ReQuest® HSV Type 1 Specific IgG

REF **01-410 96 Test Set**

IVD

For Prescription Use Only

Intended Use: For In Vitro Diagnostic Use Only. The ReQuest HSV Type 1 Specific IgG assay is an enzyme-linked immunosorbent assay (ELISA) intended for the qualitative detection of human IgG antibodies to type 1 herpes simplex virus (HSV) in human serum. The test is indicated for sexually active individuals and expectant mothers as an aid in the presumptive diagnosis of HSV-1 infection. The predictive value of a positive or negative result depends on the prevalence of HSV-1 infection in the population and the pre-test likelihood of HSV-1 infection.

The test is not FDA cleared for screening blood or plasma donors. The performance of this assay has not been established for immunocompromised patients, pediatric patients or matrices other than human serum.

Summary and Explanation of Test

Herpes simplex virus (HSV) is a ubiquitous pathogen of humans. HSV infects neonates, children and adults, and by the fourth decade of life, more than 90% of the population demonstrates antibodies to the virus (1). HSV transmission can occur through direct contact with infected secretions from either symptomatic or asymptomatic individuals.

There are two immunologically related types of herpes simplex virus. Historically, Type 1 HSV has been associated with oral infections and Type 2 HSV with genital infections. The association between virus type and site of infection is by no means specific however, and both types of HSV have been isolated from either oral or genital infections (3).

Serological tests which detect the presence of IgG antibodies to HSV provide information regarding history of previous infection. IgG antibodies to HSV detected in newborns are most likely of maternal origin, inasmuch as maternal IgG crosses the placenta.

The ReQuest HSV Type 1 Specific IgG test has been designed to detect IgG antibody directed against Type 1 HSV by the use of the type specific gG-1 protein of HSV-1 in an ELISA format. Test results are obtained after three thirty minute incubations.

Principle of the Test

Diluted patient samples are incubated in antigen-coated wells. HSV Type 1 antibodies, if present in the patient sample, are immobilized in the wells by binding to the antigen. Residual sample is eliminated by washing and draining, and conjugate (enzyme-labeled antibodies to human IgG) is added and incubated. If IgG antibodies to HSV-1 are present, the conjugate will be immobilized in the wells. Residual conjugate is eliminated by washing and draining, and the substrate is added and incubated. In the presence of the conjugate, the substrate is converted to a yellow end-product which is read photometrically.

Reagents

Coated Wells	Coated with recombinant HSV gG 1 antigen. 12 eight-well strips.
Well Support	One
Diluent*	25 ml (pink color). Contains a protein stabilizer.
Calibrator*	0.3 ml. Human serum. Strongly reactive for HSV Type 1 Specific IgG antibodies. Index values shown on vial label.
Positive Control*	0.3 ml. Human serum. Reactive for HSV Type 1 antibodies. Index values shown on vial label.
Negative Control*	0.3 ml. Human serum. Non-reactive for HSV Type 1 antibodies.
Conjugate	12 ml (green color). Goat anti-human IgG labeled with Alkaline Phosphatase (calf)
Substrate	12. p-nitrophenyl phosphate

Note: The substrate may develop a slight yellow color during storage. One hundred microliters of substrate should yield an absorbance value less than 0.35, when read in a microwell against air or water.

Wash Concentrate*	30 ml. Prepare Wash Solution by adding the contents of the Wash Concentrate bottle to 1 liter of distilled or deionized water. The diluted material, if kept at room temperature, must be used within 15 days after preparation.
Stop Reagent	12 ml.

* Contains sodium azide.

Store these reagents according to the instructions on the bottle labels. Do not allow them to contact the skin or eyes. If contact occurs, wash with copious amounts of water.

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Other Materials Required But Not Supplied

1. Wash bottle
2. Pipettors for dispensing 4, 100 and 200 µl
3. Timer
4. 1 or 2 L container for Wash Solution
5. Distilled or deionized water
6. Dilution tubes or microwells
7. Microwell reader capable of reading absorbance at 405 nm.

