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IMAGEN Herpes Simplex Virus (HSV)

REF K610611-2 **EN**

A direct immunofluorescence test for the detection of HSV 1 and HSV 2.

1. INTENDED USE

The IMAGEN™ Herpes Simplex Virus typing test is a qualitative direct immunofluorescence test for the detection and typing of HSV 1 and HSV 2 in cell cultures.

2. SUMMARY

Herpes simplex virus is a DNA virus containing an icosahedral nucleocapsid surrounded by a lipid containing envelope. Human HSV is classified within the family *Herpesviridae* and is a member of the sub-family *Alphaherpesvirinae*. The genus *Simplexvirus* includes two type species of human herpes simplex virus, HSV 1 and HSV 2[1].

HSV is a common and universal infection of humans, associated with a wide range of clinical diseases in both immunocompetent and immunocompromised individuals. Both HSV 1 and 2 are frequently implicated in localised vesicular infections of the skin, conjunctiva and mucous membranes of the mouth or genitalia[2,3,4]. After the primary infection has resolved the virus may exist in a latent form in nervous tissues and re-emerge under certain conditions to cause a recurrence of the symptoms. Primary infections of the central nervous system may lead to herpes encephalitis which may have a poor prognosis if not treated early[4,5]. Primary infection late in pregnancy can lead to a severe neonatal infection. These infections have high morbidity and mortality rates[4,5].

In immunocompromised patients severe and life threatening systemic infections, sometimes involving multiple organs, can occur[5].

Safe and effective anti-viral therapy is widely used for the treatment of primary and recurrent HSV infection[3,6] therefore the rapid laboratory diagnosis of HSV is important to support the management and treatment of infected patients.

Identification of HSV types plays an important role in the investigation and monitoring of infected patients[4]. The main diagnostic methods used include isolation of viable virus in cell culture monolayers inoculated with clinical samples or direct detection of virus or viral proteins in clinical samples[4,7].

Isolation of HSV from clinical samples can be accomplished in a variety of cell lines including diploid fibroblasts, rabbit kidney cells, vero cells and human amniotic cells, in which HSV will replicate rapidly and exhibit a cytopathic effect[7].

A range of techniques has been used to detect and confirm the identification of isolates and to differentiate between HSV 1 and HSV 2. These include DNA hybridisation, restriction enzyme analysis, neutralisation tests and culture in embryonated eggs[4,7]. These techniques can be complex, laborious and often inappropriate for routine use.

Immunofluorescence tests using HSV 1 or HSV 2 specific monoclonal antibodies have been described for the identification and typing of HSV isolates and for the detection of HSV in cell cultures prior to the appearance of cytopathic effect (CPE)[7,8,9].

Direct immunofluorescence tests, such as IMAGEN Herpes Simplex Virus, utilising specific monoclonal antibodies offer a rapid sensitive and specific method for the detection and differentiation of HSV 1 and HSV 2 isolates in cell culture monolayers. IMAGEN Herpes Simplex Virus utilises specific monoclonal antibodies to detect epitopes of HSV glycoproteins specific for either HSV 1 or HSV 2.

3. PRINCIPLE OF THE TEST

The IMAGEN HSV reagents contain monoclonal antibodies conjugated to fluorescein isothiocyanate (FITC). Conjugated antibodies bind specifically to conserved epitopes of either HSV 1 or HSV 2. The reagents are used in a one-step direct immunofluorescence technique. Specimens are incubated with FITC conjugated antibody reagents for 30 minutes, then excess reagent is washed off with phosphate buffered saline (PBS). The stained areas are mounted and viewed microscopically using epifluorescence illumination. If Herpes Simplex Virus types 1 or 2 are present, characteristic bright apple-green fluorescence is seen within cultured cells, contrasting with the red background staining of uninfected cells. The reagent with which specific fluorescence is observed will indicate whether the virus is HSV 1 or HSV 2.

Acknowledgement
The monoclonal antibodies used in this test were produced in the Department of Pathology, University of Cambridge, Cambridge, United Kingdom.

