September 30. 2025

miDiagnostics nv
Katrin Martens
Lead Regulatory Submissions
Gaston Geenslaan 1
Leuven, Flemish Brabant 3001
Belgium

Re: K250050
Trade/Device Name: miDiagnostics HSV-1&2 CSF Test
Regulation Number: 21 CFR 866.3307
Regulation Name: Herpes Simplex Virus Nucleic Acid-Based Assay For Central Nervous System Infections
Regulatory Class: Class II
Product Code: PGH
Dated: September 4, 2025
Received: September 4, 2025

Dear Katrin Martens:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device"

(https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

MAYRA GARCIA -S

Digitally signed by MAYRA GARCIA -S
Date: 2025.09.30 16:17:02 -04'00'

Mayra Garcia, Ph.D., M.B.A. on behalf of
Jorge L. Munoz, Ph.D.
Deputy Branch Chief
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

# Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
*See PRA Statement below.*

510(k) Number *(if known)*
K250050

Device Name
miDiagnostics HSV-1&2 CSF Test

Indications for Use *(Describe)*
The miDiagnostics HSV-1&2 CSF Test is an in vitro polymerase chain reaction (PCR) assay intended for use with the miDiagnostics PCR Platform for the qualitative detection and differentiation of HSV-1 and HSV-2 DNA in cerebrospinal fluid (CSF) samples from patients suspected of Herpes Simplex Virus (HSV) infection of the central nervous system (CNS). This test is intended as an aid in the diagnosis of HSV-1 and HSV-2 infection in the CNS.

Negative results do not preclude HSV-1 or HSV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.

The test is not intended for use as a donor screening test. The test is for professional use only.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

**CONTINUE ON A SEPARATE PAGE IF NEEDED.**

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# 510(k) Summary

**miDiagnostics HSV-1&2 CSF Test**

**Submitter**

| | |
|---|---|
| Applicant Name and Address | miDiagnostics nv<br>Gaston Geenslaan 1<br>3001 Leuven<br>Belgium |
| Contact Person | Katrin Martens |
| Date Prepared | 09/30/2025 |

**Device Information**

| Trade Name | Regulation Name | Regulation Number | Product code | Class |
|---|---|---|---|---|
| miDiagnostics HSV-1&2 CSF Test | Herpes simplex virus nucleic acid-based assay for central nervous system infections | 21 CFR 866.3307 | PGH | II |

**Intended use**

The miDiagnostics HSV-1&2 CSF Test is an in vitro polymerase chain reaction (PCR) assay intended for use with the miDiagnostics PCR Platform for the qualitative detection and differentiation of HSV-1 and HSV-2 DNA in cerebrospinal fluid (CSF) samples from patients suspected of Herpes Simplex Virus (HSV) infection of the central nervous system (CNS). This test is intended as an aid in the diagnosis of HSV-1 and HSV-2 infection in the CNS.

Negative results do not preclude HSV-1 or HSV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.

The test is not intended for use as a donor screening test. The test is for professional use only.

**Device Description**

The **miDiagnostics HSV-1&2 CSF Test** is a real-time polymerase chain reaction (PCR) assay that enables the direct amplification, detection, and differentiation of Herpes Simplex Virus 1 (HSV-1) and Herpes Simplex Virus 2 (HSV-2) DNA from cerebrospinal fluid (CSF) specimens, obtained via lumbar puncture, from individuals with signs and/or symptoms of HSV-1 or HSV-2 infection in the central nervous system (CNS).

The test consists of three major steps: (1) manual sample preparation, (2) manual PCR reaction preparation and (3) automated PCR run which includes targets amplification and detection by fluorescent probes for 1 target specific to HSV-1, 1 target specific to HSV-2 and a human DNA control target.

After manual sample and PCR reaction preparation, the miDiagnostics HSV-1&2 CSF test consists of three major steps: (1) manual sample preparation, (2) manual PCR reaction preparation and (3) automated PCR run on the miDiagnostics PCR Platform which is an automated in vitro diagnostic system for the amplification and detection of the target nucleic acid(s) using real-time polymerase chain reaction (PCR).

Description of the different components of miDiagnostics HSV-1&2 CSF Test:

(1) **miDiagnostics HSV-1&2 CSF Detection Mix**: contains a freeze-dried cake, stored in a tube, containing all the PCR components for amplification and fluorescent detection of HSV-1, HSV-2 and the Sample Processing Control (SPC). Each tube is individually packed in a pouch. The device consists of 24 pouches in a box.

