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See external label 2°C-8°C Σ=10 x 10 Cat # 421010-G

Varicella-Zoster Virus IgG IFA Kit

Cat # 421010-G

PRINCIPLE OF THE ASSAY

The Diagnostic Automation Inc. indirect fluorescent VZV antibody (IFA) test system is designed to detect circulating IgG VZV antibodies in human sera. The system employs VZV infected human fibroblast (derived from primary cultures of human foreskin) substrate cells and fluorescein isothiocyanate (FITC) labeled antihuman IgG antibody adjusted for optimum reactivity and free of non-specific background staining. The reaction occurs in two steps:

1. The first step is the interaction of VZV antibodies in patient sera with the VZV infected substrate cells.
2. The second is the interaction of FITC labeled anti-human IgG antibody with the VZV IgG antibodies attached to the VZV localized in the nucleus and/or cytoplasm of the infected substrate cells.

SPECIMEN COLLECTION

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease.
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay (24,25). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.

4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2° and 8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

EQUIPMENT AND MATERIALS

Materials required but not provided

1. Small serological, Pasteur, capillary, or automatic pipettes.
2. Small test tubes, 13 x 100mm or comparable.
3. Test tube racks.
4. Staining dish: A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing slides between incubation steps.
5. Cover slip, 24 x 60mm, thickness No. 1.
6. Distilled water.
7. Properly equipped fluorescence microscope assembly.

The following filter systems or their equivalent have been found to be satisfactory for routine use with transmitted or incident light dark-field assemblies:

TRANSMITTED LIGHT		
Light Source: Mercury vapor 200W or 50W		
Excitation Filter	**Barrier Filter**	**Red Suppression Filter**
KP490	K510 or K530	BG38
BG12	K510 or K530	BG38
FITC	K520	BG38
Light Source: Tungsten – Halogen 100W		
KP490	K510 or K530	BG38

INCIDENT LIGHT			
Light Source: Mercury Vapor 200, 100, 50 W			
Excitation Filter	**Dichroic Mirror**	**Barrier Filter**	**Red Suppression Filter**
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38
Light Source: Tungsten – Halogen 50 and 100 W			
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38

MATERIALS PROVIDED

Reactive Reagents

1. VZV (Ellen strain) antigen slides: Ten, 10-well substrate slides containing VZV infected human fibroblast cells in each well, and 70 to 90% uninfected cells as an internal control.
2. FITC-labeled goat anti-human IgG antibody: Contains 1.25% bovine albumin and counterstain. Two, 1.5mL vials (lyophilized).
3. VZV human positive control serum: One, 0.5mL vial composed of human sera (lyophilized).
4. VZV human negative control serum: One, 0.5mL vial composed of human sera (lyophilized).
5. DILUTION BUFFER Sample Diluent formulated to reduce non-specific staining. Contains 0.1% sodium azide as a preservative

Non-reactive Reagents

1. Phosphate-buffered-saline (PBS): Sufficient to prepare 4 liters.

2. Buffered Glycerol (mounting media): 3.0mL.
3. Absorbent blotters for drying the slide mask after washing procedures.

Note: These reactive reagents contain a preservative: (thimerosal, mercury derivative 0.04%).

STORAGE CONDITIONS

1. VZV Substrate Slides: Store at -20ºC or lower. Once thawed, the slides should be used immediately.
2. Goat anti-human IgG labeled with FITC: Store at 2-8ºC. Stable for 90 days after reconstitution. Frozen aliquots are stable for 6 months at -20ºC or lower.
3. Positive and Negative human VZV control sera: Store at 2-8ºC. Stable for 90 days after reconstitution. Frozen aliquots are stable for 6 months at -20ºC or lower.
4. DILUTION BUFFER: Store at 2-8ºC.
5. Phosphate-buffered-saline (PBS): Store packets at room temperature. Store reconstituted buffer at 2-8ºC.
6. Buffered glycerol (mounting media): Store at 2-8ºC.

Note:

1. All kit components are stable until the expiration date printed on the label provided the recommended storage conditions are strictly followed.
2. Do not freeze and thaw reagents or patient samples more than once. Repeated freezing and thawing destroys antibody activity. Do not store in self-defrosting freezers.

