SimulFluor®
HSV/VZV

Immunofluorescence Assay

Cat. No. 3295

FOR IN VITRO DIAGNOSTIC USE

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Intended Use

The Light Diagnostics™ SimulFluor® HSV/VZV Immunofluorescence Assay is intended for the simultaneous detection and identification of herpes simplex viruses (HSV) 1 and 2 and varicella-zoster virus (VZV) from patients with vesicular, oral, genital, or skin lesions, using direct specimens and culture confirmation. Specimens found to be negative on direct specimen examination must be confirmed with culture.

For in vitro diagnostic use.

Summary and Explanation

Herpes simplex virus (HSV) and varicella-zoster virus (VZV) are members of the Herpesviridae family. They are large, enveloped viruses, about 190 nm in diameter containing linear, double-stranded DNA. Both viruses cause a multitude of human diseases and are particularly severe in immunocompromised patients.

There are two biologically distinct serotypes of HSV classified as type 1 (HSV-1) and type 2 (HSV-2). The serotypes are closely related with extensive sequence homologies of their DNA’s. HSV is a major cause of conjunctival, respiratory, central nervous system, genital, and generalized disease (1-4). HSV infects mucocutaneous surfaces, then enters the dorsal root ganglia where further viral replication occurs, followed by a period of latency. Reactivation is accompanied by viral excretion at, or close to, the original mucocutaneous site of infection, with or without, the associated clinical signs and symptoms. Recurrent lesions are usually less severe than the primary infection.

HSV–1 causes gingivostomatitis, intense pharyngitis, tonsillitis, and occasionally encephalitis in infants and children during their primary infections. Ocular, nasal, orolabial, and oropharyngeal lesions occur in both children and adults. Due to the ubiquity of HSV-1 and its ease of spread by aerosolized droplets, fomites, and direct contact, most adults experience HSV-1 infection during their lifetime.
HSV-2 is more frequently associated with painful genital lesions, urethritis, and cervicitis in adults. If the virus is present in the birth canal around the time of delivery, either from primary or recurrent infection, severe generalized infection in the neonate may occur. Thus, maternal genital HSV infections pose a substantial risk to the fetus and newborn.

Shedding of virus at the time of delivery is frequently the route of transmission from mother to neonate. Neonatal HSV infection is generally symptomatic and often fatal, with a mortality rate in untreated cases of 70%. The clinical presentation may be localized infection of the skin, eyes and mucosa, encephalitis, or disseminated disease.

Acyclovir, famciclovir, foscarinet, and other nucleoside analogs can reduce clinical symptoms and virus shedding in oral and genital herpes, herpes encephalitis, neonatal herpes, and herpetic keratitis (5-7).

HSV can be readily recovered from clinical specimens following culture in cells lines such as HEp2, MRC-5, A549, HEK, NCI-H292, and others. Incubation time in stationary or roller cultures ranges from 1 to 7 days with evident cytopathology. Adequate specimens include eye swabs, swabs of vesicular lesions, saliva, throat swabs, cerebrospinal fluid, and tissues, as dictated by the clinical symptoms. Urine may also be a valid specimen.

VZV causes two different clinical syndromes: chickenpox (varicella) - primarily in children, and shingles (zoster) - predominantly in adults (8-10). The most notable feature of varicella is a generalized vesicular rash which is usually accompanied by fever. Complications, such as pneumonia, can occur in neonates and in immunocompromised patients with a mortality of 10 to 40%.

Zoster occurs as a reactivation of the latent varicella virus, and can result from almost any stimulus, such as fear, anxiety, etc. Pregnant women are at greater risk for varicella pneumonia than other adults. Postherpetic neuralgia is another complication of both varicella and zoster and is a significantly greater problem in immunosuppressed patients.

As with HSV, acyclovir is the drug of choice for reducing symptoms due to VZV infection (8,11,12). In 1995, a varicella vaccine was approved for use in the United States; this vaccine gives a 70 to 85% protection rate in children (10,13,14).
VZV can be isolated from clinical specimens, usually skin lesions, but also lung, eye, throat, and vesicular fluid in human diploid fibroblast cell cultures. Its cytopathology is fairly distinct from that of HSV. However, the virus is more labile than HSV and may not grow in culture unless care is taken in transporting the specimen to the lab.

The purpose of combining HSV and VZV antibodies in a single test reagent is that both viruses can cause many of the same symptoms and clinical findings. HSV must be identified to prevent the spread of the virus to neonates at birth and to prevent spread of the venereal disease to other adults. Specific identification of the virus in conjunctival specimens allows prompt treatment with acyclovir to reduce the chance of blindness.

