IMMUNODOT™
HERPES SIMPLEX VIRUS TYPING

IVD For In Vitro Diagnostic Use

INTENDED USE

ImmunoDOT Herpes Simplex Virus (HSV) Typing test is an enzyme immunoassay (EIA) detecting HSV or glycoprotein G (gG) type specific IgG antibodies. The test detects the presence or absence of past HSV exposure and specifically determines whether past infection(s) is due to HSV Type 1, Type 2 or both Type 1 and 2.

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) is 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. ImmunoDOT HSV Typing test uses HSV gG type 1 and type 2 recombinant proteins.

ASSAY PRINCIPLE

The assay uses an enzyme-linked immunoassay (EIA) dot technique for the detection of antibodies to HSV-1 gG, HSV-1 virus lysate proteins, HSV-2 gG or HSV-2 virus lysate proteins. An assay strip is incubated with dilute patient serum, allowing patient antibodies reactive with the test antigens to bind to the solid support membrane. In the second stage, the reaction is enhanced by removal of non-specifically bound materials. During the third stage, alkaline phosphatase-conjugated antihuman antibodies are allowed to react with bound patient antibodies. Finally, the strip is transferred to enzyme substrate reagent which reacts with bound alkaline phosphatase to produce an easily seen, distinct dot.

REAGENTS

Assay Strip: Antigens in order beginning with the window next to the label: Positive reagent control (human serum); [Total 1} HSV-1, [Total 2] HSV-2 lysate, [Specific 1] HSV-1 gG, [Specific 2] HSV-2 gG and Negative reagent control

Diluent (#1): Consists of buffered diluent containing protein stabilizers with <0.1% NaN_3

Enhancer (#2): Consists of sodium chloride with <0.1% NaN_3

Conjugate (#3): Consists of alkaline phosphatase conjugated goat antihuman IgG (heavy chain specific) in buffered diluent with <0.1% NaN_3

Developer (#4): Consists of 5-bromo-4-chloro-3-indolyl phosphate and p-nitro blue tetrazolium chloride in buffered diluent with <0.1% NaN_3

HSV-1 Positive Control: Consists of HSV-1 IgG positive human serum that is HSV-2 negative containing protein stabilizers and <0.1% NaN_3

HSV-2 Positive Control: Consists of HSV-2 IgG positive human serum that is HSV-1 negative containing protein stabilizers and <0.1% NaN_3

Negative Control: Consists of HSV-1 and HSV-2 negative human serum containing protein stabilizers and <0.1% NaN_3

WARNINGS AND PRECAUTIONS

For In-Vitro Diagnostic Use. ImmunoDOT reagents have been optimized for use as a system. Do not substitute other manufacturers' reagents or other ImmunoDOT Assay System 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. Analytic quality water must be used. Close adherence to the test procedure will assure optimal performance. Do not shorten or lengthen stated incubation times since these 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.

STORAGE

Store reagents and assay strips at 2-8°C. Reagents must be at room temperature (15-30°C) before use. Avoid contamination of reagents. Assuming good laboratory practices are used, opened reagents remain stable as indicated by the expiration date.

SPECIMEN COLLECTION AND HANDLING

ImmunoDOT Test is performed on serum. The test requires 50 µL of serum. Lipemic or hemolyzed serum has not been shown to be 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

Assay Strips	Conjugate (#3)
Diluent (#1)	Developer (#4)
Enhancer (#2)	Reaction Vessels
HSV-1 Positive Control Serum	Negative Control Serum
HSV-2 Positive Control Serum	

MATERIALS REQUIRED BUT NOT PROVIDED

Workstation	Timer
Pipets	Specimen collection apparatus (e.g., finger sticking device, venipuncture equipment)
Analytic quality water	Absorbent toweling to blot dry assay strips

SET-UP

1. Turn on Workstation and adjust to appropriate temperature if necessary. Refer to Workstation Instructions.
2. Remove 4 Reaction Vessels per test from the product box and insert into appropriate slots in Workstation. For the large Workstation, add water up to the fill line of the provided rinse container. For the small Workstation, use an appropriate container and sufficient water to cover all reactive windows of the assay strip.
3. Place 2 mL Diluent (#1) in Reaction Vessel #1; 2 mL Enhancer (#2) in Reaction Vessel #2; 2 mL Conjugate (#3) in Reaction Vessel #3; and 2 mL Developer (#4) in Reaction Vessel #4.
4. Appropriately label the Assay Strips.
5. If the large Workstation is used, insert the label end of the assay strip into the Strip Holder, one per groove, taking care not to touch the assay windows.

