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IDEIA Herpes Simplex Virus

REF K605711-2 Σ 96 **EN**

An amplified enzyme immunoassay for the direct detection of Herpes Simplex Virus in human clinical specimens from symptomatic patients.

1. INTENDED USE

The IDEIA™ Herpes Simplex Virus (HSV) test is a qualitative amplified enzyme immunoassay for the direct detection of Herpes Simplex Virus (HSV) in human clinical specimens from symptomatic patients.

2. SUMMARY

HSV is an enveloped, DNA containing virus morphologically similar to the other members of the genus Herpesviridae. Two antigenically distinct types are recognised, designated type 1 and type 2.

HSV types 1 and 2 are frequently implicated in superficial infections of the oral cavity, the skin, the eye and the genitalia[1,2]. Infections of the central nervous system (meningoencephalitis) and severe generalised infections in the neonate or immunocompromised patient are also seen, though more rarely. After the primary infection has resolved the virus may exist in a latent form in nervous tissue, from where it may re emerge, under certain conditions, to cause a recurrence of the symptoms.

At present, there are two main diagnostic methods for detection of HSV in clinical specimens. First, by isolation of viable virus in cultured cells followed by identification of the agent by immunological means or by electron microscopy;[1,2] second, by direct demonstration of virus antigen in clinical specimens using a fluorescein labelled monoclonal antibody or an enzyme immunoassay.

Viral isolation in cell culture monolayers is laborious, time consuming and expensive and requires a degree of technical expertise which may not be available in many laboratories.

The IDEIA HSV test is an alternative diagnostic test for direct detection of herpes virus antigen in clinical specimens. The test utilises monoclonal antibodies to detect HSV 1 and HSV 2 antigen and an enzyme amplification system to enhance the test signal[3]. The enzyme immunoassay is simple to perform and more rapid than viral isolation for the detection of HSV in clinical specimens and cell culture.

The IDEIA HSV test provides all the necessary reagents to detect HSV in clinical specimens. The test can be performed in less than two hours using conventional enzyme immunoassay instrumentation and procedures and gives excellent overall sensitivity (93.6%) and specificity (97.8%) relative to cell culture reference methods.

3. PRINCIPLE OF THE TEST

The IDEIA HSV test utilises HSV specific murine monoclonal antibodies and an enzyme amplification system[3]. HSV antigen (common to type 1 and type 2), present in a clinical specimen, is bound by murine monoclonal antibody adsorbed to the surface of the plastic microwell. Enzyme conjugated murine monoclonal antibody binds to the "captured" antigen and subsequently the enzyme catalyses the conversion of substrate to product. This product participates in a second enzyme reaction, which results in a colour change. The colour development process is stopped by the addition of acid. A colour intensity significantly above background levels is indicative of HSV antigen present in clinical specimens (see Section 9).

Acknowledgements

The monoclonal antibodies originated in the Department of Pathology, University of Cambridge, Cambridge, United Kingdom[4].

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 96 - Each kit contains sufficient materials for 96 determinations.

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

5.1. IDEIA HSV TEST CONTENTS

	One Instructions For Use booklet.
MICROTITRATION PLATE	One pouched 96 microwell plate of 12, 8 microwell break-apart strips coated with anti HSV murine monoclonal antibody. A resealable plastic pouch is provided for storage of unused microwells.

One bottle of each of the following is provided:

TRANSPORT MEDIUM	100mL Transport Medium: nonionic detergent in a buffer containing coloured dye, antimicrobial agent and an antifoaming agent.
CONTROL +	3mL Positive Control: an inactivated homogenate of HSV infected Hela cells in buffer solution containing protein and detergent.
CONTROL –	12mL Negative Control: buffer solution containing antimicrobial agent, coloured dye and an anti-foaming agent.
CONJUGATE	7mL Conjugate: alkaline phosphatase conjugated murine monoclonal antibody (Fab prime fragment) in stabilising buffer containing coloured dye and an antimicrobial agent.
WASH BUFFER (x10)	125mL Wash Buffer concentrate (x10): tris buffered solution containing detergent and an antimicrobial agent.
AMPLIFIER A	13mL Amplifier A: inorganic salts and buffered enzyme solution containing tetrazolium violet and an antimicrobial agent.
AMPLIFIER B	13mL Amplifier B: stabilised NADPH solution.
STOP SOLUTION	13mL Stop Solution: 1mol/L phosphoric acid.