**Test Principle**

Light Diagnostics™ SimulFluor® HSV/VZV Immunofluorescence Assay utilizes a single reagent for the simultaneous detection and identification of HSV and VZV. The primary component containing monoclonal antibodies, specific for both HSV 1 and 2 will bind to 155kD major capsid protein in HSV-infected cells. The secondary component containing monoclonal antibodies, specific for VZV, will bind to glycoprotein gp I and the immediate early antigen in VZV-infected cells. Unbound reagent is removed by rinsing with phosphate-buffered saline (PBS). Illumination with ultraviolet light allows visualization of the antigen-antibody complexes by fluorescence microscopy. When a FITC filter set is used, the HSV antigen-antibody complex will exhibit an apple-green fluorescence and the VZV antigen-antibody complex will fluoresce yellow-gold. Uninfected cells stain a dull red due to the presence of Evans blue in the reagent.

**Materials Provided**

1. SimulFluor® HSV/VZV - (Catalog No. 5235). One 5 mL dropper vial containing monoclonal antibodies specific for HSV antigen and specific for VZV antigen, protein stabilizer, Evans blue and 0.1% sodium azide (preservative).

2. HSV Control Slides - (Catalog No. 5093). Two slides containing one well of HSV -1 infected HEp-2 cells, one well of HSV -2 infected cells, and one well of uninfected cells.
3. **VZV Control Slides** - (Catalog No. 5088). Two slides containing one well of VZV (clinical isolate) infected human foreskin fibroblasts and one well of uninfected human foreskin fibroblasts.

4. **Phosphate-Buffered Saline (PBS)** - (Catalog No. 5087). One packet of phosphate buffered saline salts.

5. **Tween 20 / Sodium Azide Solution (100X)** - (Catalog No. 5037). One 10 mL vial containing Tween 20/sodium azide concentrate.

6. **Mounting Fluid** - (Catalog No. 5013). One 10 mL dropper vial containing Tris-buffered glycerin, a fluorescence enhancer, and 0.1% sodium azide (preservative).

**Materials Required But Not Provided**

- Acetone, reagent grade or better; stored in glass
- Deionized or distilled water
- Positive controls, for culture isolation procedures (reference HSV and VZV strains available from ATCC, Rockville, MD)
- Sodium hypochlorite solution, 0.05% (1:100 dilution of household bleach)
- Sterile shell vials with 12 mm coverslips for growth of MRC-5 or other HSV/VZV permissive cell line
- Tissue culture media (RPMI or Eagle's Minimum Essential Medium (EMEM) with precolostral bovine serum (FBS) and antibiotics or equivalent)
- Viral transport medium which is non-inhibitory to HSV/VZV (Hanks balanced salt solution (HBSS) with antibiotics or equivalent)
- Sterile PBS, pH 7.0 to 7.6
- Microscope slides, non-fluorescing
- No. 1 coverslips
- Aspirator device with disposable sterile Pasteur pipettes
• Centrifuge capable of 700 to 950 x g with biohazard buckets and adapters for shell vials
• Cytocentrifuge capable of depositing cell suspensions on slides
• Fluorescence microscope with 100 watt mercury or halogen lamp, appropriate filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm), 100x and 400x magnification (dry objective)
• Optional: Filter combination for TRITC (excitation peak = 550nm, emission peak = 570nm) **NOTE:** Switching from a FITC to a TRITC filter will cause apple green fluorescence to disappear and yellow-green fluorescence to fluoresce hot pink, aiding in confirmation of non-specific staining.
• Forceps
• Humid chamber
• Incubator, 37 ± 1°C
• Syringe and needle or other implement to remove coverslip from shell vial
• Ultrasonic water bath
• Vortex mixer or sonicator

**Stability and Storage**
When stored at 2° to 8°C, the SimulFluor® HSV/VZV Immunofluorescence Assay kit is stable up to the expiration date printed on the kit label. Do not freeze or expose to elevated temperatures. Discard any remaining reagents after the kit expiration date.
Warnings and Precautions

- For *In Vitro* Diagnostic use
- The sodium azide (NaN₃) used as a preservative in the SimulFluor® reagent, PBS/Tween, and Mounting Fluid is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides (15,16). Upon disposal, flush with large volumes of water to prevent build-up in plumbing.
- Pooling or alteration of any reagent may cause erroneous results.
- Do not substitute reagents from other manufacturers.
- Incubation times or temperatures other than those specified may give erroneous results. Any such change must be validated by the user.
- Do not allow shell vials or slides to dry at any time during the staining procedure.
- Handle all specimens and materials, coming in contact with them as potentially infectious and dispose of with proper precautions. Decontaminate with 0.05% sodium hypochlorite.
- Do not mouth pipette reagents.
- Acetone is extremely flammable and harmful if swallowed or inhaled. Keep away from heat, sparks or flame. Avoid breathing vapor. Use adequate ventilation.
- Avoid contact with Evans blue (present in the SimulFluor® reagent) as it is a potential carcinogen. If skin contact occurs, flush with large volumes of water.
- Mounting Fluid contains a fluorescence enhancer that may be destructive to mucous membranes. Avoid direct skin or mucous membrane contact. If contact occurs, flush with large volumes of water.
Specimen Collection

Accurate detection of HSV/VZV is dependent upon proper sample collection, transport and storage. Specimens for direct detection and culture isolation should be obtained from the base of a vesicular lesion using a Dacron swab. Vigorous scraping at the exposed base of the lesion is necessary to collect an adequate sample. Avoid contamination of the specimen with blood to prevent the possibility of a false negative result from serum antibody binding. Vesicular fluid, extracted with a sterile needle and syringe, is an ideal sample for isolation but inadequate for direct detection. Swab samples should be used immediately for making direct cell smears or placed in viral transport medium. Specimens should be transported on wet ice, and cultured or prepared for direct detection as soon as possible. Because VZV is very labile, specimens should transport to the laboratory as quickly as possible and inoculated into culture within 12 hours of collection. If long term storage is necessary, freezing at ≤ -70°C is recommended.