4. DEFINITIONS

The following symbols have been used throughout the product information.

REF	Product code and catalogue number
	Consult the instructions for use
Σ N	Contains sufficient for <N> tests
	Manufactured by
IVD	*In vitro* diagnostic medical device
	Use by
LOT	Batch Code
	Storage temperature limitations

5. REAGENTS PROVIDED

Σ 50 - Each kit contains sufficient materials to test 50 cell culture preparations.

- The shelf life of the kit is as indicated on the outer box label.

5.1. IMAGEN HSV TEST CONTENTS

Instructions For Use .

POSITIVE CONTROL SLIDE 2 x 2 well Positive Control Slides containing acetone fixed human fibroblast cells infected with either HSV 1 or HSV 2.

One bottle of each of the following:

MOUNTING FLUID 3mL of Mounting Fluid. The mounting fluid contains a photobleaching inhibitor in a glycerol solution (pH 10.0).

REAGENT 1 1.4mL of IMAGEN HSV Type 1 Reagent. The reagent contains purified murine monoclonal antibodies specific to HSV 1, conjugated to FITC. The conjugates are prepared in a protein stabilised buffer solution (pH 7.5) containing Evans blue dye as counterstain and 15mmol/L sodium azide as preservative.

REAGENT 2 1.4mL of IMAGEN HSV Type 2 Reagent. The reagent contains purified murine monoclonal antibodies specific to HSV 2, conjugated to FITC. The conjugates are prepared in a protein stabilised buffer solution (pH 7.5) containing Evans blue dye as counterstain and 15mmol/L sodium azide as preservative.

5.2. PREPARATION, STORAGE AND RE-USE OF KIT COMPONENTS

In order to ensure optimal kit performance, it is important that all unused kit components are stored according to the following instructions.

5.3. POSITIVE CONTROL SLIDES - POSITIVE CONTROL SLIDE

Postive Control Slides are provided individually in sealed foil pouches filled with nitrogen. Store unused slides at 2-8°C. The slide should be left for 5 minutes at room temperature (15-30°C) before opening.

Stain the slide immediately after opening.

5.4. MOUNTING FLUID - MOUNTING FLUID

Ready to use. Store unused Mounting Fluid at 2-8°C. The mounting fluid should be left at room temperature (15-30°C) for 5 minutes before use.

5.5. REAGENT 1 AND 2 - REAGENT 1/REAGENT 2

Ready to use. Store unused Reagents 1 and 2 at 2-8°C. Reagents 1 and 2 should be stored in the dark at 2-8°C and left at room temperature (15-30°C) for 5 minutes before use.

6. ADDITIONAL REAGENTS

6.1. REAGENTS

Acetone (for fixation).

Phosphate buffered saline (PBS) pH 7.5 for washing stained specimens and for specimen preparation.

6.2. ACCESSORIES

General
Teflon coated glass microscope slides with single 6mm diameter well (100 slides per box) available from your local distributor (Code No. S611430-6).

IMAGEN HSV Postive Control Slide (Code No. S611030-2).

For Culture Confirmation
Sterile swabs, viral transport medium and a container suitable for collection, transportation and culture of HSV.

Cell culture lines recommended for culture and isolation of HSV.

7. EQUIPMENT REQUIRED BUT NOT PROVIDED

Precision pipette and disposable tips to deliver 25µL

Wash bath

Coverslips suitable to cover 6mm diameter well

Non-fluorescing immersion oil

Epifluorescence microscope with filter system for FITC (maximum excitation wavelength 490nm, mean emission wavelength 520nm) and lenses for x200-x500 magnification

Incubator at 37°C

8. PRECAUTIONS

IVD - For *in vitro* diagnostic use. Anyone performing an assay with this product must be trained in its use and must be experienced in laboratory procedures.

8.1. SAFETY PRECAUTIONS

8.1.1 The IMAGEN HSV reagents contain 15mmol/L sodium azide, which is a poison. Sodium azide may react with copper and lead plumbing systems to form explosive metal azides. Always dispose of materials containing azide by flushing with large quantities of water.

