ZosterGent™
Varicella-Zoster Virus (VZV) Identification Reagent

INSTRUCTIONS FOR USE

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CONTENTS

1 INTENDED USE

The ZosterGent™ Varicella-Zoster Virus (VZV) Identification Reagent is intended as an aid for the diagnosis of VZV infection. This reagent can be used for the direct detection of VZV in vesicle smears by the direct fluorescent antibody technique and for the confirmation of the presence of VZV in cell culture isolates from clinical specimens.

2 SUMMARY AND EXPLANATION OF TEST

VZV, a member of the human herpesvirus family, causes two distinct clinical manifestations: childhood chickenpox (varicella) and shingles (zoster). Varicella is the outcome of the primary infection with VZV, whereas, zoster is the result of VZV reactivation from latently infected sensory ganglia which occurs predominantly in aging and immunosuppressed individuals. Both varicella and zoster are more common and more severe with underlying malignancies, steroid use, transplant patients and other immunocompromised individuals and patients undergoing cytotoxic therapy or radiation therapy[1,2].

Identification of VZV can be accomplished by cultivation in tissue culture cells followed by the appearance of characteristic cytopathic effects (cpe). Confirmation of VZV isolates in cell culture can be achieved by the immunofluorescent test[3,4]. In addition, diagnosis of VZV can be accomplished by the staining of infected basal

epithelial cells from a vesicle smear by a direct fluorescent assay[5]. Viro Varicella-Zoster Virus Identification Reagent provides a simple, rapid and accurate means for the direct detection and confirmation of VZV from clinical specimens or cell culture isolates.

3 PRINCIPLE OF TEST

The ZosterGent™ Varicella-Zoster Virus (VZV) Identification Reagent is a direct immunofluorescence assay in which a solution containing fluorescein isothiocyanate (FITC) conjugated VZV-specific monoclonal antibodies[6,7] are reacted with cells suspected for VZV infection. The results are visualized by fluorescence microscopy. Positive cells expressing VZV proteins show a strong apple-green cytoplasmic fluorescence as well as cell membrane staining, whereas, negative cells show a reddish brown counterstaining.

4 REAGENT PROVIDED

One vial (3ml) of prediluted FITC-conjugated VZV mix monoclonal antibodies (MAbs) in 20 mM Na2PO4 buffer containing 0.01% carrier protein (BSA), 0.05% sodium azide as preservative and 0.02% Evans blue counterstain. VZV monoclonal antibodies in this reagent consist of whole immunoglobulin molecules and are directed against VZV glycoproteins gE, gB, gH, gI and VZV immediate early protein encoded by VZV gene 62. Due to the presence of Fc portion of VZV

IgG molecules, this reagent may also bind to structural protein A of Staphylococcus aureus or other microorganisms which may be present in specimens tested by this reagent.

5 MATERIALS REQUIRED BUT NOT PROVIDED

The following materials are required for sample preparation and visualization, but not provided with the ZosterGent™ Varicella-Zoster Virus (VZV) Identification Reagent solution.

a) Fluorescence microscope with filter system for fluorescein isothiocyanate (FITC); excitation wavelength = 495nm, emission wavelength = 520nm, magnification = 100 X to 400 X.

b) Humidified incubator (37°C ± 1°C).

c) Viral transport media for the collection of vesicle smears.

d) Tabletop centrifuge or microfuge.

e) Centrifuge or microfuge tubes.

f) Immunofluorescence assay (IFA) slides.

g) Acetone, high performance liquid chromalography (HPLC).

h) Phosphate-buffered saline (PBS) containing 120 mM NaCl, 2.7 mM KC1 and 10 mM Na2HPO4, pH 8,0.

i) Buffered glycerol mounting fluid (20% glycerol in 1 X PBS, pH 7.5 to pH 8.5).

j) Coverslips No.1 thickness.

k) Cell scraper.

l) Deionized autoclaved water.

m) Clean bottle.

n) 3-ml plastic transfer pipettes.

o) Timer.

p) Waste container with a 1:10 dilution of household bleach (0.5% sodium hypochlorite).

