



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY AND INSTRUMENT**

**I Background Information:**

**A 510(k) Number**

K252484

**B Applicant**

Roche Molecular Systems, Inc.

**C Proprietary and Established Names**

cobas HCV

**D Regulatory Information**

Product Code(s)	Classification	Regulation Section	Panel
MZP	Class II	21 CFR 866.3170 - Nucleic Acid-Based Hepatitis C Virus Ribonucleic Acid Tests	MI - Microbiology

**II Submission/Device Overview:**

**A Purpose for Submission:**

To obtain clearance of cobas HCV on the upgraded cobas 6800/8800 Systems 2.0.

**B Measurand:**

Hepatitis C virus (HCV) RNA

**C Type of Test:**

Nucleic acid amplification test

### **III Intended Use/Indications for Use:**

#### **A Intended Use(s):**

cobas HCV is an in vitro nucleic acid amplification test for both the detection and quantitation of hepatitis C virus (HCV) RNA, in human EDTA plasma or serum, of HCV antibody positive or HCV-infected individuals. Specimens containing HCV genotypes 1 to 6 are validated for detection and quantitation in the assay.

cobas HCV is intended for use as an aid in the diagnosis of HCV infection in the following populations: individuals with antibody evidence of HCV with evidence of liver disease, individuals suspected to be actively infected with HCV antibody evidence, and individuals at risk for HCV infection with antibodies to HCV. Detection of HCV RNA indicates that the virus is replicating and therefore is evidence of active infection.

cobas HCV is intended for use as an aid in the management of HCV-infected patients undergoing anti-viral therapy. The assay can be used to measure HCV RNA levels at baseline, during treatment, at the end of treatment, and at the end of follow up of treatment to determine sustained or non-sustained viral response. The results must be interpreted within the context of all relevant clinical and laboratory findings.

cobas HCV has not been approved for use as a screening test for the presence of HCV in blood or blood products.

Assay performance characteristics have been established for individuals treated with certain direct-acting antiviral agents (DAA) regimens. No information is available on the assay's predictive value when other DAA combination therapies are used.

#### **B Indication(s) for Use:**

Same as Intended Use.

#### **C Special Conditions for Use Statement(s):**

Rx - For Prescription Use Only

#### **D Special Instrument Requirements:**

For use on the cobas 5800/6800/8800 Systems

### **IV Device/System Characteristics:**

#### **A Device Description:**

cobas HCV assay is a quantitative test performed on the cobas 5800 System, cobas 6800 System or cobas 8800 System. The test detects and quantitates HCV RNA in EDTA plasma or serum of infected patients. Dual probes are used to detect and quantify but not discriminate genotypes 1–6. The viral load is quantified against a non-HCV armored RNA quantitation standard (RNA-QS),

which is introduced into each specimen during sample preparation. The RNA-QS also functions as an internal control to assess substantial failures during the sample preparation and PCR amplification processes.

cobas HCV is based on fully automated sample preparation (nucleic acid extraction and purification) followed by reverse-transcription and PCR amplification.

## B Principle of Operation:

Nucleic acid from patient samples, external controls, and added armored RNA-QS molecules are simultaneously extracted by addition of proteinase and lysis reagent to the sample. The released nucleic acid is purified using magnetic glass particles.

Selective amplification of target nucleic acid from the sample is achieved by the use of target virus-specific primers which are selected from highly conserved regions of HCV. Selective amplification of RNA-QS is achieved by the use of sequence-specific primers which are selected to have no homology with the HCV genome. A thermostable DNA polymerase enzyme is used for both reverse-transcription and PCR amplification. The target and RNA-QS sequences are amplified simultaneously utilizing a universal PCR amplification profile. The cobas HCV master mix contains dual detection probes specific for the HCV target sequences and one for the RNA-QS. The probes are labeled with target-specific fluorescent reporter dyes allowing simultaneous detection of HCV target and RNA-QS in two different target channels.

During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5'-to-3' nuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the viral targets and RNA-QS.

