510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K142738

B. Purpose for Submission:

New device

C. Measurand:

Target DNA sequences from Herpes Simplex Virus type 1 (HSV-1) and Herpes Simplex Virus type 2 (HSV-2)

D. Type of Test:

Real-time PCR DNA amplification assay

E. Applicant:

QIAGEN

F. Proprietary and Established Names:

artus® HSV-1/2 QS-RGQ MDx Kit

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3305

2. Classification:

   Class II

3. Product code:

   OQO

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The artus HSV-1/2 QS-RGQ MDx Kit is an *in vitro* real-time PCR DNA amplification assay performed on the QIAsymphony RGQ MDx system for the direct qualitative detection and differentiation of herpes simplex virus (HSV-1 and HSV-2) DNA in genital or oral vesicular lesions from male and female patients suspected of HSV infection.

   The assay is intended for use as an aid in diagnosis of HSV infection in symptomatic patients.

   Warning: The artus HSV-1/2 QS-RGQ MDx Kit is not FDA-cleared for use with cerebrospinal fluid (CSF) or for prenatal screening.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   The *artus* HSV-1/2 QS-RGQ MDx Kit is performed on the QIAsymphony RGQ MDx system

I. Device Description:

The *artus* HSV-1/2 QS-RGQ MDx Kit is an *in vitro* PCR assay for the qualitative detection and differentiation of nucleic acids encoding the Glycoprotein D and UL30 genes isolated from HSV-1 and HSV-2 DNA present in genital or oral lesions from male and female patients. Samples are extracted and prepared for PCR using the QIAsymphony SP/AS instrument with the QIAsymphony DSP Virus/Pathogen Mini Kit. Amplification and detection are carried out using the *artus* HSV-1/2 QS-RGQ MDx Kit with the Rotor-Gene Q MDx (RGQ MDx) and Rotor-Gene AssayManager software. The presence of a HSV-1 or HSV-2 target sequence is indicated by the fluorescent signal generated through the use of fluorescently labeled oligonucleotide probes. The probes do not generate a signal unless they are specifically bound to the amplified product. The amplification cycle at which fluorescent signal is detected by the RGQ MDx is inversely proportional to the HSV-1 and/or HSV-2 target concentration present in the original specimen. A plasmid construct containing DNA

unrelated to HSV-1 and HSV‑2 is introduced into each specimen during sample preparation to serve as an internal control. Run as a separate control, the positive control serves to demonstrate that the HSV-1/2 PCR reagents are functional. In addition, the positive control functions as a process control, to demonstrate that sample preparation has proceeded correctly during the run.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   MultiCode®-RTx Herpes Simplex Virus 1 & 2 Kit (Eragen)

2. Predicate 510(k) number(s):

   K100336

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indications for use	The artus HSV-1/2 QS-RGQ MDx Kit is an *in vitro* real-time PCR DNA amplification assay performed on the QIAsymphony RGQ MDx system for the direct qualitative detection and differentiation of herpes simplex virus (HSV-1 and HSV-2) DNA in genital or oral vesicular lesions from male and female patients suspected of HSV infection. The assay is intended for use as an aid in diagnosis of HSV infection in symptomatic patients. Warning: The artus HSV-1/2 QS-RGQ MDx Kit is not FDA-cleared for use with cerebrospinal fluid (CSF) or for prenatal screening.	The MultiCode®-RTx Herpes Simplex Virus 1 & 2 Kit is a polymerase chain reaction (PCR)-based qualitative *in vitro* diagnostic test for the detection and typing of herpes simplex virus (HSV1&2) DNA in vaginal lesions. It is indicated for use in the detection and typing of HSV-1 or HSV-2 in vaginal lesion swab specimens from symptomatic female patients as an aid in the diagnosis of genital herpes infection. Warning: The device is not FDA cleared for the use with cerebral spinal fluid (CSF) or any lesions other than vaginal. The assay is not intended to be used for

Similarities		
Item	Device	Predicate
		male penile specimens, for prenatal screening, or females under the age of 18 years.
Technology	Real-time PCR	same
Target detected	HSV-1 and HSV-2 DNA	same

Differences		
Item	Device	Predicate
Sample type	Male and female genital or oral herpetic lesions	Female vaginal lesions

K. Standard/Guidance Document Referenced (if applicable):

Not Applicable

L. Test Principle:

The HSV-1/2 Master A and HSV-1/2 Master B components contain reagents and enzymes for the specific amplification of target regions within the HSV-1 and HSV-2 genomes and for the direct detection of the specific amplicon in fluorescence channels Cycling Orange and Cycling Green respectively of the Rotor-Gene Q MDx.

In addition, the *artus* HSV-1/2 QS-RGQ MDx Kit contains a second heterologous control system to identify potential failures during the assay process. This is detected as an internal control (IC) in fluorescence channel Cycling Crimson of the Rotor-Gene Q MDx.

The contents of the *artus* HSV-1/2 QS-RGQ MDx Kit are sufficient for 72 tests in one to 3 batches of 24 reactions on the QIAsymphony RGQ MDx. The Rotor-Gene Q MDx rotor holds up to 72 reaction tubes.