6 WARNINGS AND PRECAUTIONS FOR USERS

Please read this manual and follow its instructions carefully.

- For in vitro diagnostic use.
- All patients' specimens including blood and tissue samples should be considered infectious and should be handled and processed at biosafety level II according to the guidelines described in CDC-NIH Manual for Biosafety in Microbiological and Biomedical Laboratories [HHS publication # (CDC) 93-8395].
- **DO NOT** mouth pipette reagents.
- **DO NOT** use the Viro VZV Identification Reagent beyond expiration date.
- **DO NOT** let slides dry during the staining procedure.
- **DO NOT** store the stained slides in strong light.

- **DO NOT** pipette samples or reagent. Avoid contact with skin or mucous membranes.
- **DO NOT** smoke, eat or drink in areas where specimens or reagents are handled.
- Wear disposable gloves while handling samples and wash hands after assay is completed.
- Acetone is extremely flammable and harmful if swallowed or inhaled. Keep away from heat, spark or flame. Avoid breathing vapor. Use adequate ventilation
- Dispose of all materials used to perform the test by autoclaving at 121°C for at least 30 minutes. Liquid waste may be disposed by autoclaving or by mixing with 1:10 dilution of household bleach for a minimum of 60 minutes.
- Avoid splashing or generation of aerosols.
- Sodium Azide (NaN3) may react with lead and copper plumbing to form highly explosive metal azides. To dispose, flush with a large volume of water to prevent azide buildup.
- Avoid microbial contamination of reagents or incorrect results may occur. Cross contamination of samples could cause false results. Incubation time and temperatures other than those specified may give erroneous results.
- Use aseptic technique and sterile pipettes and buffers.

- Use separate pipettes or pipette tips for each sample, control and reagent. Avoid contamination with metal ions.
- In case of contact with this reagent which contains Sodium Azide and Evans Blue, immediately flush eyes or skin with copious amounts of water for at least 15 minutes. Sodium Azide is has the potential for acute toxicity and Evans Blue is a potential cancinogen.

7 STORAGE INSTRUCTIONS

Store Viro ZosterGent™ VZV Identification Reagent at 2°C - 8°C. DO NOT FREEZE.

8 PROCEDURE

8.1 Preparation of Tissue Culture Specimens

a) Scrape the infected cell cultures (exhibiting 25% to 50% CPE) into the tissue culture medium with a sterile cell scraper. In monolayer cultures of fibroblast cells including lung fibroblasts, VZV CPE first appears as small group of several swollen, rounded and refractile cells. The CPE gradually will progress linearly along the long axis of the cells. The areas of monolayer cultures infected with the virus are called foci. The infected cells in foci will eventually come off forming plaques. In monolayer cultures of epithelial cells including amnion or kidney cells, the foci

appear as small plaques with swollen, refractile and rounded cells. Multinucleated giant cells may also develop several days after appearance of CPE. Plaques are also formed in VZV-infected epithelial cells.

b) Transfer the cell suspension into a centrifuge or microfuge tube and spin at 300 X g for 5 minutes at room temperature (20°C - 30°C).

c) Remove and discard the supernatant, resuspend the cell pellet in cold PBS and spin as in step #2.

d) Resuspend the cell pellet in cold PBS with sufficient quantity to establish a light turbidity.

e) Add one drop (50ul) of the cell suspension to each well of an immunofluorescence assay (1FA) slide. Carefully cover only the area within each well (cross-contamination of samples could cause erroneous results).

f) Air dry the slides at room temperature (20°C - 30°C) for a minimum of 30 minutes. Avoid blotting the slides onto paper towels or filter papers since this may result in contamination of samples with fibers which in turn may result in nonspecific binding to Viro VZV Identification Reagent.

g) Fix the slides in cold acetone for 10 minutes and airdry slides.

h) The slides may then be stained within 24 hours or stored desiccated at 2°C to 8°C for one week or at -70°C for up to 6 months.

