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IMMUNOWELL™

HERPES SIMPLEX VIRUS (HSV)TYPE 2 (RECOMBINANT PROTEIN) IGG TEST

Product No. 3590

IVD For *In Vitro* Diagnostic Use

SEE CALIBRATION VALUES, TABLE 2, PAGE 3

INTENDED USE

The test is an ELISA method for the qualitative detection of IgG antibody to herpes simplex virus (HSV) glycoprotein G antigen (gG) in human serum. In conjunction with ImmunoWELL Herpes Simplex Virus Type 1 IgG Test, the test is indicated for testing sexually active adults or expectant mothers for aiding in the presumptive diagnosis of HSV infection.

SUMMARY AND EXPLANATION

There are two herpes antigenic types (1) (2). "Definitive diagnosis of genital herpes infections is fundamental to the management of patients and the development of strategies to prevent transmission to partners and neonates" (3). Such diagnosis has proven inaccurate when based solely on clinical history and impression (4). Instead, virus, antigen or nucleic acid detection and classification is used for patients presenting with lesions or type-specific serological tests may be used when lesions are absent.

For type specific serology, either western blot (5) (6) (7) or assays or type specific protein (8) (9) are used. Acceptable type specific classification is not possible using whole virus lysate, the commonly used antigen of early HSV serology kits. The most commonly used type specific protein is glycoprotein G. ImmunoWELL HSV typing tests use HSV gG type 1 and type 2 recombinant proteins.

ASSAY PRINCIPLE

The ImmunoWELL Test utilizes an EIA microtiter plate technique for the detection of antibodies. Serum is added to antigen coated microtiter wells and allowed to react. After removal of unbound antibodies, horseradish peroxidase-conjugated antihuman IgG antibodies are allowed to react with bound antibodies. The bound peroxidase reacts with tetramethylbenzidine (TMB), the chromogenic substrate, developing a color. Finally, the substrate reaction is stopped and the optical density is read with a micro-well spectrophotometer.

REAGENTS

Reaction Wells coated with type specific HSV-2 gG recombinant protein.
Specimen Diluent - 0.01 M phosphate buffered saline (PBS, pH 6.2-7.6) and carrier protein and <0.1% NaN_3
HSV-2 IgG Calibrators (3) - human anti-gG prediluted, ready for use in Specimen Diluent
HSV-2 IgG Positive Control - human anti-gG serum containing <0.1% NaN_3
HSV-2 Negative Control - nonreactive human serum containing <0.1% NaN_3
Wash Buffer Concentrate - a 20X concentrate of 0.01 M PBS (pH 6.2-7.6) and 0.05% Tween
Conjugate - peroxidase-conjugated goat antihuman IgG in PBS (pH 6.2-7.6) and carrier protein containing preservatives
Substrate - Contains tetramethylbenzidine (TMB)
Stop Solution - 0.5 N Hydrochloric acid

WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use: ImmunoWELL reagents have been optimized for use as a system. Do not substitute other manufacturers' reagents or other ImmunoWELL Test reagents. Dilution or adulteration of these reagents may also affect the performance of the test. Do not use any kits beyond the stated expiration date. Close adherence to the test procedure will assure optimal performance. Do not shorten or lengthen stated incubation times since this may result in poor assay performance.

Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. It may be harmful if enough is ingested (more than supplied in kit). On disposal of liquids, flush with a large volume of water to prevent azide build-up (10). This dilution is not subject to GHS, US HCS and EU Regulation 2008/1272/EC labeling requirements.

The safety data sheet (SDS) is available at support.genbio.com or upon request.

⚠ **Human source material**. Material used in the preparation of this product has been tested and found non-reactive for hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus (HCV), and antibodies to human immunodeficiency virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease (11). Follow recommended

Universal Precautions for bloodborne pathogens as defined by OSHA (12), Biosafety Level 2 guidelines from the current CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (13), WHO Laboratory Biosafety Manual (14), and/or local, regional and national regulations.

RECONSTITUTION AND STORAGE

Kit is stored at 2-8°C. Assuming good laboratory practices are used, opened reagents remain stable as indicated by the expiration date.
Reaction wells are removed from the foil pouch and unused wells are resealed in the pouch using the integral zip-lock.
Wash Buffer (pH 6.2-7.6) is prepared by adding the contents of the Wash Buffer Concentrate (20X) bottle into 1 liter of distilled/deionized water. After reconstitution, the 1X solution is stored at 2-8°C. Discard when visibly turbid.
Note: In some instances the Wash Buffer Concentrate (20X) may develop crystals upon storage at 2-8°C. It is important that these crystals are completely redissolved before dilution of the Concentrate. This can be accomplished by warming the Concentrate to 37°C in a water bath with occasional mixing.