### Interpretation of Results.

Results are determined automatically by the **cobas** software and are shown in the following table:

**Table 1.** Individual Target Result Interpretation.

Result Read-Out from cobas system	Analytical Interpretation	Clinical Interpretation
Target Not Detected	HCV RNA not detected.  Report results as “HCV not detected.”	No current HCV infection For HCV Diagnosis: No further testing indicated.* For Viral Load Assessment: Routine clinical

< Titer Min	<p>HCV RNA detected but not quantified.</p> <p>Calculated titer is below the Lower Limit of Quantitation (LLoQ) of the assay. Report results as “HCV detected, less than (Titer Min)” Titer min = 15 IU/mL</p>	<p>Low-level HCV viremia, may indicate previous spontaneous or treatment-related resolution of HCV infection.</p> <p>For HCV Diagnosis: Results must be interpreted within the context of all relevant clinical and laboratory findings.* For Viral Load Assessment: Routine clinical follow-up according to national HCV guidelines.</p>
15 IU/mL ≤ Titer < 25 IU/mL	<p>HCV RNA detected and quantified. Calculated titer is within the Linear Range of the assay – greater than or equal to 15 IU/mL and less than 25 IU/mL. Report results as “(Titer) of HCV detected”.</p>	<p>Low-level HCV viremia, may indicate previous spontaneous or treatment-related resolution of HCV infection.*</p> <p>For HCV Diagnosis and Viral Load Assessment: Provide patient with appropriate counseling and link to care and treatment according to current national HCV treatment guidelines.</p>
25 IU/mL ≤ Titer ≤ Titer Max	<p>HCV RNA detected and quantified</p> <p>Calculated titer is within the Linear Range of the assay – greater than or equal to 25 IU/mL and less than or equal to Titer Max. Report results as “(Titer) of HCV detected”.</p>	<p>Current HCV Infection.</p> <p>For HCV Diagnosis and Viral Load Assessment: Provide patient with appropriate counseling and link to care and treatment according to current national HCV treatment guidelines.</p>
> Titer Max	<p>Calculated titer is above the Upper Limit of Quantitation (ULoQ) of the assay. Report results as “HCV detected, greater than (Titer Max).” Titer max = 1.00E+08 IU/mL</p>	<p>Current HCV Infection.</p> <p>For HCV Diagnosis and Viral Load Assessment: Provide patient with appropriate counseling and link to care and treatment according to current national HCV treatment guidelines.</p>

\*Repeat HCV RNA testing if the person tested is suspected to have had HCV exposure within the past 6 months or has clinical evidence of HCV disease, or if there is concern regarding the handling or storage of the test specimen.

## C Instrument Description Information:

1. Instrument Name:  
Cobas 5800/6800/8800 Systems
2. Specimen Identification:  
cobas 5800/6800/8800 Systems support multiple types of barcodes. Loaded samples are automatically moved for barcode scanning and processing.
3. Specimen Sampling and Handling:  
Specimen processing is fully automated on the cobas 5800/6800/8800 Systems.
4. Calibration:

No instrument calibration is required by the user.

5. Quality Control:

Refer to cobas HCV assay labeling.

**V Substantial Equivalence Information:**

**A Predicate Device Name(s):**

cobas HCV

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

K221007

**C Comparison with Predicate(s):**

Device & Predicate Device(s):	Predicate Device	Candidate Device
	<u>K221007</u>	<u>K252484</u>
Device Trade Name	<b>cobas</b> HCV Quantitative nucleic acid test for use on the <b>cobas</b> 5800/6800/8800 Systems	same
General Device Characteristic Similarities		
Intended Use/Indications For Use	<p><b>cobas</b> HCV is an in vitro nucleic acid amplification test for both the detection and quantitation of hepatitis C virus (HCV) RNA, in human EDTA plasma or serum, of HCV antibody positive or HCV-infected individuals. Specimens containing HCV genotypes 1 to 6 are validated for detection and quantitation in the assay.</p> <p><b>cobas</b> HCV is intended for use as an aid in the diagnosis of HCV infection in the following populations: individuals with antibody evidence of HCV with evidence of liver disease, individuals suspected to be actively infected with HCV antibody evidence, and individuals at risk for HCV infection with antibodies to HCV. Detection of HCV RNA indicates that the virus is replicating and therefore is evidence of active infection.</p> <p><b>cobas</b> HCV is intended for use as an aid in the management of HCV-infected patients undergoing anti-viral therapy. The assay can be used to measure HCV RNA levels at baseline, during treatment, at the end of treatment, and at the end of follow up of treatment to determine sustained or non-sustained viral response. The results must be interpreted within the context of all relevant clinical and laboratory findings.</p> <p><b>cobas</b> HCV has not been approved for use as a screening test for the presence of HCV in blood or blood products.</p> <p>Assay performance characteristics</p>	same

