

VZVscan™

FOR THE QUALITATIVE OR QUANTITATIVE DETERMINATION OF VARICELLA-ZOSTER VIRUS ANTIBODIES IN HUMAN SERUM

I. INTENDED USE

The **VZVscan™** (Varicella-zoster Virus) Antibody Card Test is a qualitative and quantitative latex agglutination assay that utilizes polystyrene particles sensitized with partially purified, attenuated varicella-zoster virus (VZV) antigens for the detection of total VZV antibodies in human serum.

II. SUMMARY AND EXPLANATION

Varicella-zoster virus is the etiologic agent of chickenpox (varicella) and shingles (herpes zoster). While varicella is second only to gonorrhea in incidence among reported diseases in the United States, the current role of serological diagnosis is to determine the immune status of susceptible individuals in high risk groups, such as immunocompromised patients, hospital employees, transplant recipients and pregnant women. Additionally, antibody identification can be used to help differentiate the vesiculopapular lesions produced by other diseases, including herpes simplex virus, rickettsial pox and secondary syphilis, among others. With varicella-zoster virus vaccines currently under evaluation, general antibody screening may increase in importance.

Varicella is usually a benign disease of childhood. It is characterized by an incubation period of 10-21 days (average 14 days), 1 to 2 days of viral shedding, followed by generalized eruptions of individual maculopapules rapidly becoming vesicles that may continue to appear for 4 to 5 days. Patients above 20 years of age and, to a lesser extent, those below one year of age are at increased risk of complications. IgG-specific antibodies are detectable within 4 to 5 days after the onset of symptoms, and typically are present throughout life. Therefore, a single serum sample may be assayed as positive or negative for VZV antibody as an indication of previous exposure to the virus. ANTIBODIES ARE NOT 100% PROTECTIVE AND SECONDARY INFECTIONS CAN OCCUR, ALTHOUGH RARELY.¹ (See "Limitations of the Procedure"). However, like other herpes viruses, a permanent reservoir exists in the sensory nerve ganglia and recrudescence of the primary infection may occur in the elderly or otherwise immune suppressed. This is most commonly manifest as herpes zoster (shingles), an extremely painful vesicular eruption usually limited to one or two dermatomes occurring during the lifetime of 10-20% of individuals. In the severely immune compromised, the virus can disseminate, producing a widespread cutaneous syndrome, pneumonitis or encephalitis. While cluster cases of zoster infections suggest that reinfection may occur,² the increased use of immunosuppressive therapies, which disrupt the dormant state of the virus, contributes to the increasing rate of incidence. Post herpetic neuralgia may be severe and debilitating.

In both varicella and zoster states, inapparent infections may be accompanied by a rise in antibody titer.³ In a serological study of children with no history of past disease, antibody to VZV was demonstrated in one-third of the subjects.⁴ Therefore, elicitation of a history of clinical symptoms does not necessarily accompany either a positive screen or a rise in antibody titer.

Therapies available for infection include acyclovir, alpha interferon and zoster immune globulin (ZIG). The use of ZIG as a preventative measure in children at high risk - specifically those with leukemia or lymphoma, immunodeficiency syndromes, or undergoing immunosuppressive therapy - may result in protection, subclinical infection, or occasional severe illness.⁴ It appears that the period between exposure and treatment is critical.⁵

III. PRINCIPLES OF THE PROCEDURE

The **VZVscan™** Card Test follows the established principles of latex agglutination and employs polystyrene latex particles coated with VZV antigens. When this reagent is mixed with human serum that contains antibodies to VZV, a reaction occurs that produces visible agglutination (clumping) on the test card surface. The assay is read macroscopically without the aid of mechanical devices. In the absence of VZV-specific antibodies, no agglutination occurs and a smooth, milky appearance is observed.

The **VZVscan™** Varicella-zoster Antibody Test-Latex Agglutination may be utilized to screen serum samples as positive or negative for varicella-zoster virus antibodies, or serial two-fold dilutions may be performed to obtain a titer of relative antibody level.