8.1.2 HSV on the positive control slide has been shown to be non infectious in cell culture, however, the slide should be handled and disposed of as though potentially infectious.

8.1.3 Evans blue dye is present in the reagent. This may be carcinogenic and contact with the skin should be avoided.

8.1.4 Care should be taken when using the Mounting Fluid as it may cause skin irritation. Skin should be flushed with water if contact occurs.

8.1.5 Do not eat, drink, smoke, store or prepare foods, or apply cosmetics within the designated work area.

8.1.6 Do not pipette materials by mouth.

8.1.7 Wear disposable gloves while handling clinical specimens and infected cells, and always wash hands after working with infectious materials.

8.1.8 Dispose of all clinical specimens and reagents in accordance with local legislation.

8.1.9 Safety data sheet available for professional user on request.

8.2. TECHNICAL PRECAUTIONS

8.2.1 Components must not be used after the expiry date printed on the labels. Do not mix different batch lots of reagent.

8.2.2 The reagents are provided at fixed working concentrations. Test performance will be adversely affected if reagents are modified or stored under conditions other than those detailed in Section 5.

8.2.3 Prepare fresh Phosphate Buffered Saline (PBS) as required on the day of use.

8.2.4 Avoid microbial contamination of reagents.

8.2.5 The reagents must not be frozen.

9. COLLECTION AND PREPARATION OF SPECIMENS

The collection and preparation of specimens is of fundamental importance in the diagnosis of HSV infection by cell culture. Specimens must be collected from the site of infection so that they contain as much infected material as possible.

9.1. CLINICAL SPECIMENS

Collection
Collect clinical specimens from the site of infection. Ulcers or lesions should be firmly rubbed using a plain cotton-tipped swab in order to obtain infected cells and exudate from the base of the ulcer or lesion. Vesicles should be carefully opened, the fluid collected on the swab and the base of the lesion rubbed with the swab. The swabs should be placed in the virus transport medium routinely used and sent to the laboratory as soon as possible for processing.

Inoculation of Cell Cultures
Specimens collected for the diagnosis of HSV infection should be inoculated into the cells lines routinely used in the laboratory according to established laboratory methods and examined regularly for the appearance of cytopathic effect (CPE).

Preparation of Slides
If a CPE consistent with HSV infection is observed then the cell culture monolayer should be harvested when approximately 70% of the cells are affected.

Scrape the cell sheet into the liquid culture medium using a sterile pipette. Deposit the cells by gentle centrifugation at 200g for 10 minutes at room temperature (15-30°C) and remove the supernatant. Wash the cells by resuspending the cell deposit in PBS (see Section 6.1) and repeat the centrifugation. Remove the supernatant and resuspend the cell deposit in a small volume of fresh PBS to maintain a high cell density.

Place 25µL aliquots of the suspension on to wells on teflon coated microscope slides. Two wells are required per patient specimen. Allow to air dry at room temperature (15-30°C) and fix in fresh acetone for 10 minutes at room temperature (15-30°C). If the specimen is not stained immediately, store at 4°C overnight or freeze at –20°C for longer periods.

10. TEST PROCEDURE

PLEASE REFER TO SECTION 8.2 TECHNICAL PRECAUTIONS BEFORE PERFORMING TEST PROCEDURE.

10.1. ADDITION OF REAGENTS 1 AND 2

Add 25µL of HSV 1 reagent to one 6mm diameter well of the fixed cell preparation and 25µL of HSV 2 reagent to the other 6mm diameter well preparation (see Section 9) or add to Positive Control Slide. Ensure that the reagents cover the entire well area.

10.2. FIRST INCUBATION

Incubate the slides with reagent in a **moist chamber** for **30 minutes** at **37°C**. **Do not** allow the reagent to dry on the specimen, as this will cause the appearance of non-specific staining.

10.3. WASHING THE SLIDE

Wash off excess reagent with phosphate buffered saline (PBS) (see Section 6.1) then gently wash the slide in an agitating bath containing PBS for 5 minutes. Drain off PBS and allow the slide to air dry at room temperature (15-30°C).