8.2 Preparation of Smear Specimens

a) Vigorously swab infected lesion to obtain epithelial cells. Avoid contamination of vesicle lesion specimens with blood which may result in nonspecific binding of the reagent with blood cells. In addition, contamination with blood may result in binding of patient's antibody molecules to the virus in vesicle lesions. This complex may react with Viro VZV Identification Reagent and produce false results.

b) Immediately place swabs in viral transport medium, mix well in medium and remove. Remove swab by squeezing against the inside of the transport vial.

c) Centrifuge the cell suspension at 450 x g for 5-10 minutes, remove supernatant and resuspend the cell pellet in 1 to 2 drops of PBS

d) Transfer approximately 20ul of the cell pellet onto one well of an IFA slide. Carefully cover only the area within each well (cross-contamination of samples could cause erroneous results).

e) Air dry the slides at room temperature (20°C - 30°C) for a minimum of 30 minutes.

f) Fix the slides in cold acetone for 10 minutes and airdry.

g) The slides may then be stained within 24 hours or stored desiccated at 2°C to 8°C for one week or at -70°C for up to 6 months.

8.3 Staining

a) Allow the Viro VZV Identification Reagent to warm to room temperature before use (do not dilute).

b) Add sufficient amount of reagent with free-falling drops to cover the smear. Application of excess reagent may result in spreading of solution and contamination of adjacent wells.

c) Place slides in a covered container and incubate at 37°C for 30 minutes in a humidified chamber.

d) Rinse slides briefly under a light stream of PBS from a wash bottle (do not aim the stream of PBS directly into the well).

e) Air dry slides at room temperature.

f) Add one drop of a buffered glycerol mounting fluid to each well. Apply a No. 1 thickness glass coverslip.

g) Examine each slide under a fluorescence microscope with a 495 nm excitation filter, a 520 nm emission filter and with a magnification of 200 X to 400 X. Slides can be stored in the dark at 2°C - 8°C for up to 24 hours.

9 QUALITY CONTROL

ZosterGent™ VZV Identification Reagent should be tested concurrently with known VZV positive and negative cell culture control slides. When confirming a virus isolate, uninfected cells from the same lot as those used to isolate the virus should be utilized as the negative control. For the preparation of positive and negative control cell culture slides, follow the procedure outlined in the package insert under Preparation of Tissue Culture Specimens. Control VZV positive sample (e.g., VZV strain Ellen) can be obtained from American Type Culture Collection (Cat. No: ATCC,VR-1367).

The VZV positive control slides should show cells with characteristic cytoplasmic as well as cell membrane fluorescent staining, whereas, the control negative uninfected cells should display only a reddish-brown counterstain.

When the reagent is utilized for direct detection of VZV in clinical specimens, positive and negative tissue culture cell control slides should be tested alongside patient specimens. This is to verify the performance of both the fluorescent procedure and the microscope. Fluorescent performance patterns of these controls are described above.

10 INTERPRETATION OF RESULTS

Tissue Culture Cells. Tissue culture cells that are positive for VZV will exhibit a bright apple-green fluorescence which are distributed over nucleus, cytoplasm and cell membrane with distinct

fluorescent staining at cellular junctions. Positive fibroblast as well as epithelial cell cultures display bright fluorescent staining at VZV-infected foci. Positive rounded cells show bright fluorescent staining of predominantly outer cell membrane whereas, flat positive cells display fluorescent staining of mainly cytoplasm and cell membrane. These cultures should be reported as "VZV Isolated". Negative tissue culture cells will display a faint reddish-brown background due to Evans blue counterstaining of uninfected cells. These cultures should be reported as "No VZV Isolated". For culture confirmation with 25% CPE, a positive interpretation is made when at least 2 positively cells are observed under high power (400 X) magnification. If less than 2 positive cells are seen with 25% CPE, the sample is considered negative for VZV.