IV. REAGENTS

Reagent A,	VZVscan™ Latex Antigen, polystyrene particles sensitized with partially purified, inactivated varicella-zoster antigen, with 0.2% sodium azide and 0.025% gentamicin in a glycine buffer.
Reagent B,	VZVscan™ Card Dilution Buffer, glycine buffer containing 0.1% sodium azide and 0.025% gentamicin.
Control ++,	VZVscan™ High Reactive Control (human serum), containing varicella-zoster virus antibodies, with 0.1% sodium azide.
Control +,	VZVscan™ Low Reactive control (human serum), containing varicella-zoster virus antibodies, with 0.1% sodium azide.
Control -,	VZVscan™ Nonreactive Control (human serum), nonreactive to varicella-zoster virus antibodies, with 0.1% sodium azide.

Precautions: For *in vitro* Diagnostic Use.

After review by the Centers for Disease Control and Prevention (CDC), and the U.S. Food and Drug Administration (FDA) under CLIA '88, this product has been identified as moderate complexity.

Reagents: Do not use beyond the expiration date. Upon removal from refrigeration, allow reagents to warm to room temperature (23 to 29°C) before use. DO NOT mix reagents from different kit lot numbers.

To ensure proper drop delivery when dispensing **VZVscan™ Reagent A**, the dispensing bottle must be held vertically.

Reagent A has been prepared from disrupted vaccine strain virus, which has been judged to be inactivated by bioassay procedures.

The serum controls are derived from human blood tested by an FDA-approved method for the presence of the antibody to HIV (human immunodeficiency virus) and HBsAg (hepatitis B surface antigen) and found to be nonreactive.

WARNING: Because no test method can offer complete assurance that HIV, hepatitis B virus, or other infectious agents are absent, SPECIMENS AND THESE REAGENTS SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING AN INFECTIOUS DISEASE. The Food and Drug Administration recommends such material be handled at a Biosafety Level 2. BSL 2 is referenced in the Centers for Disease Control and Prevention / National Institutes of Health (CDC/NIH) manual, *Biosafety in Microbiological and Biomedical Laboratories*.

Warning: Reagents contain sodium azide. Very toxic by inhalation, in contact with skin, and if swallowed. Contact with acids liberates very toxic gas. After contact with skin, wash immediately with plenty of water. Sodium azides may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Test Cards: Cards must be flat for proper reactions. If necessary, flatten cards by bowing back in a direction opposite to that of the curl. Care should be taken not to finger-mark the test areas, since this may result in an oily deposit and improper test results. Use each card once and discard. Store cards in the original package in a dry area at room temperature.

Reading of Test Results: Prior to reading, a brief hand rotation of the card must be made following the mechanical rotation. Results should be read promptly under a high intensity incandescent lamp holding the card at least six inches from the light source. Fluorescent lighting is generally insufficient to distinguish minimally reactive results. The use of magnification in reading test results is not recommended.

Rotation: The recommended mechanical rotation speed is 100 ± 2 rpm, but rotation between 95 and 110 rpm does not significantly affect the results obtained. The rotator should circumscribe a circle approximately two centimeters in diameter in the horizontal plane. A moistened humidifying cover should be used to prevent drying of test specimens during rotation.

Storage of Reagents: Refrigerate at 2 to 8°C. DO NOT FREEZE. Heating reagents beyond 32°C and freezing temperatures will invalidate the reactivity of the test. Reagents should be recapped and returned to refrigeration when not in use.

V. SPECIMEN COLLECTION AND PREPARATION

Whole blood should be collected by venipuncture, allowed to clot and the serum separated from the cells. The use of plasma has not been established. Heat inactivation of the serum does not adversely affect the performance of the test. Specimens with obvious microbial contamination should not be used; however, lipemia or hemolysis does not affect the performance of the test. The sera may be stored up to 48 h at 2 to 8°C, but should be frozen in a non-self defrosting freezer if longer storage is desired.

When using the **VZVscan™** Card Test in evaluating active infection, the first serum should be obtained as soon as possible after the onset of illness and a convalescent serum obtained 10 to 14 days later. Both acute and convalescent samples should be assayed at the same time.

No special preparation of the patient is required prior to specimen collection.

VI. PROCEDURES

Review "Precautions" and "Specimen Collection and Preparation" prior to performing procedures. The testing area, reagents, test specimens and test components should be at room temperature (23 to 29°C) when used.