10.4. ADDITION OF MOUNTING FLUID

Add one drop of IMAGEN Mounting Fluid to the centre of each well and place a coverslip over the Mounting Fluid and specimen ensuring that no air bubbles are trapped.

10.5. READING THE SLIDE

Examine the entire 6mm diameter well areas containing the stained cell preparations using an epifluorescence microscope. Fluorescence as described in Section 11 should be visible at x200 - x500 magnification. (For best results slides should be examined immediately after staining, but may be stored at 2-8°C, in the dark, for up to 24 hours).

11. INTERPRETATION OF TEST RESULTS

11.1. CONTROLS

11.1.1 Positive Control Slide

When stained and viewed as described in Section 10 the positive control slide should show fluorescing cells with intracellular apple-green fluorescence contrasting against a background of counterstained material. Positive control slides should be used to check that the staining procedure has been satisfactorily performed.

11.1.2 Negative Control

If a negative control slide is required, uninfected intact cells of the type used for the culture and isolation of HSV are recommended. The cells should be prepared and fixed as described in Section 9.1 and stained as described in Section 10.

11.2. CLINICAL SPECIMENS

11.2.1 Appearance of HSV infected cells

Infected cells will demonstrate apple-green fluorescent intracellular cytoplasmic granules. In some infected cells this characteristic cytoplasmic fluorescence may be accompanied by a "rimming" effect caused by staining of the cell membrane.

Uninfected cells stain red with the Evans blue counterstain.

11.2.2 Interpretation

A positive diagnosis is made when at least one fixed stained cell shows the fluorescence pattern described in Section 11.2.1 with either HSV type 1 or HSV type 2 reagent. It is recommended that at least 50 cells of the cell culture being tested are visible within each slide well area before a negative result is reported. See Section 11.2.3 if insufficient cells are present.

11.2.3 Insufficient cells

If insufficient cells are present in the slide preparation, the remainder of the cell culture sample should be centrifuged at 200g for 10 minutes at room temperature (15-30°C). Re-suspend in a smaller volume of PBS before re-distribution (25µL) on 6mm diameter well areas on a teflon coated glass microscope slide as described in Section 9.1. Alternatively, a repeat clinical specimen should be requested.

12. LIMITATIONS OF THE PROCEDURE

12.1. Use only the Mounting Fluid provided with the IMAGEN HSV test.

12.2. The visual appearance of the fluorescence image obtained may vary due to the type of microscope and light source used.

12.3. All reagents are provided at fixed working concentrations. Test performance may be affected if the reagents are modified in any way or not stored under the recommended conditions as outlined in Section 5.

12.4. It is recommended that 25µL of reagent is used to cover a 6mm diameter well area. A reduction in this volume may lead to difficulties in covering the specimen area and may reduce sensitivity.

12.5. Failure to detect HSV may be a result of factors such as collection of specimen at an inappropriate time of the disease, improper sampling and/or handling of specimen, failure of cell culture etc. A negative result does not exclude the possibility of HSV infection.

12.6. Test results should be interpreted in conjunction with information available from epidemiological studies, clinical assessment of the patient and other diagnostic procedures.

13. EXPECTED VALUES

Herpes simplex virus infection of man occurs throughout the world and over 80% of the adult population in western countries will have experienced a primary infection of which most will have been asymptomatic[10]. Following primary infection approximately 45% of individuals with oral infection and 60% of patients with genital infection will experience recurrent herpes infections[3]. HSV has been isolated from the genital tract of 0.3 to 5.4% of males and 1.0 to 8.0% of females attending clinics for sexually transmitted diseases[11,12]. Patients with ocular herpes simplex infections are a major cause for referrals to ophthalmology clinics[3].

In symptomatic primary or recurrent infection, HSV lesions may be found on the skin, mucous membranes of mouth, pharynx and genitalia, or the eye. During infection of neonates, or immunocompromised individuals, the infection may become widely disseminated affecting organs such as lung, brain, liver, spleen etc.

