IMMUNOFLOW™

HERPES SIMPLEX VIRUS (HSV) TEST

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IVD **For In Vitro Diagnostic Use**

INTENDED USE

ImmunoFLOW HSV Test detects IgG to HSV and HSV gG-2 (HSV type 2). The identification aids in the diagnosis of diseases caused by herpes simplex viruses and provides epidemiological information.

SUMMARY

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 are 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 type specific protein (8, 9) assays 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. ImmunoFLOW HSV uses HSV gG type 2 recombinant protein.

ASSAY PRINCIPLE

ImmunoFLOW is an immunoassay consisting of a cassette and three reagents. The cassette contains a paper matrix (for example, nitrocellulose) and an absorbent material. The paper matrix is manufactured with three “dots”, each contain an antigen (e.g., positive control, analyte 1, analyte 2). A body fluid (e.g., serum) is applied to the triangular opening and allowed to flow through the paper matrix into the absorbent. To assure assay specificity, a wash reagent is applied and flows into the absorbent material. Finally, gold particles attached to an immunological reagent is applied and absorbed. If specific antibody binding occurs, immunologically active gold particles will bind and cause red/pink color formation.

REAGENTS

- **Cassette:** HSV lysate (McIntyre strain), HSV gG-2 recombinant protein (E. coli origin) and reagent control
- **Wash 1:** Buffered solution with <0.1% sodium azide
- **Diluent:** Buffered saline with <0.1% sodium azide
- **Color G:** Colloidal gold conjugated to protein A in buffered saline with <0.1% sodium azide

WARNINGS AND PRECAUTIONS

This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with the potential hazards. Handle appropriately with the requisite Good Laboratory Practices. Do not eat, drink or smoke when using this product. Wear appropriate protective clothing, including lab coat, eye/face protection and disposable gloves (synthetic, non-latex gloves are recommended) while handling kit reagents and patient samples. Wash hands thoroughly after performing the test.

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. Follow recommended Universal Precautions for bloodborne pathogens as defined by OSHA (10), Biosafety Level 2 guidelines from the current CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (11), WHO Laboratory Biosafety Manual (12), and/or local, regional and national regulations.

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. This dilution is not subject to GHS, US HCS and EU Regulation 2008/1272/EC labeling requirements.

STORAGE

Store at 2-8°C. Bring reagents to room temperature (15-45°C) before use. Avoid contamination of reagents. Once the foil pouch containing one cassette is opened, it should be used within 12 hours. Other kit components are to be used prior to the expiration date.

COLLECTION AND HANDLING

ImmunoFLOW Test is performed on serum. Store samples at room temperature for no longer than eight hours. If the assay will not be completed within eight hours, freeze the specimen at or below -20ºC.

A specimen is unsuitable if it does not flow through the membrane (e.g. highly lipemic, particulate due to storage, etc.). In some cases a brief centrifugation using a table top microfuge can render the specimen suitable. If the specimen flows through the membrane, it is suitable. Serum discoloration may also affect interpretation by causing significant background color that interferes with interpretation.

PROCEDURE

MATERIALS PROVIDED

- Cassette
- Wash 1
- Diluent
- Color G

MATERIALS REQUIRED BUT NOT PROVIDED

- Specimen Collection Apparatus
- Pipette
- Control Reagents
- Timer
- Test Tube
- Camera (optional)

SET-UP

1. Remove cassette(s) from foil pouch - 1 per test.
2. Remove Diluent, Wash 1 and Color G from kit.
3. Prepare a 1:2 (50:50) dilution of the patient specimen or control using Diluent. (For example, add 200 µL patient sample into 200 µL Diluent).

ASSAY PROCEDURE

1. Add **one hundred (100) microliters (µL)** of Wash 1 to the cassette. Allow all liquid to flow through the device.
2. Add **200 µL** of 1:2 **diluted** patient or control specimen. Allow all liquid to flow through the device. (Note: If sample takes longer than one (1) minute to flow through cassette, do not continue. Using a new cassette, attempt run again. If sample continues not to flow through, this sample is not acceptable. If this commonly occurs, report problem to GenBio. Typically this is caused by highly lipemic sera, but may occur without apparent cause. See collection and handling section.)
3. Add **100 µL** of Wash 1 to the cassette. Allow all liquid to flow through device.
4. Add **100 µL** of Color G to the cassette. Allow all liquid to flow through device.
5. Add **100 µL** of Wash 1 to the cassette. Allow all liquid to flow through device.
6. Read the results within **two (2) minutes**. (A permanent record may be made using a digital camera.)

INTERPRETATION

Reactive A red or pink dot is visible

Not Reactive No dot is visible

CLINICAL INTERPRETATION

Interpretation	C	T	2
HSV-2 Reactive	+	+	+
HSV-2 Negative	+	+	-
HSV Negative	+	-	-

If dot color is equivocal, interpret as negative/non-reactive.

QUALITY CONTROL

The assay is performed at room temperature (15-45°C). Testing should be 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. Each cassette includes a reagent positive control. The background area around the dot is the reagent negative control. The reagent positive control dot is not reactive if patient specimen (serum) is not added or if one or more reagents fail. For interpretation, this control must be reactive and the background must be white to pale pink.

LIMITATIONS

- Results obtained from immune-compromised individuals should be interpreted with caution.
- The performance characteristics have not been established for any matrices other than serum.

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

ImmunoFLOW HSV detects two analytes: HSV IgG Total and HSV type 2 IgG. To measure relative performance, eighty-four sera collected from healthy U.S. subjects donating blood are used. Total HSV IgG relative sensitivity and specificity to ImmunoWELL™ HSV IgG Total and Captia™ Herpes Group IgG ELISA is shown in Table 1. HSV type 2 sensitivity and specificity to ImmunoWELL™ HSV-2 IgG and HerpeSelect® 2 ELISA s shown in Table 2.

Table 1

Kit	HSV Positive	HSV Negative
Captia™	69/70	14/14
ImmunoWELL™	69/70	14/14

One ImmunoFLOW HSV Total equivocal. Agreement is greater than 94%.

Table 2

Kit	HSV-2 Positive	HSV-2 Negative
HerpeSelect®	32/32	52/52
ImmunoWELL™	32/32	52/52

Agreement is greater than 96%.

Reproducibility is measured by testing six standards at or near the cutoff. Six replicates of each specimen are tested. Each cassette is independently interpreted by three people. The HSV Total precision at or near the cutoff is 5% and HSV-2 precision is 6%.

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