Materials Provided:		No. 254126 (30 Tests)	No. 254201 (100 Tests)
Reagent A,	VZVscan™ Latex Antigen,	0.5 mL	1.6 mL
Reagent B,	VZVscan™ Card Dilution Buffer,	5.0 mL	20.0 mL
Control ++,	VZVscan™ High Reactive Control (human serum),	0.5 mL	0.5 mL
Control +,	VZVscan™ Low Reactive Control (human serum),	0.5 mL	0.5 mL
Control -,	VZVscan™ Nonreactive Control (human serum)	0.5 mL	0.5 mL
Test Cards and test disposable and accessories		5	8

Materials Not Provided: Rotator with humidifying cover, micropipettor (25 µL delivery), centrifuge, and high intensity incandescent lamp.

Also required are the necessary equipment and labware used for preparation, storage and handling of serologic specimens.

VII. QUALITATIVE SCREENING PROCEDURE

The following procedure may be used to screen patients' sera as reactive or nonreactive for VZV antibodies, and to test the reactivity of the **VZVscan™ Latex Reagent A** utilizing the accompanying reactive and nonreactive control sera.

1. Dilution of serum samples
 - a. With a micropipettor, place 25 μ L of **Reagent B** onto a test circle.
 - b. With the same micropipettor with a new tip, place 25 μ L of the **Control +**, **Control -**, or the patient serum into **Reagent B** in the appropriate circle and mix by drawing up and down with the micropipettor 7 times. A new tip must be used with each serum sample.
 - c. Withdraw 25 μ L of this mixture and discard. The serum in the circle is now a 1:2 dilution.
 - d. Repeat steps "a" through "c" for each sample being tested.
2. Using a new plastic stirrer for each serum sample, spread the diluted serum to fill the entire circle.
3. Mix **Reagent A** by gently inverting the bottle several times. Holding in a vertical position, dispense 1 free-falling drop onto each test circle containing diluted serum.
4. Hand rotate the card 3 or 4 times back and forth to distribute the latex throughout each circle. Avoid cross contamination of test areas in adjacent circles.
5. Place the card on a rotator and rotate for 10 min under a moistened humidifying cover.
6. Immediately following mechanical rotation, read the card macroscopically in the wet state under a high intensity incandescent lamp. Gently tilt the card (3 or 4 back-and-forth motions) to help differentiate weak agglutination from no agglutination.
7. The Reactive Control should show agglutination, while the Nonreactive Control should show no agglutination.

Report as Positive:

Reactive Showing any agglutination of the **VZVscan™ Latex Antigen (Reagent A)**.

Report as Negative:

Nonreactive Suspension remains evenly dispersed, showing no agglutination of the **VZVscan™ Latex Antigen (Reagent A)**.

To eliminate the possibility of a false negative result caused by prozoning, (See "Limitations of Procedure"), the following protocol using a 1:40 serum dilution should be used:

- a. Using a micropipettor, prepare a 1:40 dilution by placing 190 μ L of **Reagent B** into a test tube (example: 12 x 75 mm).
- b. Using a micropipettor, place 10 μ L of sample serum into the test tube and mix by drawing up and down 7 times.
- c. Using a micropipettor, place 25 μ L of **Reagent B** onto a test circle.
- d. Using a micropipettor, place 25 μ L of the diluted serum into **Reagent B** in the appropriate test circle and mix by drawing the micropipettor up and down 7 times.
- e. Withdraw 25 μ L of this mixture and discard. The serum in the circle is now a 1:40 dilution.
- f. Repeat steps "a" through "e" for each sample being tested.
- g. Follow preceding steps 2 through 6.

VIII. QUANTITATIVE TITER PROCEDURE

The following procedure may be used to quantify the relative **VZVscan™** antibody level of patients' sera and the positive control serum. If the high positive control serum does not meet the labeled titer level, then the test should be repeated.

1. Dilution of serum samples
 - a. With a micropipettor, place 25 μ L of **Reagent B** onto circles 1 - 5 on the test card.
 - b. With a micropipettor, and new tip, place 25 μ L of the **Control ++**, **Control -**, or patient serum into **Reagent B** in circle 1. Mix by drawing up and down the micropipettor 7 times. This produces a 1:2 dilution of the sample. A new tip must be used with each serum sample.
 - c. Using the same micropipettor and tip, transfer 25 μ L of the 1:2 dilution directly into the buffer in circle 2, mix as before, and continue this preparation of serial two-fold dilutions through circle 5. Withdraw 25 μ L from circle 5 and discard. The dilution in circle 5 is now a 1:32 dilution of sample serum.
 - d. Repeat steps "a" through "c" for each sample being tested.

NOTE: Additional two-fold dilutions of each serum may be continued by following steps "a" through "c" in additional test card circles.