Direct Smears. Positive specimens for VZV antigens exhibit cytoplasmic as well as membrane apple-green fluorescence staining of basal epithelial cells against the red background of the counterstained uninfected basal or squamous cells. At high power field (400 X), the positive cells may appear as individual or cell aggregates. The individual positive cells may display fluorescent staining of both cytoplasm and cell membrane or may show fluorescent staining of outer cell membrane. The cell aggregates may consist of both positive and negative cells in which positive cells show cytoplasmic and cell membrane as well as outer membrane fluorescent staining. Two or more

fluorescent positive cells in the entire smear are considered positive for VZV antigens. Specimens with fewer than one cell per high power field (400 X) and not exhibiting positive fluorescence, should be reported as insufficient cells for testing. Specimens with adequate cells for testing, but not exhibiting specific fluorescence in two or more cells, are considered negative. The results from positive smears should be reported as "Reactive for VZV Antigens" and the results from negative direct smears should be reported as "Negative for VZV Antigens". The detection of VZV antigens in vesicle smears does not indicate the presence of infectious VZV in the specimen. Since the effects of antiviral therapy on VZV viability have not been established, infectious VZV particles may or may not be present in vesicle smears. In addition, since VZV primarily infect basal epithelial cells, it is important to collect these cells during vesicle smear preparation.

11 LIMITATIONS OF THE PROCEDURE

The ZosterGent™ VZV Identification Reagent is highly specific and sensitive for VZV antigens. Failure to detect VZV antigens may be a result of factors such as collection of the specimen at an improper time during the course of the disease, improper sampling and specimen transport conditions or failure of cell culture procedures. Therefore, a negative result does not exclude the possibility of a VZV infection in the patient. Bacterial or fungal contamination may

nonspecifically bind the antibody and interfere with the interpretation. Direct smear may exhibit nonspecific fluorescence due to the presence of bacteria or fungi or from the nonspecific trapping of the reagent. Vesicle smears may also exhibit nonspecific fluorescence due to contamination of specimens with blood cells during sample collection. In addition, performance of this test is dependent upon the properties of microscope including type of microscope and its lenses, age and type of ultraviolet (UV) light source and filter system for fluorescein isothiocyanate (FITC) which may all effect the performance of this test. Furthermore, prolonged exposure of the slide to UV light will result in fading the intensity of fluorescence.

12 EXPECTED VALUES

The incidence of childhood chickenpox (varicella) in the pediatric population prior to chickenpox vaccine immunization in the United States was estimated to be 4 million cases each year (a rate of 1500 / 100,000). According to one study[2], the most common age group for varicella infection are 5-9 years old with over 60% of the cases occurring in this age group. Shingles (zoster) or herpes zoster has an annual incidence of 1.2 million cases and 90% of reported cases occur in people over 45 years of age[1,2]. Of those infected with zoster, 4% will experience multiple attacks. There is little variation in the incidence of varicella or zoster on the basis of race, sex or

socioeconomic status[1,2], however, both varicella and zoster are more severe with underlying malnourishmcnt[1,2].

13 SPECIFIC PERFORMANCE CHARACTERISTICS

Performance characteristics of Viro VZV Identification Reagent for cell culture confirmation, vesicle smears and cross-reactivity were evaluated by two independent clinical laboratories, one on the West Coast and one in the Midwest.

13.1 Specificity

Cell Culture Confirmation. A total of 54 frozen samples were tested. Thirty nine different VZV clinical isolates along with 15 different clinical isolates of herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2) and cytomegalovirus (CMV) were examined by the clinical laboratory on the West Coast using Viro VZV Identification Reagent. All thirty-nine different VZV isolates exhibited positive fluorescence staining, whereas, negative results were obtained with all HSV-1, HSV-2 and CMV isolates. Human fibroblast cells were included in each test as negative control cell cultures. Staining of uninfected human fibroblast cells with Viro VZV Identification Reagent exhibited negative results.

Table 1. VZV Cell Culture Confirmation

	Reference Reagent		
	+	-	Total
Viro Research VZV Reagent +	39	0	39
-	0	15	15
Total	39	15	54

All data were collected retrospectively. A total of 39 frozen VZV clinical isolates were tested. In addition, a total of 15 frozen clinical specimens including five HSV-1, five HSV-2 and five CMV were tested. Relative Sensitivity was 100 % (39 / 39) with a 95 % Confidence Intervals of 91.0 to 100 %. Relative Specificity was 100 % (15 / 15) with a 95 % Confidence Intervals of 78.2 to 100 %. Overall Agreement was 100 % (54 / 54).

