510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I Background Information:

A 510(k) Number

K250050

B Applicant

miDiagnostics nv

C Proprietary and Established Names

miDiagnostics HSV-1&2 CSF Test

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PGH	Class II	21 CFR 866.3307 - Herpes Simplex Virus Nucleic Acid-Based Assay For Central Nervous System Infections	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

Clearance of a new device

B Measurand:

Target DNA sequences from conserved regions of the herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) genes.

C Type of Test:

Molecular diagnostic test using real-time PCR (Polymerization Chain Reaction) technology for the qualitative detection and differentiation of HSV 1 and HSV 2 DNA present in 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).

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for 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 screning test. The test is for professional use only.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

miDiagnostics PCR Platform

IV Device/System Characteristics:

A 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 on the miDiagnostics PCR Platform, 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, the Sample Processing Control (SPC).

The miDiagnostics HSV-1&2 Test includes the following components:

1. miDiagnostics HSV-1&2 CSF Detection Mix - contains a freeze-dried cake, stored in a tube, containing the PCR components for amplification and fluorescent detection of the 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, including the sample processing control SPC (RPP30, a human housekeeping

gene) that acts as an internal control, stored in a tube, for diluting the CSF specimen. The device consists of 24 tubes in the box.

3. miDiagnostics Concentration Device - consists of one bag with 24 concentration columns and 48 collection tubes to concentrate the viral material.
4. miDiagnostics HSV-1&2 CSF Algorithm - The software system interprets the fluorescence signals and reports the test results. The algorithm is embedded in the miDiagnostics Software.

The miDiagnostics PCR Platform includes the following components:

1. The miDiagnostics PCR Reader 2.0 - receives the miDiagnostics PCR Card 2.0 and is responsible for performing consecutive PCR cycles through the thermocycler.
2. The miDiagnostics PCR Card 2.0 is a single-use, disposable test accessory. The miDiagnostics PCR Card 2.0 is manually loaded with the sample mixed with PCR components of the selected miDiagnostics test (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 the 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. The 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.

B Principle of Operation:

The miDiagnostics HSV-1&2 CSF Test is a real-time polymerase chain reaction (PCR) assay that enables the direct amplification, that detects and differentiates HSV-1 and HSV-2 genomic DNA present in human CSF specimen. The miDiagnostics HSV-1&2 Dilution Buffer contains the Sample Processing Control SPC (RPP30 - Internal Control) which acts as a control for the entire process (specimen processing, amplification, and detection). Identification of HSV-1, HSV-2 and the SPC occurs using target-specific primers and fluorescent-labeled probes that hybridize to conserved regions in the viral genomes.

The SPC is used to monitor the integrity of the miDiagnostics HSV-1&2 CSF Test process, instrument, or reagent failures and PCR inhibition. The miDiagnostics HSV-1&2 CSF Algorithm compares the fluorescence signal to a predetermined cut-off to produce a qualitative result for the presence or absence of the analyte.

Table. Primer and Probe Targets

Analyte	Gene Targeted
HSV-1	Glycoprotein B
HSV-2	Glycoprotein D
SPC	Ribonuclease P/MRP subunit p30 RPP30

As described under the device description above, 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).

The manual 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.

Interpretation of Results by the miDiagnostics HSV-1&2 Algorithm:

The miDiagnostics Software automatically analyses, interprets and displays the test results on the screen as: “Detected”, “Not Detected” for HSV-1 and HSV-2 and “invalid”, as shown in below in the Table 1.

Table 1: miDiagnostics HSV-1&2 CSF Test results interpretation

Results	Interpretation	Action to be taken
HSV-1 Not Detected HSV-2 Not Detected	HSV-1 Not Detected in the specimen HSV-2 Not Detected in the specimen	Report results
HSV-1 Detected HSV-2 Not Detected	HSV-1 Detected in the specimen HSV-2 Not Detected in the specimen	Report results
HSV-1 Not Detected	HSV-1 Not Detected in the specimen	Report results

HSV-2 Detected	HSV-2 Detected in the specimen	
HSV-1 Detected HSV-2 Detected	HSV-1 Detected in the specimen HSV-2 Detected in the specimen	Report results
Invalid*	Presence or absence of HSV-1 and HSV-2 cannot be established of a test failure or due to the absence or amplification of each target (HSV-1, HSV-2 and SPC) after completion of the PCR run	A retest is required starting from '*Step 1: Prepare the sample'* of the test procedure.

C Instrument Description Information:

1. Instrument Name: miDiagnostics PCR Platform

2. Specimen Identification: Manual

3. Specimen Sampling and Handling: Manual

4. Calibration: NA

5. Quality Control:

   Sample Processing Control (Internal Control): The miDiagnostics HSV-1&2 CSF Test contains a sample processing control (SPC) which is introduced into a sample with the miDiagnostics HSV-1&2 CSF Test process, instrument, or reagent failures and PCR inhibition. Positive and/or negative results for HSV-1 and HSV-2 are considered valid and consequently reported when a SPC result is positive for the HSV-1 and HSV-2. The SPC is required to be detected for a valid negative test result of HSV-1 and HSV-2. The SPC is human genomic DNA (RPP30) purified from buffy coat of blood, purchased from a commercial source.

   Recommended External Controls: External controls, Positive Sample Control (PSC) and Negative Sample Control (NSC), are not provided with the miDiagnostics HSV-1&2 CSF Test but are required to run the test (NSC should be pooled human CSF material from healthy donors).

V Substantial Equivalence Information:

A Predicate Device Name(s):
Simplexa HSV1 & 2 Direct

B Predicate 510(k) Number(s):
K133621

C Comparison with Predicate(s):

Device & Predicate Device(s):	K250050 (Candidate)	K133621 (Predicate)
Device Trade Name	miDiagnostics HSV-1&2 CSF Test	Simplexa HSV-1& 2 Direct

General Device Characteristic Similarities		
Intended Use/Indications For 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 screning test. The test is for professional use only.	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). This test is intended as an aid in the diagnosis of HSV-1 and HSV-2 infections of 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 assay is not intended for use as a donor screening test. The assay is for professional use only. 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.
Test Principle/Technology	Real -Time Polymerase Chain Reaction	Same
Qualitative	Yes	Same

Analyte detected	Viral DNA from HSV-1 and HSV-2	Same
Specimen Type	Cerebrospinal fluid (CSF)	Same
Automated Analysis	Yes	Same
Quality Control (Internal control)	Assay contains Sample Processing Control (SPC) to detect reagent failure/or inhibition	Same
General Device Characteristic Differences		
Instrument	miDiagnostics PCR Platform	3M Integrated Cycler Studio system
External Controls (Positive and Negative)	Required but not provided	Provided

VI Standards/Guidance Documents Referenced:

Not applicable

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

1a. 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 intermediate 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) virus stocks into pooled negative human CSF matrix.

Each panel member was tested in 2 replicates by 2 operators on 2 miDiagnostic 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 2.

Table 2. miDiagnostics HSV-1&2 CSF Test - Within-laboratory Precision

Analyte	Sample	Percent Agreement with Expected Results	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 HF strain	Low Positive 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
	Moderate Positive 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 MS strain	Low Positive 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
	Moderate Positive 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-Instrument/Lot/Operator Components.

1b. 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) virus 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 3.

Table 3. miDiagnostics HSV-1&2 CSF Test – Reproducibility

Analyte	Panel Member	Percent Agreement with Expected Results	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 HF strain	Low Positive 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
	Moderate Positive 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
	High Positive	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

Analyte	Panel Member	Percent Agreement with Expected Results	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
	10x LoD[b]														
	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 MS strain	Low Positive 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
	Moderate Positive 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
	High Positive 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/Instrument Components.
[b]For site 1 and site 2, one relicate of the high positive sample type was excluded from the analysis due to operator errors

2. Linearity:

Not applicable

3. Analytical Specificity/Interference:

3a. Potential Cross-reactivity: A study was performed to evaluate the performance of the miDiagnostics HSV-1&2 CSF Test in the presence of 46 potential cross-reactivity microorganisms that are closely related, cause similar clinical symptoms, or may be present in CSF. The potential cross-reactants were spiked into negative human CSF at high concentrations and tested in triplicate. The microorganisms were tested at 10^5 Units/mL for viruses and fungi, and 10^6 Units/mL for bacteria or the highest titer available. No cross-reactivity was observed in any of the microorganisms.

Table 4. miDiagnostics HSV-1&2 CSF Test – Potential Cross-reactivity

Potential Cross-Reactant Organism	Tested Concentration	Qualitative Result (#Detected / #Total)	
		HSV-1	HSV-2
Adenovirus (type 1)	10^5 $TCID_{50}$/mL	0/3	0/3
Adenovirus (type 7A)	10^5 $TCID_{50}$/mL	0/3	0/3
BK polyomavirus	10^5 $TCID_{50}$/mL	0/3	0/3
Citrobacter freundii	10^6 CFU/mL	0/3	0/3
Citrobacter koseri	10^6 CFU/mL	0/3	0/3
Cryptococcus neoformans	10^6 CFU/mL	0/3	0/3

Cytomegalovirus (AD169)	3.12 x 10^3 $TCID_{50}$/mL	0/3	0/3
Dengue virus	10^5 copies/mL	0/3	0/3
Enterobacter aerogenes	10^6 CFU/mL	0/3	0/3
Enterovirus 71	7.78 x 10^3 $TCID_{50}$/mL	0/3	0/3
Epstein Barr virus (B95-8)	10^5 $TCID_{50}$/mL	0/3	0/3
Escherichia coli	10^6 CFU/mL	0/3	0/3
Haemophilus influenzae	10^6 CFU/mL	0/3	0/3
Haemophilus influenzae type b (MinnA)	10^6 CFU/mL	0/3	0/3
Hepatitis B	10^5 $TCID_{50}$/mL	0/3	0/3
Hepatitis C1	10^5 $TCID_{50}$/mL	0/3	0/3
HIV1 (type IIIB)	10^4 IU/mL	0/3	0/3
Human herpesvirus 6	10^5 $TCID_{50}$/mL	0/3	0/3
Human herpesvirus 7	9.36 x 10^3 $TCID_{50}$/mL	0/3	0/3
Human herpesvirus 8	1.45 x 10^5 $TCID_{50}$/mL	0/3	0/3
Influenza B	2.80 x 10^4 $TCID_{50}$/mL	0/3	0/3
Influenza A H1N1	1.46 x 10^5 $TCID_{50}$/mL	0/3	0/3
John Cunningham virus	10^5 $TCID_{50}$/mL	0/3	0/3
Klebsiella pneumoniae	10^6 CFU/mL	0/3	0/3
Listeria monocytogenes	10^6 CFU/mL	0/3	0/3
Measles	10^5 $TCID_{50}$/mL	0/3	0/3
Mumps	10^5 $TCID_{50}$/mL	0/3	0/3
Mycobacterium tuberculosis	10^6 copies/mL	0/3	0/3
Naegleria fowleri	10^6 copies/mL	0/3	0/3
Neisseria meningitides (serogroup A)	10^6 CFU/mL	0/3	0/3
Parainfluenza Virus 1	10^5 $TCID_{50}$/mL	0/3	0/3
Parainfluenza Virus 2	8.34 x 10^4 $TCID_{50}$/mL	0/3	0/3
Parainfluenza Virus 3	10^5 $TCID_{50}$/mL	0/3	0/3
Parvovirus B19	1.28 x 10^2 $TCID_{50}$/mL	0/3	0/3
Proteus mirabilis (Z050)	10^6 CFU/mL	0/3	0/3
Pseudomonas aeruginosa	10^6 CFU/mL	0/3	0/3
Rabies virus	Not available *	0/3	0/3
Rhinovirus (Type 1A)	2.82 x 10^4 $TCID_{50}$/mL	0/3	0/3
Rotavirus (Type Wa)	10^5 $TCID_{50}$/mL	0/3	0/3