	have been established for individuals treated with certain direct-acting antiviral agents (DAA) regimens. No information is available on the assay's predictive value when other DAA combination therapies are used.	
Conditions for Use	For Prescription Use	same
Sample Types	Human EDTA Plasma, Serum	same
Analyte Targets	Hepatitis C RNA genotypes 1 to 6	same
Sample Preparation Procedure	Automated	same
Amplification Technology	Real Time PCR	same
Detection Chemistry	Dual detection probes labeled with target-specific fluorescent reporter dyes allowing simultaneous detection of HCV target and RNA-QS in two different target channels. Real time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes.	same
Controls Used	RNA-QS functions as an internal control.  Three external controls: High Titer Positive, Low Titer Positive, Negative Control	same
Results Analysis	PCR cycle threshold analysis	same
<b>General Device Characteristic Differences</b>		
Instrumentation	Cobas 5800 System	Cobas 6800/8800 Systems 2.0

## VI Standards/Guidance Documents Referenced:

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline- 2nd Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

## VII Performance Characteristics:

### A Analytical Performance:

#### 1. Precision/Reproducibility:

##### A. Precision

The precision of cobas HCV was determined by analysis of serial dilutions of clinical HCV samples or of HCV positive artificial stock material (aRNA) in HCV-negative EDTA plasma. Seven dilution levels were tested in three replicates for each level in two runs across 12 days. Each sample was carried through the entire cobas HCV test procedure on 1 cobas 6800/8800 Systems 2.0 instruments and 1 cobas 6800/8800 Systems 1.4 instrument. The study was performed with three lots of cobas HCV test reagents.

The cobas HCV showed the following precision on the cobas 6800/8800 Systems 2.0 for three lots of reagents tested across a concentration range of 1.5E+01 IU/mL to 1.0E+08 IU/mL (Table 2):

**Table 2.** Within-Laboratory Precision of cobas HCV on the cobas 6800/8800 Systems 2.0

Panel Member	mean observed log10 titer (IU/mL)	Lot		Day		Run		Within-Run / Repeatability		Total / Within-Laboratory	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Panel 1	7.97	0.01	0.07	0.00	0.00	0.01	0.17	0.02	0.22	0.02	0.29
Panel 2	6.79	0.04	0.60	0.04	0.54	0.01	0.13	0.05	0.68	0.07	1.06
Panel 3	5.92	0.03	0.53	0.03	0.48	0.00	0.00	0.05	0.84	0.07	1.11
Panel 4	3.75	0.00	0.06	0.02	0.40	0.02	0.40	0.05	1.37	0.05	1.44
Panel 5	1.79	0.00	0.00	0.00	0.00	0.06	3.13	0.11	6.30	0.13	7.04
Panel 6	1.33	0.03	2.29	0.00	0.00	0.00	0.00	0.10	7.80	0.11	8.13
Panel 7	1.36	0.11	8.23	0.00	0.00	0.00	0.00	0.18	13.34	0.21	15.68

SD: Standard Deviation; %CV: Percent Coefficient of Variation

##### B. Reproducibility.

See P150015

#### 2. Linearity:

Assay linearity was assessed using the predominant genotype (GT1) in plasma. The linearity panels were prepared as serial dilutions consisting of 16 concentration levels spanning the intended linear range and including medical decision points. The dilution series were



prepared by diluting HCV armored RNA (aRNA) and an HCV positive clinical specimen in negative pooled EDTA-plasma. The Titer assignment of the study panels has been performed via Calibrator Bracketing Method. The dilution series covered a total of 36 replicates per concentration level in negative pooled EDTA plasma which were tested with 3 kit lots and tested on 3 cobas 6800/8800 Systems 2.0 over the course of 6 days.