Specimen Processing

Direct Specimen:
Direct specimen detection may be performed whenever adequate specimens are available. It is recommended that culture isolation be performed for confirmation of negatives. If insufficient sample is available to perform both direct detection and culture, submit sample for culture isolation only.

Specimen Preparation
Cellular material from the base of vesicular lesions may be used for direct immunofluorescence detection. Basal, parabasal, and intermediate cells scraped from the base of the lesion should provide an appropriate specimen. Remove the cap of the vesicle and aspirate any vesicular fluid (for use in isolation procedures). Moisten a Dacron swab with sterile water or transport medium and vigorously scrape the base of the lesion. Contamination of the swab with blood should be avoided.
**Preparation of Direct Cell Smears from Swab**

Prepare cell smears by rolling the swab across the top half of the well of a microscope slide. Roll the opposite side of the swab over the bottom half of the well and allow the slide to air dry completely. Fix the slide in chilled (2° to 8°C) acetone for 10 minutes, remove and allow to air dry completely. Slides should be stained immediately (see “Staining Procedure”) or if necessary, store slides at ≤ -20°C with desiccant.

> All specimens prepared as cell smears should be accompanied by a sample of vesicular fluid or a swab specimen for use in cell culture isolation.

**Preparation of Direct Specimens from Swabs in Transport Medium**

Immediately place the swab into transport medium, vortex, and extract any additional fluid from the swab by pressing against the inside of the transport vessel.

> Reserve an adequate sample of cellular material for use in culture isolation.

Discard the swab into sodium hypochlorite solution. Centrifuge the cell suspension at 250 to 500 x g for 5 to 10 minutes. Aspirate the supernatant and resuspend the cell pellet in a sufficient quantity of PBS to make a slightly cloudy suspension (50 to 100 µL). The quality of the slide is dependent on the cell concentration of the suspension. Heavy suspensions are difficult to read and excessively dilute suspensions may reduce sensitivity. Optimal suspensions will yield cell spots with approximately 25 cells per 200x field. Use the cell suspension to make cell spots in several 6 to 8 mm slide wells and allow the spots to air dry completely. Fix the slide in chilled (2° to 8°C) acetone for 10 minutes, remove and allow to air dry completely. Slides should be stained immediately (see “Staining Procedure”) or if necessary, store slides at ≤ -20°C with desiccant.

**Preparation of Direct Specimens Using a Cytospin**

Wash and centrifuge cell suspension as described above. Resuspend cells to at least 400 µL in PBS and apply 200 µL to each slide by centocentrifugation at 800 rpm for 4 minutes (17). Air-dry cells completely and fix with acetone as described above.
Specimens for Culture Isolation:

**Swabs** - Swab specimens (collected as described above) in transport media should be agitated or vortexed to dislodge cells from the swab. For increased cell recovery, sonicate the specimen at 8 to 12 kc/sec for up to one minute. Discard the swab into sodium hypochlorite solution. Centrifuge the specimen at 950 x g for 7 to 10 minutes, discard the supernatant fluid, resuspend in an appropriate volume of serum-free media and use this as inoculum for virus isolation.

**Vesicular Fluids** - Vesicular fluids should be collected with a sterile needle and syringe and immediately injected into transport medium. Use this as inoculum for virus isolation.

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**Virus Isolation**

*Caution: PBS with Tween 20/Sodium Azide should not be used in viral isolation procedures.*

**Shell Vial Isolation - Centrifugation Enhanced Technique:**

1. MRC-5 cells (or other HSV/VZV permissive cell line) are seeded onto coverslips in shell vials and grown to ≥ 70% confluency. Prepare at least 2 shell vials per specimen.

2. Aspirate growth medium, add 0.2 to 0.5 mL of inoculum to each vial and centrifuge at 700 to 950 x g for 60 minutes at room temperature (15 to 30°C).

3. Aspirate inoculum and refeed immediately with 1 mL of cell maintenance medium (RPMI or equivalent supplemented with gentamicin and 2% FBS).

4. Incubate at 37°C. Prepare vials for staining 24 to 120 hours after inoculation. Additional vials may be prepared for longer incubation periods.