Rubella	2.58 x 10^3 $TCID_{50}$/mL	0/3	0/3
St. Louis Encephalitis	10^5 copies/mL	0/3	0/3
Staphylococcus aureus COL	10^6 CFU/mL	0/3	0/3
Streptococcus agalactiae	10^6 CFU/mL	0/3	0/3
Streptococcus pneumoniae Z022; 19F	10^6 CFU/mL	0/3	0/3
Varicella zoster virus	10^5 $TCID_{50}$/mL	0/3	0/3
West Nile Virus	10^5 $TCID_{50}$/mL	0/3	0/3

**No official concentration was provided by the supplier. RNA extracted from the maximum volume of pathogen 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.

3b. Microbial Interference Study:

The susceptibility of the miDiagnostics HSV-1&2 CSF Test to microbial interference was evaluated using the same microorganisms listed above in Table 4. A total of 46 microorganisms were spiked either individually or as a pool into negative human CSF along with HSV-1 and HSV-2 at 3x LoD levels and tested in in triplicate. The interfering microorganisms were tested at 10^5 Units/mL for viruses and fungi, and 10^6 Units/mL for bacteria or the highest titer available. All samples spiked with HSV-1 and HSV-2 spiked were reported positive for HSV-1 and HSV-2, in the presence of the potentially interfering microorganisms. There was no interference observed for either HSV-1 or HSV-2 due to the presence of the 46 microorganisms tested with the miDiagnostics HSV-1&2 CSF Test.

3c. 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. A panel composed of eleven (11) substances were spiked individually into negative human CSF and were tested alone and in the presence of HSV-1 (HF strain), HSV-2 (MS strain) at 3x LoD levels. There was no evidence of interference (false positive or false negative results) caused by the substances tested at the concentrations shown below in the Table 5 below.

Table 5. miDiagnostics HSV-1&2 CSF Test - Interference

Interfering Substance	**Interferent Concentration**	**Clinically relevant Concentration**
Albumin	10 mg/mL	0.32 - 2.63 mg/mL
Lactate	2.2 mg/mL	0 - 0.6 mg/mL
Glucose	9.9 mg/mL	0.32 – 0.98 mg/mL

Casein	5 mg/mL	0.15 – 40 mg/mL
Hemoglobin	0.625 g/mL	<0.03 mg/mL
Immunoglobulin (IgG)	10 mg/mL	0.8 mg/mL
White Blood Cell (WBC)	2x10^6 ells/mL	0.009 - 1x 10^6 cells/mL
Whole Blood	5% (v/v)	1% (v/v)
Topical Antiseptic – Betadin	1% (v/v)	0.25% (v/v)
Antiviral Drug (Acyclovir)	2.5 mg/mL	0.022 mg/mL
Acylovir Triphosphate	0.5 mg/mL	Metabolized from up to 0.022 mg/mL

3d. Competitive Interference:

A competitive interference study was performed to evaluate the performance of the miDiagnostics HSV-1&2 CSF Test in the presence of the 2 HSV-1 and HSV-2 target analytes. The interference between HSV-1 (HF strain) and HSV-2 (MS strain) targets with the miDiagnostics HSV-1&2 CSF Test was evaluated by spiking one of the targets at high concentration (10^5 TCID50/mL) and the other target at low concentration (3x LoD) into pooled negative human CSF. No interference between the targets was observed.

Table 6. miDiagnostics HSV-1&2 CSF Test - Competitive Interference

Low conc. (3x LoD)	High Conc.	HSV-1 Positivity	HSV-1 Mean Cq (SD)	HSV-2 Positivity	HSV-2 Mean Cq (SD)
HSV-1 HF strain 60.96 $TCID_{50}$/mL	HSV-2 HF strain 10^5 $TCID_{50}$/mL	(3/3) 100.0%	36.76 (2.44)	(3/3) 100.0%	18.35 (0.11)
HSV-2 MS strain 1.68 $TCID_{50}$/mL	HSV-1 MS strain 10^5 $TCID_{50}$/mL	(3/3) 100.0%	22.47 (0.19)	(3/3) 100.0%	36.41 (1.30)

4. Assay Reportable Range:

Not Applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Not Applicable

6. Detection Limit:

The Limit of Detection (LoD) was determined for the miDiagnostics HSV-1&2 CSF Test in 2 phases:

Phase 1: LoD Range Finding - 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). The serial dilutions of each strain were made in pooled negative human CSF matrix (negative CSF). For each viral strain, 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.

Phase 2: LoD Confirmation - The preliminary LOD for each strain was subsequently confirmed with 20 replicates diluted at the preliminary LOD concentration. The confirmed LOD per strain are included in Table 7 below.

Table 7. miDiagnostics HSV-1&2 CSF Test - LoD

Virus strain	**LoD ($TCID_{50}$/mL)**	**LoD (copies/mL)**	**Number of detected samples (% of detection)**	**Mean Cq (SD) (from detetected replicates)**
HSV-1 Maclntyre	8.84	800	20/20 (100%)	37.06 ± 0.98
HSV-1 HF	20.32	1000	20/20 (100%)	36.68 ± 0.84
HSV-2 MS	0.56	400	19/20 (95%)	37.99 ± 1.55
HSV-2 G	0.43	800	20/20 (100%)	36.63 ± 0.86

7. Analytical Reactivity (Inclusivity):

The analytical reactivity of the miDiagnostics HSV-1&2 CSF Test was evaluated with four HSV-1 strains and three HSV-2 strains in addition to the strains tested for limit of detection (*i.e.*, analytical sensitivity in Table 7 above). Viral material with known concentration was spiked into negative CSF at near LOD concentration and tested in triplicates at the LoD determined for the HSV-1 and HSV-2. The miDiagnostics HSV-1&2 CSF Test was able to detect all HSV strains: at 1x or 2x LOD concentration for HSV-1 strain F, HSV-1 strain KOS, HSV-1 ATCC-2011-1, HSV-1 strain 95 (95/1906), HSV-2 strain 131596 and HSV-2 ATCC-2011-2, and at 6x LOD for HSV-2 strain HG52.

Table 8. miDiagnostics HSV-1&2 CSF Test - Analytical reactivity

Analyte	**Strain/Isolate**	**Spiked Concentration**	**Inclusive (Yes/No)**
HSV-1	F	20.32 $TCID_{50}$/mL	Yes
	KOS	40.64 $TCID_{50}$/mL	Yes
	ATCC 2011-1	20.32 $TCID_{50}$/mL	Yes
	Strain 95 (95/1906)	20.32 $TCID_{50}$/mL	Yes
HSV-2	131596	1605 copies/mL	Yes
	ATCC 2011-2	3.36 $TCID_{50}$/mL	Yes
	HG52	0.56 $TCID_{50}$/mL	Yes

8. Assay Cut-Off:

Initially, the cut-off of the miDiagnostics HSV-1&2 Test was determined using the large datasets from clinical studies, in addition to natural and contrived samples. All studies were performed to a maximum of 46 Cq cycles of amplification during feasibility study.

The clinical cut-off was further validated using a total of 22 positive samples (11 spiked with HSV-1 and 11 with HSV-2) that were prepared by spiking either HSV-1 or HSV-2 into 11 individual negative clinical CSF specimens from symptomatic patients at or near the LoD levels. In addition, 10 negative samples from same individual CSF specimens were tested without spiking. Out of the 22 contrived samples, 10 resulted in positive call for HSV-1 while 1 resulted in a negative call, and 10 resulted in a positive call for HSV-2 while 1 resulted in a negative call. Based on the results of the verification study, HSV-1 was detected between Cq of 35.05 and 39.55 and HSV-2 was detected between Cq of 34.22 and 40.08.

The combined data analysis from clinical studies and contrived studies demonstrated that the cut-off algorithm implemented for the HSV CSF test achieved a positive concordance 96.3% for HSV-1 and 97.6% for HSV2 and a negative concordance of 98.5% for HSV-1 and 99.0% for HSV-2, therefore confirming its adequacy of the cut-off.

