

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number: K111527

B. Purpose for Submission: To add an option of an additional extraction method to the MultiCode[®]-RTx Herpes Simplex Virus 1 & 2 Kit (K100336 - Cleared Device)

C. Measurand: HSV-1 and HSV-2 nucleic acids target sequences

D. Type of Test: Real Time Polymerase chain reaction (PCR)-based qualitative *in vitro* diagnostic test for the detection and typing of herpes simplex virus 1 & 2 (HSV 1&2) DNA using vaginal swab specimens

E. Applicant: EraGen Biosciences, Inc.

F. Proprietary and Established Names: MultiCode[®]-RTx Herpes Simplex Virus 1 & 2 Kit
MultiCode[®]-RTx HSV 1 & 2 Kit

G. Regulatory Information:

1. Regulation section: 21 CFR 866.3305
2. Classification: Class II
3. Product code: OQO, HSV NAAT assays, HSV PCR assays
4. Panel: Microbiology (83)

H. Intended Use:

1. Intended use(s):

The MultiCode[®]-RTx HSV 1 & 2 Kit is a polymerase chain reaction (PCR)-based qualitative *in vitro* diagnostic test for the detection and typing of herpes simplex virus (HSV 1 & 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 use with cerebral spinal fluid CSF; for any lesions other than vaginal. The assay is not intended to be used for male penile specimens, for prenatal screening, or for females under the age of 18 years.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Extraction:

- a. Roche MagNA Pure LC Total NA Kit and Magna Pure Instrument; or
- b. bioMérieux NucliSENS Nucleic Acid Extraction Reagents and easy MAG Instrument

PCR: Roche LightCycler 1.2

Software: Eragen MultiCode[®]-RTx HSV 1&2 Kit Analysis Software - 2.1 (HSV 1.1)

I. Device Description:

The EraGen 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 1 & 2 (HSV 1 & 2) DNA using vaginal swab specimens.

Patient vaginal swab specimens are collected in Copan Universal Transport Medium, or identical Copan manufactured media formulations (Becton Dickinson Universal Viral Transport Media, Copan branded Universal Transport Medium for LabCorp, and the Quest Viral Culture Media) and transported to the laboratory. An extractable sample processing control (SPC) target is added to the specimen prior to lysis. The SPC controls for specimen lysis, for recovery of extracted nucleic acid, for inhibitory substances and for PCR reagent and instrument integrity. The specimen is lysed and nucleic acid is extracted using the Roche MagNA Pure LC instrument using the Roche MagNA Pure LC Total Nucleic Acid Isolation Kit or bioMérieux NucliSENS easyMAG and the NucliSENS Nucleic Acid Extraction Reagents.

A sample of the extracted nucleic acid is added to the MultiCode[®]-RTx Herpes Simplex Virus 1 & 2 Kit reagents that contain a primer pair specific to HSV-1 and HSV-2 and a second primer pair specific to the SPC sequence. The two specific primer pairs are labeled with distinct fluorophore labels. PCR amplification is performed and assay fluorescence is monitored using the Roche LightCycler 1.2 real-time PCR instrument. Incorporation of the quencher-labeled nucleotide causes a decrease in assay fluorescence. Following amplification, the reaction is slowly heated and fluorescence is monitored. The strands of the amplification

products will separate at a specific melting temperature (T_m) that is determined by an increase in fluorescence as the strands are separated. The instrument fluorescence output is analyzed and test results are determined using the MultiCode[®]-RTx Herpes Simplex Virus 1 & 2 Kit Analysis Software. A printed results report is generated.

MultiCode[®]-RTx HSV 1&2 Kit contains HSV-1&2 Primer Mix, HSV-1&2 Reaction Buffer, Nuclease Free Water, DNA Sample Processing Control, HSV-1 Positive Control, and HSV-2 Positive Control, Analysis Software, Certificate of Analysis, and Package Insert.