ASSAY PROCEDURE

1. Add 50 µL serum to Reaction Vessel #1.
2. Prewet Assay Strip by immersing in water for 30-60 seconds.
3. Using several (5-10) quick up and down motions with the Assay Strip, mix thoroughly in Reaction Vessel #1. Let stand for 60-90 minutes.
4. Remove Assay Strip from Reaction Vessel and swish in the water. Use a swift back and forth motion for 5-10 seconds allowing for optimal washing of the Assay Strip's membrane windows.
5. Place Assay Strip into Reaction Vessel #2. Mix thoroughly with several (5-10) quick up and down motions. Let stand for 5 minutes.
6. Remove Assay Strip from Reaction Vessel #2 and swish in water as described (step #4).
7. Place Assay Strip into Reaction Vessel #3. Mix thoroughly with several (5-10) quick up and down motions. Let stand for 30-40 minutes.
8. Remove Assay Strip from Reaction Vessel #3 and swish in water as described (step #4). DO NOT remove the Assay Strip from the water.
9. Allow the Assay Strip to stand in the water for 5 minutes.
10. Remove Assay Strip from water and place into Reaction Vessel #4. Mix thoroughly with several (5-10) quick up and down motions. Let stand for 5 minutes.
11. Remove Assay Strip from Reaction Vessel #4 and swish in water as described (step #4).

12. Blot and allow Assay Strip to dry. It is imperative that tests of borderline specimens be interpreted after the Assay Strip has been allowed to dry. **A false positive dot may be identified if the assay strip is not dry when interpreted.**

READING THE ASSAY STRIP

Positive	A dot with an EASILY SEEN, distinct border is visible in the center of the window. The outer perimeter of the window must be white to pale gray.
Negative	If no dot is seen or a dot is difficult to see, interpret it as negative.

In order to minimize the possibility of "over interpreting" positive test results it is recommended that during initial validation of the assay (as may be required by the laboratory by regulation), the laboratory test a series of presumptive negative samples and each technician interpret the assay strips in a blinded fashion. Please call GenBio Technical Service for further clarification. (To report results, refer to Interpretation Section)

QUALITY CONTROL

The assay's reagent temperature is between 42-48°C. Due to heat transfer loss, the Workstation temperature is set higher. The appropriate Workstation temperature setting is listed in the Workstation's package insert. (Contact Technical Services for additional guidance if an alternate heat source is used.)

NCCLS C24-A may be consulted for guidance on appropriate quality control practices. These should be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. Unless otherwise required, it is recommended that control sera be tested upon receipt of a kit. If the control is not reactive, results should not be reported and GenBio Technical Service should be contacted before the kit is used again.

The kit uses reagent controls to assure performance each time a test is performed. The Positive Control window (well #1) contains human serum and tests reagent reactivity. It must be reactive but the intensity must not be used as a calibrator. As a negative reagent control check, the backgrounds around dots and the bottom window (#6) must be white. If the positive is not reactive or the negative is reactive, do not interpret the assay strip.

INTERPRETATION

Interpretation	Total 1	Total 2	Specific 1	Specific 2
Negative	-	-	-	-
HSV Type 1	+	+	+	-
HSV Type 2	+	+	-	+
HSV Type 1 & 2	+	+	+	+
HSV Positive Type Unknown	+	+	-	-

- All results from this and other serologies must be correlated with clinical history, epidemiological data and other data available to the attending physician in evaluating the patient.
- It is expected that some patient samples have both HSV Total 1 and 2 positive and that both Specific 1 and 2 negative. This indicates either a recent infection or that the patient never formed type specific antibody. If a recent infection, isolation, antigen or nucleic acid detection methods are indicated. Otherwise, another method such as western blot may be used. However, it is also possible that no type specific antibody is present and therefore typing is not possible.
- As with other serological tests, negative results do not rule out the diagnosis of herpes simplex disease.
- A single positive result only indicates previous immunologic exposure. Other methods such as nucleic acid, antigen or viral culture are required to establish current infection.
- It is unlikely that patterns other than presented will occur. Repeat the testing and if the pattern repeats, contact the manufacturer.

LIMITATIONS

The performance of this assay has not been established for ruling out diseases with similar symptoms, e.g., *Candida albicans*, *Bacteriodes* species, *G. vaginalis*, *Mobiluncus* species. Instead, also use culture or other appropriate methods.

EXPECTED RESULTS

It has been shown that gG specific antibody typically develops over the course of six months. Appearance is slower and later than antibodies to other, non-type specific proteins. Therefore, it is expected that some patients will be HSV antibody positive yet anti-gG negative.

