SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Antibodies to Hepatitis C Virus (Anti-HCV) Assay Antibodies to Hepatitis C Virus Control Material
Device Trade Name:	Elecsys® Anti-HCV II Immunoassay Elecsys® PreciControl Anti-HCV
Device Procode:	MZO
Applicant's Name and Address:	Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P140021

Date of FDA Notice of Approval: June 11, 2015

Priority Review: Not Applicable

II. INDICATIONS FOR USE

Elecsys Anti-HCV II Immunoassay

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 electroluminescence Immunoassay "ECLIA" is intended for use on the Roche cobas e 601 immunoassay analyzer.

Elecsys PreciControl Anti-HCV

Elecsys PreciControl Anti-HCV is used for quality control of the Elecsys Anti-HCV immunoassay on the cobas e 601 and cobas e 602 immunoassay analyzers and the Elecsys Anti-HCV II immunoassay on the cobas e 601 immunoassay analyzer.

III. CONTRAINDICATIONS

None

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the labeling for the Elecsys® Anti-HCV II Immunoassay and the Elecsys® PreciControl Anti-HCV.

V. DEVICE DESCRIPTION

Principle of Device Methodology

Elecsys Anti-HCV II Immunoassay
The Elecsys Anti-HCV II Immunoassay employs "ECLIA" technology and is a qualitative serologic, two step sandwich assay. The assay detects total antibodies to HCV in serum and plasma samples. The total duration of the assay is 18 minutes. The basic device methodology is as follows:

1. 1st incubation: 50 µL of sample, 55 µL of a reagent containing biotinylated HCV antigens, and 55 µL of a reagent containing HCV antigens labeled with a ruthenium complex react to form a sandwich complex.
2. 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
3. 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. Application of a voltage to the electrode then induces chemiluminescent emission, which is measured by a photomultiplier.
4. Results are determined automatically by the Elecsys software by comparing the electrochemiluminescence signal obtained from the sample with the cut-off value obtained by the anti-HCV calibration.

Elecsys PreciControl Anti-HCV
The Elecsys PreciControl Anti-HCV is used for quality control testing of the Elecsys Anti-HCV II Immunoassay on the cobas e 601 immunoassay analyzer. It is also used for quality control testing of the Elecsys Anti-HCV immunoassay on the cobas e immunoassay analyzers and the MODULAR ANALYTICS E170. It consists of two components: PC A-HCV1, which contains human serum negative for anti-HCV, and PC A-HCV2, which contains human serum that is positive for anti-HCV.

Kit Configurations and Components

Elecsys Anti-HCV II Immunoassay

Package Sizes

The Elecsys Anti-HCV II Immunoassay is produced in two different package sizes- one contains reagents for up to 200 tests and the other contains reagents for up to 100 tests.

Components

The Elecsys Anti-HCV II Immunoassay consists of five reagent components supplied by Roche Diagnostics in a single package as follows:

> **Component 1:** Reagent M contains streptavidin-coated microparticles (beads) at a concentration of 0.72 mg/ml in buffer with preservative
>
> The volume of Reagent M differs between the two kit configurations. The volume of Reagent M for the 100 test kit is 6.5 mL and for the 200 test kit is 12 mL.
>
> **Component 2:** The R1 reagent contains biotinylated HCV-specific antigens in buffer with preservative.
>
> **Component 3:** The R2 reagent contains HCV-specific antigens labeled with ruthenium complex in HEPES buffer with preservative.
>
> **Component 4:** A-HCV II Cal 1 is the negative calibrator and consists of human serum negative for anti-HCV antibodies with preservative.
>
> **Component 5:** A-HCV II Cal 2 is the positive calibrator and consists of human serum positive for anti-HCV antibodies with preservative.

Components 1-3 are combined in a bundled reagent pack ("rackpack") which is placed in the instrument while operational.

Elecsys PreciControl Anti-HCV

The Elecsys PreciControl Anti-HCV contains two reagents:

> **Component 1:** PreciControl 1 (PC A-HCV1) is the negative control which consists of buffered and preserved human serum matrix negative for anti-HCV antibodies. It is provided ready-to-use and should be stored at 2-8^{o}C.
>
> **Component 2:** PreciControl 2 (PC A-HCV2) is the low positive control which consists of buffered and preserved human serum matrix and is low positive for anti-HCV antibodies. It is provided ready-to-use and should be stored at 2-8^{o}C.

Calibrator

Calibrator 1 (A-HCV II Cal 1) consists of buffered and preserved human serum matrix, negative for anti-HCV antibodies. Calibrator 2 (positive) consists of buffered and preserved inactivated human serum positive for anti-HCV antibodies. The presence or absence of HCV antibodies in the sample is determined by comparing the

electrochemiluminescence signal in the reaction to the cut-off signal determined from an active Elecsys Anti-HCV calibration curve.

Interpretation of Results

Results are determined automatically by the Elecsys software by comparing the electrochemiluminescence signal obtained from the sample with the cut-off value obtained by the calibration of the assay. The result for a sample is given in the form of a cut-off index (COI = signal of sample/signal of cut-off) along with a result interpretation as follows:

Table 1: Interpretation of Anti-HCV II Testing

Initial Elecsys Anti-HCV II Assay Result			
COI	**Result**	**Interpretation of Results**	**Retest Procedure**
< 0.90	Non-reactive*	No antibodies to HCV 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.

*If 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.

Table 2: Interpretation of Repeat Anti-HCV II Testing

Final Elecsys Anti-HCV II Assay Result			
Initial Result	**Result after Retest (COI)**	**Final Results**	**Interpretation of Results**
Non-reactive	No retest required	Non-reactive*	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 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 CDC recommendations for supplemental testing.

*If 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.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are currently several FDA approved *in vitro* diagnostic tests for detecting serological markers of hepatitis C virus (HCV). The patient's medical history and thorough clinical examination, in addition to hepatitis serology, polymerase chain reaction (PCR) assays or nucleic acid testing (NAT), determination of liver enzyme levels, and biopsy of the liver, will provide further information on the status of a hepatitis C viral infection. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

VII. MARKETING HISTORY

The Elecsys Anti-HCV II Immunoassay and Elecsys PreciControl Anti-HCV are marketed in multiple countries. The device has not been withdrawn from marketing for any reason related to its safety or effectiveness. The following table provides the list of countries where the product is distributed:

Table 3: Countries in which the Elecsys Anti-HCV II Immunoassay is Marketed

ALBANIA	GREECE	PHILIPPINES
ALGERIA	GUATEMALA	POLAND
ARGENTINA	HONG KONG	REST OF SOUTH AMERICA
ARMENIA	HUNGARY	ROMANIA
AUSTRALIA	ICELAND	RUSSIAN FEDERATION
AUSTRIA	INDONESIA	RWANDA
AZERBAIJAN	IRAN	SENEGAL
BELARUS	IRAQ	SINGAPORE
BELGIUM	ISRAEL	SLOVAKIA
BRAZIL	ITALY	SLOVENIA
CAMBODIA	JAPAN	SOUTH AFRICA
CAMEROON	JORDAN	SPAIN
CANADA	KAZAKHSTAN	SUDAN
CAYMAN ISLANDS	KOREA, REPUBLIC	SWEDEN
CHILE	KOSOVO	SWITZERLAND
CHINA	KUWAIT	SYRIAN ARAB REPUBLIC
COLOMBIA	LAOS	UGANDA
COSTA RICA	MEXICO	UKRAINE
COTE D'IVOIRE	MOLDAVIA	UNITED ARAB EMIRATES
CROATIA	MONGOLIA	UNITED KINGDOM
CZECH. REPUBLIC	MOROCCO	URUGUAY
DENMARK	MYANMAR	UZBEKISTAN
DOMINICAN REPUBLIC	NETHERLANDS	VIETNAM
ECUADOR	NEW ZEALAND	YEMEN
EGYPT	NICARAGUA	TAIWAN
EL SALVADOR	NIGERIA	THAILAND

FINLAND	OMAN	TUNISIA
FRANCE	PAKISTAN	TURKEY
GEORGIA	PALESTINE	
GERMANY	PANAMA	
GHANA	PERU	

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

When used according to the instructions in the package insert, there are no known potential direct adverse effects of this device on the health of the user. Failure of the test to perform as indicated or human error during performance of the test may lead to improper patient management.