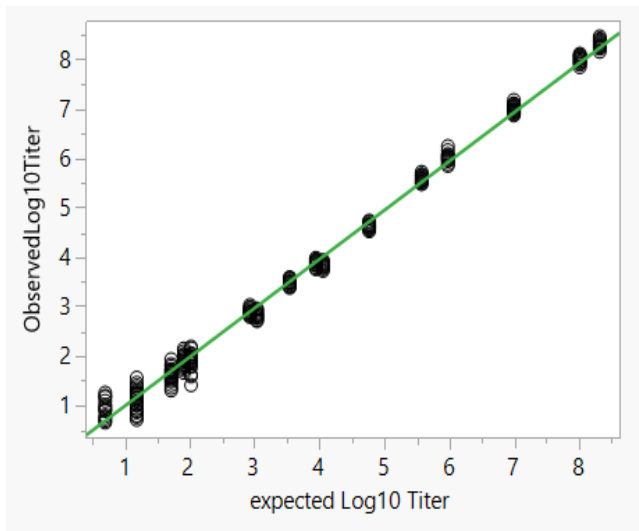
With 500 µL processing volume, **cobas** HCV is linear for EDTA plasma from 15 IU/mL to 1.00E+08 IU/mL and shows an absolute deviation from the weighted least square linear regression of less than  $\pm 0.24 \log_{10}$ . See Table 3 and Figure 1 below.

**Table 3.** cobas HCV Linearity on the cobas 6800/8800 Systems 2.0

Sample Type	Panel Member	Assigned log <sub>10</sub> Titer [IU/mL]	Relative Concentration	Mean Observed log <sub>10</sub> Titer [IU/mL]	Expected log <sub>10</sub> Titer [IU/mL]	Mean Predicted log <sub>10</sub> Titer [IU/mL]	Deviation (observed – predicted)	Acceptance criterion	Acceptance criterion met?
Armored RNA	PM1	8.18	1.00	8.32	8.32	8.23	0.09	The absolute deviation for the linear regression shall be demonstrated to be equal to or less than $\pm 0.24 \log_{10}$ for <b>cobas</b> 6800/8800 Systems 2.0.	N/A*
	PM2	7.88	0.96	8.00	8.01	7.93	0.07		YES
	PM3	6.88	0.84	7.00	7.00	6.92	0.08		
	PM4	5.88	0.72	6.01	5.98	5.92	0.10		
	PM5	5.48	0.67	5.59	5.58	5.52	0.07		
	PM7	3.88	0.47	3.89	3.95	3.90	-0.01		
	PM9	3.48	0.43	3.48	3.54	3.50	-0.03		
	PM11	2.88	0.35	2.89	2.93	2.90	0.00		
	PM13	1.88	0.23	1.93	1.91	1.89	0.04		
Clinical specimen	PM6	4.69	0.57	4.61	4.77	4.72	-0.11		
	PM8	3.99	0.49	3.83	4.06	4.01	-0.18		
	PM10	2.99	0.37	2.85	3.04	3.01	-0.16		
	PM12	1.99	0.24	1.90	2.02	2.00	-0.10		
	PM14	1.69	0.21	1.64	1.71	1.70	-0.06		
	PM15	1.16	0.14	1.15	1.18	1.17	-0.02		
	PM16	0.69	0.08	0.97	0.70	0.69	0.28		N/A*

\*Acceptance criterion is not applicable as the assigned titers for both PM1 and PM16 are not within the linear range (below ULoQ and above LLoQ).

**Figure 1.** Weighted Least Square Linear Regression (all 3 cobas 6800/8800 System 2.0)



$$\text{ObservedLog10Titer} = 0 + 0.9895621 * \text{expected Log10 Titer}$$

3. Analytical Specificity/Interference:

See P150015

4. Assay Reportable Range:

The results of the linearity study and calculation of the Lower and Upper Limits of Quantitation confirm the assay reportable range is consistent across analyzer platforms: 15 IU/mL – 1.00E+08 IU/mL.