5.2. PREPARATION, STORAGE AND RE USE OF KIT COMPONENTS

The IDEIA HSV kit format allows for testing of up to 12 batches of specimens. In order to ensure optimal kit performance, it is important that all unused kit components are stored according to the instructions.

5.2.1 Monoclonal Antibody Coated Microwells - MICROTITRATION PLATE

Open the plate pouch by cutting along the seal. Break off the required number of microwells and relocate them into the frame. Place unused microwells in the resealable plastic pouch with the desiccant, carefully reseal the pouch and store at 2-8°C. Microwells may be used for up to 6 weeks after initial opening, provided they are stored in this manner.

5.2.2 Transport Medium - TRANSPORT MEDIUM

Provided ready to use. **It is important to mix the Transport Medium prior to use.**

An anti-foaming agent in Transport Medium causes it to appear cloudy which does not affect the test and is not due to microbial contamination.

5.2.3 Positive Control - CONTROL +

Ready to use. Store unused Positive Control at 2-8°C.

5.2.4 Negative Control - CONTROL –

Ready to use. Store unused Negative Control at 2-8°C.

5.2.5 Conjugate - CONJUGATE

Ready to use. Store unused Conjugate at 2-8°C.

5.2.6 Wash Buffer Concentrate - WASH BUFFER (x10)

Provided x10 concentrate. Prepare working strength Wash Buffer by adding 1 part Wash Buffer concentrate to 9 parts of fresh deionised or distilled water. Sufficient concentrate is provided to prepare up to 100mL working strength Wash Buffer for each strip of 8 microwells. **Prepare working strength Wash Buffer as required on the day of use** (see Section 8.2.10). Store remaining concentrate at 2-8°C.

Do not store unused working strength Wash Buffer for subsequent use (see Section 8.2.10).

5.2.7 Amplifier A - AMPLIFIER A

Ready to use. Store unused Amplifier A at 2-8°C.

5.2.8 Amplifier B - AMPLIFIER B

Ready to use. Store unused Amplifier B at 2-8°C.

5.2.9 Stop Solution - STOP SOLUTION

Ready to use. Store unused Stop Solution at 2-8°C.

6. ADDITIONAL REAGENTS

6.1. REAGENTS

Fresh deionised or distilled water for preparation of Wash Buffer.

6.2. ACCESSORIES

The following products are intended for use in conjunction with IDEIA HSV Kit. Contact your local Oxoid subsidiary or distributor for further information.

IDEIA HSV Extraction Buffer 10mL (Code No. S601230-2).

IDEIA HSV Blocking Reagents (Code No. S603330-2).

IDEIA *PCE* Chlamydia/HSV Wash Buffer Concentrate (X10) 125mL (Code No. S603930-2).

IDEIA HSV Transport Medium 100mL (Code No. S605530-2).

7. EQUIPMENT

The following equipment is required:

Clean, screw capped vials

Vortex mixer

Clean absorbent paper (onto which microwells can be tapped dry)

Precision pipettes and disposable tips to deliver 50µL 1,000µL or graduated pastettes for dispensing 200µL specimen (optional)

Waste discard container with suitable fresh disinfectant

37°C incubator

Automated plate washer (optional) or suitable equipment for washing 8 microwell strips (see Section 10.4.4). **Note: If washing less than 8 test microwells in a strip using an automated washer with an 8 microwell head, it is important to completely fill the strip with blank microwells.**

Spectrophotometer or EIA plate reader able to read a 96 microwell plate of 8 microwell strips at an absorbance of 490nm with a reference at 620-650nm. (Optional, see Section 10.5, Reading the Test Results).

