# SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

## I. GENERAL INFORMATION

| | |
|---|---|
| Device Generic Name: | Hepatitis B Surface Antigen (HBsAg)<br>Hepatitis B Surface Antigen Confirmatory Test Kit<br>Hepatitis B Surface Antigen Control Material |
| Device Trade Name: | Elecsys HBsAg II<br>Elecsys HBsAg Confirmatory Test<br>PreciControl HBsAg II |
| Device Procode: | LOM |
| Applicant's Name and Address: | Roche Diagnostics<br>9115 Hague Road<br>Indianapolis, IN 46250 |
| Date(s) of Panel Recommendation: | Not Applicable |

Premarket Approval Application (PMA) Number: P160019

Date of FDA Notice of Approval: December 23, 2016

## II. INDICATIONS FOR USE

1. Elecsys HBsAg II

Immunoassay for the in vitro qualitative detection of hepatitis B surface antigen (HBsAg) in human adult and pediatric (2 to 21 years of age) serum and plasma (sodium heparin, lithium heparin, $K_2$-EDTA, sodium citrate). Assay results, in conjunction with other serological and clinical information, may be used for the laboratory diagnosis of individuals at risk for infection with HBV or with signs and symptoms of hepatitis. In addition, this assay may be used to screen for hepatitis B infection in pregnant women to identify neonates at high risk of acquiring HBV during the perinatal period.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on the **cobas e** 601 immunoassay analyzer.

2. Elecsys HBsAg Confirmatory Test

*For Elecsys HBsAg Confirmatory Test used with Elecsys HBsAg immunoassay:*
Immunoassay for in vitro qualitative confirmation of the presence of hepatitis B surface antigen in human serum and plasma (sodium heparin, $K_3$-EDTA, sodium citrate) samples repeatedly reactive when tested with the Elecsys HBsAg immunoassay. This assay is intended for use on the Elecsys and **cobas e** immunoassay analyzers.

*For Elecsys HBsAg Confirmatory Test used with Elecsys HBsAg II:*
Immunoassay for in vitro qualitative confirmation of the presence of hepatitis B surface antigen in human serum and plasma (sodium heparin, lithium heparin, $K_2$-EDTA, sodium citrate) samples repeatedly reactive when tested with Elecsys HBsAg II. This assay is intended for use on the **cobas e** 601 immunoassay analyzer.

3. PreciControl HBsAg II

PreciControl HBsAg II is used for quality control of the Elecsys HBsAg II immunoassay on the **cobas e** 601 immunoassay analyzer. The performance of PreciControl HBsAg II has not been established with any other HBsAg assay.

## III. CONTRAINDICATIONS

There are no known contraindications for use for this test.

## IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the labeling for the Elecsys HBsAg II, the Elecsys HBsAg Confirmatory Test, and the PreciControl HBsAg II.

## V. DEVICE DESCRIPTION

Principle of Device Methodology

**Elecsys HBsAg II**
The Elecsys HBsAg II is a qualitative serologic, two- incubation step assay using a sandwich test format and a total assay time of 18 minutes that enables detection of HBsAg. The assay is performed on the **cobas e** 601 immunoassay analyzer. The steps involved in the detection are as follows:

i) First incubation: 50 µL of sample, a mixture of monoclonal anti-HBsAg antibody and polyclonal anti-HBsAg antibodies labeled with a ruthenium complex, and two biotinylated monoclonal anti-HBsAg antibodies form a sandwich complex.
ii) Second incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
iii) 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 M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
iv) 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 cut-off value previously obtained by calibration.

**Elecsys HBsAg Confirmatory Test**
Elecsys HBsAg Confirmatory Test is an independent neutralization test used for further investigation of the repeatedly reactive samples. Samples confirmed by neutralization with human anti-HBs are regarded as positive for HBsAg.

**PreciControl HBsAg II**
The PreciControl HBsAg II is used for quality control testing of the Elecsys HBsAg II on the **cobas e** 601 immunoassay analyzer. It consists of two components: PC HBSAGII1, which contains human serum negative for HBsAg, and PC HBSAGII2, which contains human serum that is positive for HBsAg.

Kit Configurations and Components

**Elecsys HBsAg II**
*Package Sizes*
The Elecsys HBsAg II is produced in two different package sizes: one package contains reagents for up to 200 tests and the other contains reagents for up to 100 tests.

*Components*
**Elecsys HBsAg II**
The Elecsys HBsAg II 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 0.72mg/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:** Reagent R1 contains specific biotinylated mouse monoclonal anti-HBsAg at a concentration of 0.5 mg/L in buffer with preservative.
>
> **Component 3:** Reagent R2 contains mouse and sheep specific monoclonal and polyclonal antibodies anti- HBsAg labeled with ruthenium complex at a concentration of 1.5 mg/L in buffer with preservative.
>
> **Component 4:** HBSAG II Cal1 is the negative calibrator and consists of human serum negative for HBsAg with preservative.
>
> **Component 5:** HBSAG II Cal2 is the positive calibrator and consists of human serum positive for HBsAg with preservative.

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

**Elecsys HBsAg Confirmatory Test**
The Elecsys HBsAg Confirmatory Test is comprised of two reagents for sample pretreatment for any sample found to be repeatedly reactive:

**Component 1:** The confirmatory reagent contains specific human antibodies to HBsAg at a level > 200,000 IU/L in human serum with preservatives. The base serum is non-reactive for HBsAg, anti-HCV, and anti-HIV 1+2, with preservatives.

**Component 2:** The control reagent contains human serum with antibodies to HBsAg at a concentration < 3 IU/L. The base serum is non-reactive for HBsAg, anti-HBs, anti-HCV, and anti-HIV 1+2, with preservatives.

**PreciControl Elecsys HBsAg II**
The PreciControl HBsAg II kit is comprised of the following two reagents:

**Component 1:** The PreciControl 1 (PC HBSAGII1) is the negative control, which consists of buffered and preserved human serum matrix, negative for HBsAg.

**Component 2:** The PreciControl 2 (PC HBSAGII2) is the positive control, which consists of buffered and preserved inactivated human serum matrix and contains HBsAg at approximately 0.2 IU/mL.

**Elecsys HBsAg II calibrators**
The Elecsys HBsAg II kit is comprised of the following two reagents:

**Component 1:** Calibrator 1 (negative), HBSAG II Cal 1, consists of buffered and preserved human serum matrix negative for HBsAg.

**Component 2:** Calibrator 2 (positive), HBSAG II Cal 2, consists of buffered and preserved inactivated human serum positive for HBsAg.

The presence or absence of HBsAg in the sample is determined by comparing the electrochemiluminescence signal in the reaction to the cut-off signal determined from an active Elecsys HBsAg 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 sample/cut-off) along with a result interpretation as follows.

Samples with a COI of < 0.90 are non-reactive in the Elecsys HBsAg II assay. These samples are considered negative for HBsAg and do not require further testing. Samples with an initial COI of ≥ 1.0 are considered initially reactive. Samples with a COI of ≥ 0.90 to < 1.0 are considered borderline. All initially reactive or borderline samples should be reassayed in duplicate using the Elecsys HBsAg II assay. If COI values of < 1.0 are found in both cases, the sample is considered negative for HBsAg. Initially reactive or borderline samples giving COI values of ≥ 1.0 in two out of the three determinations are deemed repeatedly reactive. Repeatedly reactive samples must be confirmed using an independent neutralization test (Elecsys HBsAg Confirmatory Test). Samples confirmed by neutralization with human anti-HBs are regarded as positive for HBsAg.

**Table 1: Interpretation of HBsAg II Testing**

| Initial Elecsys HBsAg II Assay Result | | | |
|---|---|---|---|
| **COI** | **Initial Result** | **Interpretation of Initial Results** | **Retest Procedure** |
| **< 0.90** | Non-reactive | No HBsAg detected | No retest required. |
| **0.90 ≤ COI < 1.00** | Border | Borderline zone (undetermined) | All initially reactive or borderline samples should be retested in duplicate using the Elecsys HbsAg II |
| **≥ 1.00** | Reactive | HBsAg detected | |

**Table 2: Interpretation of Repeat HBsAg II Testing**

| Final Elecsys HBsAg II Assay Results | | | |
|---|---|---|---|
| **Initial Result** | **Result after Retest (COI)** | **Final Results** | **Interpretation of Results** |
| **Non-reactive** | No retest required | NON-REACTIVE | HBsAg not detected; does not exclude the possibility of exposure to HBV* |
| **Border** | If 2 of the 3 results have a COI < 1.0 | NON-REACTIVE | HBsAg not detected; does not exclude the possibility of exposure to HBV* |
| | If 2 of the 3 results have a COI ≥ 1.0 | REPEATEDLY REACTIVE | Presumptive evidence of HBV. Repeatedly reactive samples must be confirmed using a neutralization test (Elecsys HBsAg Confirmatory Test) |
| **Reactive** | If 2 of the 3 results have a COI ≥ 1.0 | REPEATEDLY REACTIVE | |
| | If 2 of the 3 results have a COI < 1.0 | NON-REACTIVE | HBsAg not detected; does not exclude the possibility of exposure to HBV* |

*A negative test result does not exclude with certainty a possible exposure to or an infection with HBV. Negative test results obtained for persons with a past exposure may be caused by an antigen concentration below the detection limit of this assay or the lack of reactivity of the antigens to the antibodies used in this assay.

## VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are currently several FDA approved *in vitro* diagnostic tests for detecting serological markers of hepatitis B virus (HBV). 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 B viral infection. Each alternative has its own advantages and disadvantages. Patients should fully discuss alternatives with their physicians to select the method that best meets expectations.

## VII. MARKETING HISTORY

The Elecsys HBsAg II, Elecsys HBsAg Confirmatory Test and PreciControl HBsAg II are currently marketed in multiple countries. The device has not been withdrawn to date from the market in any country for reasons relating to safety and effectiveness. The following table provides the list of countries where the product is distributed.

**Table 3: Countries in which Elecsys HBsAg II is Marketed**

| | | | |
|---|---|---|---|
| Argentina | Ecuador | Mexico | Slovakia |
| Australia | Egypt | Middle East | Slovenia |
| Austria | Finland | Myanmar | South Africa |
| Baltics | France | Netherlands | Spain |
| Belgium | Germany | New Zealand | Sweden |
| Brazil | Greece | Norway | Switzerland |
| Canada | Hong Kong | Pakistan | Taiwan |
| Central America | Hungary | Peru | Thailand |
| Chile | India | Philippines | Turkey |
| China | Indonesia | Poland | United Kingdom |
| Colombia | Italy | Portugal | Uruguay |
| Croatia | Japan | Romania | Venezuela |
| Czech Republic | Korean Republic | Russian Federation | Vietnam |
| Denmark | Malaysia | Singapore | Slovakia |

## 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 a false diagnosis and improper patient management.

The diagnosis of HBV infection requires the evaluation of the patient's blood for serological markers for HBV where a positive result is followed up with nucleic acid testing for HBV DNA.

A false non-reactive (false negative) HBsAg result may lead to a patient with hepatitis B infection 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 HBV infection to others through sexual contact or exposure to parenteral fluids, or to the neonate if the patient is pregnant. However, it is likely that if a patient is known to be at high risk of HBV infection, or in the presence of symptoms or unexpectedly elevated liver function tests, additional testing would be performed (e.g., nucleic acid testing), or serological testing would be repeated.

A false reactive (false positive) result using an HBsAg assay is not considered a patient or public health concern because a reactive result would be further investigated with the Elecsys HBsAg Confirmatory Test as a neutralization test. No treatment of the patient would be initiated until the testing was confirmed.

The risk of incorrect test results is inherent with all *in vitro* diagnostic products. In the unusual setting where a false reactive result was ultimately derived, likely subsequent testing for hepatitis B Virus (HBV) DNA by a nucleic acid test would identify the false positive result, as would atypical results from the hepatitis B serological panel (i.e., other serological assays used to identify stage of hepatitis B Virus infection).

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 PRE-CLINICAL STUDIES

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

**Establishment of Cut-off:**
The analyzer automatically calculates the cut-off based on the measurement of HBsAg II Cal1 (Cal 1) and HBsAg II Cal2 (Cal 2). The cut-off for the Elecsys HBsAg II is calculated from the signals of the negative calibrator (Cal1) and the positive calibrator (Cal2) according to the following cut-off formula:

Cut-off= (0.175 x counts Cal1) + (0.0396 x counts Cal2)

The test result is calculated in the form of a cut-off index (COI) equal to test signal/cut-off where the test signal is corrected for background.

**Verification of Cut-off:**
The cut-off was verified by testing 279 samples from blood donors and commercial sources. These samples were characterized on the Elecsys 2010 (master system) as negative and positive, respectively. The Clinical Study report provides additional cut-off validation with clinical specimens that supports the transferability of this cut-off algorithm to the **cobas e** 601 analyzer.

**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-A2.

The LoB was determined by testing five HBsAg negative serum samples, in duplicate, 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. Data analysis was based on determination of the $95^{th}$ percentile of the 60 measured values.

The LoD was determined by testing five serum samples with low analyte concentration with two lots, in duplicate, 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 where SD is the Standard Deviation

Test results were reported in IU/ml. The acceptance criteria were LoB $\leq$ 0.5 IU/ml and LoD $\leq$ 0.05 IU/ml. The LoB was determined to be 0.000 IU/ml. The LoD for two lots of reagents was determined to be 0.0034 IU/ml for one lot, and 0.0051 IU/ml for the other lot.

**High Dose Hook Effect**
Three high titer positive human samples and one human sample spiked with purified HBsAg were each diluted in a negative dilution medium (Diluent Universal) in at least 11 dilution steps to generate dilution series that cover the range from negative to high positive s/co values. The diluted samples were measured in triplicate. The acceptance criterion was no false negative results for the tested samples. At very high HBsAg concentrations, a high dose hook effect was observed; however, no false negative results were observed. The high dose hook effect was observed with concentration of HBsAg where the median s/co was > 4598. Analysis of the sample distribution in the clinical study showed that approximately 0.77% of samples had s/co values > 4598 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 HBsAg II is produced in two different package sizes: 100 and 200 test kits. The two kit sizes differ in the volumes of microparticle beads in bottle M, biotinylated antibodies R1, and ruthenylated antibodies R2. Calibrators 1 and 2 have the same filling volume. 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.

**Endogenous Interferences**
This study evaluated the effect of elevated levels of hemoglobin (from 0 to 2.2 g/dL), bilirubin (from 0 to 44 mg/dL), lipemia (intralipid) (from 0 to 2,200 mg/dL), biotin (from 0 to 44 ng/mL), and total protein (ranging from 0 to 22 g/dL) on the Elecsys HBsAg II assay.

Several interfering agents were tested using natural or spiked serum samples. Each potentially interfering endogenous agent was tested at 10 levels. All calculations were based on COI values. Samples were tested in duplicate. Percent mean recovery of the COI value of a sample spiked with an interfering substance was calculated against the respective sample without the interfering substance.

The following HBsAg samples were measured:

| Sample | Target Range (COI) |
|---|---|
| Negative | 0.228 – 0.399 |
| High negative | 0.807 – 0.902 |
| Low positive | 1.03 – 1.12 |
| Positive | 2.42 – 2.8 |

The acceptance criteria for mean recovery when compared to the initial unspiked result were:
Samples < 0.7 COI: Recovery ≤ COI (reference sample) + 0.3
Samples ≥ 0.7 COI: Mean Recovery 80 – 120%; Recovery for individual samples 70 – 130%.

The results of this study demonstrated that samples containing hemoglobin up to 2.2 g/dL, bilirubin up to 44 mg/dL, lipemia up to 2200 mg/dL, biotin up to 44 ng/mL, and total protein up to 22 g/dL test accurately with the Elecsys HBsAg II. The following levels for non-interference are claimed in the package insert:

Hemoglobin 2.2 g/dL
Bilirubin 40 mg/dL
Lipids 2,200 mg/dL
Biotin 44 ng/mL
Total protein 22 g/dL

**Matrix Effects**
Studies were conducted to verify the types of blood collection tubes that can be used with the Elecsys HBsAg II. Samples were collected into matched serum and plasma collection tubes from 43 donors and assayed in duplicate on the **cobas e** 601 immunoassay analyzer.

Forty three (43) matched pairs were collected in the evaluation of each of the following blood collection tubes:

Serum gel separation
Sodium heparin plasma
Sodium citrate plasma
Lithium heparin plasma
$K_2$-EDTA plasma

The majority of samples were processed by spiking with equivalent levels of HBsAg to cover the whole measuring range:

| | |
|---|---|
| Negative | (targeted to approximately ≤ 0.5 COI) |
| High negative | (targeted to approximately 0.93 COI) |
| Low Positive | (targeted to approximately 1.06 COI) |
| Moderate Positive | (targeted to approximately 2 – 3 COI) |

The acceptance criteria were as follows:
Samples < 0.7 COI: Recovery ≤ COI (reference sample) + 0.3
Samples ≥ 0.7 COI: Mean Recovery 80 – 120%; Recovery for individual samples 70 – 130%.

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, as indicated in tables 4 to 8 below.

**Table 4: Statistics for Serum/Plasma Comparison – Serum Gel Separation Tubes vs. Serum**

| | | | | |
|---|---|---|---|---|
| **Serum Gel Separation vs. Serum** | **Correlation Coefficient** | **0.999** | **Confidence Interval** | |
| | **N** | **43** | **Lower 95 % Cl** | **Upper 95 % Cl** |
| | **Y-intercept** | -0.005 | -0.0219 | 0.0149 |
| | **Slope** | 0.992 | 0.975 | 1.015 |

**Table 5: Statistics for Serum/Plasma Comparison – Lithium Heparin Plasma vs. Serum**

| | | | | |
|---|---|---|---|---|
| **Lithium Heparin Plasma vs. Serum** | **Correlation Coefficient** | **0.998** | **Confidence Interval** | |
| | **N** | **43** | **Lower 95 % Cl** | **Upper 95 % Cl** |
| | **Y-intercept** | 0.0570 | 0.0382 | 0.0773 |
| | **Slope** | 0.987 | 0.968 | 1.007 |

**Table 6: Statistics for Serum/Plasma Comparison – Sodium Heparin Plasma vs. Serum**

| | | | | |
|---|---|---|---|---|
| **Sodium Heparin Plasma vs. Serum** | **Correlation Coefficient** | **0.998** | **Confidence Interval** | |
| | **N** | **43** | **Lower 95 % Cl** | **Upper 95 % Cl** |
| | **Y-intercept** | 0.0482 | 0.0331 | 0.0688 |
| | **Slope** | 0.991 | 0.968 | 1.016 |

**Table 7: Statistics for Serum/Plasma Comparison – $K_2$-EDTA Plasma vs. Serum**

| | | | | |
|---|---|---|---|---|
| **$K_2$-EDTA Plasma vs. Serum** | **Correlation Coefficient** | **0.999** | **Confidence Interval** | |
| | **N** | **43** | **Lower 95 % Cl** | **Upper 95 % Cl** |
| | **Y-intercept** | 0.0322 | 0.0167 | 0.0465 |
| | **Slope** | 1.029 | 1.012 | 1.049 |

**Table 8: Statistics for Serum/Plasma Comparison – Sodium Citrate Plasma vs. Serum**

| | | | | |
|---|---|---|---|---|
| **Sodium Citrate Plasma vs. Serum** | **Correlation Coefficient** | **0.998** | **Confidence Interval** | |
| | **N** | **43** | **Lower 95 % Cl** | **Upper 95 % Cl** |
| | **Y-intercept** | 0.0377 | 0.0143 | 0.0539 |
| | **Slope** | 1.019 | 0.998 | 1.046 |

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

Serum gel separation
Sodium heparin plasma
Sodium citrate plasma
Lithium heparin plasma
$K_2$-EDTA plasma

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

**Table 9: Drugs Tested with the Elecsys HBsAg II**

| 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 |

| Compound | Concentration |
|---|---|
| Acetylsalicylic acid | 1,000 mg/L |
| Rifampicin | 60 mg/L |
| Acetaminophen | 200 mg/L |
| Ibuprofen | 500 mg/L |
| Theophylline | 100 mg/L |
| PEG interferon- alpha | 180 µg/L |
| Lamivudin | 300 mg/L |
| Entecavir | 0.5 mg/L |
| Telbivudine | 600 mg/L |
| Adefovir | 10 mg/L |

The acceptance criteria were as follows:
Samples < 0.7 COI: Recovery ≤ COI (reference sample) + 0.3
Samples ≥ 0.7 COI: Mean Recovery 80 – 120%; Recovery for individual samples 70 – 130%.

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

Since these studies were performed *in vitro*, they may not assess the potential interference that might be seen after the drugs are metabolized *in vivo*.

**HAMA Effect**
The purpose of this study was to evaluate the potential interference of human anti-mouse antibodies (HAMA) with the Elecsys HBsAg II. To determine the effect of HAMA, five negative samples were spiked with HAMA at ten concentration levels with an upper concentration of 3,200 ng/mL and tested in duplicate.

The acceptance criteria were as follows:
No false positive result up to 2,500 ng/mL HAMA.

The results fulfill the acceptance criteria. There is no influence on the Elecsys HBsAg II test up to 3,200 ng/ml HAMA.

**Carryover Study**
The use of disposable tips for sample pipetting on the **cobas e** 601 immunoassay analyzer should eliminate the 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 HBsAg negative sample was tested in triplicate with the Elecsys HBsAg II. Thereafter, a high signal generating HBV sample (≥ 2 million counts) was tested in triplicate followed again by testing of the HBsAg negative sample in triplicate. This procedure was performed seven times with six different HBsAg negative samples.

The acceptance criteria were as follows:

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 102% to 116%. The signal count values were within the acceptance criteria. This study demonstrates that there is no measurable bead or signal carryover with the Elecsys HBsAg.

**Stability Studies**

***Sample Stability Studies***

Four studies were performed to verify the stability of patient serum and plasma samples at the following concentrations:

Negative targeted to ≤ 0.5 COI
High negative targeted to 0.6 to < 0.8 COI
Low positive targeted to 1.0 to 1.4 COI
Moderate positive targeted to 2.0 to 5.0 COI

The four studies are described below:

1) Four serum and plasma samples were stored for up to 14 days at 2 to 8°C. The time points tested were 0 (unstressed), 1, 3, 7, and 14 days and samples were measured in triplicate. Recoveries after storage for 1, 3, 7, and 14 days at 2-8°C were calculated relative to the median COI at day 0.

2) Four serum and plasma samples were stored for up to 6 months at -20°C (time points tested were unstressed, after 2 weeks, and after 1, 2, 3 and 6 months) and measured in triplicate at each time point. Recoveries after storage for 2 weeks, 1 month, 2 months, 3 months, and 6 months at -20°C were calculated relative to the median COI at day 0.

3) Four serum and plasma samples were stored for 1, 2, 3 and 6 days at 25°C (measurements unstressed and after 1, 2, 3, and 6 days) and measured in triplicate. Recoveries after storage for 1 day, 2 days, 3 and 6 days at 25°C were calculated relative to the median COI at day 0.

4) Four serum and plasma samples were subjected to multiple freeze/thaw cycles (up to 6 cycles) 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 median COI of the unstressed sample.

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

The acceptance criteria were as follows:
Samples < 0.7 COI: Recovery ≤ COI (reference sample) + 0.3
Samples ≥ 0.7 COI: Mean Recovery 80 – 120%; Recovery for individual samples 70 – 130%.

The acceptance criteria were met for all serum and plasma samples. These studies indicate that serum and plasma samples may be stored for 14 days at 2-8°C, 6 months at -20°C, 6 days at 25°C, and can be subjected to 6 freeze and thaw cycles prior to testing by the Elecsys HBsAg II.

***Reagent Stability Studies***

**Reagent Real Time (Shelf Life) Stability**
Testing was performed on three lots of the 100 test kit configuration and on one lot of the 200 test kit configuration. The kits were stored at the recommended storage temperature of 2-8$^{o}$C in a temperature-controlled area for the duration of the stability studies. Testing included measurement of five human samples and the PreciControls (PC1 and PC2) in duplicate. The acceptance criteria were met and the study confirmed a claimed shelf life for the unopened Elecsys HBsAg II kit of 12 months at 2-8$^{o}$C.

**Reagent Temperature Stress Stability**
This study was conducted to determine the effect of elevated temperature stress on the Elecsys HBsAg II during transportation. The 100 and the 200 test kit configurations were stressed for one week at 25$^{o}$C (transportation is performed under cooled conditions). Four human serum samples (negative, high negative, low positive, and positive) and both 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$^{o}$C). Recoveries of the samples were calculated.

The acceptance criteria were as follows:
Samples < 0.7 COI: Recovery ≤ COI (reference sample) + 0.3
Samples ≥ 0.7 COI: Mean Recovery 80 – 120%; Recovery for individual samples 70 – 130%.

For samples < 0.7 COI, the change in COI ranged from 0.004 to 0.044. For samples > 0.7 COI, the recovery ranged from 94% to 100%. The acceptance criteria were met. This study confirms a claimed stability of the Elecsys HBsAg II reagents for 1 week at 25$^{o}$C.

**On-Board Stability - Open Reagent Pack**
This study was performed to determine the time period for which the Elecsys HBsAg II reagents can be stored on the analyzer once opened. The reagent packs of the 100 and 200 test kit configurations were stored on board for 4 weeks at 20$^{o}$C ± 3$^{o}$C. Each week, the reagent packs were checked with regard to stability of the weekly calibration. Unstressed reagent packs of the 100 and 200 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$^{o}$C) and with the reagent packs which were stressed on-board for 1, 2, 3, and 4 weeks. For each test time point, the calibration occurred seven days prior. 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.

The acceptance criteria were as follows:
Samples < 0.7 COI: Recovery ≤ COI (reference sample) + 0.3
Samples ≥ 0.7 COI: Mean Recovery 80 – 120%; Recovery for individual samples 70 – 130%.

For HBsAg negative samples, the recovery ranged from 0.005 to 0.088 COI. For HBsAg positive samples, the recovery ranged from 93% to 117%. All acceptance criteria were met for each of the time points tested. This study supports on-board reagent stability of 4 weeks at 20$^{o}$C ± 3$^{o}$C.

**Reagent Stability after First Opening**
This study was performed to determine the time period over which the Elecsys HBsAg II kits can be kept at 2-8°C once opened. One 100 test kit and one 200 test kit were opened and the **cobas e** 601 analyzer was calibrated. Four human serum samples (negative, high negative, low positive, and positive) and the two Elecsys PreciControl HBsAg II controls were tested with the opened reagent unstressed (day 0) and after 4 and 8 weeks at 2-8°C in a refrigerator. The reagent pack stability was determined by calculating the recovery (COI) of PreciControls and serum samples with stressed reagents compared to the COI results with unstressed reagents.

The acceptance criteria were as follows:
Samples < 0.7 COI: Recovery ≤ COI (reference sample) + 0.3
Samples ≥ 0.7 COI: Mean Recovery 80 – 120%; Recovery for individual samples 70 – 130%.

For HBsAg negative samples, the recovery ranged from 0 to 0.024 COI. For HBsAg positive samples, the recovery ranged from 93% to 113%. All acceptance criteria were met for each of the time points tested. The study confirms a claimed stability for the Elecsys HBsAg II reagent stored for 6 weeks, at 2-8°C after first opening.

**On-Board/Refrigerated Stability**
Stability studies were performed to determine the time period over which the Elecsys HBsAg II reagent packs can be kept in the refrigerator and, alternately moved back and forth from the refrigerator to the analyzer where they are maintained at 20°C $\pm$ 3°C (before being placed on board the analyzer, the reagent packs were stabilized for 1 hour at room temperature). Reagent packs from one 100 test kit and one 200 test kit were stored for 6 weeks in a refrigerator at 2-8°C and each week were moved on-board the **cobas e** 601 immunoassay analyzer at 20°C ± 3°C (up to 42 hours total) and then back to the refrigerator. At 1, 2, 3, 4, 5, and 6 weeks the reagent packs were checked with regard to stability.

Unstressed reagent packs (stored at 2-8$^{o}$C) of the 100-test kit and the 200-test kit were used as controls. Four human serum samples and the negative and positive PreciControl HBsAg II controls were tested in duplicate with the stressed reagent packs (alternate storage between the refrigerator and the analyzer) at weeks 1, 2, 3, 4, 5, and 6 with weekly calibration and with the unstressed reagent packs. Recovery for each sample was calculated based on COI of the stressed versus the unstressed conditions.

The acceptance criteria were as follows:
Samples < 0.7 COI: Recovery ≤ COI (reference sample) + 0.3
Samples ≥ 0.7 COI: Mean Recovery 80 – 120%; Recovery for individual samples 70 – 130%.

For HBsAg negative samples, the recovery ranged from -0.008 to 0.057 COI. For HBsAg positive samples, the recovery ranged from 94% to 118%. All acceptance criteria were met for each of the time points tested. The study confirms a claimed stability for the Elecsys HBsAg II reagents stored alternately in the refrigerator up to 6 weeks and on-board the **cobas e** 601 analyzer up to 42 hours.

***Calibration Stability Studies***

**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 HBsAg II reagent lot was tested on three separate **cobas e** 601 instruments. Four human serum samples (negative, high negative, low positive, and positive) and PreciControls 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 PreciControl HBsAg II measurements.
The acceptance criteria were as follows:
Samples < 0.7 COI: Recovery ≤ COI (reference sample) + 0.3
Samples ≥ 0.7 COI: Mean Recovery 80 – 120%; Recovery for individual samples 70 – 130%.

The studies confirm calibration stability of one month (28 days) with multiple kits from the same reagent lot. The product labeling instructs that calibration should be repeated 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 HBsAg II 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 the **cobas e** 601 analyzer was calibrated. Four human serum samples (negative, high negative, low positive, and positive) and the

Elecsys PreciControl HBsAg II 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 from unstressed reagents. Recovery for each sample (stressed/unstressed) was calculated based on COI.

The acceptance criteria were as follows:
Samples < 0.7 COI: Recovery ≤ COI (reference sample) + 0.3
Samples ≥ 0.7 COI: Mean Recovery 80 – 120%; Recovery for individual samples 70 – 130%.

For samples < 0.7 COI, the change in recovery ranged from 0.0 to 0.005. For samples ≥ 0.7 COI, the recovery ranged from 99% to 106%. The acceptance criteria were met. The studies confirm a claimed calibration stability for 7 days on-board the **cobas e** 601 when using the same reagent kit.

**Calibrator Stability after First Opening**
This study was performed to determine the time period in which the Elecsys HBsAg II calibrators can be kept at 2-8°C once opened.

A new reagent pack was opened and the **cobas e** 601 analyzer was calibrated. The opened calibrators were then tested again in duplicate after 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 90-110% recovery of signal counts for Cal 1 and Cal 2 based on unstressed signal (counts)

The percent recovery for the Cal 1 was 99% and 102%. The percent recovery for the Cal 2 was 97% and 100%. The acceptance criteria were met. The study confirms a claimed stability of 8 weeks after first opening for the Elecsys HBsAg II calibrators when stored at 2-8°C.

**On-Board Stability- Open Calibrators**
The sample rotor disk where calibrators, PreciControls, 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 Elecsys HBsAg II calibrators 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 HBsAg II 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 90%-110% recovery of signal counts for Cal 1 for Cal 2 after 2 hours at 32°C.

The percent recovery for the Cal 1 was 98% and 99%. The percent recovery for the Cal 2 was 101% and 102%. 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 analyzer.

***PreciControl HBsAg II Stability Studies***

**PreciControl Real-Time (Shelf Life) Stability**
Shelf life was determined by testing three production lots of the PreciControl HBsAg II kits stored at the recommended storage temperature of 2-8°C. The PreciControl lots were tested 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 0-0.80 of Initial COI
PC2 recovery = 80-120% of Initial COI

For PC1, the values ranged from 0 to 0.059. For PC2, the recovery ranged from 89% to 99%. The data show that PreciControl HBsAg II controls are stable for at least 13 months. The study confirms a claimed stability for the unopened PreciControl HBsAg II kits of 16 months at 2-8°C.

**PreciControl Temperature Stress Stability**
This study was conducted to determine the effect of elevated temperature stress on the PreciControl HBsAg II controls during transportation. One kit was stored at the recommended storage of 2-8°C and a second kit was stressed for one week at 35°C. The COIs of the PreciControls were assessed in duplicate before and after incubation at the indicated conditions:

The acceptance criteria were as follows:
PC1 recovery < Initial COI + 0.3
PC2 recovery = 80-120% of Initial COI.

For PC1, the values ranged from 0.036 to 0.038. For PC2, the recovery ranged from 111% to 116%. The acceptance criteria were met. The study confirms a claimed stability for the PreciControl HBsAg II controls of 1 week at 35°C.

**PreciControl Stability after First Opening**
Stability studies were performed to determine the time period over which the PreciControl HBsAg II 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 9 weeks. After 4, 8 and 9 weeks, the stressed PreciControls were tested in duplicate. Recoveries relative to the unstressed PreciControls were calculated, based on the initial values.

The acceptance criteria were as follows:

PC1 recovery < Initial COI + 0.3
PC2 recovery = 80-120% of Initial COI.

For PC1, the values ranged from 0.018 to 0.045. For PC2, the recovery ranged from 99% to 101%. The acceptance criteria were met. The study confirms a claimed stability of 8 weeks after first opening for the PreciControl HBsAg II controls when stored at 2-8°C.

**On-Board Stability for Open PreciControls**
The sample rotor disk (where calibrators, PreciControls, and samples are placed) of the **cobas e** 601 immunoassay analyzer is kept at ambient temperature (18-32°C). When the PreciControl HBsAg II reagents are placed on the rotor, the maximum temperature the controls might be exposed to is 32°C. According to the production specification, PreciControls may be used for a maximum of seven quality control procedures and should be left on the instrument only during performance of quality control.

A new Elecsys HBsAg II reagent pack and a PreciControl HBsAg II pack were opened and tested. The Elecsys HBsAg II 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 for this study was based on counts (signal).

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

For PC1, the recovery ranged from 101% to 105%. For PC2, the recovery ranged from 99% to 106%. The acceptance criteria were met. The study confirms a claimed stability for the PreciControl HBsAg II 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.

One lot of each reagent was tested with a panel of microorganisms. All reagents were plated on appropriate media prior to inoculation. Non-inoculated controls were incubated in parallel with inoculated reagents 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. Yeast and molds are to remain at or below the original inoculum during the 28-day period. USP criteria suggest that a suitable inoculum should be between $1 \times 10^5$ and $1 \times 10^6$ 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 HBsAg II for potential cross-reactivity using specimens from individuals with medical conditions unrelated to HBV infection. The study was performed by testing 269 samples. The comparison data to the comparator assay are presented in the following table:

**Table 10: Comparison of Elecsys HBsAg II (Test) and the Reference Assay Results for Subjects with Potentially Interfering Medical Conditions**

| Category | Reference HBsAg Assay Non-Reactive | | Total |
|---|---|---|---|
| | Elecsys HBsAg II | | |
| | RX[a] | NR[b] | |
| **Immune Disorders (n=40)** | | | |
| Serum Lupus Erythematosus | 0 | 10 | 10 |
| Anti-Nuclear Antibody (ANA) | 0 | 15 | 15 |
| Rheumatoid factor | 0 | 15 | 15 |
| **Non-Viral Infections (n=30)** | | | |
| Syphilis (*T.pallidum*) | 0 | 15 | 15 |
| Toxoplasmosis | 0 | 15 | 15 |
| **Viral Infection (n=149)** | | | |
| Cytomegalovirus (CMV) | 0 | 15 | 15 |
| Epstein-Barr Virus (EBV) | 0 | 15 | 15 |
| Hepatitis A Virus (HAV) | 0 | 10 | 10 |
| Hepatitis C Virus (HCV) | 0 | 11 | 11 |
| Hepatitis E Virus (HEV) | 1 | 10 | 11 |
| Human Immunodeficiency Virus (HIV) | 0 | 13 | 13 |
| Herpes Simplex Virus (HSV) | 0 | 15 | 15 |
| HTLV | 0 | 14 | 14 |
| Parvovirus B19 Infection | 0 | 15 | 15 |
| Rubella | 0 | 15 | 15 |
| Varicella Zoster (VZV) | 0 | 15 | 15 |
| **Non-Viral Liver Disease (n=40)** | | | |
| Various Cirrhosis | 0 | 8 | 8 |
| Chronic Non-Alcoholic Liver Disease | 0 | 6 | 6 |
| Steatohepatitis | 0 | 6 | 6 |
| Acute Liver Failure | 0 | 7 | 7 |
| Fatty Infiltrate of Liver | 0 | 2 | 2 |
| Autoimmune Disorder | 0 | 2 | 2 |
| Chronic Passive Congestion of Liver | 0 | 2 | 2 |
| