# Evaluation of Focus Diagnostics Simplexa™ HSV 1 & 2 Direct Sample-to-Answer Real-time PCR Assay

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## Abstract

**Introduction/Background:** The high prevalence of herpes simplex virus (HSV) infections makes it a significant public health concern. Prompt detection and differentiation of HSV infections can assist in patient management, and is especially important in cases of HSV meningoencephalitis. The Simplexa™ HSV 1 & 2 Direct assay is in development as a sample-to-answer multi-analyte detection system performed on the 3M Integrated Cycler instrument. Swabs in viral transport media and CSF specimens are loaded directly onto a Direct Amplification Disc without extraction or other specimen preparation. The goal of this study was to compare the performance of the Simplexa HSV 1 & 2 Direct assay relative to culture and to real-time PCR methods that require extracted specimens.

**Materials and Methods:** Limit of Detection (LoD) studies were performed to determine the analytical sensitivity of the assay. Inhibition, interference and cross reactivity were evaluated using a panel of bacteria, viruses and potentially interfering substances. Relative sensitivity and specificity were determined by testing blind panels of de-identified patient specimens and comparing results with those obtained using culture or a real-time PCR method that necessitates prior nucleic acid extraction.

**Results:** The LoD studies showed that the Simplexa Direct assay detected HSV-1 strains at less than 40 $TCID_{50}$/mL and HSV-2 strains at less than 5 $TCID_{50}$/mL. No inhibition, interference, or cross reactivity was observed. Relative sensitivity and specificity for swab samples were 100% (40/40) and 95.3% (102/107) for HSV-1, and 100% (38/38) and 100% (107/109) for HSV-2 samples. Relative sensitivity and specificity for CSF samples were 97.3% (36/37) and 97.9% (93/95) for HSV-1, and 94.6% (35/37) and 98.9% (94/95) for HSV-2 samples.

**Conclusion:** The Simplexa HSV 1 & 2 Direct assay was capable of directly detecting and differentiating HSV-1 and HSV-2 from un-extracted clinical specimens with performance comparable to culture or a real-time PCR method that uses upfront nucleic acid extraction. The assay and instrumentation provide a compact system for rapid (~1 hour) detection of HSV-1 and HSV-2 directly from swab and CSF samples.

## Methods

**HSV virus strains:** The following HSV stains were used in the Limit of Detection study: HSV-1 McIntyre (Virapur, San Diego, CA), HSV-1 HF (ATCC, Manassas, VA), HSV-2 G, (Virapur), HSV-2 MS (ATCC).

**Extraction and Real-time PCR Amplification and Detection:** Simplexa HSV 1 & 2 Direct (Focus Diagnostics, Cypress, CA) contains all reagents for extraction and real-time PCR. Sample extraction and real-time PCR was performed using the Direct Amplification Disc (DAD) with 50 µL of sample loaded into the sample port and 50 µL of Simplexa HSV 1 & 2 Direct Reaction Mix loaded into the reaction port. All testing was performed on the 3M Integrated Cycler instruments (3M, St. Paul, MN). Data collection and analysis was performed with Integrated Cycler Studio software.

**Limit of Detection (LoD):** The LoD for each HSV stock was determined as the lowest concentration with ≥95% detection for 20 replicates with pooled negative swab (Focus Diagnostics) and synthetic CSF (Golden West Biologicals, Temecula, CA) matrix. Synthetic CSF LoD was verified by testing against 8 replicates of human pooled CSF (Golden West Biologicals).

**Cross Reactivity:** The cross reactivity panel was contrived with $10^6$ CFU/mL of bacteria or $10^5$ $TCID_{50}$/mL of virus in either pooled negative swab or synthetic CSF matrix.

**Inhibition/Interference:** Interference panel was contrived with HSV-1 or HSV-2 at 2-4 fold above LoD concentration. Each substance was spiked into the HSV-1 or HSV-2 contrived samples and tested by Simplexa HSV 1 & 2 Direct.

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## Methods (Cont.)

**Sensitivity and Specificity:** A panel of 279 de-identified clinical specimens (Focus Diagnostics) including swab specimens with previously reported culture or PCR results and CSF specimens with previously reported PCR results were tested by Simplexa HSV 1 & 2 Direct. Simplexa HSV 1 & 2 Direct results were compared with previously reported results to determine positive and negative agreement. Discordant samples were retested by reference PCR Simplexa assay.

## Results

**Limit of Detection:** HSV-1 (McIntyre and HF strains) LoD was <40 $TCID_{50}$/mL. HSV-2 (G and MS strains) LoD was <5 $TCID_{50}$/mL.

**Table 1. Limit of Detection for HSV-1 and HSV-2 strains**

| HSV-1 McIntyre | $TCID_{50}$/mL | Mean Ct (CFR610) | Min. Ct | Max. Ct | Replicates Detected |
|---|---|---|---|---|---|
| Negative swab | 10 | 36.3 | 34.3 | 38.8 | 20/20 |
| Synthetic CSF | 10 | 36.5 | 34.4 | 40.7 | 20/20 |
| Human CSF | 10 | 34.2 | 33.6 | 35.6 | 8/8 |

| HSV-1 HF strain | $TCID_{50}$/mL | Mean Ct (CFR610) | Min. Ct | Max. Ct | Replicates Detected |
|---|---|---|---|---|---|
| Negative swab | 30 | 37.0 | 35 | 41.9 | 20/20 |
| Synthetic CSF | 30 | 36.3 | 34.6 | 38.5 | 20/20 |
| Human CSF | 30 | 36.2 | 34.3 | 38.1 | 8/8 |

| HSV-2 G strain | $TCID_{50}$/mL | Mean Ct (FAM) | Min. Ct | Max. Ct | Replicates Detected |
|---|---|---|---|---|---|
| Negative swab | 4.4 | 35.6 | 34.9 | 38.8 | 20/20 |
| Synthetic CSF | 2.2 | 36.8 | 35.3 | 39.6 | 20/20 |
| Human CSF | 2.2 | 36.4 | 35 | 37.2 | 8/8 |

| HSV-2 MS strain | $TCID_{50}$/mL | Mean Ct (FAM) | Min. Ct | Max. Ct | Replicates Detected |
|---|---|---|---|---|---|
| Negative swab | 0.14 | 34.8 | 34.2 | 35.3 | 20/20 |
| Synthetic CSF | 0.14 | 35.3 | 31.5 | 38.5 | 20/20 |
| Human CSF | 0.14 | 34.6 | 34 | 35.1 | 8/8 |

**Cross Reactivity:** No cross-reactivity was detected with the pathogens tested in Tables 2 and 3.

**Table 2. Pathogens tested for cross reactivity in swab matrix**

| | | |
|---|---|---|
| *Candida albicans* | CMV | *Gardenerella vaginalis* |
| *Chlamydia trachomatis* | Enterovirus | *Mycoplasma hominis* |
| *Escherichia coli* | EBV | *Mobiluncus mulieris* |
| *Neisseria gonorrhoeae* | VZV | *Treponema pallidum* |
| *Staphylococcus aureus* | HHV-6 | *Mycoplasma genitalium* |
| *Staphylococcus saprophyticus* | HHV-7 | *Trichomonas vaginalis* |
| *Streptococcus pyogenes* | HHV-8 | *Bacteroides fragilis* |
| Rubella virus | HPV | *Toxoplasma gondii* |

## Results (Cont.)

**Table 3. Pathogens tested for cross reactivity in CSF**

| | | |
|---|---|---|
| *Neisseria meningitidis* | HIV-1 | *St. Louis Encephalitis Virus* |
| *Haemophilus influenza B* | HIV-2 | *Mycoplasma pneumoniae* |
| *Streptococcus pneumoniae* | WNV | *Mycobacterium tuberculosis* |
| *Group B streptococcus* | Measles virus | *Borellia burgdorferi* |
| *Listeria monocytogenes* | Mumps virus | *Cryptococcus neoformans* |

**Inhibition:** No interference was detected with the substances tested in Table 4.

**Table 4. Interference substances tested in swab and/or CSF**

| Interference Substance | Matrix | Concentration tested |
|---|---|---|
| Whole Blood | swab and CSF | 10% (v/v) |
| Female Urine | swab | 10% (v/v) |
| Albumin (protein) | swab and CSF | 10 mg/mL |
| Casein (protein) | swab and CSF | 10 mg/mL |
| K-Y Brand Jelly | swab | 5% (v/v) |
| Acyclovir (Acycloguanosine) | swab and CSF | 2.5 mg/mL |
| Betadine (topical antiseptic) | swab and CSF | 5% (v/v) |
| White Blood Cell | CSF | $5.5x10^8$ WBC/mL |
| Hemoglobin | CSF | 0.625 mg/mL |

**Relative Sensitivity and Specificity of the Simplexa HSV 1 & 2 Direct Assay:** Percent positive and negative agreement with previous results for HSV-1 and HSV-2 was >95% for swab samples and >94% for CSF samples (Tables 5-9).