SPECIMEN COLLECTION AND HANDLING

ImmunoWELL Test is performed on serum. The test requires 10 µL of serum. Lipemic or hemolyzed serum has not been shown an acceptable specimen.
Store samples at room temperature for no longer than eight hours. If the assay will not be completed within eight hours, refrigerate the sample at 2-10°C. If the assay or shipment of the samples will not be completed within 48 hours, freeze at -20°C.

MATERIALS PROVIDED

Microtiter Wells in Carrier
Positive Control
Negative Control
Wash Buffer Concentrate (20X)
Specimen Diluent
Calibrators
Conjugate
Substrate
Stop Solution

MATERIALS REQUIRED BUT NOT PROVIDED

Distilled or deionized water
Microwell washer
Microwell spectrophotometer (450 nm)
Test tubes
Pipets

PERFORMANCE CONSIDERATIONS

Reproducibility in the assay is largely dependent upon the consistency with which the microwells are washed. Carefully follow the recommended washing sequence as outlined in the assay procedure.
Positive and Negative Control Sera (Undiluted) are used to assure test performance.
Calibrators (prediluted) are used to construct a standard curve.
Substrate Blank - All reagents, except serum, are added to the substrate blank well. This blank well is intended to baseline (zero) the microwell spectrophotometer.

ASSAY PROCEDURE

1. Allow all components including diluted Wash Buffer to warm to room temperature (22-27°C).
2. Determine the total number of specimens to be run. Include one blank and duplicates of calibrators (or calibrator if using the normalizing calculation) and controls in each run.
3. For each control and specimen, pipet 10 µL serum into a clean tube containing 1 mL Specimen Diluent and mix (1:100 dilution).

CAUTION: CALIBRATORS ARE PREDILUTED. DO NOT DILUTE FURTHER.

4. Determine the total number of wells to be run including blank, calibrators, controls, and specimens. Well strips can be broken to the exact number needed to conserve reagent wells. Strips need to be completed with used wells to facilitate washing procedures.
5. Add 100 µL of Specimen Diluent into the first well as a substrate blank.
6. Pipet 100 µL of the prediluted calibrators and diluted controls and specimens (step 3) into each assigned well.
7. Incubate at room temperature (22-27°C) for 60±2 minutes.
8. Aspirate the samples out of the wells.
9. Wash the wells three times by completely filling the wells with Wash Buffer (see Reconstitution and Storage) and aspirating the wells completely after washes.
10. Pipet 100 µL Conjugate into all wells.
11. Incubate the wells at room temperature (22-27°C) for 30±2 minutes.
12. Aspirate the conjugate out of the wells.

13. Wash the wells three times as described in step 9.
14. Pipet 100 µL of Substrate into each well.
15. Incubate at room temperature (22-27°C) for 30±2 minutes.
16. Add 100 µL of Stop Solution to each well.
17. Inspect the outside bottom surface of the microwells for the presence of condensation, dried buffer salts or wash solution that might interfere with the spectrophotometric reading. Carefully clean the well bottoms with a soft tissue.
18. Using the substrate blank to zero the spectrophotometer, read the optical density of each well at 450 nm within 30 minutes of completion of step 16.

QUALITY CONTROL

GenBio provides positive and negative controls with defined ranges provided in Table 2 below. The positive control value is approximately five standard deviations (absorbance) above the upper cutoff and the negative control value is less than 0.15 absorbance units. Interpretations should not be made unless the control results fall within these limits.
NCCLS C24-A should be consulted for guidance on appropriate quality control practices. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

INTERPRETATION

PROCEDURE FOR CALCULATING ACTIVITY OF SPECIMEN

Activity of the specimen may be calculated in one of two ways:

1. Construct a point-to-point standard curve using the absorbance values you observe and their corresponding assigned values. Use this curve to calculate antibody concentration of controls and specimens.
2. Calculate activity of the specimen by normalizing to the Mid Calibrator according to the following:

   $V_S = A_S \times V_{MC}/A_{MC}$
   Where:
   V_S = Value of the specimen (U/mL)
   A_S = Absorbance of the specimen
   V_{MC} = Assigned Value of the Mid Calibrator (U/mL)
   A_{MC} = Mean absorbance of the Mid Calibrator obtained in the assay

Table 1: Interpretation

	Units/mL	Interpretation
Negative	<250	Specific Antibody not detected
Equivocal	250-300	Report as negative or retest. If retested, the second result is considered final. If the repeat test is also equivocal, report as equivocal.
Positive	>300	Specific antibody detected

Table 2: Calibration Values

	Values	Units
High Calibrator Assigned Value	1805	Units/mL
Mid Calibrator Assigned Value	768	Units/mL
Low Calibrator Assigned Value	134	Units/mL
Mid Calibrator Low Limit	0.2	Absorbance
Positive Control Expected Value	262-860	Units/mL
Negative Control Expected Value	<200	Units/mL

Results should not be interpreted if calibrator absorbance is below the low limit.