Eighty-four sera collected from U.S. blood donors were tested using ImmunoDOT HSV Typing kit. These results are shown in Table 1.

Table 1

Interpretation	Percent
Negative	15.5%
HSV Type 1	36.9%
HSV Type 2	11.9%
HSV Type 1 & 2	32.1%
HSV Positive Type Unknown	3.6%

PERFORMANCE CHARACTERISTICS

Eighty-four sera collected from U.S. blood donors were tested using: ImmunoDOT HSV Typing kit [IDOT], an alternate commercial microtiter kit for specific detection of either type 1 or type 2 gG antibodies {Kit A], and two alternate commercial microtiter kits for detection of HSV lysate antibodies (Not able to specify type) [Kits B and C].

TOTAL (TRADITIONAL METHOD)

Comparison between ImmunoDOT Total 1 and 2 results and two commercial microtiter kits (Kits B and C) is shown in Table 2. Agreement is 100% and therefore sensitivity and specificity are 100%.

Table 2

Alternate Kit	ImmunoDOT	
	Negative	**Positive**
Negative	13	0
Positive	0	71

SPECIFIC (HSV SEROLOGY TYPE)

Comparison between ImmunoDOT Specific results and the alternate commercial type specific microtiter kit is shown in Table 3. One sample is HSV antibody negative using kits B and C, reported negative by ImmunoDOT but reported HSV Type 2 by the Alternate gG assay. This is considered a false positive and not shown in Table 3. Agreement between the two gG assays is 90% (75/83).

Table 3

ImmunoDOT	Alternate	Number
Negative	Negative	15 (18%)
Type 1	Type 1	32 (39%)
Type 2	Type 2	9 (11%)
Type 1 & 2	Type 1 & 2	22 (27%)
Type 1	Negative	0
Type 1	Type 2	0
Type 1	Type 1 & 2	0
Type 2	Negative	0
Type 2	Type 1	0
Type 2	Type 1 & 2	2 (2%)
Type 1 & 2	Negative	0
Type 1 & 2	Type 1	2 (2%)
Type 1 & 2	Type 2	0
Negative	Type 1	1 (1%)
Negative	Type 2	0
Negative	Type 1 & 2	0

There is 94% (78/84) agreement between the two methods. Assuming alternate test is correct (relative performance) is listed in Table 4.

Table 4

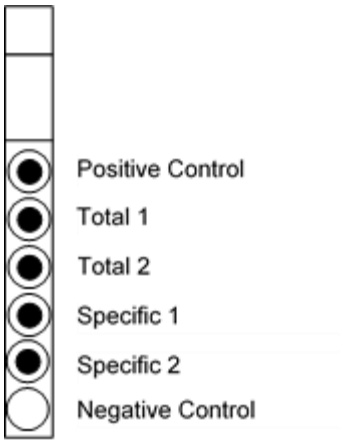
Interpretation	Relative Sensitivity	Relative Specificity
Negative	100% (15/15)	100% (15/15)
Type 1	95% (56/59)	100% (15/15)
Type 2	94% (31/33)	100% (15/15)

PRECISION

Like any visually interpreted test, dot intensity is directly related to precision. The darkest dots are most reliable while weaker reactions (less intensity) are proportionately less reliable (equivocal or borderline).

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

IMMUNODOT HSV TYPING

Set-Up

- Make sure Workstation is at temperature.
- Place reaction Vessels into slots in Workstation and add water to the rinse container.
- Place 2 mL Diluent (1) in Vessel #1; 2 mL Enhancer (2) in Vessel #2; 2 mL Conjugate (3) in Vessel #3; and 2 mL Developer (4) in Vessel #4.

Procedure

- Add 50 µL serum to Vessel #1.
- Prewet assay strip in water for 30 - 60 seconds.
- Place strip in Vessel #1, mix, let stand 60-90 min.
- Remove strip, place in water, swish 5-10 sec.
- Place strip in Vessel #2, mix, let stand 5 min.
- Remove strip, place in water, swish 5-10 sec.
- Place strip in Vessel #3, mix, let stand 30-40 min.
- Remove strip, place in water, let stand 5 min.
- Place strip in Vessel #4, mix, let stand 5 min.
- Remove strip, place in water, swish, blot, dry, and read

To place an order for ImmunoDOT products, contact your local distributor, or call GenBio directly for the distributor nearest you and for additional product information.
For assistance, please call toll-free 800-288-4368.

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