HSV can be cultured from the fluid of vesicular lesions or secretions from other infected sites (eg eyes, pharynx or genitalia). In addition, HSV can be cultured from infected tissues in disseminated herpes eg brain biopsy of patients with herpes simplex encephalitis.

14. SPECIFIC PERFORMANCE CHARACTERISTICS

14.1. REACTIVITY OF THE MONOCLONAL ANTIBODY WITH HSV TYPES 1 AND 2

The monoclonal antibodies utilised in this test have been shown to react with type specific conserved epitopes of either HSV 1 antigen or HSV 2 antigen.

14.2. SPECIFIC CHARACTERISTICS

The IMAGEN HSV typing test was evaluated at one clinical trial centre and the results compared with restriction endonuclease mapping. At this hospital centre samples were taken from various sites including nose, throat, tongue, mouth, skin, conjunctiva, perineum, urethra, penis, vulva, labia, vagina and cervix from 187 patients attending the hospital. Specimens (swabs) were placed into transport medium and transported to the laboratory for cell culture isolation of HSV. At the trial centre all 187 cell cultures were observed microscopically for typical HSV cytopathic effect and evaluated using the IMAGEN HSV reagents to determine whether HSV 1 or HSV 2 were present. HSV 1 was judged to be present if one or more cells exhibited typical fluorescence with the HSV 1 reagent. HSV 2 was judged to be present if one or more cells exhibited typical fluorescence with the HSV 2 reagent (see Section 11).

Detection of HSV
Of the 187 specimens evaluated 60 (32.1%) were positive by both reference cell culture and IMAGEN HSV typing test (Table 14.2.1). Of the positive results obtained 55.0% were from females and 45.0% from males. Distribution of positive results according to site of isolation of HSV was 83.3% genital, 13.3% oral and 3.4% from other anatomical sites.

Of the positive genital specimens 40.0% were HSV 1 and 60.0% were HSV 2. All of the positive oral specimens were HSV 1.

Positive results were obtained by both methods in all cases giving a value of 100% for correlation, specificity, sensitivity and positive and negative predictive values.

Table 14.2.1 Comparison of test results by the IMAGEN HSV test and standard methods for cell culture

TEST	RESULTS			
Cell Culture	**Pos**	**Neg**	**Neg**	**Pos**
IMAGEN HSV	**Pos**	**Neg**	**Pos**	**Neg**
No. of Specimens	60 (32.1)	127 (67.9)	0 (0)	0 (0)

( ) Expressed as a percentage of the total number of specimens tested.

Typing of HSV 1 and HSV 2
A total of 43 culture isolates of HSV were available for testing by the IMAGEN HSV test and Restriction Endonuclease Mapping (Table 14.2.2). Positive results were obtained by both methods in all cases giving a value of 100% for correlation, sensitivity and specificity.

Table 14.2.2 Comparison of typing of HSV 1 and HSV 2 using IMAGEN HSV and Restriction Endonuclease Mapping (REM)

TEST	RESULTS			
REM	**HSV 1**	**HSV 2**	**HSV 1**	**HSV 2**
IMAGEN HSV	**HSV 1**	**HSV 2**	**HSV 2**	**HSV 1**
No. of Specimens	23 (53.5)	20 (46.5)	0 (0)	0 (0)

( ) Expressed as a percentage of the total number of specimens tested.

14.3. CROSS REACTIVITY

The IMAGEN HSV typing test was performed against other viruses likely to be isolated in cell culture from human specimens. All organisms tested (Table 14.3) were negative with both IMAGEN HSV 1 and IMAGEN HSV 2 reagents.

Table 14.3 Viruses tested in the IMAGEN™ HSV test and found to be non-reactive

Adenovirus 2,3,4
Cytomegalovirus
Echovirus 11
Epstein Barr virus
Herpes zoster
Parainfluenza 1
Respiratory syncytial virus

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TECHNICAL ADVICE AND CUSTOMER SERVICE

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