IMMUNODOT™
HERPES SIMPLEX VIRUS IGM TYPING

IVD For In Vitro Diagnostic Use

INTENDED USE

ImmunoDOT Herpes Simplex Virus (HSV) IgM Typing test is an enzyme immunoassay (EIA) detecting HSV or glycoprotein G (gG) type specific IgM antibodies. Unlike HSV IgG typing, IgM does not provide optimal HSV typing results, but may offer information about a particular patient's antibody response.

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 IgM 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 Lysate and HSV-1 gG), Total 2 (HSV-2 Lysate and HSV-2 gG), Specific 1 (HSV-1 gG), Specific 2 (HSV-2 gG), and Negative reagent control

Diluent (#1): Consists of buffered diluent containing IgG absorbent (goat antihuman IgG), 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 IgM (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

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

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

Low 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.
Positive	Significantly (strongly) reactive. This reactivity is defined by using our standardized assay strip. This reactivity is one greater than window (dot) #4.
Negative	If no dot is seen or a dot is difficult to see, interpret it as negative. Each window of the assay strip is read independently.

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. A Control Kit (800-3919) containing HSV-1 IgM Positive Control, HSV-2 IgM Positive Control, and Negative Control is available separately from GenBio.

INTERPRETATION

Caution: IgM typing is less accurate than IgG typing. Total results must be positive to interpret the specific result. Also, IgG must be positive to interpret a sample as IgM positive.

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

- IgM presence may indicate infection by either serotype or reactivation of a latent HSV (either serotype). For this reason, IgM testing is not recommended as a differential diagnostic, but may offer information about a particular patient's antibody response.
- IgM reactivity DOES NOT necessarily indicate current infection since reactivation may occur.
- Confirmation of infection requires virus isolation or nucleic and identification.
- As with other serological tests, negative results do not rule out the diagnosis of herpes simplex disease.
- It is unlikely that patterns other than presented will occur. Repeat the testing and if the pattern repeats, contact the manufacturer.

PERFORMANCE CHARACTERISTICS

Fifty-seven sera collected from U.S. blood donors were tested using ImmunoDOT HSV Typing kits. These results are shown in Table 1.

Table 1

Interpretation	Result
Negative	35 (61%)
HSV Type 1	8 (14%)
HSV Type 2	12 (21%)
HSV Type 1 & 2	2 (4%)
HSV Positive Type Unknown	0

To assure specificity, 80 pediatric samples were tested. All 80 samples reported negative using the recommended interpretation criteria; however, several samples did report IgM reactive but were not IgG positive. These data reinforce the need to use the IgG result when interpreting IgM reactivity.

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).

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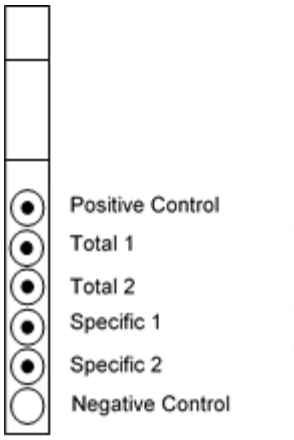

QUICK REFERENCE PROCEDURE

IMMUNODOT HSV-M 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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