AccuDiag™ Herpes Simplex Virus 1 IgG (HSV 1 IgG) ELISA Kit

REF 1401-P1

IVD See External Label 2°C 8°C 96 Tests

HSV 1 IgG ELISA	
Principle	Indirect ELISA
Detection	Qualitative
Sample	10 µL serum/plasma
Incubation Time	75 minutes
Sensitivity	93.8%
Specificity	97%
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The Diagnostic Automation Inc. AccuDiag™ Herpes Simplex Virus (HSV) 1 IgG kit is an Enzymelinked Immunosorbent Assay (ELISA) intended for qualitatively detecting the presence or absence of human IgG class antibodies to HSV-1 in human serum. In conjunction with the Diagnostic Automation Inc. AccuDiag™ Herpes Simplex Virus (HSV) 2 IgG kit, the test is indicated for testing sexually active adults or expectant mothers for aiding in the presumptive diagnosis of HSV infection. Due to the implications of positive results, it is recommended they be confirmed in a low prevalence population with Western blot. The performance of this assay has not been established for use in a pediatric population, for neonatal screening, for testing of immunocompromised patients, or for use with automated equipment. The user is responsible for establishing assay performanace in these populations and with automated equipment.

SIGNIFICANCE AND SUMMARY

Infection with HSV (Herpes Simplex Virus) types 1 and 2 occurs worldwide. HSV 2 is the most common cause of genital herpes lesions. HSV 1 infection most frequently manifests as oral lesions with gingivostomatitis and pharyngitis as the symptoms of first infection, with herpes labialis as the most frequent symptom of reactivated infection. HSV 1 has also been associated with genital lesions.[1,2,3]

Transmission is generally through contact with active lesion through sexual contact. However, some patients experience asymptomatic infection and may therefore be unknown carriers. For this reason, diagnosis through serology is important. The incubation period ranges from 1 to 26 days from time of infection to potential clinical symptoms.[1,4]

Serological diagnosis is important to determine the type of HSV infection. If a primary, intact lesion is observed, direct antigen determination, cell culture is optimal for diagnostic purposes. It is possible that the antibody may not be detectable early in an infection. It is also important to rule out other causes of lesions. Pregnant women who are at risk for infection should also be tested to prevent congenital infection of the neonate during birth.[5,6] In addition, if the IgG serology is negative, a newly infected mother should receive counseling regarding the risks and the chance of transmitting HSV to her baby in the birth canal during childbirth.[25]

A positive serological result is only indicative of past infection, as antibody titer does not always increase upon reactivation of infection. The gold standard for diagnosing an acute infection is virus isolation in tissue culture.[6,7,8,9]

The sensitivity, specificity, and reproducibility of ELISA's can be comparable to other serological tests for antibody, such as immunofluorescence, complement fixation, hemagglutination and radioimmunoassays.[16,17,18]

ASSAY PRINCIPLE

HSV 1 and HSV 2 have approximately 50% sequence homology and show considerable crossreactivity. The HSV 1 IgG ELISA uses a recombinant glycoprotein g, which is type specific for HSV 1. This allows for a rapid and less expensive sero-diagnosis of HSV 1 infection than virus isolation techniques.[9,10,11,12]

The HSV 1 IgG kit utilizes the ELISA technology where a purified recombinant HSV 1 (rHSV1) antigen is bound to the wells of a microplate. A peroxidase coupled anti-human IgG conjugate is used as the detection system.

When rHSV1 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 antigenantibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgG conjugated with horseradish peroxidase, which then binds to the antibodyantigen 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 H_2SO_4, 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.[12,13,14,15]

SPECIMEN COLLECTION & PREPARATION

1. Handle all blood and serum as if capable of transmitting infectious agents.
2. Optimal performance of the kit depends upon the use of fresh serum samples (clear, nonhemolyzed, non-lipemic, non-icteric). A minimum volume of 50 µL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture.[20]
3. Early separation from the clot prevents hemolysis of serum.
4. The National Committee for Clinical Laboratory Standards (NCCLS) provides the following recommendations for storing blood specimens (Approved Standard – Procedures for the Handling and Processing of Blood Specimens, H18-A2. 1999, Section 6.3.1.2 Storage):[21]

- Store samples at room temperature for no longer than 8 hours.
- If the assay will not be completed within 8 hours, refrigerate the sample at 2 – 10°C.
- If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C.
- Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield erroneous results.

