CE

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IVD  See external label   96 tests REF 1413-1Z

Varicella-Zoster IgM (VZV IgM)

Cat# 1413-1Z

Test	**Varicella IgM ELISA**
Method	**Enzyme Linked Immunosorbent Assay**
Principle	**Indirect ELISA : Antigen Coated Plate**
Detection Range	**Qualitative: Positive & Negative Control**
Sample	**10µL**
Specificity	**100%**
Sensitivity	**100%**
Total Time	**~ 50 min**
Shelf Life	**12-14 Months from the manufacturing date**

***** *Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.*

INTENDED USE

The Diagnostic Automation Varicella-Zoster Virus (VZV) IgM Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the detection of IgM antibody to Varicella-Zoster virus in human serum as an aid in the diagnosis of primary infection or reactivation. **For *in vitro* diagnostic use. High complexity test.**

Introduction

Varicella, more commonly known as Chickenpox and Herpes zoster are the two known clinical manifestations which can be produced by infection with a common etiologic agent, Varicella-Zoster virus (VZV) (1,2,3,4).

Chickenpox, the clinical syndrome usually produced as a result of the primary infection with VZV, is a highly contagious disease characterized by widely spread vesicular eruptions and fever. The disease is endemic in the U.S. and most commonly affects children from five to eight years of age, although adults and younger children, including newborn infants, may develop Chickenpox. Every two to five years, usually in the winter or spring, the number of cases increases to epidemic levels (1,3,4,5,6,7).

Herpes zoster is mainly a disease of adults, with most cases appearing in patients fifty years or older. Evidence suggests that this manifestation of VZV infection results from a reactivation of virus which remains latent in the sensory spinal ganglia after the primary infection rather than reintroduction of the virus into the host (8). Fever and painful localized vesicular eruptions of the skin along the distribution of the involved nerves are the most common clinical symptoms of the disease. Recurrences of Herpes zoster infection are extremely rare and can be mistaken for the similar lesions produced by Herpes simplex virus in which recurrences are common (1,2,3).

Although very rare (41 cases reported in the scientific literature from 1878 to 1973), congenital transmission of VZV may lead to severe disseminated neonatal infection with pneumonia, skin lesions, and hemorrhages and death (6,9). Infections with VZV that occur in utero during the first two trimesters of pregnancy can lead to congenital transmission of primary VZV. This route of exposure can result in a wide range of serious congenital abnormalities. The infant may have low birth weight, cutaneous scars, hypoplasia, or paralysis with muscular atrophy of limbs or rudimentary digits. Convulsions, psychomotor retardation, cortical atrophy, chorioretinitis, and cataracts can also result from congenital VZV infection (6,8). The fatality rate in cases of maternal infection with VZV near term have been reported to exceed 30% (6).

The various methods of serodiagnostic tests for the detection of VZV antibodies in a patient's serum include indirect immunofluorescence, neutralization, complement fixation and fluorescent antibody to membrane antigen (FAMA) (10,11,12,13). FAMA is generally considered the most sensitive and specific of the methods, yet requires the use of cell culture which is cumbersome to perform (14). It has been suggested by clinical and correlation studies performed by Shenab and Brunell that ELISA methodology is as sensitive and perhaps more specific than the FAMA assay (15).

The sensitivity, specificity, and reproducibility of enzyme-linked immunoassays is comparable to other serological tests for antibody, such as immunofluorescence, complement fixation, hemagglutination and radioimmunoassays (20,21,22).

Principle of the Assay

Enzyme-Linked Immunosorbent Assays (ELISA) rely on the ability of biological materials (e.g., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgM globulin conjugated with horseradish peroxidase which will bind to the

antibody- antigen complexes. The excess conjugate is removed by washing, followed by the addition of Chromogen/Substrate tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H2SO4, The contents of the wells turn yellow. The color, which is indicative of the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader (16, 17, 18, 19, 20, 21, 22).

Kit Presentation

Materials Supplied

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label.