The diagnosis of HCV infection requires the evaluation of the patient's blood for anti-HCV antibodies where a positive result is followed up with nucleic acid testing for HCV RNA.

A false non-reactive (false negative) anti-HCV result may lead to a patient with HCV going unidentified and not receiving treatment. Under these circumstances, there is a safety concern for both the patient and the public, since they may be capable of transmitting HCV infection. However, 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 performed.

A false reactive (false positive) result using an anti-HCV assay is not considered a patient or public health concern because a reactive enzyme immunoassay (EIA) result should be followed up with supplemental tests such as polymerase chain reaction (PCR) for the detection of HCV RNA. Treatment of the patient with chronic HCV infection is initiated after additional clinical, laboratory, and behavioral assessment of the patient.

The risk of incorrect test results is inherent with all *in vitro* diagnostic products. Therefore, the above potential risks are not unusual in the laboratory setting. Appropriate warnings for each of these risks are contained in the labeling and package insert instructions. Standard good laboratory practices are considered sufficient to minimize risks to the end user.

IX. SUMMARY OF PRECLINICAL STUDIES

All non-clinical studies were performed at Roche Diagnostics Laboratories using the Elecsys Anti-HCV II Immunoassay and Elecsys PreciControl Anti-HCV on the cobas e 601 immunoassay analyzer.

Establishment of the Cut-off

For the qualitative Elecsys assays, the cut-off is calculated from signals of negative calibrator, A-HCV II Cal 1 (Cal 1), and positive calibrator, A-HCV II Cal 2 (Cal 2), according to the following general cut-off formula:

a x (Cal 1 – b x Cal 1) + c x (Cal 2 – b x Cal 1) + d = cut-off (CO)

For the Elecsys Anti-HCV II Immunoassay on the cobas e 601, the values were set to: a = 0.001, b = 0, c = 0.145, d = 0. Therefore, for the Elecsys Anti-HCV II Immunoassay on the cobas e 601, the cut-off formula was set to:
Cut-off (CO) = (0.001 x Cal1) + (0.145 x Cal2).

Verification
The cut-off was verified on the cobas e 601 by testing a total of 1336 samples obtained from blood donors, seroconversion panels, performance panels, and diluted positive samples. Receiver Operator Curve (ROC) analysis was performed to verify the cut-off and determine sensitivity and specificity. The external clinical studies serve as validation of the determined cut-off.

Limit of Blank and Limit of Detection
The Limit of Blank (LoB) and Limit of Detection (LoD) were determined in accordance with the CLSI guideline EP17-A.

The LoB was determined by testing five Anti-HCV negative samples (for each of serum and sodium heparin plasma) in single determination with two lots of reagents on 3 days with 2 runs per day on 2 cobas e 601 analyzers. For each lot, there was a total of 60 measured values for each of serum and sodium heparin plasma.

The LoD was determined by testing five serum samples and five sodium heparin samples with low analyte concentration with two lots in single determination over 3 days with 2 runs per day on 2 cobas e 601 analyzers. For each lot, there was a total of 60 measured values. The LoD was calculated as:
LoD = LoB + 1.653 x SD

Test results were reported in cut-off index (COI = sample signal/cut-off value). The acceptance criteria were: LoB < 0.5 COI LoD < 0.9 COI. The LoB was determined to be 0.039 COI. The LoD for two lots of reagents was determined to be 0.046 COI and 0.071 COI for serum and 0.05 COI and 0.044 COI for sodium heparin plasma.

High Dose Hook Effect
Three high titer positive samples were diluted in human anti-HCV negative serum in a minimum of 11 dilution steps to generate a dilution series that covered the range from negative to high positive COI values. The samples were measured in triplicate. The acceptance criterion was no false negative results for the tested samples. At very high anti-HCV concentrations, a high dose hook effect was observed; however, no false negative results were observed. The concentrations at which the high dose hook effect was observed were extremely high (COI > 173). Analysis of the sample distribution in the clinical study showed that approximately 1.6% of samples had a COI > 173 and none of these samples showed a reversal in results interpretation from positive to negative.

Equivalency Study for the 100 and 200 Reagent Test Kits
The Elecsys Anti-HCV II Immunoassay is produced in two different package sizes: 100 and 200 test kits. All components have the same filling volume except for the volume of microparticle beads in bottle M. This study tested two high negative and two low positive samples with reagent packages that were at different stages of use (full and almost depleted). Experiments were carried out on two different cobas e 601 immunoassay analyzers. Samples were measured in 21 replicates. The results for the median, mean, minimum and maximum COI values as well as the % CV obtained for each of the concentrations demonstrated equivalence of the 100 and 200 reagent test kits. There was also no observed effect of the volume of microparticle beads in bottle M.

Endogenous Interferences
This study evaluated the effect of elevated levels of hemoglobin (from 0 to 1.1 g/dL), bilirubin (from 0 to 73 mg/dL), lipemia (intralipid) (from 0 to 2,200 mg/dL), biotin (from 0 to 70 ng/mL), and total protein (ranging from 0 to 22 g/dL) on the Elecsys Anti-HCV II assay. Each potentially interfering endogenous agent was tested at 10 levels. All calculations were based on COI. Samples were tested in duplicate. Mean recovery of COI values of samples spiked with interfering substance were compared with the COI values for the respective samples without the interfering substance.

The following HCV antibody samples were measured:

Negative sample	< 0.6 COI
High negative sample	target 0.8 COI, range 0.6 – 1.0 COI
Low positive sample	target 1.2 COI, range 1.0 – 1.4 COI
Positive sample	> 1.4 COI

The acceptance criteria for mean recovery when compared to the initial unspiked result were:

Samples < 1.0 COI: Spiked COI = Unspiked COI ± 0.2 COI
Samples ≥ 1.0 COI: Spiked COI = Unspiked COI ± 20%

The results of this study demonstrated that samples containing hemoglobin up to 1.1 g/dL, bilirubin up to 73 ng/dL, lipemia up to 2200 mg/dL, biotin up to 49 ng/mL, and total protein up to 22 g/dL should test accurately with the Elecsys Anti-HCV II Immunoassay. The following levels for non-interference are claimed in the package insert:

Hemoglobin	1.0 g/dL
Bilirubin	66 mg/dL
Lipemia	2,000 mg/dL
Biotin	44 ng/mL
Total protein	20 g/dL

Matrix Effects
Studies were conducted to verify the types of blood collection tubes that can be used with the Elecsys Anti-HCV II Immunoassay. Samples were collected into matched serum and plasma collection tubes from 40 donors and assayed in triplicate on the cobas e 601

immunoassay analyzer. Forty matched pairs were collected in the evaluation of each of the following blood collection tubes:

Serum gel separation
Plasma gel separation
Lithium heparin plasma
Sodium heparin plasma
K2 EDTA plasma
Sodium citrate plasma

The samples were processed by spiking with equivalent levels of Anti-HCV to cover the whole measuring range:

Negative	targeted to ≤ 0.5 COI
High negative	targeted to 0.6 to < 1.0 COI
Low Positive	targeted to 1.0 to 1.4 COI
Positive	targeted to > 1.6 to 3.0 COI

The acceptance criteria were as follows:

samples < 1.0 COI:	Matrix COI = Serum COI ± 0.2 COI
samples ≥ 1.0 COI:	Matrix COI = Serum COI ± 20%. Statistical analysis must show no overall trend of bias > 15% per sample type.

Statistical evaluations were performed to analyze the COI data for overall bias using orthogonal linear regression, which will reveal any relevant overall proportional bias. The slope, the lower and upper confidence interval limits, correlation and intercept were calculated.