(2) **miDiagnostics HSV-1&2 CSF Dilution Buffer**: contains 300 µL HSV CSF Dilution Buffer for diluting the CSF specimen. It also contains a SPC (RPP30, a human housekeeping gene). The device consists of 24 tubes packed in one box.

(3) **miDiagnostics Concentration Device**: consists of a combination of the miDiagnostics Concentration Column and 2 miDiagnostics Collection Tubes, to concentrate the viral material. The device consists of one bag with 24 miDiagnostics Concentration Columns and 48 miDiagnostics Collection Tubes.

(4) **miDiagnostics HSV-1&2 CSF Algorithm**: The software system that interprets fluorescence signals and reports test results. The miDiagnostics HSV-1&2 CSF Algorithm is embedded in the miDiagnostics Software.

The **miDiagnostics PCR Platform** is an automated *in vitro* diagnostics medical device intended to be used in combination with a selected miDiagnostics test for the amplification and detection of the target nucleic acid(s) using real-time polymerase chain reaction (PCR). The miDiagnostics PCR Platform is intended to be used by healthcare professionals (trained laboratory personnel) in laboratory environment.

Description of the different components of miDiagnostics PCR Platform:

(1) **miDiagnostics PCR Reader 2.0** receives the miDiagnostics PCR Card 2.0 and is responsible for performing consecutive PCR cycles through thermocycling. The miDiagnostics PCR Reader 2.0 monitors the consequent polymerase chain reaction's progress with a four-channel camera system. It also contains a 2D barcode reader to identify the loaded miDiagnostics PCR Card 2.0 and is connected to the miDiagnostics Computer, from which it receives test-specific parameters for the PCR and to which it sends the camera images that contain the raw data of the PCR.

The **firmware** is an embedded software system running on the miDiagnostics PCR Reader 2.0 microprocessor with as main functionalities the thermocycling and the triggering of the camera.

(2) **miDiagnostics PCR Card 2.0** is a single-use, disposable test accessory. The miDiagnostics PCR Card 2.0 is manually loaded with a small volume of sample mixed with PCR components of the selected miDiagnostics test (so called reaction mixture). Once loaded, the miDiagnostics PCR

Card 2.0 is manually inserted in the miDiagnostics PCR Reader 2.0. The miDiagnostics PCR Card 2.0 includes a fluidic chip with the reaction chamber that enables a very fast polymerase chain reaction driven by the miDiagnostics PCR Reader 2.0. Each miDiagnostics PCR Card 2.0 is individually packed in a pouch. The device consists of 24 pouches in a box.

(3) **miDiagnostics Computer** connects to the miDiagnostics PCR Reader 2.0 via two USB-cables and can connect to the customer's network via ethernet or WiFi. It interacts with a user via a touchscreen, has an integrated barcode scanner that allows the user to scan the sample consumable identifiers, and it runs the miDiagnostics Software.

The **miDiagnostics Software** is the software of the miDiagnostics PCR Platform, that is installed on the miDiagnostics Computer. It controls the miDiagnostics PCR Reader 2.0, retrieves the camera captures, hosts the test specific algorithm that interprets the fluorescence signals, reports the test results, and provides a user interface for the operator to interact with the miDiagnostics PCR Platform.

**Principle of operations**

The test consists of three major steps: (1) manual sample preparation, (2) manual PCR reaction preparation and (3) automated PCR run which includes targets amplification and detection by fluorescent probes for 1 target specific to HSV-1, 1 target specific to HSV-2 and the SPC. The SPC is a human DNA control target and is used to verify that miDiagnostics HSV-1&2 CSF Test was executed successfully and that no PCR inhibition has occurred.

The sample preparation starts out with adding 200 µL CSF to the miDiagnostics HSV-1&2 CSF Dilution Buffer. The diluted specimen is then transferred to the miDiagnostics Concentration Device and centrifuged at high speed for 5 minutes. The remaining volume in the miDiagnostics Concentration Column is washed with molecular grade water (DNase & RNase free) and centrifuged again at high speed for 5 minutes. The sample is then heated for 2 minutes at 95°C. Thereafter, an aliquot of the sample is mixed with the miDiagnostics HSV-1&2 CSF Detection Mix and loaded on the miDiagnostics PCR Card 2.0, to fill the reaction chamber of the fluidic chip.

The miDiagnostics PCR Card 2.0 is manually inserted in the miDiagnostics PCR Reader 2.0 for amplification and detection of HSV-1, HSV-2 and the SPC. The miDiagnostics PCR Reader 2.0 rapidly heats and cools the chip to drive a polymerase chain reaction inside the reaction chamber of the fluidic chip. Via the transparent optical window in the miDiagnostics PCR Card 2.0 's bottom, the progress of the reaction is measured by a camera system in the miDiagnostics PCR Reader 2.0, that sends the raw images to the miDiagnostics Computer for analysis by the miDiagnostics Software. The miDiagnostics HSV-1&2 CSF Algorithm (hosted by miDiagnostics Software) automatically interprets the fluorescence signals and reports the test results.

**Substantial Equivalence**

*Table 1: Substantial Equivalence Comparison.*

| **Device Trade Name** | **Predicate Device: (K133621)**<br>**Simplexa HSV 1 & 2 Direct** | **New Device: (K250050)**<br>**miDiagnostics HSV-1&2 CSF Test** |
|---|---|---|
| **General Device Characteristic Similarities** | | |
| **Intended Use / Indications for Use** | The Focus Diagnostics Simplexa™ HSV 1 & 2 Direct is intended for use on the 3M Integrated Cycler instrument for the qualitative detection and differentiation of HSV-1 and HSV-2 DNA in cerebrospinal fluid (CSF) samples from patients suspected of Herpes Simplex Virus (HSV) infections of the central nervous system (CNS).<br><br>This test is intended as an aid in the diagnosis of HSV-1 and HSV-2 infections of the CNS.<br><br>Negative results do not preclude HSV-1 or HSV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.<br><br>The assay is not intended for use as a donor screening test.<br><br>The assay is for professional use only.<br><br>The Positive Control is intended to be used as a control with the Simplexa™ HSV 1 & 2 Direct. This control is not intended for use with other assays or systems. | The miDiagnostics HSV-1&2 CSF Test is an in vitro polymerase chain reaction (PCR) assay intended for use with the miDiagnostics PCR Platform for the qualitative detection and differentiation of HSV-1 and HSV-2 DNA in cerebrospinal fluid (CSF) samples from patients suspected of Herpes Simplex Virus (HSV) infection of the central nervous system (CNS). This test is intended as an aid in the diagnosis of HSV-1 and HSV-2 infection in the CNS.<br><br>Negative results do not preclude HSV-1 or HSV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.<br><br>The test is not intended for use as a donor screening test.<br><br>The test is intended for professional use only. |
| **Targets** | Herpes Simplex Virus 1 (HSV-1) DNA<br>Herpes Simplex Virus 2 (HSV-2) DNA | Same |
| **Specimen type** | Cerebrospinal fluid (CSF) | Same |
| **Analyte detected** | DNA | Same |

| Device Trade Name | Predicate Device: (K133621) Simplexa HSV 1 & 2 Direct | New Device: (K250050) miDiagnostics HSV-1&2 CSF Test |
|---|---|---|
| **Technology** | Real-time Polymerase Chain Reaction | Same |
| **Result interpretation** | Automated | Same |
| **PCR Control** | Assay contains Control to detect reagent failure and/or inhibition | Same |
| **General Device Characteristic Differences** | | |
| **Instrumentation** | 3M Integrated Cycler Studio system | miDiagnostics PCR Platform |
| **External Controls** | The Positive Control is provided and intended to be used as a control with the Simplexa™ HSV 1 & 2 Direct. This control is not intended for use with other assays or systems. | External control materials are not provided with the miDiagnostics HSV-1&2 CSF Test. External controls are required to run with the test. |

**Performance**

**Analytical performance**

Analytical Sensitivity (Limit of Detection)

The Limit of Detection (LOD) for the miDiagnostics HSV-1&2 CSF Test was determined using two HSV-1 strains (MacIntyre and HF strains) and two HSV-2 strains (MS and G strains). Serial dilutions of each strain were made in pooled negative human CSF matrix (negative CSF) near their LOD during feasibility. Tests were performed over the course of 4 days, with 2 reagent batches and 9 miDiagnostics PCR Platforms. Ten replicates at each dilution series were tested per viral strain per reagent batch with a total of 20 replicates per strain. The concentration at which ≥ 95% of replicates were detected was the preliminary LOD for that strain. The preliminary LOD for each strain was subsequently confirmed with 20 replicates diluted at the preliminary LOD concentration using the worst performing reagent batch. The confirmed LOD per strain is listed in Table 2.

*Table 2: Limit of Detection (LOD) summary table for the miDiagnostics HSV-1&2 CSF Test.*

| Virus Strain | LOD ($TCID_{50}$/mL) | LOD (copies/mL) | #Detected/#Total (% of Detection) |
|---|---|---|---|
| HSV-1 MacIntyre | 8.84 | 800 | 20/20 (100%) |
| HSV-1 HF | 20.32 | 1000 | 20/20 (100%) |
| HSV-2 MS | 0.56 | 400 | 19/20 (95%) |
| HSV-2 G | 0.43 | 800 | 20/20 (100%) |

Analytical Reactivity (Inclusivity)

The analytical reactivity of the miDiagnostics HSV-1&2 CSF Test was evaluated with four HSV-1 and three HSV-2 strains, see Table 3. Viral material with known concentration was spiked into negative CSF at near LOD concentration and assessed in triplicates. Testing with increasing concentrations was continued until all strains generated "Detected" results for all three replicates. The miDiagnostics HSV-1&2 CSF Test was able to detect all HSV strains.

*Table 3: Analytical reactivity (Inclusivity) summary table for the miDiagnostics HSV-1&2 CSF Test.*

| Analyte | Virus strain | Spiked concentration |
|---|---|---|
| **HSV-1** | HSV-1 strain F | 20.32 $TCID_{50}$/mL |
| | HSV-1 strain KOS | 40.64 $TCID_{50}$/mL |
| | HSV-1 ATCC-2011-1 | 20.32 $TCID_{50}$/mL |
| | HSV-1 strain 95 (95/1906) | 20.32 $TCID_{50}$/mL |
| **HSV-2** | HSV-2 strain 131596 | 1605 copies/mL |
| | HSV-2 strain HG52 | 3.36 $TCID_{50}$/mL |
| | HSV-2 ATCC-2011-2 | 0.56 $TCID_{50}$/mL |

Analytical Specificity

*Cross-reactivity*

The cross-reactivity of the miDiagnostics HSV-1&2 CSF Test was evaluated by testing 46 microorganisms that may be present in CSF. The cross-reactants were spiked into negative CSF at high concentrations and assessed in triplicate. No cross-reactivity was observed in any of the microorganisms listed in Table 4.

*Table 4: Summary table of microorganisms tested for cross reactivity for the miDiagnostics HSV-1&2 CSF Test.*

| Microorganism | Concentration tested |
|---|---|
| Adenovirus (type 1) | $10^5$ $TCID_{50}$/mL |
| Adenovirus (type 7A) | $10^5$ $TCID_{50}$/mL |
| BK polyomavirus | $10^5$ $TCID_{50}$/mL |
| *Citrobacter freundii* | $10^6$ CFU/mL |
| *Citrobacter koseri* | $10^6$ CFU/mL |
| *Cryptococcus neoformans* | $10^6$ CFU/mL |
| Cytomegalovirus (AD169) | 3.12 x $10^3$ $TCID_{50}$/mL |