Kit contents

***artus* HSV-1/2 QS-RGQ MDx Kit**		**(72)**
Catalog no.		**4526346**
Number of reactions		**72**
Blue	HSV-1/2 Master A	3 x 330 µl
Violet	HSV-1/2 Master B	3 x 600 µl
Green	HSV-1/2 Internal Control	3 x 540 µl

Red	HSV-1/2 Positive Control	3 x 330 µl
White	HSV-1/2 Negative Control	3 x 350 µl
artus *HSV-1/2 QS-RGQ MDx Kit Instructions for Use (Handbook)*		1

Materials Required but Not Provided

The following is a list of some of the major materials required but not provided with the kit.

1. Equipment for sample preparation and assay setup:

   QIAsymphony SP (module of the QIAsymphony RGQ MDx)*

   QIAsymphony AS (module of the QIAsymphony RGQ MDx)*

   QIAsymphony software version 4.0

2. Equipment for PCR

   Rotor-Gene Q MDx (module of the QIAsymphony RGQ MDx)*

   Rotor-Gene AssayManager® version 1.0 (module of the QIAsymphony RGQ MDx)

Interpretation of Results

The *artus* HSV-1/2 QS-RGQ MDx Kit Assay Profile automatically analyzes samples, positive and negative controls, and run results. Every sample and control displays an independent result for each target: HSV-1, HSV-2, and Internal Control. Each result is reported as "Signal detected", "No signal", or "INVALID".

All targets for the positive control and negative control must be valid to confirm that the assay status is successful and that the test results may be reported. If any target of the positive control or negative control is invalid, results for every sample in the run will display "INVALID"; the entire assay run must then be retested.

The positive control must report a "Signal detected" result for HSV-1, HSV-2, and Internal Control. The negative control must report a "Signal detected" result for Internal Control and "No signal" for the specified HSV-1 and HSV-2 target.

It is expected that in some positive HSV-1/2 samples the Internal Control PCR may be inhibited due to competition from amplifying HSV-1/2, which will cause a "No signal" or "INVALID" result for Internal Control.

Summary of results interpretation

Target result				HSV detected in sample
HSV-1	HSV-2	Internal Control	Sample status	
Signal detected	Signal detected	Signal detected/ No signal/ INVALID	Valid	Yes
Signal detected	No signal	Signal detected/ No signal/ INVALID	Valid	Yes
No signal	Signal detected	Signal detected/ No signal/ INVALID	Valid	Yes
No signal	No signal	Signal detected	Valid	No
No signal	No signal	No signal/ INVALID	Invalid	Error, retest sample
Signal detected	INVALID	Signal detected/ No signal/ INVALID	Valid	Yes
INVALID	Signal detected	Signal detected/ No signal/ INVALID	Valid	Yes
INVALID	No signal	No signal/ INVALID	Invalid	Error, retest sample
No signal	INVALID	No signal/ INVALID	Invalid	Error, retest sample
No signal	INVALID*	Signal detected	Valid	Error, retest sample
INVALID*	No signal	Signal detected	Valid	Error, retest sample

*If a target is reported as "INVALID"and the flag says CT_ABOVE_ACCEPTED_RANGE, this sample does not need to be retested and is considered "No signal", if the Internal Control is valid.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The precision of the *artus* HSV-1/2 QS-RGQ MDx Kit was assessed using a seven-member precision panel consisting of 2 strains of HSV: HSV-1 MacIntyre and HSV-2 MS, diluted in M4RT viral transport medium. Panel members were formulated with a single HSV strain present (HSV-1 or HSV-2) at three concentrations; Positive (~2-3X LoD), Low Positive (1X LoD), and High Negative (<1X LoD, targeting a 20-80% positivity rate). A seventh panel member (Negative) was prepared using M4RT viral transport medium only. The data obtained were used to determine the mean C_T, standard deviation (ST DEV) and the coefficient of variation (%CV) for each target and the internal control.

For the within laboratory repeatability study, the seven-member panel was tested in replicates of three, once a day for a total of twelve days. The testing was conducted by two alternating operators using one QIAsymphony RGQ MDx (QS-RGQ MDx) instrument platform and one reagent kit lot.

Within-Laboratory Repeatability Study Results

Sample	HSV-1			HSV-2			IC			Detected/ Total
	Mean C_T	ST DEV	%CV	Mean C_T	ST DEV	%CV	Mean C_T	ST DEV	%CV	
HSV-1 Positive	32.56	0.32	0.97%	N/A	N/A	N/A	31.79	0.64	2.00%	35/35*
HSV-1 Low Positive	33.89	0.35	1.03%	N/A	N/A	N/A	31.85	0.66	2.07%	36/36
HSV-1 High Negative	37.52	0.63	1.69%	N/A	N/A	N/A	31.73	0.59	1.87%	23/36
HSV-2 Positive	N/A	N/A	N/A	33.73	0.41	1.22%	31.72	0.63	1.98%	36/36
HSV-2 Low Positive	N/A	N/A	N/A	36.73	1.05	2.86%	31.69	0.63	2.00%	36/36
HSV-2 High Negative	N/A	N/A	N/A	38.35	1.01	2.64%	31.82	0.57	1.80%	9/36
HSV Negative	N/A	N/A	N/A	N/A	N/A	N/A	31.88	0.52	1.64%	0/36

* Total number of samples is less than 36 due to exclusion of 1 invalid sample

To measure site-to-site reproducibility, the 7-member panel was run by 2 users at each of 3 external sites. Each of the 2 users performed 5 runs on alternating testing days for a total of 90 test results per panel member. Panel members were tested in replicates of 3 that were randomized and blinded to the user. A single QIAsymphony RGQ MDx instrument platform and one lot of the *artus* HSV-1/2 QS-RGQ MDx Kit were used at each site to conduct the study.