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

See P150015

6. Detection Limit:

The Limit of Detection (LoD) was assessed with a sample panel prepared by diluting an HCV secondary standard in HCV negative pooled EDTA plasma. The dilution series was prepared with 5 HCV concentration levels including 2 levels below the expected LoD, 1 level near the expected LoD, and 2 levels above the expected LoD. The panel was tested with 3 kit lots, on 5 days, on 3 cobas 6800/8800 Systems 2.0 and 1 cobas 6800/8800 System 1.4 over multiple runs, with multiple operators and with multiple replicates per run. A summary of the LoD results is provided in the table below.

**Table 4.** Results Summary cobas HCV LoD HCV on the cobas 6800/8800 System 1.4 and the cobas 6800/8800 System 2.0

Platform	LoD by PROBIT analysis (95% Hit Rate) Lower & Upper 95% CI	Target: HCV
cobas 6800/8800 Systems 2.0	PROBIT analysis (95% Hit Rate) Lower & Upper 95% CI	<b>14.01</b> IU/mL (95% CI: 11.33-18.91)
	LoD by Hit Rate	<b>15</b> IU/mL

	log10 PROBIT 95%	1.15
<b>cobas 6800/8800 Systems 1.4</b>	PROBIT analysis (95% Hit Rate) Lower & Upper 95% CI	<b>11.86 IU/mL</b> (95% CI: 9.61-16.11)
	LoD by Hit Rate	<b>15 IU/mL</b>
	log10 PROBIT 95%	1.07
<b>log10 PROBIT 95% Delta</b> (cobas 6800/8800 Systems 2.0–cobas 6800/8800 Systems 1.4)		<b>0.07</b>
<b>Acceptance Criterion met/not met</b> ( $\pm 0.2 \text{ Log}_{10}$ )		<b>Met</b>

7. Assay Cut-Off:

See P150015

8. Accuracy (Instrument):

N/A

9. Carry-Over:

See P150015

## B Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparison study was conducted using 155 archived HCV positive plasma specimens and 30 HCV negative individual plasma specimens. Some of the individual positive specimens were diluted to achieve the desired number of samples at specified concentrations: ~50 HCV specimens between 1.5 E+01 to 3E+03 IU/mL, ~50 specimens between 3E+03 to 5E+05 IU/mL and ~50 specimens between 5E+05 to 1E+08 IU/mL. Each specimen was tested on three cobas 6800/8800 Systems 2.0 and on one cobas 6800/8800 System 1.4 at one external site using three reagent lots.

Results:

For the 30 samples with target not detected (TND) results by cobas 6800/8800 Systems 1.4, the NPA was 100% (30/30).

All of the positive samples were within the linear range of 1.5E+1 IU/mL to 1E+8 IU/mL by cobas 6800/8800 Systems 1.4 (155/155=100%) and by cobas 6800/8800 Systems 2.0 (465/465=100%). Among the three categories (low, medium, and high viral loads) within the linear range, the test results between the two Systems were highly concordant.

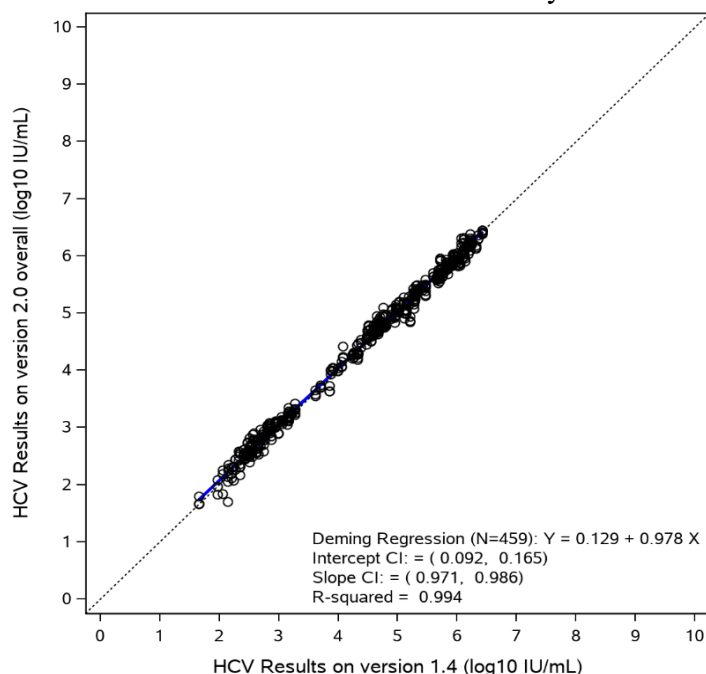
Table 5 summarizes the parameter estimates (slope and intercept) of Deming regression between cobas 6800/8800 Systems 2.0 and cobas 6800/8800 Systems 1.4 by instrument.