The studies support the use of the following blood collection tubes:

Serum gel separation
Plasma gel separation
Lithium heparin plasma
Sodium heparin plasma
K2 EDTA plasma
Sodium citrate plasma

Drug Interferences

Eighteen common therapeutic drugs and three hepatitis antiviral drugs were tested for potential interference. Each drug was spiked into a negative, high negative, low positive and moderate positive Anti-HCV sample. The spiked samples were evaluated in triplicate at the following drug concentrations:

Table 4: Drugs Tested with the Elecsys Anti-HCV II Immunoassay

Compound	Concentration
Acetyl cysteine	150 mg/L
Ampicillin-Na	1,000 mg/L
Ascorbic acid	300 mg/L
Ca- Dobesilate	200 mg/L
Cyclosporine	5 mg/L
Cefoxitin	2,500 mg/L
Heparin	5,000 U/L
Intralipid	10,000 mg/L
Levodopa	20 mg/L
Methyldopa+ 1.5	20 mg/L
Metronidazole	200 mg/L
Phenylbutazone	400 mg/L
Tetracycline	50 mg/L
Acetylsalicylic acid	1,000 mg/L
Rifampicin	60 mg/L
Acetaminophen	200 mg/L
Ibuprofen	500 mg/L
Theophylline	100 mg/L
PEG interferon	0.036 µg/mL
Ribavirin	1200 mg/L
Interferon-alpha2α	6,000 IE/mL

Acceptance criteria: The mean COI of the drug-spiked sample must be within the range of mean COI of the non-spiked sample ± 10%.

Each drug was found to not interfere at the claimed concentration.

Since these studies were performed *in vitro*, they do not assess the potential interference when the drug is metabolized *in vivo*.

HAMA Effect

This assay is a double antigen sandwich assay where recombinant antigens and peptide antigens are used. No mouse derived monoclonal antibodies were used. Potential HAMA effects can therefore be excluded by design.

Carryover Study

On the cobas e 601 immunoassay analyzer, the use of disposable tips for sample pipetting eliminates any risk of sample carryover by design. However, a study was performed to determine the extent of bead carryover and the associated residual risk for signal carryover in the instrument's measuring cell caused by a high signal-generating sample.

An anti-HCV negative sample was tested in triplicate using the Elecsys Anti-HCV II

Immunoassay. Thereafter, a high signal generating HCV sample (≥ 2 million counts) was tested in triplicate followed again by testing of the anti-HCV negative sample in triplicate. This procedure was performed seven times with seven different anti-HCV negative samples.

Acceptance criteria: The deviation of the first signal value of the negative sample after the high-signal-generating-sample should be within 75-125% of the median signal of the triplicate measurements before the high-signal-generating-sample.

The percent recovery ranged from 98% to 103%. The signal values were within the acceptance criteria. This study demonstrates that there is no measurable signal carryover with the Elecsys Anti-HCV II immunoassay.

Sample Stability Studies

Sample Stability
Four studies were performed to verify the stability of patient serum and plasma samples under several conditions at the following concentrations:

Negative	targeted to ≤ 0.5 COI
High negative	targeted to 0.6 to < 1.0 COI
Low positive	targeted to 1.0 to 1.4 COI
Moderate positive	targeted to 1.6 to 3.0 COI

The four studies are described below:

1) Twelve serum and plasma samples were stored for up to 7 days at 2 to 8°C. The time points tested were 0 (unstressed), 3, 6, and 7 days and samples were measured in triplicate. Recoveries after storage for 3, 6, and 7 days at 2-8°C were calculated relative to the mean COI at day 0.
2) Twelve serum and plasma samples were stored for up to 3 months at -20°C (time points tested were unstressed, after 2 weeks, and after 1, 2, and 3 months) and measured in triplicate at each time point. Recoveries after storage for 2 weeks, 1 month, 2 months, and 3 months at -20°C were calculated relative to the mean COI at day 0.
3) Twelve serum and plasma samples were stored for up to 3 days at 25°C (time points tested were unstressed and after 1, 2, and 3 days) and measured in triplicate. Recoveries after storage for 1 day, 2 days, and 3 days at 25°C were calculated relative to the mean COI at day 0.
4) Twelve serum and plasma samples were subjected to multiple freeze/thaw cycles (up to 6) and measured in triplicate. Measurements were performed with fresh samples and after 1, 2, 3, 4, 5, and 6 freeze/thaw cycles. The recovery after 1, 2, 3, 4, 5, and 6 cycles of freeze/thaw was calculated based on the mean COI of the unstressed sample.

The recovery (COI or %) was calculated from the mean of the triplicate measurements of the stressed versus the unstressed conditions. Recovery after storage for each test was calculated based on the mean COI.

The acceptance criteria were as follows:

samples < 1.0 COI: Stressed COI = Unstressed COI ± 0.2 COI
samples ≥ 1.0 COI: Stressed COI = Unstressed COI ± 20%

The acceptance criteria were met for all serum and plasma samples. These studies indicate that serum and plasma samples may be stored for 7 days at 2-8°C, 3 months at -20°C, 3 days at 25°C, and can be subjected to 6 freeze/thaw cycles prior to testing by the Elecsys Anti-HCV II Immunoassay.

Reagent Stability Studies

Reagent Real Time (Shelf Life) Stability
Testing was performed on three lots of the 200 test kit configuration and on one lot of the 100 test kit configuration. The kits were stored at the recommended storage temperature of 2-8°C in a temperature-controlled area for the duration of the ongoing stability studies. The measured intervals started with the production date, and measurements were performed at least in the middle of the shelf life and one month after the claimed expiration date. Testing included measurement of the internal control samples (ICS) and the PreciControls (PC1 and PC2) in duplicate measurements. The acceptance criteria were met and the study confirms a claimed shelf life for the unopened Elecsys Anti-HCV II Immunoassay kit of 10 months at 2-8°C.

Reagent Temperature Stress Stability
This study was conducted to determine the effect of elevated temperature stress on the Elecsys Anti-HCV II Immunoassay during transportation. A reagent kit of the 100 test kit configuration and the 200 test kit configuration were stressed for one week at 25°C. Four human serum samples (negative, high negative, low positive, and positive) and the two PreciControls were then measured in duplicate with the stressed reagent kits and compared to the results from the testing performed with the corresponding unstressed reagent kits (stored at 2-8°C). Recoveries of the samples were calculated.

The acceptance criteria were as follows:

Samples < 1.0 COI: Stressed COI = Unstressed COI ± 0.2 COI
Samples ≥ 1.0 COI: Stressed COI = Unstressed COI ± 20%

For samples < 1.0 COI, the change in COI ranged from 0.002 to 0.014. For samples > 1.0 COI, the recovery ranged from 101% to 103%. The acceptance criteria were met. This study confirms a claimed stability of the Elecsys Anti-HCV II reagent for 1 week at 25ºC.

On-Board Stability - Open Reagent Pack

This study was performed to determine the time period for which the Elecsys Anti-HCV II Immunoassay reagents can be stored on the analyzer once opened. The reagent packs of the 200 and 100 test kit configurations were stored on board for 31 days at 20°C ± 3°C. Each week, the reagent pack was checked with regard to stability of the weekly calibration. Unstressed reagent packs of the 200 and 100 test kit configurations were opened and calibrated. Four human serum samples and the positive and negative PreciControls were tested with the unstressed reagent packs (stored at 2-8°C) and with the reagent packs which were stressed on-board for 8, 15, 22, 29, or 31 days). For each test time point, the calibration occurred seven days prior, except for day 31. Recovery for each sample was calculated based on the COI value with the stressed reagent pack compared with the COI value with the unstressed reagent pack.

Acceptance criteria:

Samples < 1.0 COI: Stressed COI = Unstressed COI ± 0.2 COI
Samples ≥ 1.0 COI: Stressed COI = Unstressed COI ± 20%

For samples < 1.0 COI, the recovery ranged from 0 to 0.169. For samples > 1.0 COI, the recovery ranged from 95% to 105%. All acceptance criteria were met for each of the time points tested. This study supports on-board reagent stability of 31 days.

On-Board/Refrigerated Stability

Stability studies were performed to determine the time period over which the Elecsys Anti-HCV II Immunoassay reagents can be kept in the refrigerator and, alternately, on the analyzer. One 100 test kit and one 200 test kit reagent packs were stored in a refrigerator at 2-8°C and alternately on-board the cobas e 601 immunoassay analyzer at 20°C ± 3°C (up to 80 hours total) to simulate on-board stress. Each week the reagents were checked with regard to stability of the weekly calibration.

A new reagent pack was opened and calibrated. Four human serum samples and the negative and positive Elecsys PreciControl Anti-HCV controls were tested in duplicate with the on-board reagent at weeks 1, 2, 3, 4, 5, 6, and 7 with weekly calibration.