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 following reagents contain sodium azide (15mmol/L), which is a poison; Transport Medium, Conjugate, Wash Buffer Concentrate, Positive Control, Negative Control and Amplifier A reagent. Sodium azide may react with copper and lead plumbing systems to form explosive metal azides. Always dispose of azide-containing materials by flushing with large quantities of water.

8.1.2 The Stop Solution contains 1mol/L phosphoric acid. Avoid eye and skin contact by wearing protective clothing and eye protection.

8.1.3 The Positive Control contains inactivated HSV antigen, which has been shown to be non-infectious. However, the control must be handled and disposed of as though potentially infectious. The Positive Control also contains a detergent (1%v/v). Skin contact should be avoided.

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

8.1.5 Do not pipette materials by mouth.

8.1.6 Wear disposable gloves while handling clinical specimens and always wash hands after working with potentially infectious materials.

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

8.1.8 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 or interchange different batches/lots of reagents.

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 5.2.

8.2.3 Avoid contamination of reagents.

8.2.4 When using the dropper bottle method ensure all controls and reagents are added in the same way. (Performance of the kit may be adversely affected if a combination of pipette and dropper methods is used).

8.2.5 Avoid multiple sampling from amplification reagents. Transfer the required amount into a suitable clean vessel. Do not return excess reagent to the dropper bottle.

8.2.6 Use separate disposable pipettes or pipette tips for each specimen, control or reagent (if not using dropper bottles) in order to avoid cross contamination of either specimens, controls or reagents which could cause erroneous results.

8.2.7 Store deionised or distilled water for dilution of concentrated reagents in clean containers to prevent microbial contamination.

8.2.8 Ensure that no cross-contamination occurs between microwells at all stages of the test. It is essential that the Conjugate is not allowed to contaminate other reagents or equipment. If the dropper method is not used then reserve a separate pipette for dispensing Conjugate and a separate pipette for dispensing the amplifier reagents. Avoid touching or splashing the rim of the microwell with Conjugate. Conjugate allowed to dry on to the rim of the microwell may adversely affect the performance of the test.

8.2.9 Microwells cannot be re-used.

8.2.10 Do not store unused working strength Wash Buffer for subsequent use. When not in use Wash Buffer reservoirs should be rinsed with deionised or distilled water and left to dry.

8.2.11 The enzyme amplification system is a highly sensitive detector of alkaline phosphatase molecules. **It is very important that all non bound Conjugate is removed by thorough washing of microwells before addition of amplification reagents.** Careful washing of microwells is achieved by using techniques detailed in Section 10.4.4. Inefficient washing may lead to incorrect results.

8.2.12 Manual or automated washing equipment must be free of microbial contamination, be correctly calibrated and maintained according to the manufacturers instructions.

8.2.13 Phosphate Buffered Saline (PBS) and other wash solutions containing phosphate must not be used with the assay, to prevent inhibition of the Conjugate enzyme which may affect test performance. Washing equipment used with phosphate based wash solutions must be thoroughly flushed using deionised or distilled water prior to priming with working strength IDEIA *PCE* Chlamydia/HSV Wash Buffer (Code No. S603930-2).

9. COLLECTION AND PREPARATION OF SPECIMENS

Specimens collected in the following ways can be tested in the IDEIA HSV kit:

Specimens collected in IDEIA HSV Transport Medium (see Section 9.3.1)
Specimens collected in Transport Medium for viral isolation in cell culture monolayers (see Section 9.3.2).

9.1. TRANSPORT MEDIUM

If IDEIA HSV Transport Medium is to be used for specimen collection dispense 1mL aliquot of specimen Transport Medium into clean, screw-capped vials. The 1mL aliquots can be stored at 2-8°C for 12 months or expiry date stated on vial label. Store at room temperature (15-30°C) for 6 months.