Site-to-Site Reproducibility Study Results

Panel member	Site	HSV-1/HSV-2			IC			Detected/ Total
		Mean C_T	ST DEV	%CV	Mean C_T	ST DEV	%CV	
HSV-1 Positive	Site 1	31.40	1.04	3.30%	30.87	0.35	1.12%	27/27
	Site 2	31.44	0.35	1.10%	30.80	0.37	1.19%	29/29
	Site 3	31.20	0.32	1.03%	30.45	0.30	0.98%	26/26
	Overall	31.35	0.66	2.09%	30.71	0.38	1.24%	82/82*
HSV-1 Low Positive	Site 1	33.01	0.40	1.20%	30.91	0.34	1.11%	30/30
	Site 2	32.78	0.44	1.34%	30.76	0.38	1.24%	30/30
	Site 3	32.37	0.89	2.74%	30.47	0.35	1.13%	30/30
	Overall	32.72	0.66	2.03%	30.71	0.40	1.29%	90/90
HSV-1 High Negative	Site 1	36.34	0.88	2.41%	30.85	0.29	0.93%	19/30
	Site 2	36.27	0.74	2.05%	30.67	0.40	1.29%	15/30
	Site 3	36.77	1.06	2.88%	30.44	0.30	1.00%	17/30
	Overall	36.46	0.92	2.51%	30.66	0.37	1.20%	51/90
HSV-2 Positive	Site 1	33.19	0.36	1.08%	30.89	0.30	0.99%	30/30
	Site 2	32.85	0.35	1.05%	30.75	0.35	1.15%	30/30
	Site 3	32.46	0.33	1.03%	30.47	0.29	0.95%	30/30
	Overall	32.83	0.45	1.38%	30.70	0.36	1.17%	90/90
HSV-2 Low Positive	Site 1	36.12	0.96	2.65%	30.84	0.29	0.95%	30/30
	Site 2	35.53	0.66	1.85%	30.73	0.32	1.04%	30/30
	Site 3	35.81	0.92	2.58%	30.49	0.30	0.97%	30/30
	Overall	35.82	0.88	2.46%	30.69	0.33	1.08%	90/90
HSV-2 High Negative	Site 1	38.09	0.55	1.44%	30.91	0.31	0.99%	5/30
	Site 2	37.69	2.30	6.09%	30.71	0.37	1.20%	5/30
	Site 3	37.27	N/A	N/A	30.50	0.32	1.04%	1/30
	Overall	37.83	1.52	4.01%	30.71	0.37	1.20%	11/90
HSV Negative	Site 1	N/A	N/A	N/A	30.88	0.30	0.96%	0/30
	Site 2	N/A	N/A	N/A	30.72	0.36	1.16%	0/30
	Site 3	N/A	N/A	N/A	30.53	0.32	1.04%	0/30
	Overall	N/A	N/A	N/A	30.71	0.35	1.14%	0/90

* Total number of samples is less than 90 due to exclusion of invalid samples

b. Linearity/assay reportable range:

Not applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Traceability: Not applicable

Stability studies:

On-Board Reagent Stability

Two HSV strains (HSV-1, McIntyre strain and HSV-2, MS strain from Zeptometrix Corporation (ZMC) were diluted to 2-3X LoD in M4RT viral transport medium. The samples and reagents were incubated on-board the QIAsymphony SP/AS for 65 minutes before initiating the QIAsymphony SP/AS run. Following completion of the QIAsymphony AS run, assembled PCRs were held in the instrument for an additional 65 minutes before transferring the assembled reactions to the RGQ MDx instrument and initiating PCR. All 35 replicates were detected for both HSV-1 and HSV-2 after 65 minutes of incubation before initiating the QIAsymphony SP/AS run followed by 65 minutes of incubation before initiating the PCR reaction in the RGQ MDx instrument.

Sample Stability

One HSV-1 Isolate #15 strain and one HSV-2 MS strain, both from ZMC, were used to prepare contrived positive samples to assess specimen stability. Both strains were prepared in M4RT viral transport medium plus matrix at 2-3X LoD (54.6 $TCID_{50}$/mL for HSV-1 Isolate #15 and 2.94 $TCID_{50}$/mL for HSV-2 MS Strain) and ≥10X LoD (190 $TCID_{50}$/mL for HSV-1 Isolate #15 and 12 $TCID_{50}$/mL for HSV-2 MS Strain). Contrived negative specimens were also prepared, consisting of M4RT viral transport medium plus matrix alone. Six replicates of each strain at 2-3X LoD were stored at 2ºC to 8ºC, -15ºC to -10ºC, and -30ºC to -20ºC. Three replicates of each strain at ≥10X LoD as well as the negative specimen were also stored at the stated temperatures. Samples were removed at defined time intervals for testing. Testing was performed with samples stored at both ends of the temperature range (-15ºC to -10ºC and -30ºC to -20ºC) to support a specimen stability claim at -30ºC to -10ºC. A specimen was considered to be stable for a given time point/condition if the HSV-1 or HSV-2 target was detected for each positive sample replicate tested. The data from this study supports the HSV-1/2 specimen stability for 30 days at -30°C to -10°C and 7 days at 4°C and up to three freeze-thaw cycles in M4RT.

Transport Stability

The artus HSV-1/2 QS-RGQ MDx Kit is a frozen product, stored at -30°C to -10°C and shipped on dry ice. The storage of the artus HSV-1/2 QS-RGQ MDx Kit at alternating temperatures for different time intervals was tested to simulate storage and transport conditions on dry ice for 72 hours. Individual kit lot materials were cycled from required storage conditions (-30°C to -10°C freezer) to shipping conditions (-90°C to -70°C freezer) and back to required storage conditions (-30°C to -10°C freezer), before testing the kit reagents. The artus HSV-1/2 QS-RGQ MDx Kit was tested at Day 0 in order to establish baseline performance. All replicates in each run (following 1X, 2X and 3X temperature cycles) passed stability testing.

Controls:

HSV-1/2 Internal Control:

The internal control consists of synthetic plasmid DNA. The Internal Control is input into the lysis buffer at a low concentration so that it is susceptible to the presence of potential inhibitors present in the sample matrix. Its function is to monitor the integrity of PCR reagents as well as the integrity of the PCR process without interfering with the target specific PCR.

External controls:

HSV-1/2 positive and negative controls are loaded onto the QIAsymphony SP before DNA purification in place of a patient sample. They monitor the efficiency of sample preparation and assist in identifying contamination during sample preparation and/or the downstream assay. Positive Control should always be positive for HSV-1 and HSV-2 targets. Negative control should always be negative for HSV-1 and HSV-2 targets. However, the negative control may exhibit a signal in some cases but will still be considered negative as long as the signal obtained is outside the negative control C_T cutoff.

d. Detection limit:

Analytical Sensitivity (Limit of Detection):

The limit of detection (LoD) was assessed for the *artus* HSV-1/2 QS-RGQ MDx Kit using two strains of HSV-1 (MacIntyre and Isolate #15 from ZMC) and two strains of HSV-2 (MS and Isolate #2 from ZMC). The LoD is defined as the HSV titer ($TCID_{50}$/mL) detected with a probability of 95% or greater and was determined by probit analysis. The results, representative of the analytical sensitivity of the *artus* HSV-1/2 QS-RGQ MDx Kit, are summarized in the following table.

Limit of Detection

Strain	LoD (95 % CI) $TCID_{50}$/mL
HSV-1 Macintyre	4.42×10^0 (2.81×10^0 - 9.14×10^0)
HSV-1 Isolate 15	1.82×10^1 (0.96×10^1 - 5.47×10^1)
HSV-2 MS	9.78×10^{-1} (6.6×10^{-1} - 2.01×10^0)
HSV-2 Isolate 2	1.91×10^2 (1.26×10^2 - 3.55×10^2)

Analytical Reactivity:

The analytical reactivity of the *artus* HSV-1/2 QS-RGQ MDx Kit was assessed to determine whether the kit could detect a broad range of HSV-1 and HSV-2 strains. Strains were obtained from ZMC. A total of 39 strains (20 HSV-1 and 19 HSV-2) were diluted in M4RT viral transport medium to 2–3X LoD and tested with the *artus* HSV-1/2 QS-RGQ MDx Kit. The intended HSV-1 or HSV-2 was detected in all strains tested.

Strains Tested in Analytical Reactivity

Organism	Part No.	Organism	Part No.
HSV-1, Isolate #2	0810183CF	HSV-2, Isolate #3	0810204CF
HSV-1, Isolate #3	0810184CF	HSV-2, Isolate #4	0810205CF
HSV-1, Isolate #4	0810185CF	HSV-2, Isolate #5	0810206CF
HSV-1, Isolate #5	0810186CF	HSV-2, Isolate #6	0810207CF
HSV-1, Isolate #6	0810187CF	HSV-2, Isolate #7	0810208CF
HSV-1, Isolate #7	0810188CF	HSV-2, Isolate #8	0810209CF
HSV-1, Isolate #8	0810189CF	HSV-2, Isolate #9	0810210CF
HSV-1, Isolate #9	0810190CF	HSV-2, Isolate #10	0810211CF
HSV-1, Isolate #10	0810191CF	HSV-2, Isolate #11	0810212CF
HSV-1, Isolate #11	0810192CF	HSV-2, Isolate #12	0810213CF
HSV-1, Isolate #12	0810193CF	HSV-2, Isolate #13	0810214CF
HSV-1, Isolate #13	0810194CF	HSV-2, Isolate #14	0810215CF
HSV-1, Isolate #14	0810195CF	HSV-2, Isolate #15	0810216CF
HSV-1, Isolate #15	0810196CF	HSV-2, Isolate #16	0810217CF
HSV-1, Isolate #16	0810197CF	HSV-2, Isolate #17	0810218CF
HSV-1, Isolate #17	0810198CF	HSV-2, Isolate #18	0810219CF
HSV-1, Isolate #18	0810199CF	HSV-2, Isolate #19	0810220CF
HSV-1, Isolate #19	0810200CF	HSV-2, Isolate #20	0810221CF
HSV-1, Isolate #20	0810201CF	HSV-2, Isolate #21	0810222CF
HSV-1, Isolate #21	0810202CF		

e. Analytical specificity:

Cross Reactivity:

A panel of microorganisms that may be present in patient specimens was tested to determine whether these microorganisms interfered with the detection of HSV-1 or HSV-2 or were cross-reactive with the *artus* HSV-1/2 QS-RGQ MDx Kit. Organisms were tested at a target concentration of approximately 1 x 10^6 CFU/ml for bacteria (with the exception of *Neisseria gonorrhea*, which was tested at 3.5 x 10^5 CFU/mL due to the low titer of the stock culture) and fungi or ≥1 x 10^5 $TCID_{50}$/ml for viruses separately in the presence of 2–3X LoD of each of three HSV strains: HSV-1 MacIntyre, HSV-1 Isolate #15, or HSV-2 MS. None of the potential interfering organisms cross-reacted or interfered with the detection of any of the HSV strains by the *artus* HSV-1/2 QS-RGQ MDx Kit.

Organisms Tested in Cross Reactivity and Microbial Interference

Organism	Source ID
Acinetobacter calcaceticus	ATCC 51432
Acinetobacter lwoffi	ATCC 17925
Adenovirus type 2	ZMC 0810110CF
Bacteroides fragilis	ZMC 0601533
Candida albicans	ATCC 10231
Candida glabrata	ZMC Z007
Candida guilliermondii	ZMC Z008
Candida krusei	ZMC Z009
Candida lustaniae	ATCC 42720
Candida parapsilosis	ZMC Z011
Candida tropicalis	ZMC Z012
Chlamydia trachomatis	ATCC VR-885
Cytomegalovirus	ZMC 0810003CF
Enterobacter cloacae	ATCC 13047
Enterovirus	ZMC 0810047CF
Epstein-Barr Virus	ZMC 0810008CF
Escherichia coli	ATCC 23571
Fusobacterium nucleatum	ATCC 25586
Gardnerella vaginalis	ATCC 14019
Haemophilus ducreyi	ATCC 700724D-5
Human Genomic DNA	Promega G3041
Human Herpes Virus 6	ZMC 0810003CF
Human Herpes Virus 7	ZMC 0810071CF
Human papilloma virus 16	ATCC 45113
Human papilloma virus 18	ATCC 45152
Herpes Simplex Virus 1 (HSV-1), isolate 20	0810201CF
Herpes Simplex Virus 2 (HSV-2), isolate 20	0810221CF
Klebsiella pneumoniae	ATCC 13883
Lactobacillus acidophilus	ATCC 4356
Mobiluncus curtsii	ATCC 43063
Mobiluncus mulieris	ATCC 35240
Moraxella catarrhalis	ATCC 8176
Mycoplasma hominis	ATCC 23114D
Neisseria gonorrhea	ATCC 9793
Neisseria meningitides	ATCC 13077
Prevotella melaninogenica	ATCC 25845
Rubella Virus	ZMC 0810048CF
Simian Virus type 40 (SV40)	VRMC-2

Organism	Source ID
Staphylococcus aureus (MRSA)	ATCC 43300
*Staphylococcus aureus (*MSSA)	ATCC 29213
Staphylococcus epidermidis (MRSE)	ATCC 51625
Staphylococcus saprophyticus	ATCC 15305
Streptococcus mitis	ZMC Clinical isolate
Streptococcus mutans	ZMC Z072
Streptococcus pneumoniae	ZMC 19F-Z022
Streptococcus pyogenes	ATCC 8669
Streptococcus salivarius	ATCC 13419
Toxoplasma gondii	ZMC 0810007CF
Treponema pallidum	ATCC 632912
Trichomonas vaginalis	ATCC PRA-98D
Varicella-Zoster Virus (VZV)	ZMC 0810171CF

Target Carryover Study:

Absence of carryover between samples for the entire workflow was demonstrated by performing 5 runs with alternating high positive ($\geq 1.0 \times 10^5$ $TCID_{50}$/mL) and negative samples. All samples were detected correctly, generating a carryover rate of 0.0%.

Interfering Substances:

A panel of 24 substances that may be present in oral/genital patient specimens was tested to determine whether these substances interfered with the performance of the *artus* HSV-1/2 QS-RGQ MDx Kit. Two strains of HSV: HSV-1 MacIntyre and HSV-2 MS, were diluted to approximately 2–3X LOD in M4RT viral transport medium and spiked with each potentially inhibitory substance. None of the substances showed an inhibitory effect on the detection of HSV-1 or HSV-2 by the *artus* HSV-1/2 QS-RGQ MDx Kit.

Potentially Interfering Substances Tested

Substance	Potential Interferent/Active Ingredient	Concentration Of Substance Added To Reaction	Concentration Of Active Ingredient Added To Reaction
Whole blood with EDTA	Hemoglobin, lactoferin	100%	5% v/v
Buffy coat	White blood cells	100%	5% v/v
Acyclovir	Acycloguanosine	5 mg/mL	5 mg/mL
Albumin	Albumin	5 mg/mL	5 mg/mL
Casein	Casein	5 mg/mL	5 mg/mL

Substance	Potential Interferent/Active Ingredient	Concentration Of Substance Added To Reaction	Concentration Of Active Ingredient Added To Reaction
Female urine*	Urea	100%	Not listed
Male urine*	Urea	100%	Not listed
K-Y® Brand jelly	Glycerin, hydroxyethyl cellulose, chlorhexidine, gluconate, gluconolactone, methylparaben, sodium hydroxide	100%	Not listed
Douche*	Octoxynol-9, citric acid, sodium benzoate, disodium EDTA	100%	Not listed
Spermicide*	Nonoxynol-9	100%	7%
Yeast·Gard®*	*Candida albicans* ×27 HPUS†, *Candida parapsilosis* ×27 HPUS, *Pulsatilla* ×27 HPUS	100%	Not listed
Monistat® 1*	Miconazole nitrate	100%	2% v/v
Vagisil® Cream*	Benzocaine, resorcinol	100%	Benzocaine 20% Resorcinol 3%
Monistat 3*	Miconazole nitrate	100%	2% v/v
Triconazole 1*	Triconazole	100%	6.5% v/v
Rite Aid Feminine Wash, Sensitive Skin*	Ammonium laureth sulfate, ammonium lauryl sulfate, decyl glucoside,cocamidopropyl betaine	100%	Not listed
Clotrimazole-7 vaginal cream*	Clotrimazole	100%	1% v/v
Anti-itch cream*	Benzocaine	100%	5% v/v
Listerine® antiseptic mouthwash*	Eucalyptol, menthol, methyl salicylate, thymol	100%	Eucalyptol 0.920% Menthol 0.042% Methyl salicylate 0.060% Thymol 0.064%
Abreva®*	Docosanol	100%	10% v/v
Carmex® lip balm*	Menthol, camphor, phenol	100%	Menthol 0.7% Camphor 1.7% Phenol 0.4%
Releev® cold sore treatment*	Benzalkonium chloride	100%	0.13% v/v

Substance	Potential Interferent/Active Ingredient	Concentration Of Substance Added To Reaction	Concentration Of Active Ingredient Added To Reaction
Lip Clear® lysine+*	Zinc oxide	100%	1.2% v/v
Toothpaste*	Stannous fluoride	100%	0.454% v/v

*: Applied directly to sample by swab.
†: HPUS: Homeopathic Pharmacopeia of the United States.

f. Assay cut-off:

See limit of detection.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable, see clinical study

b. Matrix comparison:

Not Applicable

3. Clinical studies:

a. Clinical Sensitivity:

See other clinical supportive data

b. Clinical specificity:

See other clinical supportive data

c. Other clinical supportive data (when a. and b. are not applicable):

Prospective Study:
The performance of the *artus* HSV-1/2 QS-RGQ MDx Kit was evaluated at 3 testing sites in 2013-2014, using samples from 5 geographically diverse locations within the United States. A total of 662 male and female genital or oral lesion swabs (510 genital and 152 oral) prospectively collected from symptomatic patients was evaluated. Results from the *artus* HSV-1/2 QS-RGQ MDx Kit were compared to results obtained from the ELVIS® (Enzyme Linked Virus Inducible System) HSV ID and D3 Typing Test System (Diagnostic Hybrids, Athens, OH).

Ninety-six (96) prospective specimens identified as HSV-2 positive by ELVIS viral culture were removed from the initial 510 genital specimens for the calculation of the HSV-1 clinical performance. As a result, there were 566 samples (414 genital and 152 oral) used to determine clinical performance for HSV-1. The clinical performance for HSV-1 and HSV-2 mucocutaneous lesions was also assessed. Prospectively collected samples that were classified as mucocutaneous included oral, cervical, vaginal, rectal, and any sample taken from a vesicle or lesion. The performance information is presented in the following tables:

HSV-1 Results for Genital Samples (N=414)

HSV-1		ELVIS		
		POS	NEG	Total
artus HSV-1/2 QS-RGQ MDx Kit	POS	69	21*	90†
	NEG	3**	321	324
	TOTAL	72	342	414
		95% CI		
Sensitivity – 95.8% (69/72)		88.5% – 98.6%		
Specificity – 93.9% (321/342)		90.8% – 96.0%		
Positive Predictive Value – 76.7%		67.0% – 84.2%		
Negative Predictive Value – 99.1%		97.3% – 99.7%		
Prevalence – 17%		14% – 21%		

*15 of the 21 discordant specimens (*artus* HSV-1/2 QS-RGQ MDx Positive, ELVIS Negative) were positive for HSV-1 by alternative PCR followed by bi-directional sequencing.

**All 3 discordant specimens (*artus* HSV-1/2 QS-RGQ MDx Negative, ELVIS Positive) were negative for HSV-1by alternative PCR followed by bi-directional sequencing.

† 5 samples were undetermined for ELVIS out of a total of 95 HSV-1 *artus* positive genital samples.

HSV-2 Results for Genital Samples (N=510)

HSV-2		ELVIS		
		POS	NEG	Total
artus HSV-1/2 QS-RGQ MDx Kit	POS	93	37*	130
	NEG	3**	377	380
	TOTAL	96	414	510
		95% CI		
Sensitivity – 96.9% (93/96)		91.2% – 98.9%		
Specificity – 91.1% (377/414)		87.9% – 93.5%		
Positive Predictive Value – 71.5%		63.3% – 78.6%		
Negative Predictive Value – 99.2%		97.7% – 99.7%		
Prevalence – 19%		16% – 22%		

*27 of the 32 discordant specimens (*artus* HSV-1/2 QS-RGQ MDx Positive, ELVIS Negative) were positive for HSV-2 by alternative PCR followed by bi-directional sequencing. The remaining 5 samples were not available for discordant analysis.

**All 3 discordant specimens (*artus* HSV-1/2 QS-RGQ MDx Negative, ELVIS Positive) were negative for HSV-2 by alternative PCR followed by bi-directional sequencing.

HSV-1 Results for Oral Samples (N=152)

HSV-1		ELVIS		
		POS	NEG	Total
artus HSV-1/2 QS-RGQ MDx Kit	POS	45	19*	64
	NEG	3**	85	88
	TOTAL	48	104	152
			95% CI	
Sensitivity – 93.8% (45/48)			83.2% – 97.9%	
Specificity – 81.7% (85/104)			73.2% – 88.0%	
Positive Predictive Value – 70.3%			58.2% – 80.1%	
Negative Predictive Value – 96.6%			90.5% – 98.8%	
Prevalence – 32%			25% – 39%	

*13 of the 19 discordant specimens (*artus* HSV-1/2 QS-RGQ MDx Positive, ELVIS Negative) were positive for HSV-1 by alternative PCR followed by bi-directional sequencing.

** 2 discordant specimens (*artus* HSV-1/2 QS-RGQ MDx Negative, ELVIS Positive) were negative for HSV-1 by alternative PCR followed by bi-directional sequencing. The remaining 1 specimen was unavailable for discordant analysis testing.

HSV-2 Results for Oral Samples (N=152)

HSV-2		ELVIS		
		POS	NEG	Total
artus HSV-1/2 QS-RGQ MDx Kit	POS	0	0	0
	NEG	0	152	152
	TOTAL	0	152	152
			95% CI	
Sensitivity – N/A			N/A	
Specificity – 100% (152/152)			97.5% – 100%	
Positive Predictive Value – N/A			N/A	
Negative Predictive Value – 100%			97.5% – 100%	
Prevalence – 0%			0% – 2%	

HSV-1 Mucocutaneous Samples (N=281)

HSV-1		ELVIS		
		POS	NEG	Total
artus HSV-1/2 QS-RGQ MDx Kit	POS	62	23	85
	NEG	4	192	196
	TOTAL	66	215	281
			95% CI	
Sensitivity – 93.9% (62/66)			85.4% – 97.6%	
Specificity – 89.3% (192/215)			84.5% – 92.8%	
Positive Predictive Value – 72.9%			62.7% –81.2%	
Negative Predictive Value – 98.0%			94.9% – 99.2%	
Prevalence – 23%			19% – 29%	

HSV-2 Mucocutaneous Samples (N=320)

HSV-2		ELVIS		
		POS	NEG	Total
artus HSV-1/2 QS-RGQ MDx Kit	POS	37	16	53
	NEG	2	265	267
	TOTAL	39	281	320
			95% CI	
Sensitivity – 94.9% (37/39)			83.1% – 98.6%	
Specificity – 94.3% (265/281)			91.0% – 96.5%	
Positive Predictive Value – 69.8%			56.5% – 80.5%	
Negative Predictive Value – 99.3%			97.3% – 99.8%	
Prevalence – 12%			9% – 16%	

HSV-2 Oral Retrospective Sample Study and Contrived Sample Study:
A retrospective study was conducted using oral samples for HSV2 detection. A total of 38 oral retrospective specimens were tested with the *artus* HSV-1/2 QS-RGQ MDx assay and ELVIS HSV ID and D^3 Typing Test System. There were no HSV-2 positive specimens detected in 38 oral specimens.

A contrived specimen study was performed to provide additional performance data for detection of HSV-2 in oral samples. A panel of seventy (70) individual samples consisting of 15 HSV-1/2 negative oral samples, 10 HSV-1 positive oral samples, and 45 HSV-1/2 negative oral samples spiked with HSV-2 at a concentration from 3X LoD to 1000X LoD was tested with the *artus* HSV-1/2 QS-RGQ MDx Kit. The HSV-1 positive oral samples and HSV-1/2 negative oral samples obtained from the method comparison study were used to make the panel. All samples were randomized and blinded to the operator prior to testing. HSV-2 was detected in all 45 contrived samples at all concentrations tested, supporting the claim for detection of HSV-2 in oral samples by the *artus* HSV-1/2 QS-RGQ MDx Kit.

4. Clinical cut-off**:**

   Not Applicable

5. Expected values/Reference range:

   The observed expected values for HSV-1 and HSV-2 during a multi-center clinical trial were calculated for the artus HSV-1/2 QS-RGQ MDx kit. The expected values for the patients ages 18 and older are shown for genital lesion samples and for all ages for the oral samples. The observed prevalence rates for HSV-1 were estimated as 18.6% (91/489) for genital samples and 42.1% (64/152) for oral samples. The prevalence rates for HSV-2 were estimated as 26.2% (128/489) for genital samples and 0% (0/152) for oral samples. Gender and age distribution is provided in the following tables.