Warnings and Precautions

1. For in vitro diagnostic use.
2. Test samples, Calibrator(s), Controls and the materials that contact them, should be handled as potential biohazards. The calibrators and controls have been found to be negative for HIV, hepatitis B surface antigen and HCV antibodies by licensed tests. However, because no method can offer complete assurance that HIV, hepatitis B virus, HCV or other infectious agents are absent, these materials should be handled at the Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual "Biosafety in Microbiological and Biomedical Laboratories", 5th Edition, 2007 and CLSI / NCCLS Approved Guideline, "Protection of Laboratory Workers from Occupationally Acquired Infections", 3rd Edition.
3. The concentrations of HSV Type 1 Specific IgG antibody in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.
4. Avoid contact with open skin.
5. Never pipette by mouth.
6. Multiple test reagents contain sodium azide. Azides are reported to react with lead and copper in plumbing to form explosive metal azides. When disposing of solutions containing sodium azide, flush drains with large volumes of water to minimize the build-up of metal-azide compounds.
7. Do not interchange reagents from different reagent lots, except for Wash Concentrate, Substrate and Stop Reagent.
8. Do not use reagents beyond their stated expiration date.
9. Incubation times recommended in the Test Procedure section should be adhered to.
10. Unused Coated Wells should be kept in their re-sealable bag with desiccant, and stored in the refrigerator.

Specimen Collection

If serum specimens are not tested within 8 hours, they should be stored at 2 to 8° C for up to 48 hours. Beyond 48 hours specimens should be stored at -20° C. or below. Serum specimens stored for up to seventeen months at -20° C., showed no significant changes in index values upon retest. Samples may be frozen and thawed once. Samples that are hemolyzed, icteric, lipidemic or grossly contaminated samples or contain visible particulate matter should not be used. Samples should not be heat-inactivated before testing.

Test Procedure

Allow all reagents and patient samples to reach room temperature before use. Return them promptly to refrigerator after use. The test procedure follows:

1. Prepare 1:51 dilutions of test samples, Calibrator, Positive and Negative Controls, in the test set Diluent. For example: add 4 µl of sample to 200 µl of Diluent in a dilution well or tube, and mix well.
2. Place an appropriate number of Coated Wells in the Well Support.
3. Transfer 100 µl of each diluted Calibrator, Control and patient sample to the wells.

Note: Include one well which contains 100 µl of Diluent only. This will serve as the reagent blank and will ultimately be used to zero the photometer before reading the test results.

4. Incubate the wells at room temperature (20 to 25 °C) for 30 ± 5 minutes.
5. Wash wells four times, drain thoroughly.
6. Place 100 µl of Conjugate into each well.
7. Incubate the wells at room temperature for 30 ± 5 minutes.
8. Wash the wells four times, drain thoroughly.
9. Place 100 µl of Substrate into each well.
10. Incubate at room temperature for 30 ± 5 minutes.
11. Place 100 µl of Stop Reagent into each well.
12. Read and record the absorbance of the contents of each well at 405 nm against the reagent blank. For dichromatic instruments use reference filters 620-640. Adjust the photometer to zero absorbance against the reagent blank. Readings should be made within 2 hours after the reactions have been stopped.

Calculation of Results

Determine the Index value for each test sample (or Control) using the following formula:

$$\frac{\text{Calibrator Index}}{\text{Calibrator Absorbance}} \times \text{Test Sample Absorbance} = \text{Test Sample Index}$$

If the Calibrator is run in duplicate, use the average absorbance value to calculate results.

Test Validation Criteria (Quality Control)

1. The Calibrator, Positive and Negative Controls must be included in each test run.
2. The absorbance value of the Calibrator must be at least 0.15, when read against the reagent blank.
3. The absorbance value of the reagent blank should be less than 0.35.
4. The Negative Control must have an Index value less than 0.9.
5. The Positive Control must have an Index value within the range printed on the label.

Cut-off determination:

The ReQuest HSV Type 1 Specific IgG test cut-off value was initially obtained when 139 serum specimens shown to be negative by another commercial test were assayed by the ReQuest HSV Type 1 Specific IgG test. The cut-off value was subsequently challenged using a panel of 100 masked and coded serum specimens, which had been well characterized by EIA tests and western blots.

Interpretation of Results

Index	Result	Interpretation
≤ 0.9	Negative	No HSV-1 IgG antibodies detected. Patient is presumed not to have had a previous HSV-1 infection.
$0.9 < X < 1.0$	Equivocal	Obtain an additional sample for re-testing
≥ 1.0	Positive	IgG antibody to HSV-1 detected.

Notes:

1. A single positive result only indicates previous immunologic exposure; the level of antibody response may not be used to determine active infection or disease stage.

2. When equivocal results are obtained, another specimen should be obtained ten to fourteen days later, and tested in parallel with the initial specimen. If the second specimen is also equivocal, the patient is negative for primary or recent infection, and equivocal for antibody status. If the second sample is positive, the patient can be considered to have previous experience with HSV-1 infection.