2. Using a new plastic stirrer for each serum sample, start at circle 5 and spread the diluted serum to fill the entire circle. Proceed to the next lower dilution circles (4, 3, 2, 1) until each is spread.

3. Mix **Reagent A** by gently inverting the bottle several times. Holding in a vertical position, dispense 1 drop onto each test circle containing diluted serum.
4. Hand rotate the card 3 or 4 times back and forth to distribute the latex throughout each circle. Avoid cross contamination of test areas in adjacent circles.
5. Place the card on a rotator and rotate for 10 min under a moistened humidifying cover.
6. Immediately following mechanical rotation, read the card macroscopically in the wet state under a high intensity incandescent lamp. Gently tilt the card (3 or 4 back-and-forth motions) to help differentiate weak agglutination from no agglutination. Weak agglutination is defined as a rough appearance where aggregated latex is visible. No agglutination is defined as a smooth milky appearance.
7. The antibody titer is the inverse of the ratio of the last positive test circle (e.g., 1:32 becomes a titer of 32).

IX. INTERPRETATION OF TEST RESULTS

Qualitative Test: The single serum specimen assayed is reported as positive for VZV antibody when any agglutination (clumping) is visible without the aid of mechanical devices. In the absence of agglutination in the 1:2 and the 1:40 dilution, the specimen is reported as negative for VZV antibody.

Quantitative Test: Report reactivity in terms of highest dilution showing any agglutination of the **VZVscan™** Latex Reagent. Specimens showing no agglutination at any dilution should be reported as nonreactive. When comparing paired sera collected from 10 to 14 days apart, the two samples should be assayed at the same time. A four-fold rise in the convalescent serum compared to the acute serum or seroconversion from a negative to a positive result is indicative of active or recurrent infection.⁶

Quality Control: The reactive controls are formulated to produce definite agglutination within the labeled dilutions. Do not report control endpoints as "Reactive" unless definite agglutination is observed, assuring that the antigen antibody system is performing properly within the test environment. The nonreactive control should show no agglutination. If controls do not produce appropriate response, the test is invalid. Weak or no agglutination of the 1:2 dilution of the High Reactive Control may be the result of the prozone phenomenon.

At a minimum, the Low Reactive Control and Nonreactive Control should be run each day that the kit is used. Local, regional or other laboratory regulations may apply which supercedes insert directions for testing of positive and/or negative controls.

X. LIMITATIONS OF THE PROCEDURE

Patients with positive titers due to naturally occurring immunity may become reinfected. A reinfection incidence of 6% was observed in one study.¹ Rare patients screened positive at 1:2 are negative at 1:4 dilution. These patients are probably at higher risk for infection with infectious VZV individuals with 1:8 or greater dilution of VZV-specific immunoglobulin in their serum; however, this is not proven. When screening high risk individuals with no recollection of prior infection, performing quantitative titers may provide additional information useful for clinical decision making.

Serum antibody titers are not recommended for the demonstration of acute (active) infection because of the lack of detectable antibody in early infection, the potential prozoning phenomenon at serum dilutions less than 1:40, as well as the lack of antibody response in some immunosuppressed patients. Direct testing for virus or antigen in the lesions is preferable in these cases.

The instance of a prozoning phenomenon in serum containing unusually high VZV antibody levels cannot be ruled out. If confirmation of a nonreactive serum is desired, such as in high-risk patients, the sample should be reassayed using a 1:40 dilution in Specimen Dilution Buffer as described under "Qualitative Screening Procedure".

The test should be run between 23 to 29°C. Temperatures outside this range may cause false positive reactions.

Cards held too close to a high intensity lamp may be exposed to excessive heat. This may cause a false positive reaction due to the drying of the reagents.

The presence of passive immune globulin in sera collected from recipients of varicella zoster immune globulin (VZIG) or blood products received by the patient within the previous 3 to 6 months may result in positive test results. Therefore, prior infection of VZV may not be identifiable.

Test results from cord blood or newborns should be interpreted with caution due to the passive transfer of IgG from the mother.

Primary infections by other herpes viruses can cause a corresponding increase in antibody level from a previous VZV infection.^{7,8}

Seroconversion or a four-fold or greater rise in antibody titer are the classic methods of evaluating patient status regarding recent exposure to the virus. However, test results should be evaluated in conjunction with clinical symptoms, patient history and other laboratory findings to establish a diagnosis.