EXPECTED RESULTS

HSV IgG prevalence is primarily dependent on age, sexual activity and social economic status. It can range from near zero in the very young to above 50% (HSV, type 2) and above 90% (HSV, type 1) in older, sexually active subjects.

PERFORMANCE CHARACTERISTICS

Historically HSV IgG is detected using cell lystates containing all soluble antigens. Since most proteins are not type specific, this test method is typically used to identify past HSV infection, but does not reliably distinguish type 1 versus type 2 infections. Sera collected from eighty-four U.S. blood donors were evaluated using western blot methodology performed at the University of

Washington, Seattle, Washington, and an alternate gG typing EIA test kit. ImmunoWELL's relative sensitivity is 100% (32/32) and the specificity is 98% (51/52).

PRECISION DATA

Precision was determined by testing seven samples as triplicates in three different runs. Values are expressed as coefficient of variation (percent).

Table 3: Assay Precision

	Mean (units/mL)	Within Run	Between Run
A	1434	3%	3%
B	2027	2%	3%
C	622	2%	4%
D	1799	2%	5%
E	190	6%	9%
F	771	5%	7%
G	1462	4%	5%

BIBLIOGRAPHY

1. **Nahmias, A and Dowdle, W.** Antigenic and biologic differences in herpesvirus hominis. *Prog Med Virol.* 1968, Vol. 10, p. 110.
2. **Schnewels, K E.** Serologische untersuchungen zur typendifferenzierung des herpesvirus hominis. *Z Immunitaetsforsch.* 1962, Vol. 124, p. 24.
3. **Ashley, R L and Wald, A.** Genital herpes: review of the epidemic and potential use of type-specific serology. *Clin Microbiol Rev.* 1999, Vol. 12, 1, p. 1.
4. **Brown, Z A, et al.** A comparison between detailed and simple histories in the diagnosis of genital herpes complicating pregnancy. *Am J Obstet Gynecol.* 1995, Vol. 172, p. 1299.
5. **Bernstein, D I, Lovett, M A and Bryson, Y J.** Serologic analysis of first-episode nonprimary genital herpes simplex virus infection. *Am J Med.* 1984, Vol. 77, p. 1055.
6. **Ho, d WT, et al.** Indirect ELISA for the detection of HSV-2 specific IgG and IgM antibodies with blycoprotein G (gG-2). *J Virol Methods.* 1992, Vol. 36, p. 249.
7. **Holmberg, S D, et al.** Prior herpes simplex virus type 2 infection as a risk factor for HIV infection. *JAMA.* 1988, Vol. 259, p. 1048.
8. **McGeoch, D J, Moss, H W and McNab, D.** DNA sequence and genetic content of the HindIII region in the short unique component of herpes simplex virus type 2 genome: identification of the gene encoding glycoprotein G, and evolutionary comparisons. *J Gen Virol.* 1987, Vol. 68, p. 19.
9. **Rapoport, T A.** Protein translocation across and integration into membranes. *Crit Rev Biochem.* 1986, Vol. 20, p. 73.
10. **US Centers for Disease Control.** *Manual Guide – Safety Management No. CDC–22 Decontamination of Laboratory Sink Drains to Remove Azide Salts.* Atlanta : Centers for Disease Control, 1976.
11. —. *HHS Publication No. (CDC) 93-8395, 3rd ed: Biosafety in Microbiological and Biomedical Laboratories.* Washington DC : US Government Printing Office, 1993.
12. **US Department of Labor, Occupational Safety and Health Administration.** *29 CFR Part 1910.1030, Occupational safety and health standards, bloodborne pathogens.*
13. **US Department of Health and Human Services.** *HHS Publication No. (CDC) 21-11: Biosafety in Microbiological and Biomedical Laboratories. 5th ed.* Washington DC : US Government Printing Office, 2009.
14. **World Health Organization.** *Laboratory Biosafety Manual 3rd ed.* Geneva : World Health Organization, 1991.

QUICK REFERENCE PROCEDURE

IMMUNOWELL HSV-2 IGG

- Prepare Wash Buffer from Wash Concentrate.
- Dilute each control and specimen 1:100 in Specimen Diluent.
- Add 100 µL of Specimen Diluent into the first well as a substrate blank.
- Pipet 100 µL of the prediluted calibrators and diluted controls and specimens into coated microwells and incubate 60 minutes at room temperature.
- Aspirate microwells and wash microwells three times with Wash Buffer.
- Pipet 100 µL of Conjugate into microwells and incubate 30 min at room temperature.
- Aspirate microwells and wash microwells three times with Wash Buffer.
- Pipet 100 µL of Substrate into microwells and incubate 30 min at room temperature.
- Pipet 100 µL Stop Solution into microwells and read results at 450 nm.

To place an order for ImmunoWELL products, contact your local distributor, or call GenBio directly for the distributor nearest you and for additional product information.
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