510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I Background Information:

A 510(k) Number
K221693

B Applicant
Roche Diagnostics

C Proprietary and Established Names
Elecsys Anti-HCV II

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
MZO	Class II	21 CFR 866.3169 Hepatitis C virus antibody tests	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:
Clearance for the updated Elecsys Anti-HCV II assay that improve tolerance to biotin interference.

B Measurand:
Antibodies to Hepatitis C virus

C Type of Test:
Electrochemiluminescence immunoassay (ECLIA)

III Intended Use/Indications for Use:

A Intended Use(s):
Immunoassay for the in vitro qualitative detection of antibodies to hepatitis C virus (HCV) in human adult and pediatric (ages 18 months through 21 years) serum and plasma (potassium EDTA, lithium heparin, sodium heparin, and sodium citrate). Assay results, in conjunction with other laboratory results and clinical information, may be used to aid in the presumptive diagnosis of HCV infection in persons with signs and symptoms of hepatitis and in persons at risk for hepatitis C infection. The test does not determine the state of infection or associated disease.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on cobas e immunoassay analyzers.

B Indication(s) for Use:
See Intended Use above.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:
The electrochemiluminescence immunoassay "ECLIA" is intended for use on cobas e immunoassay analyzers.

IV Device/System Characteristics:

A Device Description:
The Elecsys Anti-HCV II immunoassay requires the use of reagents specific for (A) Anti-HCV II immunoassay and (B) the recommended PreciControl Anti-HCV. The reagent and calibrators are packaged together in the Elecsys Anti-HCV II assay kit, while the PreciControl Anti-HCV is packaged separately.

(A) The ***Elecsys Anti-HCV II immunoassay kit*** consists of five components:

- Three reagent cassettes/bottles (components M, R1, and R2) - are combined in the so-called "rackpack," a bundle of the three reagent bottles, that is placed on the instrument as a single unit

Name	Description
M#	Streptavidin-coated microparticles
R1#	Biotinylated antigens
R2#	Ruthenylated antigens

- Two calibrators (components negative Cal1 and positive Cal2) – These ready-to-use calibrators are used to calibrate the Elecsys Anti-HCV II immunoassay on the cobas e immunoassay analyzers.

(B) The ***PreciControl Anti-HCV kit*** consists of two ready-to-use reagents:

- PreciControl Anti-HCV 1 contains human serum, negative for anti-HCV
- PreciControl Anti-HCV 2 contains inactivated positive human serum for anti-HCV in a low positive concentration.

Name	Description
PC A-HCV1	PreciControl Anti-HCV 1, negative for anti-HCV antibodies
PC A-HCV2	PreciControl Anti-HCV 2, positive for anti-HCV antibodies

Interpretation of Results
The interpretation of results is presented below:

Initial Elecsys Anti-HCV II assay			
COI	***Result***	***Interpretation of results***	***Retest procedure***
< 0.90	Non-reactive	No antibodies to HCV were detected	No retest required
0.90 ≤ COI < 1.00	Border	Borderline zone (undetermined)	Retest in duplicate with the Elecsys Anti-HCV II assay
≥ 1.00	Reactive	Antibodies to HCV detected	Presumptive HCV infection, follow CDC recommendations for supplemental testing

e) Please note, per www.CDC.gov: If a patient is known to be at high risk of HCV infection, or is symptomatic, and the physician's suspicion of HCV infection is high, HCV RNA testing is often employed and is of diagnostic value, even after an initial negative anti-HCV test result.

All initially reactive or borderline samples are being instructed to be redetermined in duplicate using the Elecsys Anti-HCV II assay. If no reactivity is found in both cases, the sample is negative for anti-HCV. If the result from either of the two measurements is reactive or borderline, then the sample is *repeatedly reactive*. Repeatedly reactive samples must be investigated by supplemental methods (e.g. immunoblot or detection of HCV RNA). If one or both measurements remain borderline, the analysis of a follow-up sample is recommended.