1. **Purified Varicella-Zoster virus antigen coated microassay plate:** 96 wells, configured in twelve 1x8 strips, containing ALTERNATING strips of inactivated ANTIGEN and CONTROL ANTIGEN, stored in a foil pouch with desiccant. (96T: two plates)
2. **Calibrator**: Human serum or defibrinated plasma. Sodium azide (<0.1%) and pen/strep (0.01%) added as preservatives, with kit specific factor printed on vial label. The Calibrator is used to calibrate the assay to account for day-to-day fluctuations in temperature and other testing conditions. (96T:one vial, 0.4 mL) *
3. **Positive Control:** Human serum or defibrinated plasma. Sodium azide (<0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Positive Control is utilized to control the positive range of the assay. (96T: one vial, 0.4 mL) *
4. **Negative Control:** Human serum or defibrinated plasma. Sodium azide (<0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label.The Negative Control is utilized to control the negative range of the assay (96T: one vial, 0.4 mL) *
5. **Horseradish-peroxidase (HRP) Conjugate**: Ready to use. Goat anti-human IgM, containing proclin (0.1%) and gentamicin as preservatives. (96T: two bottles, 16 mL each)
6. **Serum Diluent Type ll:** Ready to use, contains proclin (0.1%) as preservative. (96T: two bottles, 30mL each)
7. Absorbent Solution: Ready to use. Contains goal/sheep antihuman IgG with protein stabilizers and proclin (0.1%) as preservative. (96T: two bottles, 12mL each)
8. **Wash Buffer Type 1 (20X concentrate):** dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween-20 and proclin (0.1%) as a preservative. (96T: one bottle,50 mL)
9. **Chromogen/Substrate Solution Type l:** Tetramethylbenzidine (TMB), ready to use. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells. (96T: two bottles, 15mL each)
10. **Stop Solution:** Ready to use, contains a 1N H_2SO_4 solution. (96T: two bottles, 15mL each)

***Note: serum vials may contain excess volume.**

The following components are not kit lot # dependent and may bwe used interchangeably within the DAI ELISA IgM assays: Serum Diluent Type ll, Chromogen/Substrate Solution Type l, Wash Buffer Type 1, and Stop Solution. Please check that the appropriate DAI Reagent Type (Type 1, Type 11, etc.) is used for the assay.

Additional Requirements

1. Wash bottle, automated or semi-automated microwell plate washing system.
2. Micropipettes, including multichannel, capable of accurately delivering 10-200 µL volumes (less than 3% CV).
3. One liter graduated cylinder.
4. Paper towels.
5. Test tube for serum dilution.
6. Reagent reservoirs for multichannel pipette.
7. Pipette tips.
8. Distilled or deionized water (dH_2O), CAP (College of American Pathology) Type 1 or equivalent. (36,37)
9. Timer capable of measuring to an accuracy of +/- 1 second (0-60 minutes).

10. Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL dH_2O).
11. Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the Operator's Manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.
    Note: Use only clean, dry glassware.

Storage and Stability

1. Store unopened kit between 2° and 8° C. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Unopened microassay plates must be stored between 2 and 8° C. Unused strips must be immediately resealed in a sealable bag with desiccant and returned to storage between 2 and 8° C.
3. Store HRP Conjugate and Absorbent Solution between 2 and 8° C.
4. Store the Calibrator, Positive and Negative Controls between 2 and 8° C.
5. Store Serum Diluent Type II and 20X Wash Buffer Type I between 2° and 8° C.
6. Store the Chromogen/Substrate Solution Type I between 2 and8° C. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells.
7. Store 1X (diluted) Wash Buffer Type I at room temperature (21 to 25° C) for up to 5 days, or up to 1 week between 2 and 8° C.

Note: If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit. Refer to package label for expiration date. Precautions were taken in the manufacture of this product to protect the reagents from contamination and bacteriostatic agents have been added to the liquid reagents. Care should be exercised to protect the reagents in this kit from contamination. Do not use if evidence of microbial contamination or precipitation is present.