Note: It is important to vortex or mix Transport Medium thoroughly prior to dispensing or use. The Transport Medium contains an antifoaming agent, which causes the medium to appear cloudy. This does not affect the test and is not due to microbial contamination. The Transport Medium contains a strong detergent, which renders the specimen unsuitable for cell culture.

9.2. SPECIMEN COLLECTION

A good sampling technique is essential for the optimal detection of HSV in clinical specimens. Specimens from a clinical lesion should be collected by qualified individuals using a sterile swab. Specimens must be collected so as to contain as much infected material as possible. Cotton or Dacron® tipped sterile swabs with plastic or compressed paper stems are recommended.

Creams, ointments, lotions, ice, alcohol, betadine solution, zinc, or a recent sitz bath all reduce viral yield significantly[8]. Use of such remedies should be avoided, if possible, prior to specimen collection or be reported to the physician when the lesion is sampled.

9.2.1 Clinical specimens

Ulcers should be firmly rubbed with the swab in order to pick up infected cells and exudate from the base of the ulcer. Vesicles should be carefully opened, the fluid collected on the swab, and the base of the lesion rubbed with the swab. Place the swab in a clean screw-capped vial with relevant Transport Medium. Pustular lesions should be treated as for vesicles; the crusts may be added to the Transport Medium in a screw-capped vial or sent dry. Specimens may be stored at 2-8°C for no longer than 3 days.

Rectal or ano-rectal specimens if contaminated by faecal material may give rise to false positive results. These can be confirmed using IDEIA HSV Blocking Reagents (Code No. S603330-2).

9.2.2 Cervical specimens in symptomatic patients

Firmly swab any visible lesions, otherwise swab cervix. Withdraw the swab without touching the vaginal surface and place in a screw-capped vial with relevant Transport Medium. Specimens may be stored at 2-8°C for no longer than 3 days.

9.3. PROCESSING OF SPECIMENS

9.3.1 Swabs in IDEIA HSV Transport Medium

Vortex samples prior to testing and proceed to Section 10.2.

9.3.2 Swabs in Transport Medium for Viral Isolation

IDEIA HSV Extraction Buffer (Code No. S601230-2) is required for processing swabs collected in laboratory Transport Medium for viral isolation. Samples should only be processed for IDEIA HSV testing after inoculation of cell culture monolayers as treatment with concentrated extraction buffer renders the specimen unsuitable for viral isolation.

Add 100µL of extraction buffer (Code No. S601230-2) to 900µL of viral Transport Medium. Process sample according to the procedure outlined in Section 10.2.

Do not use the Transport Medium provided in the kit for processing swabs in viral Transport Medium as this will not give optimal extraction of HSV antigen from the swab.

10. TEST PROCEDURE

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

10.1. PREPARATION OF CONTROLS

Negative Control

Vortex mix the Negative Control for **a minimum of 15 seconds**. Add the reagent directly to appropriate microwells.

Positive Control

Vortex mix the Positive Control for **1 minute**. Add the reagent directly to the appropriate microwell. If required, an additional control with a lower level of reactivity may be tested to monitor kit performance.

10.2. TREATMENT OF SPECIMENS AND CONTROLS

Vortex mix all processed specimens (see Section 9.3) and controls for 15 seconds prior to addition to microwells.

10.3. STORAGE OF TREATED SPECIMENS

Specimens may be stored at -20°C for up to 4 weeks after processing. If testing specimens that have been frozen, allow to thaw to room temperature (15-30°C) then vortex vigorously for a minimum of 1 minute immediately prior to testing.

10.4. ASSAY PROCEDURE

It is recommended that consistent methods, for addition of reagents to the microwells, are used throughout the test procedure ie either pipette tips, dropper bottles or automated probes. For small batches of tests the use of the reagent dropper bottles avoids multiple entry of pipette tips to reagent bottles.

10.4.1 Specimen and Control Addition

Locate the required number of microwells into the microwell holder. Add 200µL of specimens to the appropriate microwells. Add 6 drops (or 200µL) of Negative Control and Positive Control to separate microwells. (At least three Negative Control microwells and one Positive Control microwell should be included with each batch of specimens tested).