J. Substantial Equivalence Information:

1. Predicate device name(s): MultiCode[®]-RTx Herpes Simplex Virus 1 & 2 Kit
2. Predicate Numbers (s): K100336

Comparison with predicate: The purpose of this submission was to add an option of another extraction method to the MultiCode[®]-RTx Herpes Simplex Virus 1 & 2 Kit that was cleared under K100336. The performance of the MultiCode[®]-RTx HSV 1&2 kit using the bioMérieux NucliSENS easyMAG extraction method was compared to the performance of the device using the Roche MagNA Pure LC extraction method.

Similarities

Device Characteristic	EraGen Biosciences MultiCode [®] -RTx Herpes Simplex Virus 1 & 2 Kit (New Device)	EraGen Biosciences MultiCode [®] -RTx Herpes Simplex Virus 1 & 2 Kit (Predicate Device – K100336)
Intended Use	<p>The MultiCode[®]-RTx HSV 1&2 Kit is a polymerase chain reaction (PCR)-based qualitative <i>in vitro</i> diagnostic test for the detection and typing of herpes simplex virus (HSV 1 & 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.</p> <p>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 male penile specimens, for prenatal screening, or females under the age of 18 years.</p>	Same

Device Characteristic	EraGen Biosciences MultiCode®-RTx Herpes Simplex Virus 1 & 2 Kit (New Device)	EraGen Biosciences MultiCode®-RTx Herpes Simplex Virus 1 & 2 Kit (Predicate Device – K100336)
Identification and Typing of HSV-1 and HSV-2	Yes	Same
Assay Results	Qualitative	Same
Packaging	The product is supplied in labeled, sterile tubes. The outer container is a labeled box.	Same

Difference

Device Characteristic	EraGen Biosciences MultiCode®-RTx Herpes Simplex Virus 1 & 2 Kit (New Device)	EraGen Biosciences MultiCode®-RTx Herpes Simplex Virus 1 & 2 Kit (Predicate Device – K100336)
Target Extraction Method	Roche MagNA Pure LC Total Nucleic Acid Isolation Kit; or bioMérieux NucliSENS Nucleic Acid Extraction Reagents	Roche MagNA Pure LC Total Nucleic Acid Isolation Kit

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

1. Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices, May 11, 2005.
<http://www.fda.gov/cdrh/ode/guidance/337.pdf>
2. CLSI EP17-A, “Protocols for Determination of Limits of Detection”.

L. Test Principle:

Following nucleic acid extraction from a patient swab specimen, a fluorophore-labeled PCR primer pair amplifies a segment of the glycoprotein B gene of HSV-1 and HSV-2. The sample processing control (SPC) will also be amplified by a distinct fluorophore-labeled PCR primer pair unless there are sample processing errors, inhibitory substances in the PCR reaction, reagent failure, or instrument malfunction.

The MultiCode®-RTx system is based on an expanded genetic alphabet technology, consisting of 2'-deoxy-5-methyl-isocytidine (iC) and 2'-deoxy-isoguanosine (iG) nucleotide bases also known as isobases. The isobases pair specifically with each other and not with natural nucleotides. In addition isobases are efficiently incorporated during PCR. The isobase pair allows site-specific incorporation of a dabcyI quencher directly adjacent to a fluorophore-labeled primer. During PCR amplification, a quencher-modified iGTP is incorporated by the polymerase opposite an iC and a fluorophore reporter attached to a PCR primer. If target is present and is amplified, assay fluorescence decreases with every cycle as amplification product accumulates. The decrease in assay

fluorescence is monitored in real time using the Roche LightCycler 1.2 instrument. Following PCR, the amplification products are thermally denatured and assay fluorescence is monitored. The strands of the amplification products are separated and assay fluorescence increases, thus determining the melting temperature (T_m) profile of the amplicon. The sequences between the PCR primer binding sites of the HSV-1 and HSV-2 amplicons have different base compositions that are distinguished by their different melting temperatures using the MultiCode[®]-RTx HSV 1&2 Analysis Software.

Interpretation of Control Results:

A. Validation of Assay Run: The MultiCode[®]-RTx HSV 1&2 Kit Analysis Software automatically determines results for the controls based on the amplification cycle threshold (Ct) value and the melting temperature (T_m) value.

B. Valid Assay Run:

- For a valid run, the following conditions must be met:

Control ID	Assay Result	F1			F2	
		Ct	HSV-1 T_m	HSV-2 T_m	Ct	SPC T_m
HSV-1 Positive Control	Pass	+	+	N/A	N/A	N/A
HSV-2 Positive Control	Pass	+	N/A	+	N/A	N/A
Negative Control	Pass	-	-	-	+	+
		+	-	-	+	+

A “+” symbol in the Ct column indicates that the amplification curve crosses the amplification threshold. A “+” symbol in the T_m column indicates that a melt peak is observed in the appropriate temperature range. A “-” symbol in the Ct column indicates that the amplification curve does not cross the amplification threshold. A “-” symbol in the T_m column indicates that no melt peak was observed in the appropriate temperature range. N/A indicates that the result is not dependent on the value.

- The clinical report displays only the Assay Result column.
- If the HSV-1 Positive Control fails, no data will be available for the HSV-2 Positive Control or the Negative Control because the criteria for Pass/Fail are based on the results of the HSV-1 Positive Control.

C. Invalid Assay Run: If the conditions for a Valid Assay Run are not met, the run must be repeated. Start from the original specimen(s), repeat the target extraction using a new Negative Control, and repeat the reaction setup using new Positive Controls.

Interpretation of Specimen Results:

The MultiCode[®]-RTx HSV 1&2 Kit Analysis Software automatically determines

results for the specimens based on the amplification cycle threshold (Ct) and the melting temperature (T_m) value.

Clinical Report Call Logic

Clinical Report	Input				
	F1			F2	
	Ct	HSV-1 T _m	HSV-2 T _m	Ct	SPC T _m
HSV-2 Positive	+	-	+	-	-
HSV-2 Positive	+	-	+	-	+
HSV-2 Positive	+	-	+	+	-
HSV-2 Positive	+	-	+	+	+
HSV-1 Positive	+	+	-	-	-
HSV-1 Positive	+	+	-	-	+
HSV-1 Positive	+	+	-	+	-
HSV-1 Positive	+	+	-	+	+
HSV-1&2 Positive	+	+	+	-	-
HSV-1&2 Positive	+	+	+	-	+
HSV-1&2 Positive	+	+	+	+	-
HSV-1&2 Positive	+	+	+	+	+
Negative	+	-	-	+	+
Negative	-	-	-	+	+

A “+” symbol in the Ct column indicates that the amplification curve crosses the amplification threshold. A “+” symbol in the T_m column indicates that a melt peak is observed in the appropriate temperature range. A “-” symbol in the Ct column indicates that the amplification curve does not cross the amplification threshold. A “-” symbol in the T_m column indicates that no melt peak was observed in the appropriate temperature range. N/A indicates that the result is not dependent on the value.

- A sample is Positive if both the Ct and T_m are positive in the F1 channel irrespective of the Ct and T_m in the F2 channel.
- A sample is Negative if the Ct and T_m are negative in the F1 channel and the Ct and T_m are positive in the F2 channel.

Invalid/Fail Report Call Logic

Invalid/Fail Report	Input				
	F1			F2	
	Ct	HSV-1 T _m	HSV-2 T _m	Ct	SPC T _m
Invalid	-	-	+	-	-
Invalid	-	-	+	-	+
Invalid	-	-	+	+	-
Invalid	-	-	+	+	+
Invalid	-	+	-	-	-
Invalid	-	+	-	-	+
Invalid	-	+	-	+	-
Invalid	-	+	-	+	+
Invalid	-	+	+	-	-
Invalid	-	+	+	-	+
Invalid	-	+	+	+	-
Invalid	-	+	+	+	+
Fail	+	-	-	-	-
Fail	+	-	-	-	+
Fail	+	-	-	+	-
Fail	-	-	-	-	-
Fail	-	-	-	-	+
Fail	-	-	-	+	-

A “+” symbol in the Ct column indicates that the amplification curve crosses the amplification threshold. A “+” symbol in the T_m column indicates that a melt peak is observed in the appropriate temperature range. A “-“ symbol in the Ct column indicates that the amplification curve does not cross the amplification threshold. A “-“ symbol in the T_m column indicates that no melt peak was observed in the appropriate temperature range. N/A indicates that the result is not dependent on the value.