3. Values obtained with different manufacturer's assay methods may not be used interchangeably. The magnitude of the reported IgG index value cannot be correlated to an endpoint titer. The magnitude of results above the cut-off is not an indicator of total antibody present.

Limitations

The results obtained with the ReQuest HSV Type 1 Specific IgG test serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.

Serological procedures for HSV are not intended to replace viral isolation and identification. The results of serological tests should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

A negative serological test does not exclude the possibility of past infection. Following primary HSV infection, antibody may fall to undetectable levels and then be boosted by later clinical infection with the same, or heterologous virus type. Such an occurrence may lead to incorrect interpretations of seroconversion and primary infection, or negative antibody status. In addition, samples obtained too early during primary infection, may not contain detectable antibody. Some persons may fail to develop detectable antibody after herpes simplex virus infection.

The performance characteristics of the ReQuest HSV Type 1 Specific IgG test have not been established with immunocompromised patients, pre-transplant patients, neonatal or cord blood, or matrices other than serum.

Specific antibodies to either type of HSV do not confer immunity, and will not protect against future infections.

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Interference by RF, ANA and HAMA has not been assessed, and therefore the user is responsible for the evaluation.

Limited Cross-reactivity has been evaluated in specimens containing antibodies to Human Papilloma Virus, Chlamydia trachomitis, and Neisseria gonorrhea. Caution should be used when interpreting positive results in patients with these antibodies.

Expected Values

The performance of the ReQuest HSV Type 1 Specific IgG test was assessed with masked, archived, unselected sera from 164 sexually active adults and from 242 expectant mothers. The reference method was a commercial Immunoblot test. The observed prevalence and the hypothetical predictive values for the two populations are shown below in Tables 1 and 2. The positive predictive value will decrease proportionally with the prevalence of HSV infection as shown below in Table 2. The calculations were based on the ReQuest HSV Type 1 Specific IgG test having a sensitivity of 92.3% and a specificity of 91.7% for the sexually active adults; and a sensitivity of 93.3% and a specificity of 89.4% for the expectant mothers.

Table 1 Observed Prevalence of HSV 1 IgG Antibodies in Sexually Active Adults and Expectant Mothers

Population	Sero-Status	Observed Prevalence
		ReQuest HSV 1 Type Specific IgG
Sexually Active Adults	Negative	47.0% (77/164)
	Positive	53.0% (87/164)
Expectant Mothers	Negative	48.8% (118/242)
	Positive	51.2% (124/242)

Table 2 Observed Prevalence of HSV 1 IgG Antibodies in Sexually Active Adults (N=164)

Age in Years	Gender	Positive		Equivocal		Negative		Total
		N	%	N	%	N	%	
18-20	F	7	39	0	0	11	61	18
	M	1	20	1	20	3	60	5
21-30	F	21	50	1	2	20	48	42
	M	4	40	0	0	6	60	10
31-40	F	12	60	0	0	8	40	20
	M	3	30	1	10	6	60	10
41-50	F	17	77	0	0	5	23	22
	M	5	38	1	8	7	54	13
51-60	F	8	73	0	0	3	27	11
	M	4	80	0	0	1	20	5
61-70	F	3	75	0	0	1	25	4
	M	1	33	0	0	2	67	3
71-80	F	1	100	0	0	0	0	1
	M	0	0	0	0	0	0	0
81-89	F	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0
Total		87	53	4	2	73	45	164

Table 3 Observed Prevalence of HSV 1 IgG Antibodies in Expectant Mothers (N=242)

Age in Years	Positive		Equivocal		Negative		Total
	N	%	N	%	N	%	
18-20	10	46	0	0	12	54	22
21-30	71	48	1	1	76	51	148
31-40	40	59	2	3	26	38	68
41-50	3	75	0	0	1	25	4
Total	124	51	3	1	115	48	242

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Table 4 Prevalence versus Hypothetical Predictive Values

Prevalence	Sexually Active Adults		Expectant Mothers	
	PPV	NPV	PPV	NPV
50%	91.7%	92.2%	89.7%	93.0%
40%	88.1%	94.6%	85.4%	95.2%
30%	82.7%	96.5%	79.0%	96.9%
25%	78.8%	97.3%	75.6%	97.5%
20%	73.5%	97.9%	68.8%	98.2%
15%	66.2%	98.5%	60.8%	98.7%
10%	55.3%	99.1%	49.4%	99.2%
5%	36.9%	99.6%	31.7%	99.6%

Comparative Testing

One hundred and sixty-four serum samples, from sexually active adults, that were submitted for HSV serology to a clinical laboratory in the Southeastern United States, were prospectively collected, masked, archived, and tested using the ReQuest HSV Type 1 Specific IgG test and a commercial HSV 1 Immunoblot test. The results of this comparative test are shown in Table 5 below.

Table 5

Relative Sensitivity and Specificity of the ReQuest HSV 1 Type Specific IgG Test, in Comparison with an HSV 1 Immunoblot Test, in Parallel Tests of 164 Sexually Active Adults Whose Sera Had Been Submitted for Herpes Simplex Virus Serology.

ReQuest HSV 1 Type Specific IgG Result						
	Positive	Equivocal	Negative		% Agreement*	95% C.I.*
HSV 1 Immunoblot Result						
Positive	84	1	6	Sensitivity	92.3	85.0 to 96.2
Negative	3	3	67	Specificity	91.7	83.2 to 96.2

Two hundred and forty-two serum samples, from expectant mothers, that were submitted for HSV serology to clinical laboratories in the Northeastern and Southeastern United States, were prospectively collected, masked, archived, and tested using the ReQuest HSV Type 1 Specific IgG test and a legally marketed, HSV 1 Immunoblot test. One hundred and ninety-eight (82%) of the specimens were obtained during the first trimester, nineteen (8%) during the second trimester and twenty-five (10%) during the third trimester of pregnancy. The results of this comparative test are shown in Table 6 below.

Table 6

Relative Sensitivity and Specificity of the ReQuest HSV 1 Type Specific IgG Test, in Comparison with a Commercial HSV 1 Immunoblot Test, in Parallel Tests of 242 Expectant Mothers Whose Sera Had Been Submitted for Herpes Simplex Virus Serology.

ReQuest HSV 1 Type Specific IgG Result						
	Positive	Equivocal	Negative		% Agreement*	95% C.I.*
HSV 1 Immunoblot Result						
Positive	111	3	5	Sensitivity	93.3	87.3 to 96.6
Negative	13	0	110	Specificity	89.4	87.1 to 93.7

CDC Panel Results

The following information was obtained with the Centers for Disease Control and Prevention (CDC) serum panel for HSV serology assays, which was tested in-house by the ReQuest HSV Type 1 Specific IgG test. The results are presented as a means to convey further information on the performance of this assay with a masked, well characterized serum panel. This does not imply an endorsement by the CDC.

Percent Agreement with the CDC Panel
The panel consists of 46 HSV-1 IgG positive and 54 HSV-1 IgG negative samples. The results of this study are shown below in Table 7.

Table 7 ReQuest HSV Type 1 Specific IgG Results Obtained with the CDC HSV Panel of 100 Sera

		ReQuest HSV Type 1 Specific IgG			
		Positive	Equivocal	Negative	Total
CDC HSV 1 Result	Positive	42	2	2	46
	Negative	0	1	53	54
	Total	42	3	55	100

Reproducibility/Precision

Six serum specimens (2 negative, 1 equivocal and 3 positive) and the ReQuest HSV Type 1 Specific IgG Positive and Negative Controls, were assayed in triplicate, on three separate occasions, at Quest International and at two external independent laboratories. The results are summarized below in Table 8.

Table 8 Results of Intra-assay, Inter-assay and Inter-laboratory Precision Tests Performed at Quest International and at Two External, Independent Laboratories. The Means, Standard Deviations and Coefficients of Variation were calculated from The ReQuest Index values.

Name of Analyte Panel Member	Sample N	Mean Index	Intra-assay		Inter-assay		Inter-laboratory		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%
ReQuest Positive Serum Control	27	2.0	0.12	5.8	0.13	7.2	0.17	8.5	0.14	7.2
ReQuest Negative Serum Control	27	0.3	0.03	10.1	0.08	23.7	0.12	39.0	0.08	24.2
Negative Sample # 1	27	0.3	0.02	7.0	0.03	10.7	0.12	43.0	0.05	20.1
Negative Sample # 2	27	0.5	0.01	2.6	0.03	5.6	0.09	17.7	0.04	8.7
Equivocal Sample	27	1.0	0.08	8.6	0.10	10.7	0.15	15.1	0.11	11.4
Positive Sample # 1	27	1.8	0.12	6.7	0.16	8.9	0.23	12.7	0.17	9.5
Positive Sample # 2	27	1.9	0.11	5.8	0.18	9.5	0.21	11.2	0.17	8.8
Positive Sample # 3	27	2.6	0.11	4.3	0.20	7.6	0.43	16.6	0.25	9.5

Cross Reactivity

A cross-reactivity study was performed to determine if samples from various disease states and other potentially cross-reacting agents interfere with the test results. The samples were determined to be positive for IgG antibodies directed against taxonomically related viruses and other related pathogens by other legally marketed devices. Human Papilloma Virus, Chlamydia trachomitis, and Neisseria gonorrhea samples were from individual patients with confirmed sexually transmitted infections. The samples were also tested for Type 1 HSV antibody by another legally marketed device. Only those samples that tested negative for Type 1 HSV antibody by the legally marketed device were included in the study. The results of this study are shown below in Table 9.