10.4.2 Conjugate Addition

After addition of all specimens and controls add 1 drop (or 50µL) of Conjugate to each microwell. **Avoid dipping pipette or dropper bottle tip into microwells when dispensing Conjugate as this may lead to cross-contamination between microwells. Also avoid touching or contaminating tops or rims of microwells with Conjugate as this may adversely affect the performance of the test.**

10.4.3 First Incubation

Place a lid over the plates and incubate the microwells at **35-37°C** in a **moist box** for **90 minutes**.

10.4.4 Washing the Microwells

The microwells should be washed using freshly prepared working strength Wash Buffer (see Section 5.2.6).

The washing technique is critical to the test performance (see Section 8.2.10) and should be carried out so as to ensure complete filling (with a minimum of **350µL** of working strength Wash Buffer) and emptying of the microwells.

Four wash cycles are essential, by either automated or manual washing techniques, which should include a 2 minute soak period during the second wash or a total of a 2 minute soak period during the complete cycle.

Manual washing

If washing microwells manually, aspirate or shake out the contents of the microwells and using freshly prepared Wash Buffer, ensure complete filling and emptying of microwells. Between each wash step remove all remaining Wash Buffer by tapping the inverted microwells on to clean absorbent paper. Manual washing efficiency is further ensured if the Wash Buffer is delivered at an angle so as to produce a vortex in the microwells. After the final wash, the plate should be inverted and tapped on absorbent paper to remove the last traces of Wash Buffer.

Automated Washing

Automated washers should be programmed to complete 4 wash cycles and to incorporate the equivalent of 2 minutes soaking time during the complete washing cycle. Washers must be correctly calibrated to ensure complete filling and emptying of microwells between each wash. After the final wash, the plate should be inverted and tapped on absorbent paper to remove the last traces of Wash Buffer.

10.4.5 Amplifier Addition

Add 2 drops (or 100µL) of Amplifier A to each microwell.

Add 2 drops (or 100µL) of Amplifier B to each microwell.

Avoid touching microwells with dropper tips or pipette tips when dispensing Amplifier A and B as this may lead to cross-contamination between microwells.

10.4.6 Second Incubation

Place a lid over the plates and incubate the microwells at **35-37°C** in a **moist box** for **30 minutes**.

10.4.7 Stopping the Reaction

Add 2 drops (or 100µL) of Stop Solution to each microwell. Ensure thorough mixing in the microwells. The coloured product is stable for 30 minutes. **Do not expose to direct sunlight** as photobleaching of the coloured product may occur.

10.5. READING THE TEST RESULTS

10.5.1 Visual Reading

The microwells may be assessed visually up to 30 minutes after addition of the Stop Solution. It is recommended that microwells in which the colour intensity is difficult to interpret when compared to the Negative Control are also read photometrically (see Section 10.5.2).

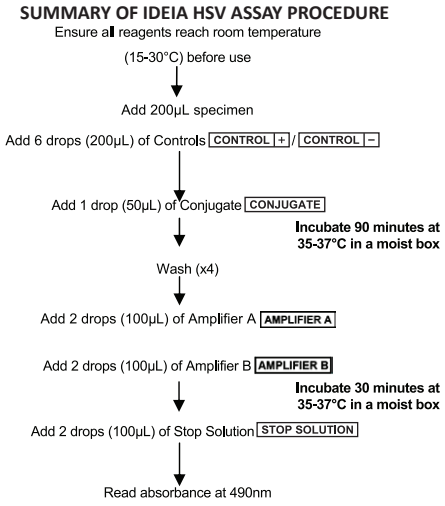
10.5.2 Photometric Reading

The microwells should be read photometrically within 30 minutes after addition of the Stop Solution. Mix the contents of the microwells and read the absorbance of each microwell using a suitable spectrophotometer or EIA plate reader set at 490nm. Ensure that the bottoms of the microwells are clean

before reading and check that no foreign matter is present in the microwells. The reader should be blanked on air (ie with no plate in the carriage) before the plate is scanned.