QUALITY CONTROL

1. Negative Control: No fluorescent staining in the negative control represents a negative reaction.
2. Positive Control: The positive control is standardized to yield a 3+ to 4+ apple-green granular fluorescence in the nucleus and cytoplasm of the infected cells. Between 10% and 30% of the cells in any one field are infected. The remaining cells in the same field (70-90%) are uninfected and serve as a “within-field” negative control.
3. Invalid Test: Unless the above reactions are noted as described (positive and negative controls), the subsequent patient results are invalid and should not be reported.
4. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

PROCEDURE – STEPWISE

Preparation of Reagents

1. Phosphate-buffered-saline (PBS): Empty contents of one buffer packet into one liter of distilled water. Mix until all salts are thoroughly dissolved.
2. VZV Human Positive and Negative Control Sera: Reconstitute with 0.5mL of distilled water. Represents a 1:10 screening dilution. Use as reconstituted. Do not dilute.
3. FITC-labeled Conjugate: Reconstitute with 1.5mL distilled water. Alternatively, aliquot in 0.5mL amounts and store in small tubes at -20ºC or lower if the total volume of conjugate is not to be used within 90 days.

Note:

1. The controls are intended to be used undiluted. As an option, users may titrate the positive control(s) to endpoint. In such cases, the control(s) should be diluted two-fold in PBS. When evaluated by DAI an endpoint dilution is established and printed on the positive control vial (± one

dilution). It should be noted that due to variations within the laboratory (equipment, etc.), each laboratory should establish its own mean titer for each lot of controls.

2. Reconstitute reagents gently but thoroughly. Reagents should be free of particulate matter. If reagents become cloudy, bacterial contamination should be suspected and thus, should be discarded.

TEST PROCEDURE

1. Remove substrate slides from the freezer and allow them to warm to room temperature (20-25ºC). Tear open the protective envelope and remove slides containing the VZV infected cells. Do NOT apply pressure to flat sides of protective envelope. Remove the other components from their appropriate storage and allow them to equilibrate to room temperature before proceeding with the test (approximately 10 minutes).
2. Prepare a 1:10 screening dilution of patient sample using 20µL of sample and 180µL of PBS. (Alternatively, you may prepare the 1:10 screening dilution in dilution buffer). Positive and negative controls should be run each time the test is performed. The controls, as rehydrated, represent the “working” dilution. Do NOT prepare serial dilutions for endpoint titers in
3. Identify each well with the appropriate patient sera or controls.
4. Spread 20µL of test or control sera over each appropriately labeled well. Be careful not to disturb the substrate cells with the pipette tip.
5. Incubate slides in a sealed, moist chamber at room temperature (20-25ºC) for 30 minutes. Do NOT allow wells to dry.
6. Take slides from the moist chamber and remove excess sera from the wells by gently rinsing slides with a stream of PBS. The multi-well slide mask requires care in directing a gentle stream of buffer away from the adjacent “row” of wells to avoid cross-contamination of samples during the first phase of the test. Do NOT direct the stream of PBS into the test wells.
7. Place slides in a Coplin jar (or similar dish) and wash in PBS for two, 5-minute intervals with a change of PBS. Gentle agitation several times during the wash will ensure proper washing technique.
8. Remove slides one-at-a-time from the PBS solution. Dry mask area with blotters provided being careful not to disturb substrate in wells. Note: DO NOT ALLOW SUBSTRATE WELLS TO DRY.
9. Place slides in a moist chamber and add 20µL of conjugate to each well.
10. Incubate slides for 30 minutes at room temperature (20-25ºC). Do NOT allow slides to dry.
11. Repeat steps 6, 7, and 8.
12. Add 3-4 drops of buffered glycerol to the mask area of each slide and coverslip. Avoid entrapment of air bubbles. Slides should be examined immediately, or within four hours at a total magnification of 250X to 400X. Slides held for any period of time prior to reading should be stored in a cool, dark environment. Care must be taken not to allow slides to dry prior to examination of the test wells. This may be accomplished by sealing the coverslips with clear nail polish.