| Microorganism | Concentration tested |
|---|---|
| Dengue virus | $10^5$ copies/mL |
| *Enterobacter aerogenes* | $10^6$ CFU/mL |
| Enterovirus 71 | $7.78 \times 10^3$ $TCID_{50}$/mL |
| Epstein Barr virus (B95-8) | $10^5$ $TCID_{50}$/mL |
| *Escherichia coli* | $10^6$ CFU/mL |
| *Haemophilus influenzae* | $10^6$ CFU/mL |
| *Haemophilus influenzae* type b (MinnA) | $10^6$ CFU/mL |
| Hepatitis B | $10^5$ $TCID_{50}$/mL |
| Hepatitis C1 | $10^5$ $TCID_{50}$/mL |
| HIV1 (type IIIB) | $10^4$ IU/mL |
| Human herpesvirus 6 | $10^5$ $TCID_{50}$/mL |
| Human herpesvirus 7 | $9.36 \times 10^3$ $TCID_{50}$/mL |
| Human herpesvirus 8 | $1.45 \times 10^5$ $TCID_{50}$/mL |
| Influenza B | $2.80 \times 10^4$ $TCID_{50}$/mL |
| Influenza A H1N1 | $1.46 \times 10^5$ $TCID_{50}$/mL |
| John Cunningham virus | $10^5$ $TCID_{50}$/mL |
| *Klebsiella pneumoniae* | $10^6$ CFU/mL |
| *Listeria monocytogenes* | $10^6$ CFU/mL |
| Measles | $10^5$ $TCID_{50}$/mL |
| Mumps | $10^5$ $TCID_{50}$/mL |
| *Mycobacterium tuberculosis* | $10^6$ copies/mL |
| *Naegleria fowleri* | $10^6$ copies/mL |
| *Neisseria meningitides* (serogroup A) | $10^6$ CFU/mL |
| Parainfluenza Virus 1 | $10^5$ $TCID_{50}$/mL |
| Parainfluenza Virus 2 | $8.34 \times 10^4$ $TCID_{50}$/mL |
| Parainfluenza Virus 3 | $10^5$ $TCID_{50}$/mL |
| Parvovirus B19 | $1.28 \times 10^2$ $TCID_{50}$/mL |

| Microorganism | Concentration tested |
|---|---|
| *Proteus mirabilis* (Z050) | $10^6$ CFU/mL |
| Pseudomonas aeruginosa | $10^6$ CFU/mL |
| Rabies virus | Not available * |
| Rhinovirus (Type 1A) | $2.82 \times 10^4$ $TCID_{50}$/mL |
| Rotavirus (Type Wa) | $10^5$ $TCID_{50}$/mL |
| Rubella | $2.58 \times 10^3$ $TCID_{50}$/mL |
| St. Louis Encephalitis | $10^5$ copies/mL |
| *Staphylococcus aureus* COL | $10^6$ CFU/mL |
| *Streptococcus agalactiae* | $10^6$ CFU/mL |
| *Streptococcus pneumoniae Z022; 19F* | $10^6$ CFU/mL |
| Varicella zoster virus | $10^5$ $TCID_{50}$/mL |
| West Nile Virus | $10^5$ $TCID_{50}$/mL |

***** No official concentration provided by the supplier. The maximum volume of the pathogen's RNA extract was spiked for testing without exceeding 20% of the total sample volume.

Cross-reactivity with Polio virus and La Crosse encephalitis virus was evaluated by an *in-silico* analysis using viral genome RNA sequences of these two viruses available in GenBank. There was no homology found between the primers and probes of the miDiagnostics HSV 1 &2 CSF test to either of the virus.

*Microbial interference*

The ability of the miDiagnostics HSV-1&2 CSF Test to detect HSV-1 and HSV-2 in the presence of other potentially inhibitory microorganisms was evaluated. A total of 46 microorganisms were spiked either individually or as a pool into HSV-1 and HSV-2 positive CSF (spiked at 3x LOD concentration). No interference was observed for either HSV-1 or HSV-2. The microorganisms and the tested concentration of each of them are listed in Table 4 and are the same that were evaluated for cross-reactivity.

*Interfering Substances*

The potential interference of substances that may be present in CSF specimens was evaluated by testing eleven substances at the concentrations listed (above normal clinical concentrations) in the table below. Negative CSF was spiked with HSV-1 and HSV-2 at 3x LOD and was tested in triplicate for each substance individually. No interference was observed for any of the substances at the concentrations listed in Table 5.

*Table 5: Summary table of potential interfering substances tested with miDiagnostics HSV-1&2 CSF Test.*

| Potential interfering substance | Interferent concentration | Clinically relevant concentration |
|---|---|---|
| Albumin | 10 mg/mL | 0.32-2.63 mg/mL |
| Lactate (L-, sodium salt) | 2.2 mg/mL | 0-0.6 mg/mL |
| D(+) Glucose | 9.9 mg/mL | 0.32-0.98 mg/mL |
| Casein | 5 mg/mL | 0.15-0.40 mg/mL |
| Hemoglobin | 0.625 mg/mL | <0.03 mg/mL |
| Immunoglobulin (IgG) | 10 mg/mL | 0.8 mg/mL |
| White Blood Cells (WBC) | 2 x $10^6$ cells/ mL | 0.009- 1 x $10^6$ cells/mL |
| Whole Blood | 5% (v/v) | <1% (v/v) |
| Betadine® | 1% (w/v) | 0.25% (v/v) |
| Acyclovir | 2.5 mg/mL | 0.022 mg/mL |
| Acyclovir Triphosphate | 0.5 mg/mL | Metabolized from up to 0.022 mg/mL |

*Competitive Interference*

The interference between HSV targets of the miDiagnostics HSV-1&2 CSF Test was evaluated by spiking one of the targets at high concentration ($10^5$ $TCID_{50}$/mL) and the other target at low concentration (3x LOD) into negative CSF. No interference between the targets was observed.