Vesicle Smears. A total of 48 vesicle smear specimens were tested. Twenty-eight specimens including 10 frozen and 18 fresh samples were tested from patients with varicella or zoster diagnosed by clinical presentation or by virus isolation. In addition, twenty specimens including 9 frozen and 11 fresh samples from ten patients with HSV-1 and ten patients with HSV-2 infections were tested and found negative when stained with Viro VZV Identification Reagent.

Table 2. Confirmation of VZV Antigens in Vesicle Smears

		Reference Reagent		
		+	-	Total
Viro Research VZV Reagent	+	28	0	28
	-	0	20	20
	Total	28	20	48

All data were collected from 28 patients with varicella or zoster. A total of 10 frozen and 18 fresh specimens were tested. In addition, from 20 patients with HSV infection, a total of 9 frozen and II fresh specimens were tested. Relative Sensitivity was 100 % (28 / 28) with a 95% Confidence Intervals of 87.7 to 100 %. Relative Specificity was 100 % (20 / 20) with a 95% Confidence Intervals of 83.2 to 100 %. Overall Agreement was 100 % (48/48).

Cross-Reactivity

The following viruses were tested and found to be negative by Viro VZV Identification Reagent: herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2). cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus 6 (HHV-6), measles virus, rubella virus, influenza virus types A and B and parainfluenza virus types 1, 2 and 3. In addition, the following cell lines demonstrated no cross-reactivity with Viro VZV Identification Reagent: human foreskin fibroblast cells (HFF), human embryonic lung

fibroblast cells (MRC-5 and WI-38), African green monkey kidney cells (VERO) and human lung carcinoma cells (A549).

Table 3: Cross-Reactivity of Viro VZV Identification Reagent with Other Viruses

Virus Identification	Stain Identification	Cross-Reactivity	No. Tested Samples
Herpes Simplex Virus type 1 (HSV-1)	76-102	-	1
Herpes Simplex Virus type 2 (HSV-2)	82-3912	-	1
Varicella-Zoster Virus (VZV)	90-7217	+	1
Cytomegalovirus (CMV)	AD169	-	1
Epstein-Barr Virus (EBV)	B-95-8	-	1
Human Herpesvirus 6 (HHV-6)	GS	-	1
Measels Virus	Edmonston	-	1
Rubella Virus	RA/27	-	1
Influenza Virus type A	1-14690 (H3N2)	-	1
Influenza Virus type B	Flu B/HK	-	1
Parainfluenza Virus	Par 1 2-12466	-	1
	Par 2 ATCC VR-92	-	1
	Par 3 ATCC VR-93	-	1

Cross-Reactivity was determined by direct flourescent antibody test using virus-infected cells and Viro Identification Reagent

Table 4: Cross-Reactivity of Viro VZV Identification Reagent with Uninfected Culture Cells

Cell Identification	Viro VZV Reagent	No. Tested Samples
Human foreskin fibroblast cells (HFF)	-	1
Human embryonic lung fibroblast cells (MRC-5)	-	1
Human embryonic lung fibroblast cells (WI-38)	-	1
African green monkey kidney cells (VERO)	-	1
Human lung carcinoma cells (A549)	-	1

Cross-Reactivity was determined by direct immunoflourescent test of uninfected cells with Viro VZV Identification Reagent

14 REFERENCES

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15 GLOSSARY OF SYMBOLS

SYMBOL DESCRIPTION

	Caution. Consult the Instructions for Use for important cautionary information such as warnings and precautions.
LOT	Lot code.
	Manufacturer Name and Address with Date of Manufacture.
	Consult Instructions for Use.
	Temperature limit. The temperature limits to which the medical device can be safely exposed.
	Use-by-date.
IVD	In Vitro Medical Device (IVD).
REF	Catalog Number.
	Indicates the total number of IVD tests that can be performed with the IVD.

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