XI. EXPECTED VALUES AND PERFORMANCE CHARACTERISTICS

Methods used to detect antibodies to VZV in human serum include: FAMA (Fluorescent Antibody to Membrane Antigen), indirect immunofluorescence, immune adherence hemagglutination, neutralization, indirect hemagglutination, complement fixation and enzyme immunoassays.

The FAMA test represents one of the oldest and most reliable antibody detection tests and is considered the standard method.

One hundred eighty serum samples, obtained from normal donors, were assayed to compare results from two commercially available ELISA test kits to the **VZVscan™** Latex Agglutination Test. There was a 97.8% overall qualitative agreement between ELISA test kits and the **VZVscan™** Latex Agglutination Tests.

Sera from 50 adults with no history of varicella were tested for antibody to VZV. The FAMA assay detected 34 positives out of 50 sera (68%), while LA and ELISA detected 36 (72%) and 30 (60%) positives respectively, yielding 92% accuracy for both tests compared to the FAMA Assay.

Absence of Cross-Reactivity with other Herpes Viruses: Fifteen adult serum samples were shown to be negative for VZV antibodies by both LA and FAMA. These serum samples were tested for antibodies to other human herpes viruses using commercial kits for antibody detection. Each serum tested positive for antibodies to at least one of the other herpes viruses (13 EBV, 5 CMV, 11 HSV). These data indicate the absence of cross reactivity between antibodies directed against other herpes viruses and the VZV antigens in the **VZVscan™** Latex Agglutination Test.

Reproducibility

The intra-assay reproducibility of the **VZVscan™** Latex Agglutination Test was examined in 5 replicate tests of 4 serum samples (1 high positive, 1 medium positive, 1 low positive, 1 negative). All replicate tests gave the same agglutination titer with each of the serum samples.

The inter-assay reproducibility was examined by determining the agglutination titers of 4 serum samples (1 high positive, 1 medium positive, 1 low positive, 1 negative) with 3 different lots of **VZVscan™** Latex Reagent. No significant variation in agglutination titers was observed from lot-to-lot of **VZVscan™** Latex Reagent.

XII. AVAILABILITY

Catalog #

254126	VZVscan™ 30 Test Kit (<i>Qualitative</i>)
254201	VZVscan™ 100 Test Kit (<i>Qualitative</i>)
278051	Macro-Vue™ Card Test Rotator (with humidifying cover), 100 ± 2 rpm, automatic timer, friction drive, Model 51-11 (110V)
277979	Macro-Vue Card Test Rotator Accessories Package, containing one 15"x 7" extension top and two humidifying covers
273310	Pipette Tips, Box 1000, 25 µL

XIII. REFERENCES

1. Gershon, A., Steinberg, S., Gelb, L., and the National Institute of Allergy and Infectious Diseases Collaborative Varicella Vaccine Study Group. Clinical reinfection with varicella-zoster virus, *J. Infect. Dis.*, 149:137-142, 1984.
2. Pallett, A., and Nicholls, N., Varicella-zoster Reactivation or reinfection? *Lancet* 1:160, 1986.
3. Boughton, C., Varicella-zoster in Sydney. I. Varicella and its complications, *Med. J. Aust.* 2:392-397, 1966.
4. Gershon, A., Steinberg, S., and Brunell, P., Zoster immune globulin: A further assessment, *N. Engl. J. Med.* 290:243-245, 1974.
5. Winsnes, R., Efficacy of zoster immunoglobulin in prophylaxis of varicella in high risk patients, *Acta Paediatr. Scand.*, 67:77-82, 1978.
6. Weller, T., Varicella and Herpes Zoster. *In: Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections*, Lennette and Schmidt, eds. American Public Health Association, Inc., Washington, D.C., 175-395, 1979.
7. Brunell, P.A., Varicella-zoster virus, Chapter 82, *Manual of Clinical Immunology*, Second edition, 1980.
8. Gallo, D., Schmidt, N. Comparison of Anticomplement Immunofluorescence and Fluorescent Antibody-to-Membrane Antigen Tests for Determination of Immunity Status to Varicella-Zoster Virus and for Serodifferentiation of Varicella-Zoster Virus and for Herpes Simplex Virus Infections, *J. of Clin. Microbiol.*, V. 14: 539-543, 1981.

TECHNICAL INFORMATION: In the United States telephone BD Diagnostic Systems Technical Services, toll free (800) 638-8663.

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