Carry-over Contamination

The risk of carry-over contamination from one test to another was evaluated by alternately testing high positive sample (HSV-1, at ≥ $10^5$ pfu/mL) followed by negative sample on a single Reader. Five operators processed a high positive sample followed by a negative sample, for a total of eight times per Reader across five different Readers. No carry-over contamination was observed throughout the study.

Within-Laboratory Precision

The miDiagnostics HSV-1&2 CSF Test within-laboratory precision was evaluated including Intermediate precision (within lab) and within run repeatability. The within-laboratory precision study included 3 panel members 2x LoD (low positive), 5x LoD (moderate positive) and negative samples. The positive panel members were prepared by spiking HSV-1 (HF strain) and HSV-2 (MS strain) stocks into pooled negative human CSF matrix.

Each panel member was tested in 2 replicates by 2 operators on 2 miDiagnostics PCR Platforms for 12 days, with 2 runs per day per operator, using 2 different lots of reagents for a total of 96 replicates of each panel member (2 replicates x 2 runs per day x 2 operators x 12 days = 96 total replicates).

The within-laboratory precision results for HSV-1 and HSV-2 are summarized below in Table 6.

*Table 6: Within-laboratory precision summary table of miDiagnostics HSV-1&2 CSF Test for HSV-1 and HSV-2*

| Analyte | Sample | Rate of Detection | Mean Cq | Within Run | | Between Run | | Between Day | | Within laboratory[a] | | Between Operator | | Total[b] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | SD | % CV | SD | % CV | SD | %CV | SD | % CV | SD | % CV | SD | % CV |
| HSV-1 | 2x LoD | 96/96 (100%) | 34.84 | 0.61 | 1.75 | 1.06 | 3.05 | 0.00 | 0.00 | 1.23 | 3.52 | 0.00 | 0.00 | 1.23 | 3.52 |
| | 5x LoD | 96/96 (100%) | 32.94 | 0.51 | 1.54 | 0.98 | 2.97 | 0.00 | 0.00 | 1.10 | 3.34 | 0.00 | 0.00 | 1.10 | 3.34 |
| | Negative | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| HSV-2 | 2x LoD | 95/96 (98.96%) | 35.92 | 0.79 | 2.19 | 0.99 | 2.75 | 0.00 | 0.00 | 1.26 | 3.51 | 0.00 | 0.00 | 1.26 | 3.51 |
| | 5x LoD | 96/96 (100%) | 34.53 | 0.71 | 2.07 | 1.11 | 3.21 | 0.00 | 0.00 | 1.32 | 3.82 | 0.00 | 0.00 | 1.32 | 3.82 |
| | Negative | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |

[a]Within Laboratory includes Within Run, Between Run, and Between Day Components
[b]Total includes Within Run, Between Run, Between Day, and Between Operator Components.

Reproducibility Study (multi-site precision)

The reproducibility of miDiagnostics HSV-1&2 CSF Test was evaluated by testing a 4 panel member panel, including a 2x LoD (low positive), 5x LoD (moderate positive), 10x LoD (high positive) and 1x LoD (high negative). The positive panel members were prepared by spiking HSV-1 (HF strain) and HSV-2 (MS strain) stocks into pooled negative human CSF matrix. Each panel member was tested with 3 replicates at 3 sites, 2 operators for 5 days (3 replicates x 3 sites x 2 operators x 5 days = 90 total replicates). The study was carried out at three sites (two external and one internal) with 2 operators, 2 lots of reagents, and 3 miDiagnostics PCR Platforms per site across 5 non-consecutive days. Each panel member was tested with 3 replicates at 3 sites, 2 operators for 5 days (3 replicates x 3 sites x 2 operators x 5 days = 90 total replicates). The reproducibility results of the miDiagnostics HSV-1&2 CSF Test for HSV-1 and HSV-2 are summarized below in the Table 7**.**