**Table 5.** Overall and by-instrument parameter estimates from Deming regression analysis.

Instrument	Number of Pairs within Linear Range	Parameter	Parameter Estimate	95% CI	r <sup>2</sup>
Overall	459	Intercept	0.13	0.09 – 0.17	0.99

		Slope	0.98	0.97 – 0.99	
1	153	Intercept	0.19	0.14 – 0.24	0.99
		Slope	0.97	0.96 – 0.98	
2	153	Intercept	0.10	0.05 – 0.16	0.99
		Slope	0.98	0.97 – 0.99	
3	153	Intercept	0.10	0.02 – 0.17	0.99
		Slope	0.99	0.97 – 1.00	

**Figure 2.** Deming regression result of the averaged log10-transformed viral load concentration across 3 cobas 6800/8800 Systems 2.0 versus log10-transformed viral load concentration from the cobas 6800/8800 Systems 1.4.



Using Deming regression analysis, an estimate of the systematic bias between the log10-transformed viral load concentration of the two Systems (cobas 6800/8800 Systems 2.0 and cobas 6800/8800 Systems 1.4) was calculated at medical decision levels for each site. Jackknife method was used to estimate the 95% CI of systematic bias (Table 6).

**Table 6.** Systematic bias at medical decision point by instrument, based on Deming regression.

Instrument	Medical Decision Point (IU/mL)	Number of Pairs within Linear Range	Medical Decision Point in log10 (IU/mL)	Predicted Value at Medical Decision Point in log10 (IU/mL)	Bias in log10 (IU/mL)
1	25	150	1.398	1.542	0.1443
	800,000	150	5.903	5.903	0.0
	6,000,000	150	6.778	6.750	-0.028
	25	150	1.398	1.472	0.074
	800,000	150	5.903	5.887	-0.016

2	6,000,000	150	6.778	6.744	-0.033
3	25	150	1.398	1.476	0.078
	800,000	150	5.903	5.922	0.019
	6,000,000	150	6.778	6.786	0.008
combined	25	150	1.398	1.496	0.098
	800,000	150	5.903	5.902	-0.001
	6,000,000	150	6.778	6.758	-0.020

A Bland-Altman plot between the results of the two Systems (cobas 6800/8800 Systems 2.0 and cobas 6800/8800 Systems 1.4) with allowable total difference (ATD) zone were created for each site and for all sites combined (Table 7). Over the entire range, 100% of the samples fell within the ATD zone and the lower bound of the one-sided 95% CI was 100%.

**Table 7.** Percentage of samples within the ATD zone and  $\pm 0.5 \log_{10}$ -range by all instruments combined.

Instrument	Viral Load Range Category	Viral Load Range (IU/mL)	Percentage of Samples within ATD Zone (95% One-Sided CI)	Percentage of Samples with difference within $\pm 0.5 \log_{10}$ (95% One-Sided CI)
Overall	Low	1.50E+01 - 3.00E+03	100% (153/153) (98.26%)	100% (153/153) (98.26%)
	Medium	3.00E+03 - 5.00E+05	100% (203/203) (98.68%)	100% (203/203) (98.68%)
	High	5.00E+05 - 1.00E+08	100% (103/103) (97.44%)	100% (103/103) (97.44%)
	Overall		100% (459/459) (99.41%)	100% (459/459) (99.41%)
CI = Confidence Interval				

2. Matrix Comparison:

See P150015

**C Clinical Studies:**

1. Clinical Sensitivity:

See P150015

2. Clinical Specificity:

See P150015

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

N/A

**D Clinical Cut-Off:**

See P150015

**E Expected Values/Reference Range:**

See P150015

**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.