation of Immunoglobulin M (IgM) Essentially Free of IgG From Serum in Systems Requiring Assay of IgM-Type Antibodies Without Interference From Rheumatoid Factor. J. Clin. Micro. 12:451-454, 1980.
15. Gispen, R., J. Nagel, B. Brand-Saathof, S. DeGraff, Immunofluorescence Test for IgM Rubella Antibodies in Whole Serum After Absorption with Specific Anti-gamma Fc. Clin. Exp. Immunol., 22:431-437, 1975.
16. Joassin, L., M. Reginster, Elimination of Nonspecific Cytomegalovirus Immunoglobulin M Activities in the Enzyme-Linked Immunosorbent Assay by Using Antihuman Immunoglobulin G., J. Clin. Microbiol. 23:576-581, 1986.
17. Lyerla, H.C., F.T. Forrester, The Immunofluorescence (IF) Test, in: Immunofluorescence Methods in Virology, USDHHS, Georgia, 71-81, 1979.
18. Westmoreland, D., J.F. Watkins, The IgG Receptor Induced by Herpes Simplex Virus: Studies Using Radioiodinated IgG, J. Gen. Virol., 24:167-178, 1974.
19. 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.
20. Berg, P.A., I. Roitt, D. Doniach, H.M. Cooper, Mitochondrial Antibodies in Primary Biliary Cirrhosis, Immunol. 17:281-293, 1969.
21. Chernesky, M.A., C.G. Ray, T.F. Smith, Laboratory Diagnosis of Viral Infections, Cumitech 15, ASM, Washington, D.C., March 1982.
22. Schmidt, N.J., Update on Class-specific Viral Antibody Assays, Clinical Immunology Newsletter, 5 (6) :81-85, June, 1984.
23. Preissner, C.M., S.P. Steinberg, A.A. Gershon, and T.F. Smith, Evaluation of the Anticomplement Immunofluorescence Test for Detection of Antibody to VZV, J. Clin. Microbiol. 16:373-376, 1982.
24. Rawls, W.E., Herpes Simplex Virus Types 1 and 2 and Herpesvirus Simiae., in: Diagnostic Procedures For Viral, Rickettsial And Chlamydial Infections, Fifth Ed., Lennette, E.H., and N.J. Schmidt (eds), American Public Health Association, Inc., Washington, D.C., 337-338, 1979.
25. Cremer, N.E., V.L. Devlin, J.L. Riggs, and S.J. Hagens, Anomalous antibody responses in viral infection: Specific stimulation or polyclonal activation? J. Clin. Microbiol. 20:468-472, 1984.
26. Weller, T.H., Varicella and Herpes Zoster, in: Diagnostic Procedures for Virol, Rickettsial and Chlamydial Infections, fifth ed., Lennette, E.H., and N.J. Schmidt (eds), American Public Health Association, Inc., Washington, D.C., 379-398, 1979.
27. Gardner, P.S., J. McQuillin, Rapid Virus Diagnosis: Application of Immunofluorescence, In: Detection of Virus Specific IgM by Immunofluorescence, Butterworth, Boston, 259-287, 1980.
28. Landry, M.L. and G.D. Hsiung, Diagnosis of Dual Herpes Virus Infection: Varicella Zoster and Herpes Simplex Virus in: The Human Herpesviruses, A.J. Nahmias, W.R. Dowdle, and R.F. Chinazi, (eds), Elseiver, New York: 652-653, 1981.
29. Joncas, J., et.al., Dual Congenital Infection with the Epstein-Barr Viruses (EBV) and the Cytomegalovirus (CMV) in: The Human Herpesviruses, A.J. Nahmias, W.R. Dowdle, and R.F. Schinazi, (eds), Elseiver, New York: 614, 1981.
30. Forsgren, M. and A. Demissie, IgM Responses to EBV/CMV in Cytomegalovirus and Epstein-Barr Infections in: The Human Herpesviruses, A.J. Nahmias, W.R. Dowdle, and R.F. Schinazi, (eds), Elseiver, New York: 662, 1981.
31. Ho, Monto, Cytomegalovirus, Biology and Infection, Plenum Medical Book Co., New York: 44, 133 and 169, 1982.
32. Rossier, E., H.R. Miller, P.H. Phipps, Rapid Viral Diagnosis by Immunofluorescence, An Atlas and Practical Guide, Univ. of Ottawa Press, Ottawa, 127-137, 1989.
33. Gershon, A.A., et.al., Varicella-Zoster Virus in: Laboratory Diagnosis of Viral Infections, Lennette, E.H. (ed), Marcel Dekker, Inc., New York, 751,1992.
34. Gershon, A.A., et.al., VZV in: Manual of Clin. Micro., Murray, P.R. (ed), ASM Press, 895, 1995.
35. Data on file, Bion, Des Plaines, Illinois.

AESKU.BION
AN AESKU.GROUP COMPANY

MANUFACTURER

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