   *Note: Specimens with high viral load may exhibit staining as early as 12 hours post infection.*

5. Aspirate the maintenance medium and gently wash shell vials 3 times with 1 mL of PBS. *Caution: PBS should not be directed onto the monolayer but allowed to run down the side of the vial.*
6. Aspirate the PBS from the shell vial and fix as follows: add 1 mL of chilled (2° to 8°C) acetone (use caution, acetone is extremely flammable and harmful if swallowed or inhaled), aspirate, and immediately refill with 1 mL of chilled acetone. Fix for 10 minutes at 2° to 8°C.

7. Aspirate the acetone and allow the shell vials to dry completely. Rinse with 1 mL PBS, aspirate, and stain. If storage is necessary, add 1 mL of PBS / sodium azide, cap tightly and store at 2° to 8°C.

**Standard Tube Culture Isolation Procedure:**

1. MRC-5 cells (or other HSV/VZV permissive cell line) are grown to ≥ 70% confluency in culture tubes.

2. Aspirate the growth medium, add 0.2 to 0.5 mL of inoculum and place on a slant rack or in a roller drum for 1 hour at room temperature, or manually rotate every few minutes for 60 minutes at room temperature.

3. Aspirate the inoculum and add sufficient maintenance medium to completely cover the cell monolayer. Incubate at 37°C.

4. Examine the monolayer daily for cytopathic effect (CPE). When the CPE is ≥ 2+ (10 to 50 foci), aspirate the medium from the culture and gently rinse the monolayer 3 times with 1 mL of PBS.

5. Add 0.5 mL of PBS and scrape the culture tube to remove the cell monolayer, resuspending the cells in PBS.

6. If necessary, centrifuge the cell suspension at 250 x g for 10 minutes at room temperature.

7. Resuspend the cell pellet in PBS to make a slightly cloudy suspension (50 to 100 µL).

8. Use the cell suspension to make cell spots in several 6 to 8 mm slide wells and allow the spots to air dry completely.

9. Fix the slide in chilled (2° to 8°C) acetone for 10 minutes (use caution, acetone is extremely flammable and harmful if swallowed or inhaled).
10. Remove the slide from the acetone and allow to air dry completely.

11. The slide should be stained immediately. If storage is necessary, the slides should be kept at ≤ -20°C, with desiccant.  

**Note:** A sample of each lot of the cell lines used for cell culture should be inoculated with reference HSV and VZV strains to ensure infection sensitivity and appropriate CPE. HSV cytopathic effects are detected by observation of foci of enlarged, refractile cells in the monolayer. Typical VZV cytopathic effects are the formation of small, discrete foci of rounded swollen refractile cells. Uninoculated cell cultures should also be grown and examined daily. These will act as a control for normal cell morphology and could be useful in detecting early CPE. Unless these control cell cultures show appropriate growth, the results of the cell culture isolation should be considered invalid.

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**Staining Procedure**

**Reagent Preparation:**

PBS / Tween - Dissolve the contents of the PBS packet in 950 mL of deionized or distilled water. Add the contents of the Tween 20 / Sodium Azide vial to the PBS; mix thoroughly; Q.S. to one liter with deionized or distilled water. Transfer to a clean labeled storage container and cap tightly; store at room temperature. Discard if PBS becomes turbid or forms a precipitate. All other reagents are provided ready to use.

**Suggested Direct Immunofluorescence (Staining) Procedure:**

1. Allow the acetone fixed control slide and/or test slide and reagents to equilibrate to room temperature.  

   **Note:** Do not allow slides to dry at any time during the staining procedure.

2. Add sufficient DFA Reagent to cover the cells; 1 drop for cell spots and 4-6 drops for shell vials.

3. Incubate the slide at 37°C for 30 minutes in a humid chamber.
4. Rinse the slide gently with a squirt bottle of PBS/Tween 20 for 10-15 seconds to remove excess monoclonal antibody solution, taking care to direct the stream away from the well. For shell vials: aspirate reagent from vial and gently wash each shell vial 3 times with 1mL PBS/Tween 20.

5. Shake off excess reagent from the slide and carefully dry the area surrounding the cell spot.

6. Mount under a coverslip using an aqueous Mounting Medium pH 8.5, (Catalog No. 5013 or equivalent). For shell vials: Aspirate PBS/Tween from shell vials. Raise each coverslip using a bent needle affixed to a small syringe and carefully remove with forceps. Mount each coverslip CELL SIDE DOWN on a glass slide with Mounting Fluid.

7. Wipe excess fluid from the edges of the slide.

   **Note:** For best results, read slides immediately after preparation. If slides are to be stored after staining, store at 2°C to 8°C, in a secure container in the dark.

8. Examine slides, using a fluorescence microscope at 160-200x for cells exhibiting fluorescence. Detailed examination may be carried out at 400x magnification.

   **Note:** Performance of the fluorescence microscope is of critical importance in achieving satisfactory test results. While objectives, bulb intensity and wattage, and filters may affect results, use of a positive control will verify functioning of reagents, culture methodology and microscope.
Interpretation of Results

Quality Control

Shell vials inoculated with reference HSV strains, VZV strains and uninoculated cultures should be maintained and tested in parallel to ensure proper culture isolation and staining procedures. The control slides supplied with the kit are intended to demonstrate the proper function of kit components and procedures during immunofluorescence staining.