*Table 7: Reproducibility summary table of miDiagnostics HSV-1&2 CSF Test for HSV-1 and HSV-2*

| Analyte | Panel Member | Rate of Detection | Mean Cq | Within Run | | Between Run | | Between Day | | Between Lot | | Between Site | | Total[a] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | SD | % CV | SD | % CV | SD | % CV | SD | % CV | SD | % CV | SD | % CV |
| HSV-1 | 2x LoD | 90/90 (100%) | 35.16 | 1.06 | 3.02 | 0.58 | 1.65 | 0.00 | 0.00 | 0.30 | 0.86 | 0.51 | 1.44 | 1.35 | 3.83 |
| | 5x LoD | 90/90 (100%) | 33.41 | 0.96 | 2.87 | 0.00 | 0.00 | 0.52 | 1.56 | 0.52 | 1.56 | 0.24 | 0.72 | 1.23 | 3.69 |
| | 10x LoD[b] | 88/88 (100%) | 31.48 | 0.83 | 2.63 | 0.00 | 0.00 | 0.53 | 1.69 | 0.11 | 0.35 | 0.19 | 0.61 | 1.01 | 3.20 |
| | High Negative | 50/90 (55.56%) | 38.16 | 1.34 | 3.50 | 1.20 | 3.15 | 0.00 | 0.00 | 0.40 | 1.05 | 0.00 | 0.00 | 1.84 | 4.82 |
| HSV-2 | 2x LoD | 90/90 (100%) | 36.36 | 1.62 | 4.46 | 0.00 | 0.00 | 0.33 | 0.91 | 0.29 | 0.80 | 0.26 | 0.72 | 1.70 | 4.68 |
| | 5x LoD | 90/90 (100%) | 34.56 | 0.79 | 2.28 | 0.95 | 2.74 | 0.17 | 0.49 | 0.27 | 0.78 | 0.23 | 0.66 | 1.30 | 3.75 |
| | 10x LoD | 88/88 (100%) | 32.74 | 0.85 | 2.59 | 0.00 | 0.00 | 0.61 | 1.88 | 0.00 | 0.00 | 0.21 | 0.63 | 1.07 | 3.26 |
| | High Negative | 33/90 (36.67%) | 38.72 | 2.11 | 5.45 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.11 | 5.45 |

[a]Total includes Within Run/Day, Between Run/Day, Between Lot, and Between-Site Components.
[b]For site 1 and site 2, one replicate of the high positive sample was excluded from the analysis due to operator errors.

**Clinical performance characteristics**

Performance characteristics of the miDiagnostics HSV-1&2 CSF Test were established in prospective (Cohort 1) and retrospective (Cohort 2) clinical studies conducted at 4 participating testing sites in the United States and United Kingdom. The specimens were tested with the miDiagnostics HSV-1&2 CSF Test and the comparator method.

A total of 362 specimens were enrolled and tested from August 2024 to December 2024 at 4 participating testing sites to evaluate the clinical performance of the miDiagnostics HSV-1&2 Test. A total of 325 specimens were included for the clinical performance of the miDiagnostics HSV-1&2 CSF Test. Thirtyseven (37) specimens did not meet the inclusion criteria and were excluded from the studies. The CSF specimens of individuals of all sexes, all ethnicities and all ages, with signs and/or symptoms of HSV-1 or HSV-2 infection in the CNS, that were eligible according to the inclusion and exclusion criteria, were enrolled. The CSF specimens were either collected prospectively (Cohort 1) or originating from a biorepository, and thus retrospective (Cohort 2), and each CSF specimen originated from a unique subject.

The gender and age demographic of individuals for prospective and retrospective studies are presented in Table 8 and Table 9 below.