Age Distribution for Genital Specimens

Age (Years)	Total # of specimens	HSV-1 Positive	HSV-2 Positive	Negative
18-20	48	11	13	24
21-30	167	32	40	95
31-40	111	18	31	62
41-50	69	12	15	42
51-60	48	10	15	23
61-70	31	4	12	15
71-80	11	4	2	5
81-90	3	0	0	3
91-97	1	0	0	1
Total	489	91/489	128/489	270/489

Age Distribution for Oral Specimens

Age (Years)	Total # of specimens	HSV-1 Positive	HSV-2 Positive	Negative
< 1	1	1	0	1
1-10	21	15	0	6
11-20	20	6	0	14
21-30	25	8	0	17
31-40	23	9	0	14
41-50	16	3	0	13
51-60	14	5	0	9
61-70	15	6	0	9
71-80	11	6	0	5
81-90	2	2	0	0
91-97	4	3	0	1
Total	152	64/152	0/152	88/152

Positive and Negative Predictive Value: Hypothetical positive and negative predictive values (PPV & NPV) for the *artus* HSV-1/2 QS-RGQ MDx kit are shown in the table below. These calculations are based on hypothetical prevalence and overall sensitivity and specificity per specimen type as determined in the clinical study.

For HSV-1, these calculations are based upon an overall sensitivity and specificity of 96% and 94%, respectively, for genital swabs and 94% and 82%, respectively, for oral swabs.

For HSV-2, these calculations are based upon an overall sensitivity and specificity of 97% and 91%, respectively, for genital swabs and 0.0% and 100%, respectively, for oral swabs.

PPV was calculated using: (Sensitivity x Prevalence) / (Sensitivity x Prevalence + [1 - Specificity] x [1 - Prevalence]).

NPV was calculated using: (Specificity x [1 - Prevalence]) / ([1 - Sensitivity] x Prevalence + Specificity x [1 - Prevalence]).

Positive and Negative Predictive Values (PPV & NPV) for the *artus* assay based on sample type

Prevalence (%)	Genital Swabs				Oral Swabs			
	HSV-1		HSV-2		HSV-1		HSV-2	
	PPV (%)	NPV (%)	PPV (%)	NPV (%)	PPV (%)	NPV (%)	PPV (%)	NPV (%)
2	24.6%	99.9%	18.0%	99.9%	9.6%	99.9%	N/A	98.0%
5	45.7%	99.8%	36.2%	99.8%	21.6%	99.6%	N/A	95.0%
10	64.0%	99.5%	54.5%	99.6%	36.7%	99.2%	N/A	90.0%
20	80.0%	98.9%	72.9%	99.2%	56.6%	98.2%	N/A	80.0%
30	87.3%	98.2%	82.2%	98.6%	69.1%	97.0%	N/A	70.0%
40	91.4%	97.2%	87.8%	97.8%	77.7%	95.3%	N/A	60.0%
50	94.1%	95.9%	91.5%	96.8%	83.9%	93.2%	N/A	50.0%

N/A = Not Applicable

Mucocutaneous Lesions: A subset of samples from the clinical study was identified as mucocutaneous. Those classified as mucocutaneous included: oral, cervical, vaginal, rectal. The following table lists the specific locations for the mucocutaneous lesions that were reported as such in the study along with the total number of samples from each specific location and number of positives.

Mucocutaneous Lesion Sites

Breakdown of Mucocutaneous Samples		HSV-1 Culture Positive	HSV-1 *artus* Positive	HSV-1 Concordant Positive	HSV-2 Culture Positive	HSV-2 *artus* Positive	HSV-2 Concordant Positive
Location	Total						
Bottom Lip	1						
Cervical	21	3	3	2	1	2	1
Clitoral	2						
Corners Of Lips	1						
Penis foreskin	1						
Genital	8	2	3	2		1	
Groin Vesicles	1						
In-Mouth	1	1	1	1			
Vaginal Introitus	1						
L Nares	1						
Labia	47	3	5	3	9	12	9
Labia Major	1				1	1	1
Left Labia	5				1	1	1
Left Outer Labia	1						
Left Upper Palate	1						
Left Vulvar	1						

Breakdown of Mucocutaneous Samples		HSV-1 Culture Positive	HSV-1 *artus* Positive	HSV-1 Concordant Positive	HSV-2 Culture Positive	HSV-2 *artus* Positive	HSV-2 Concordant Positive
Location	Total						
Lip	31	17	20	17			
Mons Pubic, Clitoris	1					1	
Mouth	35	10	14	10			
Oral Blister	3		2				
Palate	2	1					
Penile Lesion	2					1	
Rectal	3				1	1	1
Right Labia Papule	2				1	1	1
Right Side Of Mouth	1	1	1	1			
Throat	7	2	1	1			
Tongue	6	2	3	2			
Tooth	1	1	1	1			
Ulcer In Mouth	1	1	1	1			
Upper Hard Palate	1		1				
Upper Lip	4	2	3	2			
Urethral	8		1		2	2	2
Urogenital	4	1	1	1			
Vagina	65	11	11	10	17	20	15
Vaginal Rectal	9	1	2	1		1	
Vesicle	6		2			1	
Vulva	34	7	9	7	6	8	6
Total	320	66	85	62	39	53	37

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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