Note: Scan the entire coverslip or slide well for the presence of cell associated fluorescence.

When viewed using a FITC filter set, a positive reaction for HSV is indicated by a bright apple-green fluorescence in the cytoplasm and/or cell membrane. A positive reaction for VZV is indicated by yellow-gold fluorescence in the nucleus and/or cytoplasm of the infected cells. The pattern of fluorescence is finely granular and may be present in basal, parabasal or intermediate cells.

Direct Specimen:

When viewed using a FITC filter set, a positive reaction for HSV is indicated by a bright apple-green fluorescence in the cytoplasm and/or cell membrane of the infected epithelial cells. A positive reaction for VZV is indicated by yellow-gold fluorescence in the nucleus and/or cytoplasm of the infected epithelial cells. The pattern of fluorescence is finely granular and may be present in basal, parabasal or intermediate cells.

Positive staining for HSV and/or VZV is represented by the presence of 2 or more epithelial cells exhibiting specific fluorescence. A presumptive negative result is indicated by the absence of fluorescence in a minimum sampling of 20 epithelial cells. An inadequate sample is indicated by fewer than 20 epithelial cells present in the sample and the test is considered invalid.
**Caution:** Fluorescent staining of cell fragments, due to trapping of the conjugate in such debris, should be ignored. If the positive and negative controls cannot be clearly distinguished, the test should be considered invalid. A negative result does not rule out HSV and/or VZV infection. The negative result may be due to a variety of factors such as: inadequate sample, improper specimen collection and handling, improper culture technique, use of inappropriate cell line or temperature during isolation, or other factors mentioned in the “Troubleshooting” section. All presumptive negative results should be reported as “No virus observed”. It is useful to examine the negative cells prior to the positive cells to determine if there is non-specific staining.

**Cell Culture Isolation / Confirmation:**

Examine the control slides first to ensure proper culture isolation and staining procedures. If the controls do not function adequately, the assay is considered invalid.

When viewed using a FITC filter set, a HSV positive reaction is indicated by a bright apple-green fluorescence in the cytoplasm and/or cell membrane of the infected cells. A VZV positive reaction is indicated by a yellow-gold fluorescence in the nucleus, cytoplasm and/or cell membrane of the infected cells.

Positive staining for HSV and/or VZV is represented by the presence of at least 2 or more intact cells exhibiting specific fluorescence. A presumptive negative reaction is indicated by the absence of fluorescence and presence of a dull red color due to the Evans blue counterstain.

**Confirmation of VZV Staining**

The yellow-gold staining of VZV-infected cells can be confirmed by examination using a TRITC filter set. Under a TRITC filter, HSV–infected cells will not exhibit fluorescence staining while VZV-infected cells will exhibit bright pink fluorescence staining.
Limitations

- A negative result on direct specimen testing does not rule out the possibility of infection with HSV or VZV and must be confirmed by culture isolation.
- A negative result in cell culture does not rule out HSV or VZV infection. A negative result may be due to a variety of factors such as: time of collection during infection, inadequate sample, improper specimen collection and handling, improper culture technique, use of an inappropriate cell line or temperature during isolation, or other factors mentioned in the “Troubleshooting” section.
- The use of a 10x objective (100x magnification) may not provide sufficient magnification to see cell morphology and staining pattern, particularly for cells infected in VZV.
- The monoclonal antibodies used in this kit were prepared using prototype strains and may not detect all antigenic variants or new strains of HSV and/or VZV, or those that have undergone changes in the target epitope region.
- Direct specimen staining procedures detect HSV and/or VZV antigen from vesicular smears and cannot be used to determine the viability of HSV and/or VZV.
- VZV is a very labile agent. Culture should be performed within 12 hours of collection of samples. If necessary, freezing at ≤ -70°C is recommended.
- Protein A, produced by certain bacteria, will bind the Fc portion of the monoclonal antibody used in the Light Diagnostics™ SimulFluor® HSV/VZV. Bacterial contamination would be identifiable in a tissue culture isolate and such samples should be eliminated from analysis. Staining however, could be differentiated by size and morphology. The presence of Staphylococcus aureus (producing protein A) will result in fluorescence of the cell wall which is bright, small (0.8-1.0µm) and spherical.
- The performance characteristics for this test have not been established for monitoring therapy.
- Performance of the fluorescence microscope is of critical importance in achieving satisfactory test results. While objectives, bulb intensity and wattage and filters may affect results, use of appropriate controls will verify functioning of reagents, culture methodology and microscope.
Expected Values

Site 1
At a hospital laboratory that also acts as a regional reference lab in the northeastern US, 203 specimens were submitted for HSV or VZV testing between December, 1997 and June, 1998. HSV isolates were not sub-typed. HSV was isolated from 58 specimens for an overall prevalence of 28.6%, while VZV was identified in 21 specimens for a prevalence of 10.3%. Sixty-five (32%) of the samples were from genital sites, 16 (7.9%) were from the oral cavity (including lips, throat, etc), 111 (54.7%) were skin lesions and 4 (2%) were ocular specimens. The remaining samples were from various tissues or site was not recorded. The relative prevalence of HSV or VZV from each specimen type are indicated below.