- A sample is INVALID if the T_m is positive and the Ct is negative in the F1 channel. This condition warrants repeat testing using the extracted nucleic acid. If the result is INVALID again, then the specimen needs to be re-extracted and tested.
- A sample is FAIL if the T_m is negative in the F1 channel, and the Ct or T_m is negative in the F2 channel. This condition warrants repeat testing following extraction of another aliquot of the specimen. If the result is again a FAIL, then the specimen needs to be re-collected and tested.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Extraction using the bioMérieux NucliSENS easyMAG:

The Precision/Reproducibility of the MultiCode[®]-RTx HSV 1&2 Kit was evaluated using the bioMérieux NucliSENS easyMAG extraction method for the specimens at 3 U.S. clinical laboratories. A panel was prepared containing 6 simulated HSV-1 and HSV-2 samples that included High Negative, Low Positive (near the assay limit of detection) and Moderate Positive samples. The panel, along with external HSV-1 and HSV-2 positive and negative controls, was extracted with bioMérieux NucliSENS easyMAG and assayed in duplicate using MultiCode[®]-RTx HSV 1&2 Kit. Kit positive and negative controls were included with each assay run. Panels and controls were tested at each site by 1 operator, 1 time each per day for 5 days (N = 315). Results of the Precision/Reproducibility studies for the MultiCode[®]-RTx HSV 1&2 assay are presented from three sites in the tables below.

**Reproducibility Panel Results from bioMérieux NucliSENS_easyMAG
extraction – Site #1**

Target	Site #1			
	Agreement - Expected Results / Number Tested	Avg T _m ¹	% Coefficient of Variation	Avg % Deflection ²
HSV-1 Positive Control	5/5	84.7	0.60	69.4
HSV-2 Positive Control	5/5	87.8	0.42	57.8
Negative Control	5/5	77.8	0.70	-2.0
HSV-1 Extractable Control ¹	10/10	84.4	0.69	66.5
HSV-2 Extractable Control	10/10	87.3	0.70	53.9
HSV-1/HSV-2 Negative Extractable Control	10/10	77.3	0.73	-2.2
HSV-1 High Negative*	10/10	77.3	0.65	6.5
HSV-1 Low Positive	10/10	84.1	0.54	73.6
HSV-1 High Positive	10/10	84.2	0.45	94.3
HSV-2 High Negative*	10/10	77.2	0.61	2.8
HSV-2 Low Positive	10/10	87.1	0.50	60.8
HSV-2 High Positive	10/10	87.1	0.40	91.8

* The High Negative target is expected to be negative ~95% of the time (results are positive <5% of the time)

¹No detectable HSV-1 or HSV-2 signal was generated by HSV-1/HSV-2 Negative Extraction Control, Extractable HSV-1/HSV-2 Negative Control or HSV-1 and HSV-2 High Negative Targets and therefore the T_m of the Sample Processing Control (SPC) is reported.

² % Deflection is the individual sample deflection obtained during the melt curve analysis expressed as a percent of the maximum deflection of the melt curve in that assay run. Average % deflection is the average deflection for that panel member across all the 5 runs for the site.

**Reproducibility Panel Results from bioMérieux NucliSENS easyMAG
extraction – Site #2**

Target	Site #2			
	Agreement - Expected Results / Number Tested	Avg T _m ¹	% Coefficient of Variation	Avg % Deflection ²
HSV-1 Positive Control	5/5	84.5	0.10	72.9
HSV-2 Positive Control	5/5	87.4	0.13	64.2
Negative Control	5/5	77.7	0.07	-3.1
HSV-1 Extractable Control ¹	10/10	84.2	0.49	75.7
HSV-2 Extractable Control	10/10	87.0	0.52	52.0
HSV-1/HSV-2 Negative Extractable Control	10/10	77.3	0.46	-3.1
HSV-1 High Negative *	10/10	77.3	0.50	7.5
HSV-1 Low Positive	10/10	83.9	0.37	77.3
HSV-1 High Positive	10/10	84.0	0.23	94.3
HSV-2 High Negative *	10/10	77.2	0.48	-0.1
HSV-2 Low Positive	10/10	86.7	0.32	65.4
HSV-2 High Positive	10/10	86.8	0.16	92.7

* The High Negative target is expected to be negative ~95% of the time (results are positive <5% of the time)

¹No detectable HSV-1 or HSV-2 signal was generated by HSV-1/HSV-2 Negative Extraction Control, Extractable HSV-1/HSV-2 Negative Control or HSV-1 and HSV-2 High Negative Targets and therefore the T_m of the Sample Processing Control (SPC) is reported.

² % Deflection is the individual sample deflection obtained during the melt curve analysis expressed as a percent of the maximum deflection of the melt curve in that assay run. Average % Deflection is the average deflection for that panel member across all the 5 runs for the site.

**Reproducibility Panel Results from bioMérieux NucliSENS_easyMAG
extraction – Site #3**

Target	Site #3			
	Agreement - Expected Results / Number Tested	Avg T _m ¹	% Coefficient of Variation	Avg % Deflection ²
HSV-1 Positive Control	5/5	84.80	0.00	57.1
HSV-2 Positive Control	5/5	88.0	0.22	54.0
Negative Control	5/5	78.1	0.11	-2.4
HSV-1 Extractable Control ¹	10/10	84.7	0.34	72.1
HSV-2 Extractable Control	10/10	87.6	0.40	59.5
HSV-1/HSV-2 Negative Extractable Control	10/10	77.8	0.39	-2.8
HSV-1 High Negative*	10/10	77.7	0.43	7.6
HSV-1 Low Positive	10/10	84.5	0.40	80.4
HSV-1 High Positive	10/10	84.5	0.29	95.1
HSV-2 High Negative*	10/10	77.7	0.46	8.8
HSV-2 Low Positive	10/10	87.4	0.37	70.9
HSV-2 High Positive	10/10	87.4	0.22	95.5

* The High Negative target is expected to be negative ~95% of the time (results are positive <5% of the time)

¹No detectable HSV-1 or HSV-2 signal was generated by HSV-1/HSV-2 Negative Extraction Control, Extractable HSV-1/HSV-2 Negative Control or HSV-1 and HSV-2 High Negative Targets and therefore the T_m of the Sample Processing Control (SPC) is reported.

² % Deflection is the individual sample deflection obtained during the melt curve analysis expressed as a percent of the maximum deflection of the melt curve in that assay run. Average % Deflection is the average deflection for that panel member across all the 5 runs for the site.

**Reproducibility Panel Consolidated Results across all 3 sites for Roche MagNA
Pure LC and bioMérieux NucliSENS easyMAG extractions**

Target	Agreement with Expected Results - All sites Roche MagNA Pure LC				Agreement with Expected Results - All sites <u>bioMérieux NucliSENS</u> easyMAG			
	Total Positive	Total Tested	Total Agreement	95 % C. I.	Total Positive	Total Tested	Total Agreement	95 % C. I.
HSV-1 Positive Control	30	30	100%	88.4% - 100.0%	15	15	100%	78.2% - 100.0%
HSV-2 Positive Control	30	30	100%	88.4% - 100.0%	15	15	100%	78.2% - 100.0%
Negative Control	30	30	100%	88.4% - 100.0%	15	15	100%	78.2% - 100.0%
HSV-1 Extractable Control	89	90	98.9%	93.9% - 100.0%	30	30	100%	88.4% - 100.0%
HSV-2 Extractable Control	89	90	98.9%	93.9% - 100.0%	30	30	100%	88.4% - 100.0%
HSV-1/HSV-2 Negative Extractable Control	90	90	100%	95.9% - 100.0%	30	30	100%	88.4% - 100.0%
HSV-1 High Negative*	90	90	100%	95.9% - 100.0%	30	30	100%	88.4% - 100.0%
HSV-1 Low Positive	89	90	98.9%	93.9% - 100.0%	30	30	100%	88.4% - 100.0%
HSV-1 High Positive	90	90	100%	95.9% - 100.0%	30	30	100%	88.4% - 100.0%
HSV-2 High Negative*	90	90	100%	95.9% - 100.0%	30	30	100%	88.4% - 100.0%
HSV-2 Low Positive	90	90	100%	95.9% - 100.0%	30	30	100%	88.4% - 100.0%
HSV-2 High Positive	90	90	100%	95.9% - 100.0%	30	30	100%	88.4% - 100.0%

* The High Negative target is expected to be negative ~95% of the time (results are positive <5% of the time)

b. *Linearity/assay reportable range:*
N/A

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

Assay Controls: Assay controls are same as described in the Decision Summary of Original 510k submission (K100336).

d. *Detection limits:*

Comparison of LoD and LoB for Roche MagNA Pure LC and bioMérieux NucliSENS easyMAG extracted specimens:

LoD and LoB study was performed at EraGen Biosciences, Inc. to determine the analytical LoD and LoB for Roche MagNA Pure LC and bioMérieux NucliSENS easyMAG extracted specimens.

LoD and LoB were determined using a new lot of quantified (TCID₅₀/mL) cultures of HSV-1 and HSV-2 serially diluted in Copan Universal Transport Media. Each viral strain was extracted using the Roche MagNA Pure LC or bioMérieux NucliSENS easyMAG and tested in replicates of 23 per concentration of virus. The results are presented below:

LoD for HSV-1 extracted with a Roche MagNA Pure LC and bioMérieux NucliSENS easyMAG

Concentration (TCID ₅₀ /mL)	Roche MagNA Pure LC			bioMérieux NucliSENS easyMAG			
	Positive /Total	Positivity	95% C. I.	Positive /Total	Positivity	95% C. I.	
2.70 x 10 ⁷	23/23	100.00%	85.18-100.00%	23/23	100.00%	85.18-100.00%	
2.70 x 10 ⁶	23/23	100.00%	85.18-100.00%	23/23	100.00%	85.18-100.00%	
2.70 x 10 ⁵	23/23	100.00%	85.18-100.00%	23/23	100.00%	85.18-100.00%	
2.70 x 10⁴	23/23	100.00%	85.18-100.00%	23/23	100.00%	85.18-100.00%	LoD
2.70 x 10³	0/23	0.00%	0.00-14.82%	0/23	0.00%	0.00-14.82%	LoB

LoD for HSV-2 extracted with a Roche MagNA Pure LC and bioMérieux NucliSENS easyMAG

Concentration (TCID ₅₀ /mL)	Roche MagNA Pure LC			bioMérieux NucliSENS easyMAG			
	Positive /Total	Positivity	95% C. I.	Positive /Total	Positivity	95% C. I.	
7.02 x 10 ⁴	23/23	100.00%	85.18-100.00%	23/23	100.00%	85.18-100.00%	
7.02 x 10 ³	23/23	100.00%	85.18-100.00%	23/23	100.00%	85.18-100.00%	
7.02 x 10²	22/23	95.65%	78.05-100.00%	23/23	100.00%	85.18-100.00%	LoD
7.02 x 10¹	0/23	0.00%	0.00-14.82%	0/23	0.00%	0.00-14.82%	LoB

Note: The LoD value for HSV-1 was shown higher than HSV-2. This could be due to mismatch in the forward primer because a single set of forward and reverse HSV primers was used to amplify the target region from HSV-1 and HSV-2. The HSV Forward primer is a perfect match with the HSV-2 glycoprotein B sequence but contains a one base mismatch with the HSV-1 glycoprotein B sequence. The mismatch is in the center of the primer with 10 bases 3' to the mismatch. The predicted T_m for the forward primer to HSV-1 is 57.4°C; the predicted T_m for the forward primer to HSV-2 is 59.8°C. It is likely that in the initial rounds of PCR, HSV-2 is amplified more efficiently than HSV-1 which could result in a small increase in analytical sensitivity for HSV-2.