Alternatively if the spectrophotometer or EIA plate reader allows for the use of a reference wavelength (at 620-650nm), dual wavelength reading should be performed which will eliminate any potential interference caused by aberrations, such as dirt or marks, on the optical surface of the microwells.

10.6. SUMMARY OF IDEIA HSV ASSAY PROCEDURE



11. QUALITY CONTROL AND INTERPRETATION OF TEST RESULTS

11.1. NEGATIVE CONTROL

As detailed in Section 10.4.1 (Specimen and Control Addition), three Negative Control microwells must be included in each assay.

Visual Determination

All Negative Control microwells should be colourless or only slightly pink. If this is not the case test results should not be determined visually. Results should be read photometrically or the test repeated.

Photometric Determination

Individual Negative Control absorbance values must be less than or equal to 0.30 absorbance units. Individual Negative Control absorbance values must fall within ± 0.05 absorbance units of the mean of the three Negative Controls. If one Negative Control absorbance value falls outside the accepted range, exclude this value and recalculate the mean of the remaining two. If two Negative Control absorbance values are unacceptable the test must be repeated.

Calculation of the cut-off value

The cut-off value is calculated by adding 0.15 to the mean Negative Control absorbance value.

11.2. POSITIVE CONTROL

As detailed in Section 10.4.1 (Specimen and Control Addition), one Positive Control microwell must be included in each assay.

Visual Determination

The Positive Control microwell should be a red/magenta colour clearly distinguishable from the Negative Controls. If this is not the case the test results should not be determined visually. Results should be read photometrically or the test repeated.

Photometric Determination

The Positive Control microwell must have an absorbance value of greater than 0.50 absorbance units. If this is not the case the test should be repeated.

11.3. SPECIMENS

Visual Determination

Any specimen giving a red/magenta colour more intense than that of the Negative Controls is positive. Any specimen giving colour equal to or less than the colour of the Negative Controls is negative. Any specimen giving a pale pink coloration close to that of the Negative Controls should be read photometrically or retested. Alternatively the patient should be resampled.

Photometric Determination

Clinical specimens having absorbance values greater than the cut-off value are positive (see Section 11.1). A result within 0.015 absorbance units of the cut-off value should be interpreted cautiously, and the test repeated or the patient resampled. Patient results should not be reported if controls are outside the expected values.

11.4. INTERPRETATION AND VERIFICATION OF TEST RESULTS

11.4.1 Interpretation of Test Results

The following table is a summary of the recommended interpretation and reporting of results.

Table 11.4 Summary of Intepretation of Results and Recommended Reporting

Result	Interpretation	Reporting Recommendations
OD > CO + 0.015	Positive*	Presumptive HSV antigen (No blocking test performed)
OD = CO ± 0.015	Equivocal*	Unable to determine result. Retest
OD < CO - 0.015	Negative	No HSV antigen detected

OD = Optical Density (Absorbance units)
CO = Cut-off = Mean of Negative Controls + 0.15 Absorbance units
* Positive and equivocal results should be verified.

11.4.2 Verification of Test Results

If verification of specimens shown to be reactive in the IDEIA HSV test is considered necessary, use of the IDEIA HSV Blocking Reagents (Code No. S603330-2) is recommended. The blocking test for verification of results offers an additional means of quality controlling aspects of specimen collection.

12. PERFORMANCE LIMITATIONS

12.1. Specimen quality is crucial to the success of all diagnostic tests. Specimens collected must contain as much viral antigen as possible (see Section 9). Thus a negative result does not exclude the possibility of an HSV infection.

12.2. For optimal test performance IDEIA HSV Transport Medium or Extraction Buffer should be used. The performance characteristics of this test using other culture transport media have not been validated.

12.3. The performance characteristics of this test for use in asymptomatic patients, cerebral spinal fluid, tissue biopsy or eye exudate specimens and for tissue culture confirmation have not been validated.