REAGENTS

Materials provided with the kit

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

1. Purified Recombinant HSV gG1 protein coated micro assay plate: 96 wells, configured in twelve 1x8 strips, stored in a foil pouch with desiccant. (96T: one plate)
2. Serum Diluent Buffer Type I: Ready for use. Contains ProClin® (0.1%) as a preservative, pH 7.5 + 0.2 (96T: one bottle, 30 mL)
3. Cutoff Calibrator (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 source material for the Cutoff Calibrator is different than the controls. (96T: one vial)*
4. High Positive Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives. The High Positive Control is utilized to control the upper dynamic range of the assay. (96T: one vial, 0.4 mL)*
5. Low Positive Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives. The Low Positive Control is utilized to control the range near the cutoff of the assay. (96T: one vial, 0.4 mL)*
6. Negative Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives. The Negative Control is utilized to control the negative range of the assay. (96T: one vial, 0.4 mL)*
7. Horseradish-peroxidase (HRP) Conjugate: Ready to use. Goat anti-human IgG, containing ProClin® (0.1%) and gentamicin as preservatives. (96T: one bottle, 16 mL)
8. Chromogen/Substrate Solution Type I: 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 and would render the plate unusable. (96T: one bottle, 15 mL)
9. Wash Buffer Type I (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)
10. Stop Solution: Ready to use, contains a 1N H_2SO_4 solution. (96T: one bottle, 15 mL)

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

Materials required but not provided

- Wash bottle, automated or semi-automated microwell plate washing system
- Micropipettes, including multichannel, capable of accurately delivering 10-200 µL volumes (less than 3% CV)
- One Liter Graduated cylinder
- Paper towels
- Test tubes for serum dilution
- Reagent reservoirs for multichannel pipettes
- Pipette tips
- Distilled or deionized water (dH2O), CAP (College of American Pathology) Type I or equivalent.[21,22]
- Timer capable of measuring to an accuracy of +/- 1 second (0 to 60 minutes)
- Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL dH2O)
- 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 Operators' manual or contact the instrument manufacturer to establish linearity performance specifications of the reader

Note: Use only clean, dry glassware.

REAGENT PREPARATION

1. All reagents must be removed from refrigeration and allowed to come to room temperature (21 to 25°C) before use. 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 I to 1 L with distilled and/or deionized H_2O. Mix well.

ASSAY PROCEDURE

1. Place the desired number of strips into a microwell frame. Allow six (6) Control/Cutoff Calibrator determinations (one Negative Control, three Cutoff Calibrators, one High Positive Control and one Low Positive Control) per run. A reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Control/Calibrator configuration. Return unused strips to the sealable bag with desiccant, seal and immediately refrigerate.

Example Configuration:

Plate Location	Sample Description	Plate Location	Sample Description
1A	RB	2A	Patient #2
1B	NC	2B	Patient #3
1C	Cal	2C	Patient #4
1D	Cal	2D	Patient #5
1E	Cal	2E	Patient #6
1F	HPC	2F	Patient #7
1G	LPC	2G	Patient #8
1H	Patient #1	2H	Patient #9

RB = Reagent Blank – Well without serum addition run with all reagents. Utilized to blank reader.
NC = Negative Control
Cal = Calibrator
HPC = Positive Control

LPC = Negative Control

2. Dilute test sera, Cutoff Calibrator and control sera 1:21 (e.g., 10 µL + 200 µL) in Serum Diluent. Mix well. (For manual dilutions it is suggested to dispense the Serum Diluent into the test tube first and then add the patient serum.)
3. To individual wells, add 100 µL of the appropriate diluted Cutoff Calibrator, Controls and patient sera. Add 100 µL of Serum Diluent to reagent blank well. Check software and reader requirements for the correct reagent blank well configuration.
4. Incubate each well at room temperature (21 to 25 °C) for thirty (30) +/- 2 minutes.
5. 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 to remove all liquid. Repeat the wash procedure two (2) times (for a total of three [3] washes) for manual or semi-automated equipment or four (4) times (for a total of five [5] washes) for automated equipment. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.