*Table 8: Prospective Study - Age and Gender Distribution of individuals*

| Age Category | Female | | | | Male | | | |
|---|---|---|---|---|---|---|---|---|
| | HSV-1 | HSV-2 | Not detected | Total | HSV-1 | HSV-2 | Not detected | Total |
| From birth to 1 year | 0 | 0 | 4 | 4 | 0 | 0 | 3 | 3 |
| >1 to 2 years | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 |
| >2 to 12 years | 0 | 0 | 3 | 3 | 0 | 0 | 2 | 2 |
| >12 to 21 years | 0 | 0 | 4 | 4 | 0 | 0 | 4 | 4 |
| >21 to 60 years | 1 | 1 | 41 | 43 | 1 | 1 | 35 | 37 |
| >60 years | 0 | 0 | 35 | 35 | 0 | 1 | 35 | 36 |
| **Total** | **1** | **1** | **89** | **91** | **1** | **2** | **79** | **82** |

*Table 9: Retrospective Study - Age and Gender Distribution of individuals*

| Age Category | Female | | | | Male | | | |
|---|---|---|---|---|---|---|---|---|
| | HSV-1 | HSV-2 | Not detected | Total | HSV-1 | HSV-2 | Not detected | Total |
| From birth to 1 year | 0 | 0 | 2 | 2 | 0 | 0 | 2 | 2 |
| >1 to 2 years | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| >2 to 12 years | 0 | 0 | 2 | 2 | 0 | 0 | 1 | 1 |
| >12 to 21 years | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 |
| >21 to 60 years | 1 | 19 | 34 | 54 | 4 | 5 | 24 | 33 |
| >60 years | 4 | 3 | 16 | 23 | 10 | 2 | 17 | 29 |
| **Total** | **5** | **23** | **56** | **84** | **14** | **8** | **46** | **68** |

Performance of the miDiagnostics HSV-1&2 Test is presented as Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) with the comparator method in the tables 10 to 13 below.

*Table 10: HSV-1 Prospective Samples.*

| Candidate Device | Comparator Method | | |
|---|---|---|---|
| | Detected | Not Detected | Total |
| Detected | 0 | 2* | 2 |
| Not Detected | 0 | 171 | 171 |
| Total | 0 | 173 | 173 |
| PPA: NA | | | |
| NPA: 171/173 (98.84%); (95% CI: 95.88% - 99.68%) | | | |

* Two false positive specimens were reported to be HSV-1 negative by Standard of Care PCR testing. No clinical diagnoses were available.

*Table 11: HSV-1 Retrospective Samples.*

| Candidate Device | Comparator Method | | |
|---|---|---|---|
| | Detected | Not Detected | Total |
| Detected | 16 | 3* | 19 |
| Not Detected | 0 | 133 | 133 |
| Total | 16 | 136 | 152 |
| PPA: 16/16 (100.00%); (95% CI: 80.64% - 100.00%) | | | |
| NPA: 133/136 (97.79%); (95% CI: 93.72% - 99.25%) | | | |

* All 3 false positive specimens were reported to be HSV-1 negative by Standard of Care PCR testing. Two of them were clinically diagnosed with HSV-1 infection, and one subject was clinically diagnosed with Herpes Meningoencephalitis.

*Table 12: HSV-2 Prospective Samples*

| Candidate Device | Comparator Method | | |
|---|---|---|---|
| | Detected | Not Detected | Total |
| Detected | 2 | 1* | 3 |
| Not Detected | 0 | 170 | 170 |
| Total | 2 | 171 | 173 |
| PPA: 2/2 (100.00%); (95% CI: 34.24% - 100.00%) | | | |
| NPA: 170/171 (99.42%); (95% CI: 96.76% - 99.90%) | | | |

* One false positive specimen was reported to be HSV-2 negative by Standard of Care PCR testing. No clinical diagnosis was available.

*Table 13: HSV-2 Retrospective Samples*

| Candidate Device | Comparator Method | | |
|---|---|---|---|
| | Detected | Not Detected | Total |
| Detected | 29 | 2* | 31 |
| Not Detected | 0 | 121 | 121 |
| Total | 29 | 123 | 152 |
| PPA: 29/29 (100.00%); (95% CI: 88.31% - 100.00%) | | | |
| NPA: 121/123 (98.37%); (95% CI: 94.27% - 99.55%) | | | |

* The 2 false positive specimens were reported to be HSV-2 positive by Standard of Care PCR testing. One subject was clinically diagnosed with Herpes Meningitis. No clinical diagnosis was available for the other subject.

**CONCLUSION STATEMENT**

The analytical and clinical study results demonstrate that miDiagnostics HSV-1&2 CSF Test on the miDiagnostics PCR Platform performs comparably to the predicate device in detecting HSV-1 & 2 DNA and supports a substantial equivalence decision.

The information submitted in this premarket notification is complete and supports a substantial equivalence decision.