Final Elecsys Anti-HCV II assay			
Initial result	***Result after retest (COI)***	***Final results***	***Interpretation of results***
Non-reactive	No retest required	NON-REACTIVEe)	Antibodies to HCV were not detected; does not exclude the possibility of exposure to HCV
Border	If 2 of the 3 results have a COI < 1.00	NON-REACTIVE	Antibodies to HCV were not detected; does not exclude the possibility of exposure to HCV
	If 2 of the 3 results have a COI ≥ 1.00	REACTIVE	Presumptive evidence of antibodies to HCV. Follow CDC recommendations for supplemental testing.
Reactive	No retest required	REACTIVE	Presumptive evidence of antibodies to HCV. Follow

Final Elecsys Anti-HCV II assay			
Initial result	***Result after retest (COI)***	***Final results***	***Interpretation of results***
			CDC recommendations for supplemental testing.

B Principle of Operation:

This assay is based on the sandwich principle. The total duration of the assay is 18 minutes.

- 1st incubation: 50 µL of sample, 55 µL of a reagent containing biotinylated HCV specific antigens and 55 µL of a reagent containing HCV specific antigens labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy))

C Instrument Description Information:

1. Instrument Name:
   cobas e immunoassay analyzers

2. Specimen Identification:
   See P140021.

3. Specimen Sampling and Handling:
   See P140021.

4. Calibration:
   See P140021.

5. Quality Control:
   See P140021.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Elecsys Anti-HCV II Immunoassay

B Predicate 510(k) Number(s):

P140021

C Comparison with Predicate(s):

Device & Predicate Device(s):	K221693	P140021
Device Trade Name	Elecsys Anti-HCV II	Elecsys Anti-HCV II
General Device Characteristic Similarities		
Intended Use/Indications For Use	Immunoassay for the in vitro qualitative detection of antibodies to hepatitis C virus (HCV) in human adult and pediatric (ages 18 months through 21 years) serum and plasma (potassium EDTA, lithium heparin, sodium heparin, and sodium citrate). Assay results, in conjunction with other laboratory results and clinical information, may be used to aid in the presumptive diagnosis of HCV infection in persons with signs and symptoms of hepatitis and in persons at risk for hepatitis C infection. The test does not determine the state of infection or associated disease. The electrochemiluminescence immunoassay "ECLIA" is intended for use on cobas e immunoassay analyzers.[1]	Immunoassay for the in vitro qualitative detection of antibodies to hepatitis C virus (HCV) in human adult and pediatric (ages 18 months through 21 years) serum and plasma (potassium EDTA, lithium heparin, sodium heparin, and sodium citrate). Assay results, in conjunction with other laboratory results and clinical information, may be used to aid in the presumptive diagnosis of HCV infection in persons with signs and symptoms of hepatitis and in persons at risk for hepatitis C infection. The test does not determine the state of infection or associated disease. The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers.
Technology	Same	ECLIA
Test Format	Same	Sandwich
Test Type	Same	Qualitative
Application Time	Same	18 min

Assay Protocol	Same	1st Incubation: R1+R2+sample 2nd incubation: Addition of streptavidin-coated microparticles (beads)
Pipetting Volume (samples)	Same	cobas e 601 49 µL
Pipeting Volume (beads)	Same	cobas e 601 36µL
Pipetting volume (R1)	Same	cobas e 601 55 µL
Pipetting volume (R2)	Same	cobas e 601 56µL
Buffer Composition R1	Same	HEPES
	Addition of 300 mM NaCl and 0.05% SDS	Not added
Antibodies used in R1	Same	Same
Buffer Composition R2	Same	HEPES
Antigens used in R2	Same	1) rec. HCV-NS3-sulfoBPRu(NHS) 2) HCV, core(9-48) (BPRu(UE)MH) 3) HCV,NS4(KN4.1-BPRu)amid
Reporting of values	Same	COI and result message (nonreactive, borderline, or reactive) COI < 0.9 = non-reactive 0.90 ≤ COI < 1.00 = borderline COI ≥ 1.0 = reactive
General Device Characteristic Differences		
Antigens used in R1	1) rec. HCV-NS3-(PEG)24-Bi 2) no change 3) no change	1) rec. HCV-NS3-hyBi(DSS) 2) HCV, core(9-48) (Bi(UE)25MH)

		3) HCV,NS4(KN4.1-Bi)amid
Interference elimination for streptavidin	Streptavidin rec. Mutein Polymer	None
Antibodies used in R2	Anti-Biotin Antibody fragment specific for free, unconjugated Biotin ("scavenger antibody")	Same
Biotin tolerance	1200 ng/mL	44 ng/mL
Calibrators	Preservative: MIT / Oxypyrion	Preservative: Bronidox

[1] - The Elecsys instruments were discontinued and are therefore no longer included in the Intended Use.

VI Standards/Guidance Documents Referenced:

Standard/Guidance Documents referenced are below:

- Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition CLSI EP05-A3 7-251
- Interference Testing in Clinical Chemistry CLSI EP07 3rd Edition 7-275
- Measurement Procedure Comparison and Bias Estimation Using Patient Samples CLSI EP09c-ED3
- Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline CLSI EP25-A 7-235
- User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline - Second Edition. CLSI EP12-A2 7 – 152

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Roche conducted a single-analyzer and a multi-analyzer precision study.

Within laboratory *single-analyzer precision study* was determined using 1 reagent lot of Elecsys Anti-HCV II assay reagents, calibrators and controls in a protocol based on CLSI (Clinical and Laboratory Standards Institute) EP05-A3. The test sample panel consisted of 5 human serum samples of different CMV IgG concentrations (see Table X). The negative sample (HS 1) consists of a human serum pool. Panel members HS 2 – HS 5 were prepared by a human serum pool or unique negative serum samples spiked with unique anti-HCV positive serum from individual donors. Samples and assay controls were measured in duplicates in each of the 2 runs per day, for 12 days with one reagent lot on the cobas e 601 Analyzer. Calibration of the assay was performed according to the package insert.

The single-analyzer precision study followed a 12 days x 2 runs x 2 replicates study design. The acceptance criteria for this study is as follows:

Table 1: Acceptance criteria for the single-analyzer precision study

Sample concentration	*Acceptable Variance*
≤ 0.5 COI	SD ≤ 0.12 COI
> 0.5 - < 1.0 COI	SD ≤ 0.10 COI
≥ 1.0 COI	CV ≤ 10%

The following results were obtained for the single-analyzer precision study:

Table 2: Overall Statistical Analysis of the Single-Analyzer Precision Study of Elecsys Anti-HCV II on cobas e 601 analyzer

Sample	**Mean [COI]**	**Repeatability (within-run)**		**Intermediate Precision (within-lab)**	
		SD [COI] (95% UCL)	**CV (%) (95% UCL)**	**SD [COI] (95% UCL)**	**CV (%) (95% UCL)**
HS 1	0.038	0.0009 (0.001)	2.50 (3.30)	0.0009 (0.001)	2.50 (3.00)
HS 2	0.776	0.026 (0.034)	3.40 (4.40)	0.034 (0.042)	4.30 (5.40)
HS 3	1.06	0.013 (0.017)	1.20 (1.60)	0.020 (0.026)	1.80 (2.40)
HS 4	1.99	0.035 (0.046)	1.80 (2.30)	0.048 (0.062)	2.40 (3.10)
HS 5	5.65	0.085 (0.112)	1.50 (2.00)	0.113 (0.145)	2.00 (2.60)
PC 1	0.048	0.0009 (0.001)	1.90 (2.50)	0.0009 (0.001)	1.90 (2.30)
PC 2	3.34	0.043 (0.057)	1.30 (1.70)	0.069 (0.093)	2.10 (2.80)
HS = human serum, PC = PreciControl Anti-HCV UCL = Upper Confidence Limit					

Roche concluded that the results of the precision studies performed with Elecsys Anti-HCV II were acceptable as the observed %CV was less than 4.5%.

An additional within laboratory multi-analyzer precision study was conducted internally (Roche Diagnostics, Penzberg) on three different cobas e 601 analyzers with one reagent lot. This study was conducted with 6 human sera samples and two levels of the PreciControl Anti-HCV. The following table shows the sample panel:

Table 3: Details of sample panel

Sample Source	**Sample**	**Mean**	**N**
Human serum pool	HS 01	0.040	90
	HS 02	0.796	90

A human serum pool or unique negative serum samples spiked with unique anti-HCV positive serum from individual donors	HS 03	0.827	90
	HS 04	1.21	90
	HS 05	2.05	90
	HS 06	5.85	90
Manufactured controls	PC 1	0.05	90
	PC 2	3.24	90

HS = human serum
PC = PreciControl Anti-HCV

The multi-analyzer study followed the 5 days x 2 runs x 3 replicates study design. The acceptance criteria for this study are as follows:

Table 4: Acceptance criteria for the multi-analyzer precision study

Sample concentration	***Acceptable Variance***
≤ 0.5 COI	SD ≤ 0.15 COI
> 0.5 - < 1.0 COI	SD ≤ 0.15 COI
≥ 1.0 COI	CV ≤ 15%

The results of the multi-analyzer study are presented below:

Table 5: Overall Statistical Analysis of the Multi-Analyzer Study of Elecsys Anti-HCV II on cobas e 601 analyzer

Sample	**Mean**	**N**	**Repeatability**		**Between-Run**		**Between-Day**		**Between-Device**		**Reproducibility**	
			SD	**CV [%]**	**SD**	**CV [%]**	**SD**	**CV [%]**	**SD**	**CV [%]**	**SD**	**CV [%]**
HS 01	0.040	90	0.001	2.2	0.000	0.8	0.000	1.1	0.001	2.0	0.001	3.3
HS 02	0.796	90	0.021	2.6	0.006	0.7	0.016	2.0	0.000	0.0	0.027	3.4
HS 03	0.827	90	0.009	1.0	0.010	1.2	0.014	1.7	0.007	0.9	0.021	2.5
HS 04	1.21	90	0.022	1.8	0.006	0.5	0.024	2.0	0.000	0.0	0.033	2.7
HS 05	2.05	90	0.024	1.2	0.022	1.1	0.033	1.6	0.025	1.2	0.052	2.6
HS 06	5.85	90	0.068	1.2	0.073	1.2	0.107	1.8	0.091	1.6	0.172	2.9
PC 1	0.05	90	0.001	1.5	0.000	1.0	0.001	1.5	0.001	1.8	0.001	2.9
PC 2	3.24	90	0.028	0.9	0.031	1.0	0.055	1.7	0.015	0.5	0.071	2.2

HS = human serum
PC = PreciControl Anti-HCV

According to Roche the results (see above) of the multi-analyzer precision study performed with Elecsys Anti-HCV II fulfilled the designated specifications.

2. Linearity:
   Not applicable.

3. Analytical Specificity/Interference:

To evaluate the effect of elevated levels of hemoglobin, bilirubin, lipemia (intralipid), biotin, and total protein on the Elecsys Anti-HCV II assay with four anti-HCV samples (negative, high negative, low positive and positive samples) were spiked with the potential interferents. Samples containing hemoglobin, bilirubin, intralipid, total protein, and rheumatoid factor were tested in duplicate on the cobas e601 analyzer with an acceptance criteria of sample recovery of ± 0.2 COI for samples <1.0 COI and 80-120% of unspiked result COI of samples ≥1.0 COI.

The purpose of this study was to evaluate potential endogenous interference with biotin measured on the cobas e 601 analyzer for anti-HCV concentrations at clinically relevant concentrations. For biotin, serum samples that contain biotin at a concentration of 1200 ng/mL demonstrate less than or equal to 10 % bias in COI values.

The acceptance criteria of ± 0.20 COI for negative samples and ± 10% for positive samples was used to assess the result so the biotin interference study. The results of the biotin interference are were tested up to the 3600 ng/mL and no impact was observed at the requested claim of 1200 ng/mL.