The acceptance criteria were as follows:

samples < 1.0 COI: Stressed COI = Unstressed COI ± 0.2 COI.
samples ≥ 1.0 COI: Stressed COI = Unstressed COI ± 20%

For samples < 1.0 COI, the change in recovery ranged from 0 to 0.052. For samples > 1.0 COI, the recovery ranged from 90% to 107%. The acceptance criteria were met. The study confirms a claimed stability for the Elecsys Anti-HCV II Immunoassay reagent stored alternately in the refrigerator up to 7 weeks and on-board the cobas e 601 analyzer up to 80 hours.

Reagent Stability after First Opening

This study was performed to determine the time period over which the Elecsys anti-HCV II Immunoassay kits can be kept at 2-8°C once opened. One 100 test kit and one 200 test kit

were opened and calibrated on the cobas e 601 analyzer. Four human serum samples (negative, high negative, low positive, and positive) and the two Elecsys PreciControl Anti-HCV controls were tested with the opened reagent unstressed (day 0) and after 8 weeks at 2-8°C. The reagent pack stability was determined by calculating the recovery (COI) of PreciControls and serum samples with stressed reagent compared to the COI results with unstressed reagent.

The acceptance criteria were as follows:

samples < 1.0 COI	Stressed COI = Unstressed COI ± 0.2 COI
samples ≥ 1.0 COI	Stressed COI = Unstressed COI ± 20%

For samples < 1.0 COI, the change in recovery ranged from 0.001 to 0.054. For samples > 1.0 COI, the recovery ranged from 100% to 107%. The acceptance criteria were met. The study confirms a claimed stability for the Elecsys Anti-HCV II Immunoassay reagent stored for 8 weeks at 2-8°C after first opening.

Calibration Stability Studies

On-Board Stability- Open Calibrators

The sample rotor disk where calibrators, PreciControl controls, and samples are placed in the cobas e 601 immunoassay analyzer is kept at ambient temperature (18 to 32°C). As the calibrators are placed on the rack during calibration, the maximum temperature the calibrators of the Elecsys Anti-HCV II immunoassay might be exposed to is assumed to be 32°C, which is the upper limit of the specification for the ambient temperature of the cobas e 601 analyzer.

A pair of Elecsys Anti-HCV II Immunoassay calibrators were opened and stored at 32°C. After 2 hours of incubation at 32°C, the calibrators were tested in duplicate together with a pair of unstressed calibrators. Recovery for each calibrator was calculated based on counts (signal).

Acceptance criteria were 75%-125% recovery of signal counts for Cal 1 and 90%-110% recovery of signal counts for Cal 2 after 2 hours at 32°C.

The percent recovery for the duplicate Cal 1 was 102% and 103%. The percent recovery for the duplicate Cal 2 was 100% and 101%. The acceptance criteria were met. The study confirms a claimed stability of 2 hours for the calibrators to be open and on-board the cobas e 601.

Calibrator Stability after First Opening

This study was performed to determine the time period in which the Elecsys Anti-HCV II Immunoassay calibrators can be kept at 2-8°C once opened.

A new reagent pack was opened and calibrated. The opened calibrators were then tested again in duplicate after 4 and 8 weeks stored at 2–8°C. Calibrator stability was

determined by calculation of the recovery of the calibrator signals (counts) of opened calibrators compared to the signals (counts) for unstressed calibrators.

Acceptance criteria were 75-125% recovery of signal counts for Cal 1 and 90-110% recovery of signal counts for Cal 2 after 2 hours at 32°C.

The percent recoveries for the duplicates were as follows:

Cal 1: 4 weeks - 104% and 106%; 8 weeks - 99% and 101%
Cal 2: 4 weeks - 102% and 103%; 8 weeks - 96% and 99%

The acceptance criteria were met. The study confirms a claimed stability of 8 weeks after first opening for the Elecsys Anti-HCV II Immunoassay calibrators when stored at 2-8°C.

Lot Calibration Stability
This study was performed to verify the claim that one calibration can be used for one month with multiple reagent packs of the same lot. One Elecsys Anti-HCV II Immunoassay reagent lot was tested on three separate cobas e 601 instruments. Four human serum samples (negative, high negative, low positive, and positive) and negative and positive PreciControl Anti-HCV were tested in duplicate. Calibration was performed with unstressed reagent on Day 1. After 29 days, unstressed reagent of the same lot was run again using the initial unstressed calibration to demonstrate stability of the initial calibration, and stability of the control measurements.

The acceptance criteria for samples < 1.0 COI was a recovery at day 29 within ± 0.2 COI of the initial COI. The acceptance criteria for samples ≥ 1.0 COI was a recovery at day 29 of 80-120% of the initial COI.

The studies confirm calibration stability of one month (28 days) with multiple kits from the same reagent lot. The product labeling instructs a repeat of calibration at 28 days when using the same reagent lot.

Reagent Pack On-Board Calibration Stability
This study was performed to test the stability of the weekly calibration. An Elecsys Anti-HCV II Immunoassay reagent pack was tested unstressed (stored at 2-8°C) and after storage on-board the cobas e 601 at 20 ± 3°C for one week.

A new reagent pack was opened and calibrated. Four human serum samples (negative, high negative, low positive, and positive) and the Elecsys PreciControl Anti-HCV controls (PC 1 and PC 2) were tested in duplicate with the unstressed reagent and after the reagent pack was stored for 1 week on-board using the calibration of unstressed reagents. Recovery for each sample (stressed/unstressed) was calculated based on COI.

The acceptance criteria were as follows:

samples < 1.0 COI	Stressed COI = Unstressed COI ± 0.2 COI
samples ≥ 1.0 COI	Stressed COI = Unstressed COI ± 20%

For samples < 1.0 COI, the change in recovery ranged from 0.001 to 0.032. For samples > 1.0 COI, the recovery ranged from 94% to 108%. The acceptance criteria were met. The studies confirm a claimed calibration stability for 7 days on the cobas e 601 when using the same reagent kit.

PreciControl Anti-HCV Stability Studies

PreciControl Real-Time (Shelf Life) Stability

Shelf life was determined by testing three production lots of the Elecsys PreciControl Anti-HCV control kits stored at the recommended storage temperature of 2-8°C. The PreciControl lots were tested, at a minimum, after production, in the middle of the shelf life and one month after expiry. The PreciControls were measured in duplicate.

Acceptance criteria were as follows:

PC1	recovery within 0-0.3 of initial COI
PC2	recovery = 70-130% of initial COI

For PC1, the values ranged from 0.039 to 0.127. For PC2, the recovery ranged from 94% to 105%. The data show that PreciControl Anti-HCV controls are stable for at least 22 months. The study confirms a claimed stability for the unopened Elecsys PreciControl Anti-HCV kits of 18 months at 2-8°C.

PreciControl Temperature Stress Stability

This study was conducted to determine the effect of elevated temperature stress on the Elecsys PreciControl Anti-HCV controls during transportation. One kit was stored at the recommended storage of 2-8^{o}C and a second kit was stressed for one week at 35^{o}C. The COIs of the controls were assessed in duplicate before and after incubation at the indicated conditions:

The acceptance criteria were as follows:

samples < 1.0 COI	Stressed COI = Unstressed COI ± 0.2 COI
samples ≥ 1.0 COI	Stressed COI = Unstressed COI ± 20%

For PC1, the change in recovery was 0.004. For PC2, the recovery was 92%. The acceptance criteria were met. The study confirms a claimed stability for the Elecsys PreciControl Anti-HCV controls of 1 week at 35°C.

PreciControl Stability after First Opening

Stability studies were performed to determine the time period over which the Elecsys PreciControl Anti-HCV controls can be kept at 2-8°C once opened.

A new PreciControl kit pack was opened, tested on day 0 (unstressed reference), and then stored at 2-8°C for 8 weeks. After 4 and 8 weeks, the stressed PreciControls were tested in duplicate. Recovery relative to the unstressed PreciControl kit pack, based on the initial value, was calculated.

The acceptance criteria were as follows:

samples < 1.0 COI	Stressed COI = Unstressed COI ± 0.2 COI
samples ≥ 1.0 COI	Stressed COI = Unstressed COI ± 20%

For PC1, the change in recovery was 0.007. For PC2, the recovery was 100%. The acceptance criteria were met. The study confirms a claimed stability for the Elecsys PreciControls after first opening of 8 weeks when stored at 2-8°C.

On-Board Stability for Open PreciControls

An Elecsys Anti-HCV II Immunoassay reagent pack and an Elecsys PreciControl Anti-HCV pack were opened and tested. The Elecsys Anti-HCV II Immunoassay reagent pack was stored at 2-8°C, and the opened PreciControls stored at 32°C. In 6 one-hour intervals, the stressed PreciControls were tested in duplicate. Recovery was calculated based on counts (signal).

The acceptance criterion was 90-110% recovery of signal counts.

For PC1, the recovery ranged from 98% to 99%. For PC2, the recovery ranged from 97% to 104%. The acceptance criteria were met. The study confirms a claimed stability for the Elecsys PreciControl Anti-HCV of up to 5 hours on-board the cobas e 601 analyzer.

Antimicrobial Effectiveness Testing

Antimicrobial effectiveness testing (AET) was performed according to United States Pharmacopoeia (USP) chapter 51. Testing was performed with all reagents of the Elecsys Anti-HCV II Immunoassay and the Elecsys PreciControl Anti-HCV.

One lot of each reagent was tested with a panel of microorganisms. All reagents were plated on appropriate media prior to inoculation, and non-inoculated controls were incubated in parallel and plated at each time point.

After inoculation, samples were plated on appropriate media on Days 0, 7, 14, and 28. To pass USP criteria, the bacterial concentration is to be reduced to < 0.1 % of the original inoculum by day 14, and remain at or below this level until day 28. For yeast and molds, these are to remain at or below the original inoculum during the 28 day period. USP criteria suggest that a suitable inoculum should be between 1×10^5 and 1×10^6 organisms per mL.

All reagents met the USP requirements for antimicrobial effectiveness testing.

In addition to these studies, each lot of components is checked for microbial contamination as part of the QC Release Testing Procedure. Microbial contaminants at a level which would compromise product performance would also fail quality assurance criteria listed in the stability specifications. No microbial outgrowth has been observed in components stored at elevated temperatures, relative to 2-8°C storage, in previous accelerated stability studies.

Analytical Specificity/Cross-Reactivity

A study was conducted to evaluate the Elecsys Anti-HCV II Immunoassay for potential cross-reactivity with specimens from individuals with medical conditions unrelated to HCV infection. The study was performed by testing 283 samples. The comparison data to the comparator assay are presented in the following table:

Table 5: Comparison of Elecsys Anti-HCV II Assay (Test) and the Reference Assay Results for Subjects with Potentially Interfering Medical Conditions

Category	Reference Assay				Total
	Reactive		Non-Reactive		
	Elecsys Anti-HCV II Assay				
	RX [a]	NR [b]	RX	NR	
Immune Disorders					
AMA anti-mitochondrial ab	3[c]	0	0	12	15
ANA anti-nuclear ab	0	0	0	6	6
Rheumatoid factor	0	0	0	11	11
Non-Viral Infections					
E. coli infection	0	0	0	12	12
Syphilis	0	0	0	11	11
Toxoplasmosis	0	0	0	11	11
Viral Infection					
Cytomegalovirus (CMV)	0	0	0	12	12
Dengue Fever	0	0	0	12	12
Epstein-Barr Virus	0	0	0	11	11
Hepatitis A Virus (HAV)	0	0	0	10	10
Hepatitis B Virus (HBV)	0	0	0	10	10
Hepatitis D Virus	8[c]	0	0	10	18
Hepatitis E Virus	0	0	0	24	24
Human Immunodeficiency Virus (HIV)	1	0	0	10	11
Herpes Simplex Virus (HSV)	0	0	0	12	12
Kunjin Fever	0	0	0	1	1
Murray Valley/Australian Encephalitis	0	0	0	4	4
Parvovirus B19 Infection	0	0	0	12	12
Rubella	0	0	0	12	12
Varicella Zoster (VZV)	0	0	0	12	12
West Nile Disease	0	0	0	12	12
Non-Viral Liver Disease					
Alcohol Liver Disease	0	0	0	5	5
Autoimmune Hepatitis	0	0	0	6	6
Primary Biliary Cirrhosis	0	0	0	7	7
Other Non-Viral Liver Disease	0	0	0	13	13

Category	Reference Assay				Total
	Reactive		Non-Reactive		
	Elecsys Anti-HCV II Assay				
	RX [a]	NR [b]	RX	NR	
Vaccination					
HAV Vaccination	0	0	0	10	10
HBV Vaccination	0	0	0	10	10
Flu Vaccination	0	0	0	9	9

[a] RX = Reactive

[b] NR = Non-reactive

[c] Specimens were not further tested to determine HCV status because there is no FDA approved test that has been shown to not cross-react.

Precision

A precision study was conducted to evaluate repeatability and the intermediate precision of within-laboratory precision according to CLSI guideline EP5-A2.

Internal Precision

A six-member precision panel consisting of 4 human serum (HS) pools (one negative, one high negative, one low positive, and one positive) and two Elecsys PreciControl Anti-HCV controls (one positive and one negative) was measured in duplicate determinations in two runs per day for 12 days. The measurements were performed on one cobas e 601 analyzer, at one site, with one reagent lot, performing weekly rack pack calibration, and spanning at least two calibration cycles. Repeatability and within-laboratory precision were calculated according to EP5-A2. Repeatability precision ranged from 0.866 to 4.01 % CV. Within-laboratory precision ranged from 2.33 to 4.34 % CV as shown in the following table:

Table 6: Internal Precision Data

Sample	n	Mean	Repeatability		Within-laboratory precision	
		COI	SD	%CV	SD	%CV
HS, Negative	96	0.037	0.001	1.86	0.001	3.12
HS, High Negative	96	0.930	0.037	4.01	0.039	4.23
HS, Low Positive	96	1.11	0.013	1.20	0.029	2.59
HS, Positive	96	2.80	0.035	1.25	0.065	2.33
PreciControl A-HCV1	96	0.054	0.002	3.39	0.002	4.34
PreciControl A-HCV2	96	4.47	0.039	0.866	0.123	2.75

(External Precision)

Imprecision results collected on three cobas e 601 analyzers at external sites were based on three lots of reagents (A, B, and C) with two lots tested at each site: AB, BC, AC. PreciControl Anti-HCV materials (A-HCV1 and A-HCV2), four near cut-off human serum pools (lower negative HS07, higher negative HS01, lower positive HS02, higher positive HS04), and a moderately positive human serum pool (HS06) were tested in

replicates of 3 in 2 runs/day for 5 days according to the CLSI documents EP15-A2 and EP5-A2. Data from all 3 reagent lots were combined to determine SD and percent CV for repeatability (within-run), between-run, between-day, between-lot, between-site and reproducibility. The cut-off index (COI) and standard deviation (SD) of the results are summarized in the following table:

Table 7: Reproducibility (External Precision) Data

Sample		**HS07**	**HS02**	**HS01**	**HS04**	**HS06**	**A-HCV1**	**A-HCV2**
N		180	180	180	180	180	180	180
Mean COI [a]		0.730	1.034	1.037	1.330	2.660	0.056	4.025
Repeatability	SD [b]	0.008	0.010	0.014	0.014	0.032	0.001	0.075
	%CV	1.1	1.0	1.3	1.1	1.2	2.6	1.9
Between-run	SD	0.013	0.019	0.016	0.025	0.040	0.001	0.067
	%CV	1.8	1.9	1.5	1.9	1.5	1.0	1.7
Between-day	SD	0.010	0.000 [c]	0.013	0.010	0.041	0.000 [c]	0.152
	%CV	1.3	0.0	1.2	0.8	1.6	0.7	3.8
Between-lot	SD	0.068	0.109	0.048	0.138	0.111	0.002	0.356
	%CV	9.3	10.6	4.6	10.4	4.2	4.4	8.8
Between-site	SD	0.038	0.047	0.077	0.056	0.193	0.000 [c]	0.271
	%CV	5.2	4.5	7.4	4.2	7.3	0.0	6.7
Reproducibility	SD	0.080	0.121	0.094	0.153	0.232	0.003	0.483
	%CV	10.9	11.7	9.0	11.5	8.7	5.2	12.0

[a] COI - Cut-off index
[b] SD - Standard deviation
[c] Set of variables had a SD of zero because variance contributed by particular component was below stated significant figure.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The safety and effectiveness of the Elecsys Anti-HCV II Immunoassay was determined by a clinical trial consisting of the following studies:

Clinical Study:

A. Study Design

The purpose of this study was to evaluate the clinical performance of the Elecsys Anti-HCV immunoassay on the cobas e 601 analyzer with specimens from patients at increased risk (at-risk) or with signs and symptoms of HCV infection. Agreement of the Elecsys Anti-HCV II assay was assessed relative to a patient infected status algorithm.