Table 1. Prevalence of HSV and VZV in Different Specimen Sources

<table>
<thead>
<tr>
<th></th>
<th>Genital</th>
<th>Oral</th>
<th>Skin</th>
<th>Ocular</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV</td>
<td>33.9%</td>
<td>25.0%</td>
<td>27.9%</td>
<td>0</td>
<td>14.3%</td>
</tr>
<tr>
<td>VZV</td>
<td>1.5%</td>
<td>0</td>
<td>16.2%</td>
<td>25.0%</td>
<td>14.3%</td>
</tr>
</tbody>
</table>

Oral: lip, mouth, palette
Other: tissue biopsy, axilla, tibia, or unknown

Site 2
At a large hospital on the west coast, 283 specimens were submitted for HSV or VZV testing between October, 1997 and June, 1998. HSV was isolated from 83 specimens (51 HSV-2 and 32 HSV-1) for an overall prevalence of 21.7%, while VZV was identified in 17 specimens for a prevalence of 4.4%. One hundred and sixty-two (42.3%) samples were from genital sites, 109 (28.5%) from the oral cavity (including lips, throat, etc), 90 (23.5%) were skin lesions and 10 (2.6%) were ocular specimens. The remaining specimens were derived from various tissues and bronchial lavage. The relative prevalence of HSV or VZV isolated from each specimen type are indicated in the table below.

Table 2. Prevalence of HSV and VZV in Different Specimen Sources

<table>
<thead>
<tr>
<th></th>
<th>Genital</th>
<th>Oral</th>
<th>Skin</th>
<th>Ocular</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1</td>
<td>7.4%</td>
<td>23.9%</td>
<td>5.5%</td>
<td>20.0%</td>
<td>54.5%</td>
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<tr>
<td>HSV-2</td>
<td>15.4%</td>
<td>1.8%</td>
<td>5.5%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VZV</td>
<td>0%</td>
<td>0.9%</td>
<td>17.7%</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Oral includes lip, mouth, gum, throat specimens
Other: tissue sections, bronchial lavage,
**Specificity and Cross Reactivity**

The **Light Diagnostics™ SimulFluor® HSV/VZV** was evaluated to determine specificity. A representative sample of various microorganisms was tested demonstrating the following results:

**Table 1. Specificity**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herpes simplex virus 1 ATCC VR733/735</td>
<td>+ (green staining)</td>
</tr>
<tr>
<td>Clinical isolates (9)</td>
<td>+ (green staining)</td>
</tr>
<tr>
<td>Herpes simplex virus 2 ATCC VR734</td>
<td>+ (green staining)</td>
</tr>
<tr>
<td>Clinical isolates (8)</td>
<td>+ (green staining)</td>
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<tr>
<td>Varicella zoster virus Oka strain</td>
<td>+ (gold staining)</td>
</tr>
<tr>
<td>Cytomegalovirus Clinical isolate 70-35</td>
<td>-</td>
</tr>
<tr>
<td>Human herpes virus 6 strain Z-29</td>
<td>-</td>
</tr>
<tr>
<td>Epstein-Barr virus Hu. Lymph. P3HR1</td>
<td>-</td>
</tr>
<tr>
<td>Adenovirus CDC strains V5002</td>
<td>-</td>
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<tr>
<td>Influenza A Clinical isolate</td>
<td>-</td>
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<tr>
<td>Influenza B Clinical isolate</td>
<td>-</td>
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<tr>
<td>Mumps CDC V5004</td>
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<tr>
<td>Parainfluenza 1 CDC V6004</td>
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<tr>
<td>Parainfluenza 2 CDC V7003</td>
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<tr>
<td>Parainfluenza 3 CDC V5003</td>
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<td>Parainfluenza 4 ATCC strain VR-1378</td>
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<tr>
<td>Respiratory syncytial virus CDC strain A2</td>
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<td>Rubella VR315 strain M-33</td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
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<tr>
<td><em>Trichomonas vaginalis</em></td>
<td>-</td>
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<tr>
<td><em>Bordetella bronchiseptica</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Bordetella pertussis</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Chlamydia trachomatis</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Chlamydia pneumonia</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>-</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Streptococcus pneumonia</em></td>
<td>-</td>
</tr>
<tr>
<td>Organism</td>
<td>Result</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>-</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>-</td>
</tr>
<tr>
<td>Mycoplasma hominis</td>
<td>-</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>-</td>
</tr>
<tr>
<td>Corynebacterium diphtheriae</td>
<td>-</td>
</tr>
<tr>
<td>Legionella micdadei</td>
<td>-</td>
</tr>
<tr>
<td>Legionella pneumophila</td>
<td>-</td>
</tr>
<tr>
<td>Neisseria Meningitidis</td>
<td>-</td>
</tr>
<tr>
<td>Branhamella catarrhalis</td>
<td>-</td>
</tr>
</tbody>
</table>

Five host cell cultures were also tested to ensure no cross-reactivity using the Light Diagnostics™ SimulFluor® HSV/VZV. MRC-5, A549, Vero, LLC-Mk₂ and HEp-2 cells were tested, and no cross reactivity was observed. Staphylococcus aureus, (Cowan strain), contaminated cultures were tested using the Light Diagnostics™ SimulFluor® HSV/VZV kit to determine the staining activity to the protein A producing bacteria. Staining of S. aureus appeared as bright, small, spherical points of fluorescence.