Precautions

1. For *in vitro* diagnostic use.
2. The human serum components used in the preparation of the Controls and Calibrator in this kit have been tested by an FDA approved method for the presence of antibodies to human immunodeficiency virus 1 & 2 (HIV 1&2), hepatitis C (HCV) as well as hepatitis B surface antigen and found negative. Because no lest method can offer complete assurance that HIV, HCV, hepatitis B virus, or other infectious agents are absent, specimens and human-based reagents should be handled as if capable of transmitting infectious agents.
3. The Centers for Disease Control & Prevention and the National be handled at the Biosafety Level 2 (23).
4. The components in this kit have been quality control tested as a Master lot unit. Do not mix components from different lot numbers except Chromogen/Substrate Solution Type I, Stop Solution, Wash Buffer Type 1, and Serum Diluent Type ll. Do not mix with components from other manufactures.
5. Do not use reagents beyond the stated expiration date marked on the package label.
6. All reagents must be at room temperature (21 to 25°C) before running assay. Remove only the volume of reagents that is needed. **Do not pour reagents back into vials as reagent contamination may accur.**
7. Before opening Control and Calibrator vials, tap firmly on the benchtop to ensure that all liquid is at the bottom of the vial.
8. Use only distilled or deionized water and clean glassware.
9. Do not let wells dry during assay: add reagents immediately after completing wash steps.
10. Avoid cross-contamination of reagents. Wash hands before and after handling reagents. **Cross-contamination of reagents and/or samples could cause false results.**
11. If washing steps are performed manually, wells are to be washed three times. Up to five wash cycles may be necessary if a washing manifold or automated equipment is used.
12. **Sodium azide inhibits Conjugate activity. Clean pipette tips must be used for the Conjugate addition so that sodium azide is not carried over from other reagents.**
13. It has been reported that sodium azide may react with lead and copper in plumbing to form explosive compounds. When disposing, flush drains with water to minimize build-up of metal azide compounds.
14. Never pipette by mouth or allow reagents or patient sample to come into contact with skin. Reagents

containing proclin, sodium azide, and TMB may be irritating. Avoid contact with skin and eyes. In case of contact, flush with plenty of water.

15. If a sodium hypochlorite (bleach) solution is being used as a disinfectant, do not expose to work area duting actual test procedure because of potential interference with enzyme activity.
16. Avoid contact of Stop Solution (1N sulfuric acid) with skin or eyes. If contact occurs, immediately flush area with water.
17. **Caution:** Liquid waste at acid pH must be neutralized prior to adding sodium hypochlorite (bleach) solution to avoid formation of poisonous gas. Recommend disposing of reacted, stopped plates in biohazard bags. See Precaution 3.
18. The concentrations of anti-Varicella-Zoster Virus in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

Specimen Collection and Storage

1. Handle all blood and serum as if capable of transmitting infectous agents (23).
2. Optimal performance of the kit depends upon the use of fresh serum samples (clear, non-hemolyzed , non-lipemic, A minimum volume of 50 µL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture (34). Early separation from the clot prevents hemolysis of serum.
3. Store serum between 2° and 8° C if testing will take place within two days. If specimens are to be kept for longer periods, store at -20° C or colder. Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperty stored or are subjected to multiple freeze-thaw cycles may yield erroneous results.
4. The NCCLS provided recommendations for storing blood specimens (Approved Standard Procedures for the Handing and Processing of Blood Specimens. H18-A. 1990) (34).

Methods for Use

Preparation for the Assay

1. All reagents must be removed from refrigeration and allowed to come to room temperature before use (21° to 25° C). Return all reagents to refrigerator promptly after use.
2. All samples and controls should be vortexed before use.
3. Dilute 50 mL of the 20X Wash Buffer Type l to 1 L with distilled and/or deionized H_2O. Mix well.

Serum Treatment

Solid phase immunoassays for the detection of virus-specific IgM are known to be sensitive to interfering factors. This kit overcomes interference by treating smples prior to running the assay. The Absorbent Solution diminished competing virus-specific IgG, which would be responsible for false negative reactions. False positive are similarly minimized by removing the IgG, this neutralizing the bound rheumatoid factor in the samples.

Assay Procedure for Serum Absorption

1. In a set of test tubes, dilute Calibrator, Controls and patient samples 1:41 in Serum diluent Type ll (i..e., 400 µL Serum diluent Type ll + 10 µL of Calibrator, Control or serum sample). Mix well. Note: The Calibrator must be run in duplicate, therefore make two separate dilutions.
2. Transfer 150 µL of the 1:41 dilution (Step 1) of the Calibrators, Control, and patient samples to a second set of test tubes, add 150 µL Absorbent Solution to each test tube. Mix well. **This will yield enough for 1 antigen well and 1 cotnrol antigen well. (Final diltion 1:81)**
3. Incubation all absorbent dilutions at room temperature (21° to 25°C) for **20 minutes +/- 5 minutes.**

Assay Procedure

1. Place the desired number of strips into a microwell frame. The Calibrator, Positive Control, Negative Control, and each patient sample must be run in both an antigen and a control antigen coated well. A reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Control/Calibrator configurations. Return unused strips to the sealable bag with desiccant, seat and immediately refrigerate.

Example Configuration:

Plate Location	Sample Description	Plate Location	Sample Description
1A	RB	2A	Blank Well
1B	NC (Ag)	2B	NC (C Ag)
1C	Cal (Ag)	2C	Cal (C Ag)
1D	Cal (Ag)	2D	Cal (C Ag)
1E	PC (Ag)	2E	PC (C Ag)
1F	Patient # 1 (Ag)	2F	Patient # 1 (C Ag)
1G	Patient # 2 (Ag)	2G	Patient # 2 (C Ag)
1H	Patient # 3 (Ag)	2H	Patient # 3 (C Ag)

RB = Reagent Blank- Well without serum addition run with all reagents. Utilized to blank reader.
NC = Negative Control
Cal = Calibrator
PC = Positive Control
(Ag) = Antigen Coated Strip
(C Ag) = Control Antigen Coated Strip

2. To corresponding wells of the antigen strip and the control antigen strip, add 100 µL of absorbed and diluted patient sera, Calibrator and Control sera. Add 100 µL of Serum Diluent Type II to the reagent blank well. Check software and reader requirements of the correct reagent blank well configuration.
3. Incubate each well at room temperature (21° to 25° C) for **20 minutes +/- 2 minutes.**
4. Aspirate or shake out liquid from all wells. Using semi-automated or automated washing equipment add 250-300 µL of diluted Wash Buffer to each well. Aspirate or shake out and turn plate upside down and blot on paper toweling to remove all liquid. Repeat the wash procedure two times (for a total of three washes) for semiautomated equipment or four times (for a total of five washes) for semiautomated equipment. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.

****IMPORTANT NOTE:** Regarding steps 4 to 7 – insufficient or excessive washing wll result in assay variation and will affect validity of results. Therefore, for best results the use of semi-automated or automated equipment set to deliver a volume to completely fill each well (250-300 µL) is recommended. A total of five (5) washes may be necessary with automated equipment. Complete removal of the Wash Buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.

5. Add 100 µL Conjugate to each well, including to each well, including the reagent blank well. Avoid bubbles upon addition as they may yield erroneous resultes.
6. Incubate each well at room temperature (21° to 25° C) for **20 minutes +/- 2 minutes.**
7. Repeat wash as described in Step 4**
8. Add 100 µL Chromogen/Substrate solution (TMB) solution to each well, including reagent blank well, maintaining a constant rate of addition across the plate.
9. Incubate each well at room temperature (21° to 25° C) for **10 minutes +/- 2 minutes.**
10. Stop reaction by addition of 100 µL of Stop Solution (1N HSO) following the same order of Chromogen/Substate addition, including reagent blank well. Tap the plate gently along the outsides to mix contents of the wells. The plate may be held up to one (1) hour after addition of the Stop Solution before reading.
11. The developed color should be read on an ELISA plate reader equipped with a 450nm filter. If dual wavelength is used, set the reference filter to 600-650. The instrument should be blanked on air. The reagent blank must be less than 0.150 Absorbance at 450 nm. If the reagent blank is $\geq$0.150, the run must be repeated. Blank the reader on the reagent blank well and then continue to read the entire plate. Dispose of used plates after readings have been obtained.

Quality Control

For the assay to be considered valid the following conditions must be met:

1. Calibrator and Control must be run with each test run.
2. Reagent blank (when read against air blank) must be < 0.150 Absorbance (A) at 450 nm.
3. Negative Control must be ≤0.250 A at 450 nm (when read against reagent blank).
4. Each Calibrator must be ≥0.300 A at 450 nm (must read against regent balnk).
5. Positive Control must be ≥0.250 A at 450 nm (when read against reagent blank).
6. The ISR for the Positive and Negative Controls should be in their respective ranges printed on the vial labels. If the Control values are not within their respective ranges, the test should be considered invalid and the test should be repeated.
7. Additional Controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
8. Refer to NCCLS C24A for guidance on appropriate Quality Control practices (35).
9. If above criteria are not met on repeat testing, contact DAI Technical Services.

Interpretation

Calculations

1. For each patient sample and Controls/Calibrator, subtract the control antigen well absorbance from the antigen well absorbance. This is the Delta value.
2. Mean Delta Calibrator O.D. (Optical Density) - Calculate the mean Delta absorbance value for the Calibrator from the two Calibrator determinations.
3. Correction Factor - To account for day-to-day fluctuations in assay activity due to room temperature and timing, a Correction Factor is determined by DAI for each lot of kits. The Correction Factor is printed on the Calibrator vial.
4. Cutoff Calibrator Value - The Cutoff Calibrator Value for each assay is determined by multiplying the Correction Factor by the mean Delta Calibrator O.D. determined in Step 2.
5. ISR Value - Calculate an Immune Status Ratio (ISR) for each specimen by dividing the Delta specimen O.D. value by the Cutoff Calibrator Value determined in Step 4.

Example:

Delta O.D's obtained for Calibrator = 0.38, 0.42
Mean Delta O.D. for Calibrator = 0.40
Correction factor = 0.50
Cutoff Calibrator Value = 0.50 x 0.40 = 0.20
Delta O.D. obtained for patient sera = 0.60
ISR Value = 0.60/0.20 = 3.00

Analysis

1. The patients' ISR (Immune Status Ratio) values are interpreted as follows:

ISR Value	Results	Interpretation
≤ 0.90	Negative	No significant level of detectable IgM antibody to Varicella-Zoster Virus.
0.91-1.09	Equivocal	Samples should be retested. See number 2 below.
≥ 1.10	Positive	Significant level of detectable IgM antibody to Varicella-Zoster Virus. Indicative of current or recent infection.

2. Samples that remain equivocal after repeat testing should be retested on an alternate method, e.g., immunofluorescence assay (IFA).

Expected Values

Expected values may vary depending upon patient age and climate (31). In temperate climates, varicella is a childhood disease. Peak incidence is between March and May (32). In the United States, 32% of a varicella cases occur between the ages of 1 and 4 years, and 50% between the ages of 5 and 9 (31). By the age of 16 nearly all members of a population have anti-VZV IgG (33). In tropical and semitropical climates, varicella typically occurs much later (31).

An IgM response is seen in 100% of primary VZV infections and a rise in the IgM level is seen in 50 to 80% of active herpes zoster cases depending on the test method used (27,28,29,30). IgM antibodies to VZV are generally detectable 2-5 days after onset of rash, reaching peak levels at 8-11 days, dropping to undetectable levels within a few weeks of onset.

Limitations of Use

1. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
2. The results of ELISA immunoassays performed on serum from immunosuppressed patients must be interpreted with caution.
3. Samples that remain equivocal after repeat testing should be retested by an alternate method, e.g., Immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken.
4. Negative test results cannot rule out the possibility of an active herpes zoster infection, but positive results are confirmatory.
5. Specific IgG may compete with the IgM for sites and may result in a false negative. Conversely, rheumatoid factor in the presence of specific IgG may result in a false positive reaction. The Absorbent Solution diminishes competing virus-specific IgG and minimizes rheumatoid factor interference in samples. Studies indicate that the maximum amount of IgG which can be removed by the kit Absorbent Solution is in excess of the expected high end of the normal range for IgG > 1340 mg/dL.
6. Some antinuclear antibodies have been found to cause a false positive reaction on some ELISA tests.
7. It is strongly recommended that neonate's and mother's serum samples be tested in parallel. The presence of IgM antibody in the neonate's serum can be considered indicative of congenital infection only if there has not been placental leakage. Additionally, if the infant has a congenital infection, the IgM antibody (and IgG antibody) level may persist or rise, whereas if the source of the antibody is maternal, the neonate's antibody level will drop in parallel to the half-life of that immunoglobulin.
8. Results of this test should be interpreted by the physician in the light of other clinical findings and diagnostic procedures. A definitive diagnosis is made by isolation of VZV in tissue culture.
9. This test is not intended for the determination of immune status. It is intended for the determination of a person’s antibodyresponse to indicate active infection to VZV (primary or reactivation) and not as an indication of immunity.
10. Cord blood specimens are not recommended for use with this product. Performance characteristics have not been established for cord blood.
11. Samples obtained too early during primary infection (within 5 days after onset of rash) may not contain detectable antibodies (10,24). If VZV infection is suspected a second sample should be obtained 2-3 weeks later (25) and tested in parallel with the first specimen to look for seroconversion which is indicative of a primary infection.
12. Varicella may be a severe illness in newborn infants whose mothers have varicella at delivery (26).
13. User should be aware of possible IgM antibody interference from Epstein-Barr virus.

Performance Characteristics

Sensitivity and Specificity

The DAI Varicella-Zoster Virus IgM ELISA was evaluated in comparison to a commercially available IFA VZV IgM test kit. The study population of 124 samples was comprised of randomly collected sera from apparently healthy ambulatory donors as well as positive samples from independent clinical laboratories in the Northeastern U.S. An equivocal by the ELISA test is considered indeterminate and that result (1 total) was omitted from the calculations for relative sensitivity and specificity. The results are as follows:

Table 1
Relative Sensitivity & Specificity

DAI ELISA				Relative Sensitivity	Relative Specificity
		(+)	(-)		
IFA	(+)	31	0	100%	100%
	(-)	0	92		

Cross-Reactivity

Method

A study was performed to determine the cross-reactivity of the DAI VZV IgM ELISA with 19 samples, negative for VZV by IFA, which tested positive by other commercially available kits for: HSV-1 (2), HSV-2 (1), EBV (1), CMV (2), measles (2), rubella (1), antinuclear antibody (ANA) (5), and rheumatoid factor (RF) (5).

Results

Negative DAI Varicella-Zoster Virus IgM ELISA test results in 18 samples (EBV + sample tested equivocal) indicate an absence of cross-reactivity of the DAI VZV IgM ELISA with other members of the Herpesvirus family, other IgM antibodies non-specific to VZV and with RF and ANA.

Precision

A study was performed to document typical assay precision with the DAI VZV IgM ELISA kit. The mean, SD, and %CV, were calculated for Intra- and Inter- assay, and Inter-lot Precision.

Intra-Assay Precision

Table 2 presents the results of five (5) samples individually pipetted in groups of twenty (20) in a single assay.

Table 2
Intra-Assay Precision for DAI VZV IgM ELISA

	n	Mean ISR	Std Dev	%CV
Serum 1	20	5.05	0.221	4.4%
Serum2	20	5.04	0.225	4.5%
Serum3	20	4.41	0.194	4.4%
Serum4	20	0.24	0.012	4.9%
Serum5	20	0.36	0.035	9.6%

Inter-Assay Precision

Table 3 presents the summary of the Inter-Assay precision data determined by replicate testing of five (5) samples individually pipetted in groups of five (5) on three (3) consecutive days.

Table 3
Inter-Assay Precision for DAI VZV IgM ELISA

	n	Day 1	Day 2	Day 3	Mean ISR	Std Dev	%CV
Serum 1	3	4.91	4.67	5.49	5.02	0.348	7.0%
Serum 2	3	4.95	4.71	5.33	5.00	0.261	5.2%
Serum 3	3	4.60	4.32	4.83	4.59	0.237	5.2%
Serum 4	3	0.22	0.21	0.19	0.21	0.017	8.0%
Serum 5	3	0.32	0.29	0.35	0.33	0.050	15.5%

Inter-Lot Precision

Table 4 presents the summary of the lot to lot precision data determined by replicate testing of five (5) samples individually pipetted in groups of five (5) using three (3) different lots of reagent.

Table 4
Inter-Lot Precision for DAI VZV IgM ELISA

	n	Lot 1	Lot 2	Lot 3	Mean ISR	Std Dev	%CV
Serum 1	3	5.89	5.11	6.00	5.67	0.411	7.3%
Serum 2	3	5.60	5.14	5.97	5.57	0.371	6.7%
Serum 3	3	5.16	4.63	4.95	4.91	0.290	5.9%
Serum 4	3	0.13	0.18	0.15	0.15	0.023	15.1%
Serum 5	3	0.47	0.44	0.53	0.48	0.054	11.3%

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