**Table 5. Swab clinical specimens**

| Previous Result | Swab | | CSF |
|---|---|---|---|
| | Culture | PCR | PCR |
| HSV-1 | 40 | 0 | 37 |
| HSV-2 | 38 | 0 | 37 |
| Negative | 49 | 20 | 58 |
| **Total** | 147 | | 132 |

**Table 6. Concordance for HSV-1 swab clinical specimens**

| Simplexa™ \ Previous Results | HSV-1 Positive | HSV-1 Negative | Total | HSV-1 Swab % Agreement |
|---|---|---|---|---|
| **HSV-1 Positive** | 40 | 5[a] | 45 | % Positive Agreement 100% (40/40) |
| **HSV-1 Negative** | 0 | 102 | 102 | % Negative Agreement 95.3% (102/107) |
| **Total** | 40 | 107 | 147 | |

[a]Specimen 43, previous reported as negative by PCR, was initially detected as HSV-1 positive by Simplexa with Ct 40.8. This specimen was negative upon repeat testing.

Specimens 68 and 108, previously reported as HSV-2 positive culture, were initially detected as both HSV-1 and HSV-2 positive by Simplexa. HSV-1 Ct were 41.4 and 38.2 respectively. These specimens were detected only as HSV-2 upon repeat testing

Specimens 100 and 123, previous reported as negative by culture, were detected as HSV-1 positive by Simplexa with Ct 35.3 and 34.6 respectively. Reference PCR assay also reported HSV-1 positive with Ct 33.89 and 34.16 respectively.

## Results (Cont.)

**Table 7. Concordance for HSV-2 swab clinical specimens**

| Simplexa™ \ Previous Results | HSV-2 Positive | HSV-2 Negative | Total | HSV-2 Swab % Agreement |
|---|---|---|---|---|
| **HSV-2 Positive** | 38 | 2[b] | 40 | % Positive Agreement 100% (38/38) |
| **HSV-2 Negative** | 0 | 107 | 107 | % Negative Agreement 98.2% (107/109) |
| **Total** | 38 | 109 | 147 | |

[b]Specimens 55 and 107, previously reported as negative by culture, were detected as *HSV-2 positive by Simplexa with Ct 35.8 and 39.6 respectively. Reference PCR assay also reported HSV-2 positive with Ct 38.2 and 41.24 respectively.*

**Table 8. Concordance for HSV-1 CSF clinical specimens**

| Simplexa™ \ Previous Results | HSV-1 Positive | HSV-1 Negative | Total | HSV-1 CSF % Agreement |
|---|---|---|---|---|
| **HSV-1 Positive** | 36 | 2[c] | 38 | % Positive Agreement 97.3% (36/37) |
| **HSV-1 Negative** | 1[d] | 93 | 94 | % Negative Agreement 97.9% (93/95) |
| **Total** | 37 | 95 | 132 | |

[c]*Specimen 213 and 268, previously reported as negative, were initially detected as HSV-1 positive by Simplexa with Ct 42.1 and 38.3 respectively. Both specimens were negative upon repeat testing.*

[d]*Specimen 229, previously reported as HSV-1 positive, was detected as negative by Simplexa. Reference PCR assay also reported as negative.*

**Table 9. Concordance for HSV-2 CSF clinical specimens**

| Simplexa™ \ Previous Results | HSV-2 Positive | HSV-2 Negative | Total | HSV-2 CSF % Agreement |
|---|---|---|---|---|
| **HSV-2 Positive** | 35 | 1[f] | 36 | % Positive Agreement 94.6% (35/37) |
| **HSV-2 Negative** | 2[e] | 94 | 96 | % Negative Agreement 98.9% (94/95) |
| **Total** | 37 | 95 | 132 | |

[e]*Specimens 247 and 269, previously reported as HSV-2 positive, were initially detected as negative by Simplexa. Both specimens were detected as HSV-2 with Ct 39.4 and 38.4 respectively upon repeat.*

[f]*Specimen 417, previously reported as HSV-1 positive, was detected as HSV-1 and HSV-2 positive by Simplexa. HSV-2 Ct was 41.8.*

## Conclusions

- Simplexa HSV 1 & 2 Direct is a simple and rapid molecular test. The assay provides answers ~1 hour without a separate extraction system.
- This assay was capable of directly detecting and differentiating HSV-1 and HSV-2 from un-extracted specimens.
- Performance was comparable to that obtained with culture or a real-time PCR method with upfront nucleic acid extraction
- Simplexa HSV 1 & 2 Direct is in development. It is not currently available for sale, and is not FDA cleared.

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