**Performance Characteristics**

The performance of the Light Diagnostics™ SimulFluor® HSV/VZV on direct specimen tests and on culture confirmation was compared to two predicate devices for the detection of HSV-1, HSV-2, and VZV in culture. Studies were performed at two sites in the United States: Site 1 was in the Northeast and performed during October, 1997 through June, 1998; and Site 2 was on the west coast and performed from October, 1997 through January, 1998.

**Clinical Accuracy**

The Light Diagnostics™ SimulFluor® HSV/VZV reagent contains monoclonal antibodies directed against VZV and HSV, but does not differentiate between HSV type 1 and HSV type 2. As a part of the clinical studies, some HSV isolates were typed by a predicate device as either HSV type 1 or HSV type 2. At Site 1, 30 HSV type 1 isolates and 25 HSV type 2 isolates were identified as HSV. At Site 2, 51 HSV type 1 isolates and 32 HSV type 2 isolates were identified. The results of the clinical evaluation are below.
Reproducibility

Reproducibility testing consisted of blinded proficiency panels of 12 well control slides. The panels were prepared using HSV infected, VZV infected and uninfected cells on fixed slides. Each 12 well slide contained 8 infected wells and 4 uninfected wells. The panels were tested at 2 clinical sites prior to the clinical evaluation of the device.

At Site 1, five technicians performed the proficiency panel resulting in the identification of 40 out of 40 positive wells and 19 out of 20 negative wells, yielding a 100% agreement for positives and 95% agreement for negatives.

At Site 2, ten technicians performed the proficiency panel resulting in the identification of 80 out of 80 positive wells and 37 out of 40 negative wells, yielding a 100% agreement for positives and 92.5% agreement for negatives. Repeat testing of 2 additional panels yielded 100% agreement of both positive and negative wells.

Clinical Evaluation

SITE 1

Two hundred and three specimens were submitted to a hospital laboratory which was also a regional reference lab for testing of HSV and VZV. Samples were submitted in viral transport media, the cells were washed, slides were made, and fixed with acetone. Slides were stained with the SimulFluor® HSV/VZV, the predicate HSV DFA and the predicate VZV DFA reagents. Specimens were then inoculated into MRC-5 or HNF cells in standard tube culture and were stained when CPE was seen.

Of the 203 specimens submitted for HSV and/or VZV testing, 36 were excluded from analysis due to inadequate specimens for direct detection.

DETECTION OF HSV BY DIRECT SPECIMEN TESTING

Thirty three specimens were positive by both direct testing and culture. Culture identified an additional 18 isolates and the SimulFluor® HSV/VZV incorrectly identified 2 specimens as positive for HSV.
Direct Specimen Testing of HSV vs Culture Isolation

<table>
<thead>
<tr>
<th></th>
<th>Culture Positive</th>
<th>Culture Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SimulFluor® HSV/VZV Positive</strong></td>
<td>33</td>
<td>2</td>
<td>35</td>
</tr>
<tr>
<td><strong>SimulFluor® HSV/VZV Negative</strong></td>
<td>18</td>
<td>78</td>
<td>96</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>51</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity = 64.7% (33/51) 95% Confidence Interval 50.1% - 77.62%
Specificity = 97.5% (78/80) 95% Confidence Interval 91.3% – 99.7%

DETECTION OF VZV BY DIRECT SPECIMEN TESTING

Twenty specimens were positive by both direct testing and culture. No additional isolates were identified by culture. SimulFluor® HSV/VZV incorrectly identified 16 specimens as positive for VZV.

Direct Specimen Detection of VZV vs Culture Isolation

<table>
<thead>
<tr>
<th></th>
<th>Culture Positive</th>
<th>Culture Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SimulFluor® HSV/VZV Positive</strong></td>
<td>20</td>
<td>16</td>
<td>36</td>
</tr>
<tr>
<td><strong>SimulFluor® HSV/VZV Negative</strong></td>
<td>0</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>20</td>
<td>112</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity = 100% (20/20) 95% Confidence Interval 83.2% - 100%
Specificity = 85.7%(96/112) 95% Confidence Interval 79.2% – 92.2%
DETECTION OF HSV BY CULTURE CONFIRMATION TESTING
Fifty-eight specimens were positive by both devices after amplification in cell culture. There were no discrepancies between the SimulFluor® HSV/VZV and the predicate device results.