4. Assay Reportable Range:
   See P140021.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):
   a. Traceability:
      See P140021.

   b. Reagent Stability:
      Roche conducted the following reagent stability studies with the biotin updated Elecsys Anti-HCV II assay and obtained the following claims:

Table 6: Stability Claim

Study	Claim
On board reagent stability	31 days
Open kit reagent stability	57 days (8 weeks)
Real-time stability	12 months at 2-8°C
Calibration lot stability	28 days
Onboard reagent stability	7 days

6. Detection Limit:
   Not applicable.

7. Assay Cut-Off:
   See P140021.

8. Accuracy (Instrument):
   See P140021.

9. Carry-Over:
   See P140021.

B Comparison Studies:

1. Method Comparison with Predicate Device:
   The Method Comparison Study was conducted to evaluate the equivalence of the current and biotin-updated Elecsys Anti-HCV II assay. One reagent lot (506983) of the current Elecsys Anti-HCV II assay and 3 reagent lots (527094, 527095 and 527096) of the biotin-updated Elecsys Anti-HCV II assay.

   A total of 219 samples were tested in this study. Native clinical and contrived samples were tested in this study to adequately represent the measuring range of Elecsys Anti-HCV II assay. Contrived samples were prepared by diluting individual positive samples with individual negative clinical samples to achieve concentrations close to cut-off.

   Based on the results of the reference assay results 91 samples were < 0.9 COI, 19 samples were between ≥ 0.9 to < 1.0 COI, 109 were ≥1.0 COI.

   The equivalence of the current assay and biotin-updated assay design was evaluated by the following:
   a. Determination of Percent Agreement
   b. Estimation of Systematic Difference
   c. Regression analysis

   Acceptance Criteria
   The percent agreements should have the lower limits of the 95% two-sided Confidence Interval ≥ 90.0%.

   The results of the method comparison study are presented below:

A. Determination of Percent Agreement

The positive and negative percent agreement (PPA and NPA, respectively) between the biotin-updated and the current assay were determined with each lot of the biotin updated Elecsys Anti-HCV II assay. The re-test sample results were counted against the performance. Presented below are the calculated positive and negative percent agreements:

Table 7: Agreement Table - Lot 506983 (reference) vs Lot 527094 (MP01)

		Current Elecsys Anti-HCV II		
		Negative	**Re-test**	**Positive**
Updated Elecsys Anti-HCV II	**Negative < 0.9 COI**	91	13	0
	Re-test ≥ 0.9 - < 1.0 COI	0	6	3
	Positive ≥ 1.0 COI	0	0	106
	Overall	91	19	109

PPA = 97.25% (106/109) [92.22% to 99.06%]

NPA = 100% (91/91) [95.95% to 100.00%]

Table 8: Agreement Table - Lot 506983 (reference) vs Lot 527095

		Current Elecsys Anti-HCV II		
		Negative	**Re-test**	**Positive**
Updated Elecsys Anti-HCV II	**Negative < 0.9 COI**	90	17	1
	Re-test ≥ 0.9 - < 1.0 COI	1	2	5
	Positive ≥ 1.0 COI	0	0	103
	Overall	91	19	109

PPA = 94.50% (103/109) [88.51% to 97.45%]

NPA = 98.90% (90/91) [94.04% to 99.81%]

Table 9: Agreement Table - Lot 506983 (reference) vs Lot 527096

		Current Elecsys Anti-HCV II		
		Negative	**Re-test**	**Positive**
Updated Elecsys Anti-HCV II	**Negative < 0.9 COI**	91	10	0
	Re-test ≥ 0.9 - < 1.0 COI	0	9	3
	Positive ≥ 1.0 COI	0	0	106
	Overall	91	19	109

PPA = 97.25% (106/109) [92.22% to 99.06%]
NPA = 100.00% (91/91) [95.95% to 100.00%]

The summary of percent agreements is presented below:

Table 10: Overall percent agreements

Reagent Lots	**Agreement Rate Positive Agreement (%) (95% CI)**	**Agreement Rate Negative Agreement (%) (95% CI)**
1	97.25% 106/109 (92.22% to 99.26%)	100.00% 91/91 (95.95% to 100.00%)
2	94.50% 103/109 (88.51% to 97.45%)	98.90% 90/91 (94.04% to 99.81%)
3	97.25% 106/109 (92.22 to 99.06%)	100.00% 91/91 (95.95% to 100.00%)
Overall (all lots combined)	96.33% (90.94% to 98.56%)*	99.63% (95.27% to 100.00%)*

*95% CI are based on the Wilson score method, which uses an independent results assumption. This CI may be overstated.

B. Estimation of Systematic Difference

Roche determined the bias at the medical decision point of 1.0 COI which is presented below:

Lots tested	Bias at medical point	95% CI [COI]
527094	-0.04	-0.06, -0.02
527095	-0.09	-0.10, -0.07
527096	-0.04	-0.05, -0.03
All lots combined	-0.05	-0.07, -0.03

Based on the bias estimation, Roche concluded that there was no systematic difference between the biotin-updated and the current Elecsys Anti-HCV II assay.

C. Regression analysis

Roche assessed the regression analysis of the biotin updated and the current Elecys Anti-HCV II assay for each of the three lots of the biotin updated Elecsys Anti-HCV II assay. The regression plots are presented below.

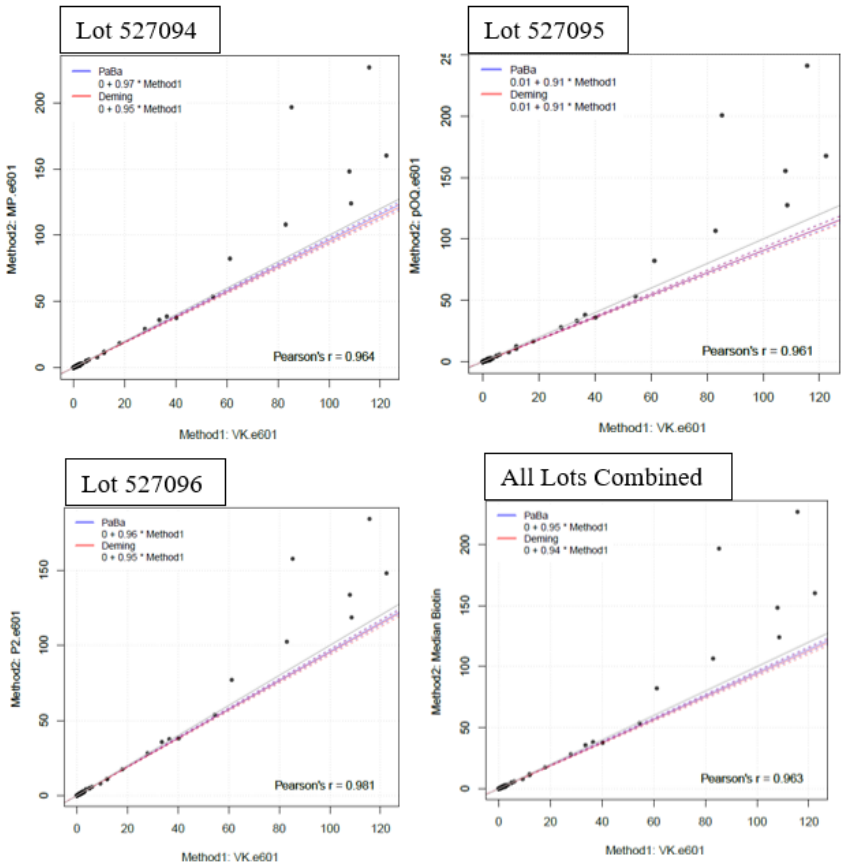

Figure: Regression analysis of the biotin updated vs the current Elecsys Anti-HCV II assay

The regression analyses for each lot of the biotin updated Elecsys Anti-HCV II assay and the combination of lots were acceptable.

Based on the percent agreement, bias estimation and the regression analyses, Roche concluded the biotin updated Elecsys Anti-HCV II assay was comparable to the current Elecsys Anti-HCV II assay.

2. Matrix Comparison:
See P140021.

C Clinical Studies:

1. Clinical Sensitivity:
See P140021.

2. Clinical Specificity:
See P140021.

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

D Clinical Cut-Off:
Not applicable.

E Expected Values/Reference Range:
See P140021.

F 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.