The LoD value for HSV-1 and HSV-2 was shown one log higher than shown in the original submission (K100336). This could be due to using a new lot of quantified (TCID₅₀/mL) cultures of HSV-1 and HSV-2.

e. Analytical specificity:

The specificity studies were not repeated and remain same as described in the Decision Summary of Original 510k submission (K100336).

f. Interfering Substances:

An Interfering Substance study was performed at EraGen Biosciences, Inc. to evaluate the effects of potential interfering substances on the MultiCode[®]-RTx HSV 1&2 Kit. A panel was prepared containing 6 substances that could reasonably be expected to be present in vaginal swab specimens. The substance panel was extracted with both Roche MagNA Pure LC and bioMérieux NucliSENS easyMAG tested near the device's Limit of Detection (LoD) for HSV-1 and HSV-2. No interference was observed in the presence of exogenous and endogenous substances in an extractable sample. The highest concentration of substances at which no interference was observed is

listed below.

Interfering Substances Panel

Substance	Concentration
Whole Blood (with EDTA)	10%
Female Urine	10%
Protein (Albumin)	10 mg/ml
Protein (Casein)	10 mg/ml
K-Y Brand Jelly	5%
Acyclovir (Acycloguanosine)	2.5 mg/ml

g. *Carry-over/Cross-Contamination*

Carry-over/Contamination studies were done with both HSV-1 and HSV-2 target, for the bioMérieux NucliSENS easyMAG extraction platform. The MultiCode[®]-RTx HSV 1&2 Kit was evaluated internally using simulated samples at the LoB and High Positive HSV-1 and HSV-2 ($\geq 10,000X$ LoD) concentrations. Alternating 22 samples of HSV-1 (high positive and LoB concentrations) and HSV-2 (high positive and LoB concentrations) were aliquoted into Sample Strips and extracted using the bioMérieux NucliSENS easyMAG extraction instrument. The high positive samples were positive for HSV-1 or HSV-2 in all cases and all LoB samples were negative for HSV-1 and HSV-2 in all cases indicating that there was no carry-over/cross-contamination of HSV-1 or HSV-2 in the LoB samples.

2. Comparison studies:

Equivalency of the two extraction platforms, Roche MagNA Pure LC and bioMérieux NucliSENS easyMAG was evaluated at EraGen Biosciences, Inc. using the MultiCode[®]-RTx HSV 1&2 Kit. The swab specimens were collected from lesions in Copan Universal Transport Medium from the patient population ranged from 18 to 85 years. These specimens were a sub-set of vaginal lesion swab specimens from the 2008-2009 prospective study collection. Each specimen was extracted using the Roche MagNA Pure LC or bioMérieux NucliSENS easyMAG and tested. A total of 111 clinical specimens were tested (30 HSV-1 reference positives, 30 HSV-2 reference positives, and 51 HSV 1&2 reference negative specimens).

Equivalence between bioMérieux NucliSENS easyMAG and Roche MagNA Pure LC extraction platforms for HSV-1

Herpes Simplex Virus Type 1 Comparison Results				
		Roche MagNA Pure LC Extraction		
		Positive	Negative	Total
bioMérieux NucliSENS easyMAG extraction	Positive	30	0	30
	Negative	0	81	81
	Total	30	81	111
Value				
		Value	95% Confidence Interval	
Positive percent agreement		100.0%	88.4 – 100.0%	
Negative percent agreement		100.0%	95.5 – 100.0%	

Equivalence between bioMérieux NucliSENS easyMAG and Roche MagNA Pure LC extraction platforms for HSV-2

Herpes Simplex Virus Type 2 Comparison Results				
		Roche MagNA Pure LC Extraction		
		Positive	Negative	Total
bioMérieux NucliSENS easyMAG extraction	Positive	30	0	30
	Negative	0	81	81
	Total	30	81	111
Value				
		Value	95% Confidence Interval	
Positive percent agreement		100.0%	88.4 – 100.0%	
Negative percent agreement		100.0%	95.5 – 100.0%	

3. Clinical Studies:

a. *Clinical Sensitivity:* N/A

b. *Clinical specificity:* N/A

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

4. Clinical Cut-off: N/A

5. Expected values/Reference range:

Expected values remain same as described in the Decision Summary of Original 510k submission (K100336).

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.