12.4. This test detects both Herpes Simplex Virus type 1 and type 2, but does not differentiate which is the infecting type.

12.5. This test should not be used as the sole means to diagnose HSV when a Caesarean Section is contemplated. Prior culture results (when available) and best clinical judgement must prevail.

12.6. This test may detect non viable or culture negative HSV or HSV antigens.

12.7. 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

Positivity rates may vary according to the prevalence for HSV in different populations, geographical location, specimen collection, handling, storage, transportation of specimens, sexual behaviour and the general health environment of the patient population under study.

Herpes simplex virus is a world-wide infection of man and over 80% of the adult population in western countries will have experienced a primary infection, many of which will have been asymptomatic[9]. Following primary infection about 45% of orally infected individuals and 60% of patients with genital herpes will experience recurrent infections[10].

Herpes simplex virus infections of the eye are the major cause of referrals to eye clinics[10]. 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 sexually transmitted disease clinics[11,12].

In symptomatic primary and recurrent infection, lesions may be found on the dermis, mucous membranes of mouth, pharynx and genitalia or the eye. During neonatal infections or infections in immunocompromised individuals, infection may become more widely disseminated infecting organs such as lung, brain, liver, spleen etc.

HSV antigen may be present and the virus cultured from the fluid of vesicular lesions as microwell as saliva 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.

The likelihood of detecting HSV decreases with time following the onset of disease and the development of lesions. The probability of viral isolation decreases as the lesion ulcerates, crusts and heals. Specimens should be collected as soon as possible after the appearance of lesions[5,6]. In one study, virus was recovered from 94% of vesicular lesions, 87% of pustular lesions, 70% of ulcers and 27% of crusted lesions[7].

14. SPECIFIC PERFORMANCE CHARACTERISTICS

All results given assume that the cell culture methods are 100% sensitive and specific.

14.1. CLINICAL STUDIES

The IDEIA HSV test was evaluated against established cell culture systems at four independent routine diagnostic laboratories.

A total of 1375 human clinical specimens were tested using the IDEIA HSV test and results were compared to the results of the cell culture system employed by the trial centres (Table 14.1). In these studies the incidence of HSV infection (by cell culture) was 12.7 to 36.9%.

Each specimen was scored positive in the IDEIA HSV test when the absorbance reading of the specimen at 492nm was greater than the recommended cut off value (see Section 11.1). A specimen was scored as positive in the cell culture when the characteristic cytopathic effect of HSV was observed.

Positive cell cultures were typed using direct immunofluorescence at two of the trial centres. Of the 37* positive specimens typed at Centre 1,15/37 (40.5%) were type 1 and 22/37 (59.5%) were type 2. At Centre 4, 14/71 (19.7%) were type 1 and 57/71 (80.3%) were type 2.

*(4 positive specimens were unavailable for typing studies).

The IDEIA HSV test result agreed with cell culture result in 1302 out of 1375 specimens, an overall agreement of 94.7%.

Resolution of 29 discrepant "false positive" IDEIA HSV test results after reference to available clinical details gives a final overall agreement of 96.8% (1331/1375).

14.2. PERFORMANCE

Sensitivity*

Overall, the sensitivity of the IDEIA HSV test was 93.6% (307/328).

Specificity*

Overall, the specificity of the IDEIA HSV test was 97.8% (1024/1047).

Predictive Values*

Overall, the positive and negative predictive values of the IDEIA HSV test were 93.0% (307/330) and 98.0% (1024/1045) respectively.

*After resolution of 29 discrepant "false positive" IDEIA HSV test results, (see Table 14.2).

14.3. ASSAY PRECISION

Table 14.2 shows the results of assay precision studies for the IDEIA HSV test.

Control suspensions of 3 levels of HSV antigen in IDEIA HSV Transport Medium were assayed in triplicate on three separate occasions (a total of 9 assays). Results are expressed in absorbance units at 492nm. The observed ranges for intra-assay and inter-assay CV were 1.2 to 6.6% and 5.0 to 8.4% respectively.