Prospective Study:

Inclusion criteria

The adult at-risk population group was required to have an increased risk (medical, occupational, sexual, or behavioral) for hepatitis, with or without symptoms of hepatitis infection. Subjects were all at least 22 years old or older.

The pediatric at-risk population group was required to have the same inclusion criteria as the adult at-risk population except the samples collected must have been collected from subjects 2 through 21 years of age.

The at-risk pregnant population of any age was included and evaluated as part of the intended use population.

Exclusion criteria

Exclusion criteria consisted of the following: Subjects younger than 22 years old (excluded from the adult population); subjects 22 years of age or older or less than 2 years of age (excluded from the pediatric population); subjects who violated any of the inclusion criteria; subjects who were unable to understand and sign the informed consent form or have a legal guardian who is willing to give consent; and adult subjects who were unable to donate approximately twenty milliliters of blood, or pediatric subjects who were unable to donate approximately 5 milliliters.

Retrospective Study:

Retrospective study specimens were obtained from commercial vendors.

Seroconversion Panels:

The panels were obtains from commercial sources.

B. Accountability of PMA Cohorts

The Elecsys Anti-HCV II Immunoassay clinical study included a total of 2,435 specimens at risk of HCV infection. There were 2,243 adult and 192 pediatric specimens, 205 of which were from pregnant subjects. All subjects were at risk of HCV infection due to lifestyle, behavior, occupation or known exposure event and included symptomatic and asymptomatic subjects. Sample collection occurred between June and December, 2013. Prospective clinical specimens were obtained from multiple collection sites in the US including Miami, FL, Los Angeles, CA, Industry, CA, San Antonio, TX , Minneapolis, MN, Baltimore, MD, and Darby, PA.

Sixteen seroconversion panels were obtained from commercial sources and tested.

C. Study Population Demographics

The Elecsys Anti-HCV II clinical study population consisted of a total of 2,435 specimens from adult (n = 2,243) and pediatric (n = 192) individuals at risk of HCV infection. A demographic summary of the overall at risk specimen population by race, age and gender is provided in the following tables:

Table 8: Demographic Summary of At-Risk Population by Race

Race	Group n	Percent %
American Indian/Alaska Native	20	0.82
Asian	22	0.90
African American/Black	1054	43.29
Caucasian/White	1278	52.48
Pacific Islander	6	0.25
Unknown	11	0.45
Other	44	1.84
Total	2435	100

Table 9: Elecsys Anti-HCV II Results by Age Range and Gender for Individuals at Risk of HCV Infection

Elecsys Anti-HCV II Results				
Age years	**Sex**	**Pos n (%)**	**Neg n (%)**	**Total n**
2 – 11	Female	1 (6.67)	14 (93.33)	15
	Male	2 (8.00)	23 (92.00)	25
12 – 21	Female	1 (2.3)	101 (97.7)	102
	Male	1 (6.2)	49 (93.8)	50
22 – 29	Female	7 (2.62)	260 (97.38)	267
	Male	7 (6.14)	107 (93.86)	114
30 – 39	Female	29 (11.7)	218 (88.3)	247
	Male	34 (19.5)	140 (80.5)	174
40 – 49	Female	59 (21.6)	214 (78.4)	273
	Male	81 (24.3)	252 (75.7)	333
50 – 59	Female	102 (40.5)	150 (59.5)	252
	Male	158 (42.5)	214 (57.5)	372
60 – 69	Female	37 (47.4)	41 (52.6)	78
	Male	59 (52.2)	54 (47.8)	113
70 – 79	Female	3 (30.0)	7 (70.0)	10
	Male	2 (28.6)	5 (71.4)	7
≥80	Female	0 (0.0)	3 (100.0)	3
	Male	0 (0.00)	0 (0.00)	0
Totals	Female	239 (19.2)	1008 (80.8)	1247
	Male	344 (28.86)	844 (71.04)	1188

Elecsys Anti-HCV II Results				
Age years	**Sex**	**Pos n (%)**	**Neg n (%)**	**Total n**
All	All	583 (23.9)	1852 (76.1)	2435

Study Results:

Results of Method Comparison Studies

The Elecsys Anti-HCV II Immunoassay was evaluated at four clinical sites located at St. Louis, MO, Miami, FL, South Bend, IN, and Louisville, KY.

The patient infected status was determined according to the following table:

Table 10: HCV Infection Status Algorithm

Reference Anti-HCV #1	Reference Anti-HCV #2	Reference Anti-HCV #3	Intermediate HCV Infection Status	FDA approved RNA test	Final HCV Infection Status
Reactive	-	-	Not Determined	Negative	Not HCV Infected
Reactive	+ or EQ*	-	Not Determined	Negative	Not Determined
	- or EQ	+			
Reactive	+ or EQ	-	Not Determined	Positive	HCV Infected
	- or EQ	+			
	-	-			
Negative	Not applicable		Not HCV Infected	Not Applicable	Not HCV Infected
Reactive	+	+	HCV Infected	Not Applicable	HCV Infected

*EQ: Equivocal result

Following testing with the reference anti-HCV assays indicated in Table 10, subjects were assigned an Intermediate HCV Status of “HCV Infected”, “Not HCV Infected”, or “Not Determined.” The first two categories did not require additional testing, however, the “Not Determined” category was further tested for HCV RNA by an FDA approved assay.

The following table compares the Elecsys Anti-HCV II Immunoassay results with the Intermediate HCV Status results according to Hepatitis Risk Group.

Table 11: Comparison of Elecsys Anti-HCV II Results to the Intermediate HCV Infection Status for the Adult Increased Risk Population

Hepatitis Ranked Risk Group	Intermediate HCV status						
	HCV infected		Not determined		Not HCV infected		
	Elecsys Anti-HCV II result						
	RX	NR	RX	NR	RX	NR	
Signs and symptoms	241	0	7	3	1	470	721
Clotting factor recipients	1	0	0	0	0	3	4
User of IV drugs	173	0	4	1	0	82	260
Dialysis	2	0	0	0	0	3	5
Transfusion/ transplant	6	0	0	0	0	21	27
High risk sex	70	0	4	5	1	684	764
Healthcare worker	4	0	0	0	1	89	94
Other risks	61	0	3	2	0	302	368
Total	557	0	18	11	3	1654	2243

The Intermediate HCV status of 29 subjects was "Not Determined." According to the algorithm, additional testing for HCV RNA was performed. The results of this testing are presented in the following table:

Table 12: Determination of the Final HCV Infection Status following HCV RNA Testing

Hepatitis Ranked Risk Group	Sample (n)	HCV RNA Result	Elecsys Anti-HCV II Result	Final HCV Infection Status
Signs and Symptoms	2	Negative	Non-reactive	Not HCV Infected
	1	Negative	Non-reactive	Not Determined
	4	Negative	Reactive	Not HCV Infected
	3	Negative	Reactive	Not Determined
User of IV Drugs	1	Negative	Non-reactive	Not HCV Infected
	3	Negative	Reactive	Not HCV Infected

Hepatitis Ranked Risk Group	Sample (n)	HCV RNA Result	Elecsys Anti-HCV II Result	Final HCV Infection Status
	1	Negative	Reactive	Not Determined
High Risk Sex	4	Negative	Non-reactive	Not HCV Infected
	1	Negative	Non-reactive	Not Determined
	3	Negative	Reactive	Not HCV Infected
	1	Positive	Reactive	HCV Infected
Other Risks	2	Negative	Non-reactive	Not HCV Infected
	2	Negative	Reactive	Not HCV Infected
	1	Negative	Reactive	Not Determined
Total	**29**			

Positive percent agreement (PPA) and negative percent agreement (NPA) between the Elecsys Anti-HCV II Immunoassay and the Final HCV Infection Status were calculated for both the at risk asymptomatic and symptomatic subjects and is shown in the following table:

Table 13: Percent Agreement between Elecsys Anti-HCV II and Final HCV Infection Status among Adult Study Subjects Ranked According to Risk for HCV Infection

Hepatitis Ranked Risk Group	Positive Percent Agreement (%)	95% Exact Confidence Interval	Negative Percent Agreement (%)	95% Exact Confidence Interval
Signs and Symptoms	99.59 (240/241)	97.71 to 99.99	98.33 (472/480)	96.74 to 99.28
Recipients of clotting factor	100.00 (1/1)	2.50 to 100.00	100.00 (3/3)	29.24 to 100.00
User of IV Drugs	100.00 (173/173)	97.89 to 100.00	95.40 (83/87)	88.64 to 98.73
Dialysis	100.00 (2/2)	15.81 to 100.00	100.00 (3/3)	29.24 to 100.00
Transfusion/Transplant	100.00 (6/6)	54.07 to 100.00	100.00 (21/21)	83.89 to 100.00
High Risk Sex	98.61 (71/72)	92.50 to 99.96	99.42 (688/692)	98.53 to 99.84
Healthcare Worker	100.00 (4/4)	39.76 to 100.00	98.89 (89/90)	93.96 to 99.97
Other Risks[a]	100.00 (61/61)	94.13 to 100.00	99.02 (304/307)	97.17 to 99.80
Total	99.64 (558/560)	98.72 to 99.96	98.81 (1663/1683)	98.17 to 99.27

[a] Other Risks included patients with any of the following: HIV infected or immunocompromised, prenatal exposure, family history of any hepatitis, living in high endemic areas for hepatitis, tattoo artists, morticians, individuals with history of incarceration, individuals sharing straw cocaine, and individuals with tattoo or body piercing.

The positive percent agreement between the Elecsys Anti-HCV II Immunoassay and the Final HCV Infection Status was 99.64% (558/560) with a 95% confidence

interval of 98.72 to 99.96%. The negative percent agreement between the Elecsys Anti-HCV II Immunoassay and the Final HCV Infection Status was 98.82% (1,663/1,683) with a 95% confidence interval of 98.17 to 99.27%. Among the various ranked risk categories, the positive percent agreement ranged from 98.61% to 100.00% with a median value of 100.00%. The negative percent agreement ranged from 95.40% to 100.00% with a median value of 99.22%.

The table below summarizes the overall agreement between the Elecsys Anti- HCV II Immunoassay and the Final HCV Infection Status for the 2,243 at-risk adult subjects.

Table 14: Elecsys Anti-HCV II Results versus HCV Infection Status Percent Agreement among the 2,243 Adult at Risk Study Subjects

	Final HCV Infection Status		
Elecsys Anti-HCV II Result	HCV Infected	Not Determined	Not HCV Infected
Reactive	558	5	15
Non-reactive	0	2	1663
Total	558	7	1678
PPA	99.64 (558/560)		
95% CI	98.72 to 99.96		
NPA	98.81 (1,663/1,683)		
95% CI	98.17 to 99.27		

The table below presents the agreement between the Elecsys Anti- HCV II Immunoassay and the Final HCV Infection Status for the 192 pediatric at risk subjects.

Table 15: Elecsys Anti-HCV II Results versus HCV Infection Status Percent Agreement among Pediatric at Risk Study Subjects

	Final HCV Infection Status		
Elecsys Anti-HCV II Result	HCV Infected	Not Determined	Not HCV Infected
Reactive	2	0	3
Non-reactive	0	0	187
Total	2	0	190
PPA	100.00 (2/2)		
95% CI	15.81 to 100.00		

	Final HCV Infection Status		
Elecsys Anti-HCV II Result	HCV Infected	Not Determined	Not HCV Infected
NPA	98.42 (187/190)		
95% CI	95.46 to 99.67		

Pediatric vs Adult Comparison (Analytical)

Because few positive subjects were identified in the pediatric at risk subjects table above, a spiking study was performed to compare the percent of analyte recovered from adult versus pediatric serum. Individual HCV-negative pediatric samples (31 samples) and HCV-negative adult samples (31 samples) were spiked with analyte from a high HCV-positive pediatric or adult sample up to 10% by volume of the sample being spiked. The level of spiking for the samples was high negative (6 samples), border zone (5 samples), and moderate positive (20 samples) in the range of 2-4 COI. All of the samples were tested in triplicate with the Elecsys Anti-HCV II Immunoassay before and after spiking. The difference in signal due to spiking was calculated by subtracting the value of the unspiked sample from the spiked sample. The obtained signal for a given concentration in the adult sample was compared to the corresponding pediatric sample. The deviation from adult to pediatric sample was calculated in percentage for each pediatric spiked sample as follows:

[Pediatric spiked COI - unspiked COI]/ [Adult spiked COI - unspiked COI] x 100%

The pediatric samples included in this study had the following age distributions: Ages 2-12: 74% and Ages 12 through 21: 26%. The acceptance criterion was a recovery of 80-120 %) compared with the reference sample.

The results are shown in the following table:

Table 16: Distribution of Percent Differences

Adult Spiked Observed Mean (Index)	Number Tested (n)	Distribution of % Bias		
		X < 10%	**10% ≤ X ≤ 20%**	**X > 20%**
Negative (0.7)	6	16.7% (1/6)	66.7% (4/6)	16.7% (1/6)
Cut-Off (0.8-1.0)	5	20.0% (1/5)	80.0% (4/5)	0.0% (0/5)
Positive (2.0-4.0)	20	15.0% (3/20)	85.0% (17/20)	0.0% (0/20)
Total	31	16.1% (5/31)	80.6% (25/31)	3.2% (1/31)

One sample out of 31 gave a result outside of the acceptance criterion with a recovery of 76% while the other 30 samples were found to be within specification. This sample was a high negative sample both before and after spiking. The results, together with recommendations that infants should not be tested prior to the disappearance of maternal antibodies, support a pediatric claim for 18 months to 21 years.

Pregnant Women

The adult and pediatric cohorts included 205 at risk pregnant women. There was one subject identified as infected (positive) and the remaining 204 were not infected (negative). The positive percent agreement between the Elecsys Anti HCV II Immunoassay results and the Final HCV Infection Status for the pregnant population was 100 % (1/1) with a 95 % confidence interval of 2.50 to 100 %. The negative percent agreement between the Elecsys Anti HCV II Immunoassay results and the Final HCV Infection Status status was 99.5 % (203/204) with a 95 % confidence interval of 97.3 to 99.99 %.

Table 17: Elecsys Anti-HCV II Results versus HCV Infection Status: Percent Agreement among the 205 at Risk Pregnant Women

	Final HCV Infection Status		
Elecsys Anti-HCV II Result	Infected	Not Determined	Not Infected
Reactive	1	0	1
Non-reactive	0	0	203
Total	1	0	204
PPA	100% (1/1)		
95% CI	2.50 to 100%		
NPA	99.5% (203/204)		
95% CI	97.3 to 99.99%		

Potential Cross-reactivity with HBV Infected Individuals

Samples were tested for Hepatitis B infection (HBV) in a population of 2,082 prospectively collected samples. HBV positive samples (n=43) were identified with commercially available FDA approved HBsAg and HBsAg Confirmatory Assays. The following table compares the Elecsys Anti-HCV II Immunoassay results with Final HCV Infection Status according to the ranking of the risk of HCV infection in these study subjects:

Table 18: Comparison of the Elecsys Anti-HCV II Results to the Final HCV Infection Status among Confirmed HBsAg Reactive Specimens (n = 43)

Hepatitis Rank Risk	**Final HCV Infection Status**				**Total**
	HCV Infected		**Not HCV Infected**		
	Elecsys Anti-HCV II Result				
	RX	**NR**	**RX**	**NR**	
Signs and Symptoms	3	0	0	9	12

User of IV Drugs	3	0	0	1	4
Transfusion/Transplan	0	0	0	1	1
High Risk Sex	0	0	0	20	20
Healthcare Worker	0	0	0	2	2
Other Risks	0	0	0	4	4
Total	6	0	0	37	43

The PPA between the Elecsys Anti-HCV II Immunoassay and the Final HCV Infection Status was 100% (6/6). The NPA was 100% (37/37).

Results from Seroconversion Commercial Panels

Seroconversion sensitivity of the Elecsys Anti-HCV II Immunoassay was evaluated by testing twenty commercially-sourced seroconversion panels and comparing the results with those obtained with an FDA approved comparator assay. The comparison of seroconversion timing for the Elecsys Anti-HCV II Immunoassay and for the comparator assay for each panel is presented in the following table:

Table 19: Results for Days to Evidence of HCV Seroconversion for Elecsys Anti-HCV II on the cobas e 601 module Compared to the Reference Assay

	Comparator Anti-HCV (Reference Test)		**Roche Elecsys Anti-HCV II**		**Difference in Days to Elecsys Anti-HCV II Reactivity vs Reference Test**
Panel ID	**NR**	**RX**	**NR**	**RX**	
6212	0	12	N/A[a]	0	1 to 12
6222	26	36	26	36	0
9041	31	62	31	62	0
9044	17	21	17	21	0
9045	32	37	26	32	1 to 5 days
9046	0	67	0	67	0
9047	21	28	21	28	0
9058	3	7	0	3	1 to 4 days
PHV911	3	14	3	14	0
PHV913	2	7	2	7	0
PHV914	9	12	5	9	1 to 3 days
PHV917	22	85	22	85	0
PHV918	16	24	16	24	0
PHV921	7	14	0	4	4 to 10 days
PHV922	10	14	10	14	0
PHV923	11	21	2	9	3 to 12 days

[a] According to Vendor Insert and Reference Test result, the sample was non-reactive on Day 0.

Genotype Detection

The study was performed to evaluate the ability of the Elecsys Anti HCV II Immunoassay to detect antibodies to various known HCV genotypes and subtypes.

Three genotyping panels from a commercial vendor were available for the genotype study and consisted of the following genotypes, as determined by the specimen vendor with commercially available HCV RNA assays: 1, 2, 3, 4, 5 and 6. The panels were tested with the Elecsys Anti HCV II Immunoassay and the reference anti-HCV assay and final results were compared. The positive samples were all detected by the Elecsys Anti-HCV II Immunoassay.

D. Safety and Effectiveness Results of the Clinical Studies

1. Safety Results

   As an *in vitro* diagnostic test, the Elecsys Anti-HCV II Immunoassay involves removal of blood from an individual for testing purposes. The test, therefore, presents no more safety hazard to an individual being tested than other tests where blood is drawn.

   False positive and false negative results are discussed in Section VIII. There were no adverse effects of the device reported while the study was conducted.

2. Effectiveness Results

   Multi-centered clinical studies were conducted in the US. The observed clinical sensitivity and specificity of the Elecsys Anti-HCV II Immunoassay were comparable to current commercially available, FDA approved assays.

   The prospective study resulted in a positive percent agreement between the Elecsys Anti-HCV II immunoassay and the patient Final HCV Infection Status in the adult at-risk population of 99.64% (558/560) with a 95% confidence interval of 98.72-99.96%. The negative percent agreement between the Elecsys Anti-HCV II immunoassay and the Final HCV Infection Status in the adult at-risk population was 99.82% (1663/1683) with a 95% confidence interval of 98.17-99.27%.

   The prospective study resulted in a positive percent agreement between the Elecsys Anti-HCV II Immunoassay and the Final HCV Infection Status in the pediatric at-risk population of 100% (2/2) with a 95% confidence interval of 15.81-100%. The negative percent agreement between the Elecsys Anti-HCV II immunoassay and the Final HCV Infection Status in the pediatric at-risk population was 98.42% (187/190) with a 95% confidence interval of 95.46-99.67%.

   Testing of 16 seroconversion panels generated data that reflected the sensitivity of the Elecsys Anti-HCV II Immunoassay. Results from the Elecsys Anti-HCV II Immunoassay were equivalent to the results of the reference assay in 10 of the 16 seroconversion panels. Seroconversion was detected by the Elecsys Anti-HCV II Immunoassay earlier than the reference assay in 6 panels.

   The specificity of the Elecsys Anti-HCV II Immunoassay when tested for potential cross reactivity with samples from individuals with various medical

conditions and with antibodies reactive with various bacteria and viruses showed minimal reactivity, thus indicating low risk of false positive results by the test device.

Overall, the clinical studies showed the effectiveness of the Elecsys Anti-HCV II Immunoassay in detecting accurately the presence of antibodies to hepatitis C virus.

3. Subgroup Analyses
   Not applicable.

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included 7 investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2.(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the FDA Microbiology Devices Advisory Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

- The Elecsys Anti-HCV II Immunoassay performance is acceptable when testing serum and plasma (potassium EDTA, lithium heparin, sodium heparin, and sodium citrate).
- There are no issues with endogenous interferents at physiological levels or with commonly administered medications.
- Samples are stable when refrigerated for 7 days (2-8°C), frozen for 3 months (-20°C), or at room temperature for 3 days (25°C). The samples can also withstand 6 freeze/thaw cycles.
- The Elecsys Anti-HCV II Immunoassay kit can withstand stress at 25°C for one week. It is stable for 8 weeks after opening when stored at 2-8°C. It is stable on-board the cobas e 601 analyzer for 31 days and when alternated from analyzer and refrigeration for 80 hours.

- The Elecsys Anti-HCV II calibrators are stable on-board the cobas e 601 analyzer for 2 hours. The calibrators are stable for 8 weeks when stored at 2-8°C. Calibration is stable for one month when using multiple kits from the same reagent lot and for 7 days when using the same reagent kit.
- The Elecsys PreciControl Anti-HCV controls can withstand stress at 35°C for one week. They are stable for 8 weeks after opening when stored at 2-8°C, and are stable on-board the cobas e 601 for 5 hours.
- The preservatives that the Elecsys Anti-HCV II assay reagents and controls contain have been shown to meet USP Chapter 51 criteria.
- The Elecsys Anti-HCV II Immunoassay demonstrated precision estimates that met internal specifications for repeatability and intermediate precision. The repeatability ranged from 1.20 to 1.25% CV for the positive samples and 1.86 to 4.01% CV for the negative samples. The within-laboratory precision ranged from 2.33 to 2.59% CV for the positive samples and 3.12 to 4.23% CV for the negative samples
- Reproducibility of the Elecsys Anti-HCV II immunoassay was acceptable from run to run, day to day, reagent lot to reagent lot, and site to site. The between-site reproducibility study ranged from 4.2 to 7.3% CV for samples with COI values > 1.0 (positives).
- The clinical performance was evaluated in an ethnically diverse population representative of the intended use population and of different HCV infected groups. The positive and negative percent agreement values obtained for the Elecsys Anti-HCV II Immunoassay relative to Final HCV Infection Status algorithm were acceptable.

B. Safety Conclusions

The adverse effects of the device are based on data collected in clinical studies conducted to support PMA approval as described above. As a diagnostic test, the Elecsys Anti-HCV II Immunoassay for use on the cobas e 601 immunoassay analyzer involves removal of blood from an individual for testing purposes. The test therefore presents no more safety hazard to an individual being tested than other tests where blood is drawn.

False positive and false negative results are discussed in Section VIII. There were no adverse effects of the device reported while the study was conducted.

C. Benefit-Risk Conclusions

The probable benefits of the device are also based on data collected in the clinical studies conducted to support PMA approval as described above. Detection of antibodies to HCV is an important component of hepatitis C diagnosis. Although it cannot be used by itself to determine the state of infection (active or resolved), a positive antibody result is followed by additional HCV RNA testing.

The risks from use of the Elecsys Anti-HCV II Immunoassay are false positive and false negative results. In both cases, risks are mitigated since HCV status would always be interpreted in conjunction with HCV RNA, serum markers of hepatic inflammation, and clinical history.

There are risks to the phlebotomist who obtains the blood and sample and to the laboratory technician; however, these are anticipated for any blood sample and do not add additional risk above that expected in laboratory practice.

In conclusion, given the available information above, the data support that for the qualitative detection of HCV antibodies in human serum or plasma by the Elecsys HCV II immunoassay, the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. The rate of false positive and false negative results is within acceptable limits compared with previously approved tests. Therefore, this device should benefit the physician in the diagnosis and management of HCV infected patients.

XIII. CDRH DECISION

CDRH issued an approval order on June 11, 2015. The final conditions of approval cited in the approval order are described below.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.
Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.
Post-approval Requirements and Restrictions: See approval order.