*****IMPORTANT NOTE: Regarding steps 5 and 8 - Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semiautomated or automated equipment set to deliver a volume to completely fill each well (250-300 µL) is recommended. A total of up to 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.

6. Add 100 µL Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.
7. Incubate each well at room temperature (21 to 25°C) for **thirty (30) +/- 2 minutes**.
8. Repeat Wash as described in Step 5.
9. Add 100 µL Chromogen/Substrate Solution (TMB) to each well, including reagent blank well, maintaining a constant rate of addition across the plate.
10. Incubate each well at room temperature (21 to 25°C) for **fifteen (15) +/- 2 minutes**.
11. Stop reaction by addition of 100 µL of Stop Solution (1N H_2SO_4) following the same order of Chromogen/Substrate solution addition, including the 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.
12. The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. 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 > 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.

INTERPRETATION

CUTOFF DETERMINATION

48 HSV Type 1 negative sera were assayed by the HSV 1 IgG ELISA test. The mean and standard deviation of the optical density readings for the sera was 0.155 and 0.073 respectively. The positive threshold for the assay was determined by adding the mean and 2.5 standard deviations, (0.155 + 2.5 (0.073) = 0.338). A positive serum was titrated to give a constant ratio of the threshold value to obtain a calibrator sera. On all subsequent assays, this sera was run and the assay calibrated by multiplying the O.D. value for the calibrator by the ratio to the cut off to obtain the cut off O.D. This value was then divided into the O.D. for the patient sera to obtain an index value. By definition, the cut off index is equal to 1.00. To account for inherent variation in the immunoassay, values of 0.91 – 1.09 were considered equivocal. Therefore, values ≤ 0.90 are considered negative and the values ≥ 1.10 are considered positive. Further analytical validation was performed using levels 15% above and 30% below the cut off.

CALCULATIONS

1. Mean Cutoff Calibrator O.D. (Optical Density) - Calculate the mean O.D. value for the Cutoff Calibrator from the three Cutoff Calibrator determinations. If any of the three Cutoff Page 3 of 5 – EN 3800-29 Rev J Calibrator values differ by more than 15% from the mean, discard that value and calculate the average of the two remaining values.
2. Correction Factor - The correction factor, determined by Diagnostic Automation Inc. for each lot of kits, is intended to account for lot-to-lot variation of the calibrator material. The Correction Factor is printed on the Cutoff Calibrator vial.
3. Cutoff Calibrator Value - The Cutoff Calibrator Value for each assay is determined by multiplying the Correction Factor by the mean Calibrator O.D. determined in Step 1.
4. ISR Value - Calculate an ISR Value for each specimen by dividing the specimen O.D. value by the Cutoff Calibrator Value determined in Step 3.

Values obtained greater than the cutoff are not necessarily indicative of the amount of antibody present.

Example:

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

ANALYSIS

1. The patients' ISR values are interpreted as follows:

ISR	Results	Interpretation
≤ 0.90	Negative	No detectable HSV 1 IgG antibody by the ELISA test. Negative results do not rule out the diagnosis of infection with HSV1. The specimen may have been drawn before appearance of detectable antibodies. Negative results in suspected early disease should be repeated in 4 – 6 weeks.
0.91 – 1.09	Equivocal	Samples that remain equivocal after repeat testing should be re-tested by an alternate method, e.g., immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken 4 to 12 weeks later and tested in parallel with the original sample.[11, 19]
≥ 1.10	Positive	Indicates presence of detectable IgG antibody to HSV 1 by the ELISA test. A positive test may be confirmed by Western Blot testing.

2. The following is a recommended method for reporting the results obtained: "The following results were obtained with the Diagnostic Automation Inc. HSV 1 IgG ELISA. Values obtained with different methods

may not be used interchangeably. The magnitude of the reported IgG level is not necessarily indicative of antibody level."

3. Since HSV 1 IgG antibodies are present in convalescent sera, a single result can not be used for diagnosis. Accurate interpretation of HSV 1 infection is based on the results from HSV 1 IgG, HSV 2 IgG and clinical profile.

QUALITY CONTROL

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

1. Cut off Calibrator and Controls must be run with each test.
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 Cutoff Calibrator must be ≥ 0.250 A at 450 nm (when read against reagent blank).
5. High Positive Control must be ≥ 0.500 A at 450 nm (when read against reagent blank).
6. The ISR values for the High Positive, Low 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 should be repeated.
7. The Negative and Positive Controls are intended to monitor for substantial reagent failure.
8. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
9. Refer to NCCLS C24-A for guidance on appropriate QC practices.[23]
10. If above criteria are not met upon repeat testing, contact Diagnostic Automation Inc. Technical Services. Do not report results.

EXPECTED RANGES OF VALUES

PREVALENCE

Within the U.S., studies have found that more than 20 percent of the population has HSV-2 and 66 percent has HSV-1.3 An outside investigator assessed Diagnostic Automation Inc. HSV 1 IgG ELISA with consented, prospectively collected and masked sera from 1) sexually active adults over the age of 14 (n = 198) and 2) expectant mothers (n = 210). The reference method was an HSV 1 Western Blot (WB) from a Pacific Northwest university. The observed prevalence and the hypothetical predictive values for the two populations are shown in the tables below. The positive predictive value (PPV) will decrease proportionally to a decrease in the prevalence of HSV infection and the negative predictive value (NPV) will decrease proportionally to an increase in the prevalence of HSV infection as reflected in the table below. The calculations are based on Diagnostic Automation Inc. HSV 1 IgG ELISA having 1) a hypothetical sensitivity of 87.93% and a hypothetical specificity of 100.00% (sexually active adults) and 2) a hypothetical sensitivity of 88.31% and a hypothetical specificity of 98.18% (expectant mothers).

Observed Prevalence in Sexually Active Adults and Expectant Mothers

Population	HSV 1 Sero-	Observed Prevalence	
	status	WB	Diagnostic Automation Inc. ELISA
Sexually Active Adults*	neg	40.8% (80/196)	48.0% (94/196)
	+	59.2% (116/196)	52.0% (102/196)
Expectant Mothers**	neg	26.3% (55/209)	34.4% (72/209)
	+	73.7% (154/209)	65.6% (137/209)

*Excludes two atypical Western Blots.
**Excludes one ELISA equivocal.

Prevalence vs. Hypothetical Predictive Values

Prevalence	Sexually Active Adults		Expectant Mothers	
	PPV*	NPV	PPV	NPV
50%	98.6	89.2	98.0	89.4
40%	97.9	92.6	97.0	92.7
30%	96.8	95.1	95.4	95.1
25%	95.9	96.1	94.2	96.2
20%	94.6	97.1	92.4	97.1
15%	92.5	97.9	89.5	97.9
10%	88.7	98.7	84.4	98.7
5%	78.7	99.4	71.9	99.4

*Assumes one discrepant sample.

Note: Sexually active adults and expectant mother populations in different geographic areas may produce different frequency distributions from the table above. Each laboratory should establish frequency distributions for their specific patient populations.

PERFORMANCE CHARACTERISTICS

% AGREEMENT POSITIVE AND % AGREEMENT NEGATIVE WITH EXPECTANT MOTHERS†

An outside investigator assessed the % agreement positive and % agreement negative with consented, coded, unselected, banked and masked sera from expectant mothers (n = 210). The reference method was an HSV 1 Western Blot (WB) from a Pacific Northwest university. Of 155 WB positives, Diagnostic Automation Inc. ELISA was 136 positive, 18 negative and 1 equivocal. Of 55 WB negatives, Diagnostic Automation Inc. ELISA was 54 negative and 1 positive.

% Agreement Positive and % Agreement Negative with Expectant Mothers†

Characteristic	% (EL/WB)*	95% Confidence Interval (CI)
% agreement positive to WB	87.74% (136/155)	82.6-92.9%‡
% agreement negative to WB	98.18% (54/55)	90.3-100.0%

† The word "% agreement" refers to comparing this assay's results with those of a similar assay. No attempt was made to correlate the assay results to disease presence or absence. No judgment can be made on the similar assay's accuracy in predicting disease.
‡ 95% CI calculated using the normal approximate method.

% AGREEMENT POSITIVE AND % AGREEMENT NEGATIVE WITH SEXUALLY ACTIVE ADULTS†

An outside investigator assessed the % agreement positive and % agreement negative with consented, unselected and masked sera from sexually active adults over the age of 14 (n = 198). The reference method was an HSV 1 Western Blot (WB) from a Pacific Northwest university. Of 116 WB positives, Diagnostic Automation Inc. ELISA was 102 positive and 14 negative. Of 80 WB negatives, Diagnostic Automation Inc. ELISA was 80 negative.

% Agreement Positive and % Agreement Negative with Sexually Active Adults (n = 198)†

Characteristic	% (EL/WB)*	95% Confidence Interval (CI)
% agreement positive to WB	87.93% (102/116)	82.0-93.9%‡
% agreement negative to WB	100.00% (80/80)	95.5-100.0%

***** Excludes two atypical Western Blots.
† The word "% agreement" refers to comparing this assay's results with those of a similar assay. No attempt was made to correlate the assay results to disease presence or absence. No judgment can be made on the similar assay's accuracy in predicting disease.
‡ 95% CI calculated using the normal approximate method.

% AGREEMENT POSITIVE AND % AGREEMENT NEGATIVE WITH A LOW PREVALENCE POPULATION (N = 184)†
An outside investigator assessed the % agreement positive and % agreement negative with unselected, banked and masked sera from a low prevalence population (n = 184). The reference method was an HSV 1 Western Blot (WB) from a Pacific Northwest university. Of 131 WB negatives, Diagnostic Automation Inc. ELISA was 128 negative, 1 positive and 2 equivocal. Of 53 WB positives, Diagnostic Automation Inc. ELISA was 42 positive, 8 negative and 3 equivocal.

% Agreement Positive and % Agreement Negative with a Low Prevalence Population (n = 184)†

Characteristic	% (EL/WB)*	95% Confidence Interval (CI)
% agreement positive to WB	79.25% (42/53)	65.9-89.2%
% agreement negative to WB	97.71% (128/131)	93.5-99.5%

†The word "% agreement" refers to comparing this assay's results with those of a similar assay. No attempt was made to correlate the assay results to disease presence or absence. No judgment can be made on the similar assay's accuracy in predicting disease.

% AGREEMENT POSITIVE WITH CULTURE POSITIVES†
An outside investigator assessed the % agreement positive using unselected, retrospective and masked sera from patients that were at least six weeks but not more than one year post clinical presentation and culture HSV 1 positive (n = 53). Reference methods included culture (infection) and an HSV 1 Western Blot (WB) (antibody) from a Pacific Northwest university. Of 53 culture positives: 1) Diagnostic Automation Inc. ELISA was 37 positive, 12 negative and 4 equivocal and, 2) WB was 44 positive and 9 negative. Of 44 WB positives: Diagnostic Automation Inc. ELISA was 36 positive, 6 negative, and 2 equivocal.

% Agreement Positive with Culture Positives (n = 53)†

Characteristic	% (EL/WB or Culture)	95% Confidence Interval (CI)
% agreement positive to culture	69.81% (37/53)	55.7-81.7%
% agreement positive to WB	81.82% (36/44)	67.3-91.8%

† The word "% agreement" refers to comparing this assay's results with those of a similar assay. No attempt was made to correlate the assay results to disease presence or absence. No judgment can be made on the similar assay's accuracy in predicting disease.

% AGREEMENT POSITIVE AND % AGREEMENT NEGATIVE TO ALTERNATE HSV 1 IGG ELISA
An outside investigator at a Pacific Northwest University assessed the % agreement positive and % agreement negative of the Diagnostic Automation Inc. AccuDiag™ HSV 1 IgG kit and an alternate HSV 1 IgG ELISA test with 200 prospective, unselected, sequentially submitted specimens.

% Agreement Positive and % Agreement Negative with Expectant Mothers (n = 210)†

Prospectively Collected, Sequential Sera		Alternate HSV 1 IgG		
Diagnostic Automation Inc. AccuDiag™ HSV 1 IgG		+	–	E
	+	92	3	0
	–	6	99	0
	E	0	0	0

	%(TBU ELISA / Alt. ELISA)	95% Confidence Interval (CI)
Percent Positive Agreement	93.88% (92/98)	87.2-97.7%
Percent Negative Agreement	97.06% (99/102)	91.6-99.4%
Percent Agreement	95.50% (191/200)	91.6-97.9%

TYPE SPECIFICITY WITH HSV 2 WESTERN BLOT POSITIVES
An outside investigator at a Pacific Northwest University assessed the type specificity using HSV 2 Western Blot positive and HSV 1 Western Blot negative sera from the above-described populations (n = 56): expectant mothers, sexually active adults, low prevalence persons, and HSV 2 culture positives. Of 56 HSV 2 Western Blot positive and HSV 1 Western Blot negative samples, Diagnostic Automation Inc. ELISA was 54 negative and 2 positive.

Type Specificity with HSV 2 Western Blot Positives (n = 56)

Characteristic	% (EL/WB)*	95% Confidence Interval (CI)
Type-specificity relative to WB	96.4% (54/56)	87.7-99.6%
Type cross-reactivity to WB	3.6% (2/56)	0.43-12.3%

AGREEMENT WITH THE CDC PANEL
A CDC serum panel was obtained and tested by Diagnostic Automation Inc. The results of this testing are presented as a means to convey further information on the performance of this assay with a masked, characterized serum panel. This does not imply an endorsement of the assay by the CDC.

The panel consisted of 58.0% HSV 1 positive and 42.0% HSV 1 negative specimens. The Diagnostic Automation Inc. HSV 1 IgG ELISA demonstrated 96.0% total agreement with the CDC results. Of the results obtained by Diagnostic Automation Inc, there was 93.1% agreement with all HSV 1 positive specimens (This includes sera that are positive for HSV 1 only and sera that are positive for both HSV 1 and HSV 2.). There was 100.0% agreement with the specimens that were HSV 2 positive only and 100.0% agreement with the specimens that were HSV negative for both 1 and 2.

PRECISION
The Diagnostic Automation Inc. HSV 1 IgG ELISA was evaluated for precision by testing six sera ten times each (within one plate) on three different days at three different sites, a Southwest Reference Laboratory, a Mid-Atlantic Health

Department, and the manufacturer. The results are summarized in Tables 1-4 below.

Table 1
HSV 1 IgG Intra and Inter Assay Precision
Study Site 1

	Assay 1 (n=10)			Assay 2 (n=10)			Assay 3 (n=10)			Inter Assay (n=30)		
Serum ID	X	S.D.	CV%	X	S.D.	CV%	X	S.D.	CV%	X	S.D.	CV%
1	2.84	0.109	4%	2.71	0.239	9%	2.81	0.152	5%	2.79	0.179	6%
2	5.28	0.113	2%	5.08	0.280	6%	5.36	0.146	3%	5.24	0.222	4%
3	3.54	0.077	2%	3.40	0.073	2%	3.62	0.096	3%	3.52	0.122	3%
4	2.27	0.117	5%	2.11	0.107	5%	2.23	0.067	3%	2.210	0.118	5%
5	0.15	0.039	26%	0.12	0.027	23%	0.12	0.013	11%	0.13	0.032	24%
6	0.09	0.036	42%	0.05	0.015	32%	0.03	0.007	21%	0.06	0.031	55%

Table 2
HSV 1 IgG Intra and Inter Assay Precision
Study Site 2

	Assay 1 (n=10)			Assay 2 (n=10)			Assay 3 (n=10)			Inter Assay (n=30)		
Serum ID	X	S.D.	CV%	X	S.D.	CV%	X	S.D.	CV%	X	S.D.	CV%
1	3.40	0.109	3%	3.32	0.196	6%	2.75	0.118	4%	3.16	0.141	4%
2	6.44	0.261	4%	6.41	0.261	4%	6.30	0.337	5%	6.39	0.286	4%
3	4.22	0.127	3%	4.20	0.136	3%	4.01	0.098	2%	4.6	0.121	3%
4	2.70	0.091	3%	2.68	0.097	4%	2.67	0.081	3%	2.68	0.090	3%
5	0.20	0.014	7%	0.19	0.011	6%	0.18	0.009	5%	0.19	0.011	6%
6	0.24	0.032	13%	0.23	0.030	13%	0.23	0.031	13%	0.24	0.025	13%

Table 3
HSV 1 IgG Intra and Inter Assay Precision
Study Site 3

	Assay 1 (n=10)			Assay 2 (n=10)			Assay 3 (n=10)			Inter Assay (n=30)		
Serum ID	X	S.D.	CV%	X	S.D.	CV%	X	S.D.	CV%	X	S.D.	CV%
1	2.82	0.107	4%	2.75	0.101	3%	3.05	0.141	4%	2.87	0.172	6%
2	5.31	0.103	2%	5.07	0.237	4%	5.46	0.127	2%	5.28	0.228	4%
3	3.47	0.063	2%	3.25	0.186	5%	3.51	0.207	6%	3.41	0.196	6%
4	2.08	0.066	3%	2.08	0.069	3%	2.12	0.056	2%	2.09	0.064	3%
5	0.19	0.040	2%	0.22	0.020	9%	0.22	0.006	3%	0.21	0.029	14%
6	0.17	0.013	8%	0.17	0.007	4%	0.16	0.010	6%	0.16	0.011	7%

Table 4
HSV 1 IgG Inter Site Precision

	Site 1	Site 2	Site 3			
Sample ID	X	X	X	X	S.D.	C.V.
1	2.79	3.16	2.87	2.94	0.195	7%
2	5.24	6.39	5.28	5.64	0.653	12%
3	3.52	4.16	3.41	3.70	0.405	11%
4	2.20	2.68	2.09	2.32	0.314	14%
5	0.13	0.19	0.21	0.18	0.042	24%
6	0.06	0.24	0.16	0.15	0.090	59%

X = Mean ISR Value
S.D. = Standard Deviation
C.V. = Coefficient of Variation

CROSS-REACTIVITY WITH TAXONOMICALLY RELATED VIRUSES

A study was performed by the manufacturer to determine the cross-reactivity of the Diagnostic Automation Inc. HSV 1 IgG ELISA test with 31 sera containing IgG antibody to taxonomically similar viruses including Cytomegalovirus (CMV), Varicella-Zoster Virus (VZV), and Epstein-Barr Virus (EBV). Of the 31 sera, 5 tested positive for CMV IgG by ELISA, 25 tested positive for VZV IgG by ELISA, and 30 tested positive for EBV IgG by ELISA. All 31 sera were negative by the Diagnostic Automation Inc. HSV 1 IgG ELISA indicating that antibodies to these viruses do not crossreact with the Diagnostic Automation Inc. HSV 1 IgG ELISA.

Because minimal cross-reactivity performance testing was done, each laboratory should consider performing testing on taxonomically related viruses and viruses, which could cause a syndrome similar to HSV such as HPV and gonorrhea. The levels tested should exceed ISR values of 3.10.

A study was performed by the manufacturer to determine the cross-reactivity of the Diagnostic Automation Inc. HSV 1 IgG ELISA test with 39 sera containing IgG antibody to related pathogens including Measles, Rubella and Syphilis. Of the 39 sera, the same 25 tested positive for Measles IgG and Rubella IgG by ELISA, and 14 tested positive for Syphilis IgG by ELISA. All 39 sera were negative by the Diagnostic Automation Inc. HSV 1 IgG ELISA indicating that antibodies to these related pathogens do not cross-react with the Diagnostic Automation Inc. HSV 1 IgG ELISA.

LIMITATIONS OF THE ASSAY

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 values obtained from this assay are intended for laboratory diagnosis only. Each treating physician must interpret the results in light of the patient's history, physical findings and other diagnostic procedures.
3. Results obtained from immunocompromised individuals should be interpreted with caution.

4. Testing of potentially cross-reacting specimens with gonorrhea, human papilloma virus (HPV) and the vector (Baculovirus) has not been performed.
5. Icteric, lipemic, hemolyzed or heat inactivated sera may cause erroneous results and are not recommended to be used, since they could possibly affect tests results.
6. Individuals in the early stages of infection may yield negative results, as seroconversion may not yet have occurred. In this case where infection is suspected, a second sample should be taken 4 to 12 weeks later and tested in parallel with the original sample.[11,19]
7. Paired sera samples should be tested concurrently to check for seroconversion.
8. The presence of continuing antibodies cannot be used as a determination of therapy success or failure.
9. A positive result is not sufficient to distinguish between current and past infection. If acute infection is suspected the serum sample should be tested on an IgM specific test, or an intact lesion isolated in cell culture is also recommended. A single serum specimen is not sufficient to distinguish between a past and current infection.
10. Kit procedures or practices outside those in this package insert may yield questionable results.
11. The performance characteristics have not been established for any matrices other than serum.
12. The prevalence of the analyte will affect the assay's predictive value.
13. The performance characteristics for this assay have not been established for pediatric specimens.
14. Assay performance characteristics for visual result determination has not been established.
15. Performance characteristics for this device have not been established for newborn screening.
16. Testing should not be performed as a screening procedure for the general population. The predictive value of a positive or negative serologic result depends on the pretest likelihood of disease being present. Testing should only be done when clinical evidence suggests the diagnosis of disease.

STORAGE CONDITIONS

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 micro assay 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 between 2 and 8°C.
4. Store the Calibrator, High Positive Control, Low Positive Control and Negative Control between 2 and 8°C.
5. Store Serum Diluent Type I and 20X Wash Buffer Type I between 2 and 8°C.
6. Store the Chromogen/Substrate Solution Type I between 2 and 8°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 one 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.

PRECAUTIONS

1. **For *in vitro* diagnostic use.**
2. Human samples and blood-derived products may be routinely processed with minimum risk using the procedures described. Human source components of this device were tested and found negative for anti-HIV (types 1 and 2), anti-HCV, and HBsAg by FDA recommended (approved/licensed) tests. Because no test method can offer complete assurance that laboratory specimens do not contain HIV, hepatitis B virus, or other infectious agents, specimens should be handled at the BSL 2 as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 3rd Edition, 1993 and NCCLS Approved Guideline M29-A, Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue.
3. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2. 19
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 I and Serum Diluent Type I. Do not mix with components from other manufacturers.
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 occur.
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 erroneous results.
11. When 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 during 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 solutions (bleach) to avoid formation of poisonous gas. Recommend disposing of reacted, stopped plates in biohazard bags. See Precaution 3.

18. The concentrations of anti-herpes simplex virus type 1 in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.
19. Performing the assay outside the time and temperature ranges provided may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.

The safety data sheet is available upon request.

WARNING
Serum Diluent, Conjugate, and Wash Buffer contain 0.1% ProClin 300R, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.
H317: May cause an allergic skin reaction.
P280: Wear protective gloves / protective clothing / eye protection / face protection.
P302 + **P352**: IF ON SKIN: Wash with plenty of soap and water.
P333 + **P313**: If skin irritation or rash occurs: Get medical advice/ attention.
P501: Dispose of contents and container in accordance to local, regional, national and international regulations.
WARNING
Serum Diluent and Controls contain < 0.1% sodium azide.
H302: Harmful if swallowed
P264: Wash thoroughly with plenty of soap and water after handling
P270: Do not eat, drink or smoke when using this product
P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell
P330: If swallowed, rinse mouth
P501: Dispose of contents/container to in accordance to local, regional, national and international regulations.

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MANUFACTURER AND BRAND DETAILS

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