14.4. CROSS REACTIVITY

The following list of organisms, at approximate concentration of 10^7 organisms/mL for non-viral cultures and approximately 50-100% CPE for viral cultures, were found to be non-reactive with the IDEIA HSV test.

Acholeplasma laidlawii	*Mycoplasma spp*
Acinetobacter spp	*Neisseria gonorrhoeae*
Aeromonas spp	*Peptococcus spp*
Bacteroides spp	*Peptostreptococcus spp*
Campylobacter spp	*Proteus spp*
Candida spp	*Pseudomonas spp*
Citrobacter spp	*Salmonella spp*
Chlamydia trachomatis	*Serratia spp*
Clostridium spp	*Shigella spp*
Cytomegalovirus	*Staphylococcus aureus* (cowan 1 strain)
Enterobacter spp	*Staphylococcus spp* (coag.neg)
Epstein Barr Virus	*Staphylococcus spp* (coag.pos)
Escherichia coli	*Streptococcus spp*
Gardnerella spp	*Trichomonas spp*
Haemophilus influenzae	*Ureaplasma urealyticum*
Klebsiella spp	*Varicella zoster virus*
Lactobacillus spp	*Veillonella spp*
Listeria spp	

Table 14.1: Comparison of Test Results by the IDEIA HSV Test and Cell Culture

STUDY	% INCIDENCE BY CULTURE	IDEIA HSV TEST: SENSITIVITY		IDEIA HSV TEST: SPECIFICITY		PREDICTIVE VALUES: POSITIVE		PREDICTIVE VALUES: NEGATIVE	
			*		*		*		*
1 (UK)	35.3% (41/116)	90.2% (37/41)	**90.2% (37/41)**	98.7% (74/75)	**98.7% (74/75)**	97.3% (37/38)	**97.3% (37/38)**	94.9% (74/78)	**94.9% (74/78)**
2 (UK)	36.9% (89/241)	89.9% (80/89)	**90.2% (83/92)**	94.1% (143/152)	**96.0% (143/149)**	89.9% (80/89)	**93.3% (83/89)**	94.1% (143/152)	**94.1% (143/152)**
3 (UK)	12.7% (98/774)	94.9% (93/98)	**95.5% (106/111)**	97.5% (659/676)	**99.4% (659/663)**	84.5% (93/110)	**96.4% (106/110)**	99.2% (659/664)	**99.2% (659/664)**
4 (US)	29.1% (71/244)	95.8% (68/71)	**96.4% (81/84)**	85.5% (148/173)	**92.5% (148/160)**	73.1% (68/93)	**87.0% (81/93)**	98.0% (148/151)	**98.0% (148/151)**
Total		**93.0% (278/299)**	**93.6% (307/328)**	**95.2% (1024/1076)**	**97.8% (1024/1047)**	**84.2% (278/330)**	**93.0% (307/330)**	**98.0% (1024/1045)**	**98.0% (1024/1045)**

* Data in these columns are those recalculated after resolution of 29 "false positive" IDEIA results. These specimens were from patients who either tested positive at an adjacent site, had HSV positive partners or had previous history of HSV.

Table 14.2: Intra and Inter-assay Reproducibility of the IDEIA HSV Test

Antigen Level	Intra-assay (n=3)									Inter-assay (n=9)		
	DAY 1			DAY 2			DAY 3					
	Mean	SD	% CV	Mean	SD	% CV	Mean	SD	% CV	Mean	SD	% CV
Negative	0.105	0.005	4.9	0.104	0.006	5.4	0.108	0.005	4.6	0.106	0.005	5.0
Low Positive	0.275	0.010	3.5	0.287	0.010	3.7	0.247	0.003	1.2	0.270	0.019	7.0
High Positive	1.279	0.079	6.2	1.205	0.080	6.6	1.100	0.037	3.4	1.195	0.100	8.4

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