9. Accuracy (Instrument):

Not applicable

10. Carry-Over:

The risk of sample carry-over contamination was evaluated with the miDiagnostics HSV-1&2 Test by alternately testing high positive samples (HSV-1 and HSV-2 at $\geq 10^5$ pfu/mL) followed by negative sample on a single Reader. A total of 5 runs were performed, each by different operators. Within each run, eight cycles of a high positive sample followed by a negative sample were measured using a single instrument per operator per run. A total of 16 data points, including 8 measurements for high positive samples and 8 measurements for negative samples, were generated for each operator. All high positive samples resulted in "Detected" calls for both HSV-1 and HSV-2 and all negative samples resulted in "Not detected" calls. No carry-over contamination was observed throughout the study.

11. Specimen Stability:

The specimen stability study was executed by spiking HSV-1 and HSV-2 in pooled human CSF material obtained from deidentified healthy donors. The material was confirmed negative for HSV-1 (MacIntyre) and HSV-2 (MS). The positive specimens were prepared by spiking HSV-1 and HSV-2 at the concentration of 1- 2x LoD and 3-4x LoD in pooled negative CSF matrix. The data supports the following CSF specimen storage conditions for testing with the miDiagnostics HSV-1&2 Test:

- Up to 24 hrs at room temperature (between 15°C to 30°C).
- Up to 7 days at 2-8 °C.
- Up to 30 days at -70°C.

12. **Comparison Studies**:

a. Method Comparison with Predicate Device:

See Section 13.C below.

b. Matrix Comparison:

Not applicable.

13. **Clinical Studies**:

a. Clinical Sensitivity:

Not applicable

b. Clinical Specificity:

Not applicable

c. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

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

A total of 362 cerebrospinal fluid (CSF) specimens were collected from patients presenting with signs and symptoms of Herpes Simplex Virus (HSV) central nervous system (CNS) infection at 4 participating sites between August 2024 and December 2024. A total of 325 specimens were included for the clinical performance of the miDiagnostics HSV-1&2 CSF Test. Thirty-seven (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 gender and age demographic of individuals for prospective and retrospective studies are presented in Table 9 and Table 10 below.

Table 9. Prospective Study - Age and Gender Distribution of individuals

Age Category	Female				Male			
	HSV-1	**HSV-2**	**Not detected**	**All**	**HSV-1**	**HSV-2**	**Not detected**	**All**

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 10. Retrospective Study - Age and Gender Distribution of individuals

Age Category	**Female**				**Male**			
	HSV-1	**HSV-2**	**Not detected**	**All**	**HSV-1**	**HSV-2**	**Not detected**	**All**
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 respect to the comparator method in the following tables below.

Table 11. HSV-1 Prospective Samples

Candidate Device	Comparator Method		Total
	Detected	Not Detected	
Detected	0	2*	2
Not Detected	0	171	171
Total	0	173	173
PPA	PPA: N/A		
NPA	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.
N/A: Not Applicable.

Table 12. HSV-1 Retrospective Samples

Candidate Device	Comparator Method		Total
	Detected	Not Detected	
Detected	16	3*	19
Not Detected	0	133	133
Total	16	136	152
PPA	PPA: 16/16 (100.00%); (95% CI: 80.64% - 100.00%)		
NPA	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 13. HSV-2 Prospective Samples

Candidate Device	Comparator Method		Total
	Detected	Not Detected	
Detected	2	1*	3
Not Detected	0	170	171
Total	2	171	173
PPA	PPA: 2/2 (100.00%); (95% CI: 34.24% -100.00%)		
NPA	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 14. HSV-2 Retrospective Samples

Candidate Device	Comparator Method		Total
	Detected	Not Detected	
Detected	29	2*	31
Not Detected	0	121	121

Total	29	123	152
PPA	PPA: 29/29 (100.00%); (95% CI 88.31% - 100.00%)		
NPA	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.

14. **Clinical Cut-Off:**

Not applicable.

15. **Expected Values/Reference Range:**

Not applicable

16. **Other Supportive Instrument Performance Characteristics Data:**

Not applicable

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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