<table>
<thead>
<tr>
<th>Culture Isolation of HSV</th>
<th>Culture Positive</th>
<th>Culture Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SimulFluor® HSV/VZV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture Positive</td>
<td>58</td>
<td>0</td>
<td>58</td>
</tr>
<tr>
<td>Culture Negative</td>
<td>0</td>
<td>124</td>
<td>124</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>124</td>
<td></td>
</tr>
</tbody>
</table>

Relative sensitivity = 100% (58/58) 95% Confidence Interval 93.8% - 100%
Relative specificity = 100% (124/124) 95% Confidence Interval 97.1% – 100%

DETECTION OF VZV BY CULTURE CONFIRMATION TESTING
Twenty specimens were positive by both devices after amplification in culture. The predicate device identified one additional isolate found negative by SimulFluor® HSV/VZV.

<table>
<thead>
<tr>
<th>Culture Isolation of VZV</th>
<th>Culture Positive</th>
<th>Culture Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SimulFluor® HSV/VZV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture Positive</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Culture Negative</td>
<td>1</td>
<td>123</td>
<td>124</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>123</td>
<td></td>
</tr>
</tbody>
</table>

Relative sensitivity = 95.2% (20/21) 95% Confidence Interval 76.2% - 99.9%
Relative specificity = 100% (123/123) 95% Confidence Interval 97% – 100%
SITE 2

Two hundred and eighty-three specimens in viral transport media were submitted to a hospital laboratory on the West Coast and tested by direct specimen for the presence of HSV and VZV. The cells were washed by centrifugation, dropped on slides, air-dried and fixed with acetone. Fixed slides were stained with the SimulFluor® HSV/VZV, the predicate HSV DFA and the predicate VZV DFA reagents. Specimens were also inoculated into MRC-5 cells in either a standard tube culture and/or shell vial. Shell vials were stained within 72 hours, while cells in tube cultures were stained once CPE was seen. Slides were stained as for the direct specimen slides.

DETECTION OF HSV BY DIRECT SPECIMEN TESTING

Of the 283 specimens submitted to a hospital laboratory for HSV and/or VZV testing, 46 had insufficient cells for direct specimen testing by, SimulFluor® HSV/VZV, and the predicate HSV DFA kit, and 1 was contaminated. These were excluded from analysis

Fourteen specimens were positive by both direct testing and culture. Culture identified an additional 3 isolates missed by the SimulFluor® HSV/VZV and the SimulFluor® HSV/VZV incorrectly identified 1 specimen as positive for HSV.

<table>
<thead>
<tr>
<th></th>
<th>Culture Positive</th>
<th>Culture Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SimulFluor® HSV/VZV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>SimulFluor® HSV/VZV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>218</td>
<td>221</td>
</tr>
</tbody>
</table>

Sensitivity = 82.4% (14/17) 95% Confidence Interval 56.6% - 96.2%
Specificity = 99.5% (218/219) 95% Confidence Interval 97.5%– 100%
DETECTION OF VZV BY DIRECT SPECIMEN TESTING

Forty-seven specimens of the 283 specimens submitted for HSV and/or VZV testing did not have adequate cells for testing both direct specimens and culture for the presence of VZV and were excluded from the analysis.

Eleven specimens were positive by the SimulFluor® HSV/VZV reagent on the direct specimen and by culture. Two specimens which were positive on direct specimen testing failed to grow in culture.

<table>
<thead>
<tr>
<th></th>
<th>Culture Positive</th>
<th>Culture Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SimulFluor® HSV/VZV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>11</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>232</td>
<td>232</td>
</tr>
</tbody>
</table>

Sensitivity = 100% (11/11) 95% Confidence Interval 71.5% - 100%
Specificity = 99.1% (232/234) 95% Confidence Interval 96.9%–99.9%

Troubleshooting

Specimen preparation is technique dependent and may affect the results obtained. In order to resolve any performance questions, all steps in the process must be analyzed.

Controls should always be included with each test to assure proper performance of procedures for HSV and VZV isolation and detection. Positive controls should be prepared with HSV and VZV laboratory strains or with known positive clinical isolates. Negative controls can utilize known laboratory negative samples.

A marked decrease in fluorescence may indicate:
1) reagent deterioration,  
2) microscopy problems or,  
3) other equipment or technique effect.

- Verify kit expiration date. If reagents are within their expiration date, verify microscope performance; re-read positive controls.
- If the problem is still not identified, verify all equipment operation as per the package insert, and repeat the test.

Contact Chemicon Technical Service at (800) 437-7500 for additional assistance.
References


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ABBREVIATED STAINING PROCEDURE

1. Allow reagents to equilibrate to room temperature.

2. Add SimulFluor® HSV/VZV reagent to cell spot (1 drop) and shell vials (4 to 6 drops).

3. Incubate for 30 minutes at 37° C.

4. Rinse thoroughly with PBS/Tween.

5. Mount slides with Mounting Fluid or remove coverslip from shell vial and mount cell side down on glass slide with Mounting Fluid.

6. Read using fluorescence microscope at 160 to 200x magnification.