Table 9. Results of ReQuest HSV Type 1 Specific IgG Tests of Samples Which Tested Positive for Antibodies Directed Against Taxonomically Related Viruses and Other Viruses and Pathogens but Negative for Type 1 HSV by Other Legally Marketed Devices.

Samples	Number of Samples	Number of Samples Testing Positive in the ReQuest HSV 1 Type Specific IgG Test
HSV 2 IgG	10	0/10
CMV IgG	11	0/11

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EBV EBNA IgG	13	0/13
EBV VCA IgG	17	0/17
VZV IgG	19	0/19
Measles IgG	20	0/20
Rubella IgG	15	0/15
Toxoplasma IgG	6	0/6
Syphilis IgG	4	0/4
Human Papilloma Virus	2	1/2
Chlamydia trachomitis	1	0/1
Neisseria gonorrhea	3	1/3

Interference

The effects of icterus, hemolysis, hyperglycemia, hyperlipidemia and hyperproteinemia on the test results were examined. Samples that were negative, weakly positive and moderately positive for antibodies to Type 1 HSV were tested with and without the addition of elevated levels of the following potential interfering substances: hemoglobin 18 g/dL, glucose 800 mg dL, cholesterol 2,720 mg/dL, globulin 28 g/dL, unconjugated bilirubin 20 mg/dL, conjugated bilirubin 20 mg/dL, human albumin 12 g/dL and ascorbic acid 3mg/dL.

No significant interference was observed in the presence of abnormally elevated levels of the potentially interfering substances tested, however the use of grossly hemolyzed, icteric or lipemic samples, as well as samples containing particulate matter or exhibiting obvious microbial contamination is not recommended and they should not be tested.

References

1. Aurelian, L. Herpes Simplex Viruses. Clinical Virology Manual, 2nd Edition, Specter, S. and G. eds. New York, Elsevier 473-497, 1992.

2. Nahmias, A.J., and Josey, W.E., Epidemiology of Herpes Simplex Viruses 1 and 2. In Viral Infections of Humans, Epidemiology and Control (Evans, A.S. ed.), New York, Plenum Medical Book Co., 253-271, 1976.

3. Nahmias, A.J., Dannenbarger, J., Wickliffe, C., and Muther, J., Clinical Aspects of Infection with Herpes Simplex Viruses 1 and 2. In The Human Herpes Viruses. An Interdisciplinary Perspective, Nahmias, A.J., Dowdle, W.R. and Schinazi, R.F. eds., New York, Elsevier, 3-9, 1981.

4. Maza, de la, L.M. and Peterson, E.M., Med. Clin. N. Am., 67: 1059-1073, 1983.

5. Gardner, M.J. and Altman, D.G., Confidence Intervals Rather Than Hypothesis Testing. Brit. Med. J., 292: 746-750, 1986.

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Symbols Glossary

Symbol	Standard Title and Number	Title of Symbol	Symbol reference #	Explanatory Text
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Manufacturer	5.1.1	Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Authorized representative in the European Community	5.1.2	Indicates the Authorized representative in the European Community.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Use-by-date	5.1.4	Indicates the date after which the medical device is not to be used.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Batch code	5.1.5	Indicates the manufacturer's batch code so that the batch or lot can be identified.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Catalog number	5.1.6	Indicates the manufacturer's catalogue number so that the medical device can be identified.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Temperature limit	5.3.7	Indicates the temperature limits to which the medical device can be safely exposed.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Consult instruction for use	5.4.3	Indicates the need for the user to consult the instructions for use.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	*In vitro* diagnostic medical device	5.5.1	Indicates a medical device that is intended to be used as an *in vitro* diagnostic medical device.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Contains sufficient for 96 tests	5.5.5	Indicates the total number of IVD tests that can be performed with the IVD kit reagents.
Rx Only	Guidance for Industry and FDA on Alternative to Certain Prescription Device Labeling Requirements	Rx Only	N/A	Caution: Federal law prohibits dispensing without prescription.

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