CALCULATIONS/REPORTING RESULTS

INTERPRETATION

1. Negative Patients: The absence of characteristic staining in the infected cells represents a negative reaction. This reaction should compare favorably with the reaction observed in the negative control.
2. Positive Patients: The number of infected cells observed with positive patient sera should closely approximate the number of infected cells seen in the positive control well. The reactivity, depending on patient antibody concentration, may vary from 1+ to 4+ apple-green granular fluorescence in both the cytoplasm and/or nucleus of the infected cells.
3. Nonspecific Reactions: Nuclear staining alone cannot be considered positive as it is not typical for the VZ immune antibody staining pattern. If all the cells (infected and uninfected) in a test field

fluoresce apple-green either in the nucleus and/or cytoplasm, an autoimmune or other antibody (i.e., Anti-HLA) reaction should be considered. Test results can still be reported only if one is able to discern characteristic VZV reactivity from the non-specific fluorescence.

Note: All positive test sera should be titered to endpoint. Serial two-fold dilutions should be prepared in PBS starting with a 1:20 (repeating the 1:10 dilution is optional) in volumes of at least 100µL. DO NOT PREPARE SERIAL DILUTIONS FOR ENDPOINT TITERS IN DILUTION BUFFER. The endpoint is the last dilution that produces positive apple-green staining (1+) in the infected cells. If, for any reason, the sample does not titer to endpoint, test results should be reported as indeterminant.

ANALYSIS OF TITERS	
SERUM TITER	**SIGNIFICANCE**
LESS THAN 1:10	No fluorescence in the substrate cells. No detectable antibody to VZ by IFA test. (See Limitations No. 5, and 6.
EQUAL TO OR GREATER THAN 1:10 (> 1+ Staining)	Positive characteristic fluorescence in varicella-zoster infected cells only (see Interpretation of Results). This indicates prior varicella infection. Patient is presumed to be immune to varicella (but not zoster). See Limitation No. 2).
FOUR-FOLD OR GREATER RISE IN TITER	A four-fold rise or greater between acute and convalescent specimens is highly suggestive of a current infection with VZV unless a concurrent HSV rise in titer is noted. If this occurs, the infection may be caused by either virus. (See Limitation No. 1).

PROCEDURE NOTES

1. For in vitro diagnostic use.
2. The thimerosal and sodium azide preservatives may be toxic if ingested.
3. DILUTION BUFFER contains sodium azide as a preservative. Sodium azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse thoroughly with water after disposing of solution containing sodium azide.
4. Remove only the amount of DILUTION BUFFER needed to perform each test run to reduce the possibility of product contamination.
5. Use DILUTION BUFFER for screening dilutions only. DO NOT PREPARE SERIAL DILUTIONS FOR ENDPOINT TITERS IN DILUTION BUFFER.
6. DILUTION BUFFER should be used only as a diluent for patient specimens.
7. DO NOT use DILUTION BUFFER to reconstitute the controls or conjugate.
8. DO NOT use DILUTION BUFFER in any of the wash steps.
9. The volume of DILUTION BUFFER supplied has been calculated to provide sufficient material for all the individual test wells included in this kit when used according to the instructions herein. The use of larger volumes for sample preparation will result in insufficient DILUTION BUFFER to allow each test well to be utilized.
10. NO U.S. STANDARD OF POTENCY.
11. The human serum controls are POTENTIALLY BIOHAZARDOUS MATERIALS. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg. and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual “Biosafety in

Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens (26).

12. Substitution of other reagents for components of this kit are to be avoided. Since the components of this kit have been tested for maximum efficiency, DAI is not responsible for test performance if reagent substitution occurs.
13. The DAI indirect immunofluorescent test uses a substrate of human fibroblast cells infected with VZV. The infected substrates are fixed and exposed to ultra-violet radiation. The fixed substrate does not contain live virus; however, as with all other viral reagents employed in any laboratory, these substrates should be considered as potentially hazardous material and should be disposed of according to local/federal regulations.
14. Kit components should not be used beyond the expiration date stated on the label.
15. All components should equilibrate to room temperature (20-25ºC) prior to use (approximately 10 minutes).
16. Microbial contamination of any of the test samples or components should be avoided due to interference with the immunofluorescence reactions.
17. The water used for reconstituting the reagents should be properly stored to prevent contamination.
18. The FITC-labeled conjugate should be stored in the dark due to acknowledged light-sensitivity.
19. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.

LIMITATIONS OF THE PROCEDURE

1. A four-fold or greater rise in antibody titer should be used to confirm a clinical diagnosis of a typical varicella or zoster infection only if a patient is tested concurrently for Herpes simplex. Heterotypic antibody responses have been reported for both of these viruses (15, 18, 19, 21). For immune status testing, this heterotypic reaction would not be classified as false-positive since previous exposure is assumed for this to occur.
2. A definitive diagnosis for patients demonstrating rises in titer for both varicella-zoster and Herpes simplex viruses must be made by isolation and/or direct identification of the 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 type is absent, or at a very low titer in the acute phase specimen; whereas antibody to the viral heterotype is already present (21).
3. Persons who have received blood products containing plasma within the past six months who present a positive test result may not have had prior varicella involvement.
4. IFA test results with the Zeus VZV immunoglobulin G should be used in conjunction with information available from the clinical evaluation as well as other diagnostic procedures.
5. The endpoint reactions may vary due to the type of microscope employed, the light source, age of bulb, filter assembly, and filter thickness.
6. If testing of a particular sample occurs early (less than five days following onset) during a primary infection, no detectable IgG may be evident. If VZ infection is suspected, a second sample should be taken at least fourteen days later and the two specimens should be tested in parallel to look for seroconversion.
7. The correlation of negative test results determined by this method (IFA) as they relate to protection from varicella infection has not yet been definitively established (22,23).

REFERENCES

1. Weller TH, Witton HM, Bell EJ. Exp. Med. 108:843, 1958
2. Weller TH, Coons AH: Proc. Soc. Exp. Biol. Med. 86:789, 1954
3. Weller TH: Viral Infections of Human: Epidemiology and Control. 2nd ed. NY: Pelnum 569-95,

1982
4. Kimura A, et al: Arch virus 36: 1, 1972
5. Esiri M, Tomlinson AH: J. Neurol. Sci. 15:25, 1972
6. Oakes JE, Iltis JP Hyman RW, et al: Virology, 82:353, 1977
7. Richards JC, Human RW, Rapp F: J. Virol. 32:812, 1979
8. Fleisher G, Henry W, McSorley M, Arbeter A, Plotkin S: Am. J. Dis. Child. 135:869-9, 1981.
9. Preblud SR: Pediatrics 68:14-7, 1981.
10. Ojeda VJ, et al: ASCP 529-532 (Vol 81, No.4), April 1984.
11. The Harvard Medical School Health Center, Vol IX. No.8 “Shingles”. June, 1984.
12. Leclair JM, Zaia JA, Levin MJ: N. Engl. J. Med. 302:450, 1980.
13. Tyzzer EE: J. Med. Res. 14:361, 1960.
14. Stevens DA, Merigan TC: J. Infect. Dis. 509, 1975.
15. Rawls WE: Chaptr 11, Viral, Rickettsial & Chlamydial Infections. Eds: Lennette EH, Schmidt NJ, 5th ed: 337-338, 1979.
16. Scott TFM: Epidemiology of Herpetic Infections. Am. J. Ophthal. 43:134-147, 1957.
17. Benyesh-Meinick: Manual of Clinical Microbiology. Eds: Lennette EH, *et al.* ASM:762-772, 1974.
18. Gallo D, Schmidt NJ: J. Clin. Microbiol. 14:539-543, 1981.
19. Preissner CM, Steinberg SP, Gershon AA, and Smith TF: J. Clin. Microbiol. 14:539-543, 1981.
20. Zeus Scientific, Inc., 1983. Available upon request.
21. Schmidt NJ: Varicella-Zoster Virus, Chap. 77, Man. Clin. Microbiol. 3rd ed. Eds: Lennette EH, Balows A, Hausler WJ, Truant JP: ASM:798-806, 1980.
22. Zala JA, Oxman MN: J. Inf. Dis. 136:519-530, 1977.
23. Williams V, Gershon A, Brunell PA: Serologic response to Varicella-Zoster membrane antigens measured by indirect immunofluorescence. J. Inf. Dis. 130:669-672, 1974.
24. Procedures for the collection of diagnostic blood specimens by venipuncture - Second Edition; Approved Standard (1984). Published by National Committee for Clinical Laboratory Standards.
25. Procedures for the Handling and Processing of Blood Specimens. NCCLS Document H18-A, Vol. 10, No. 12, Approved Guideline, 1990.
26. U.S. Department of Labor, Occupational Safety and Health Administration: Occupational Exposure to Bloodborne Pathogens, Final Rule. Fed. Register 56:64175-64182, 1991.

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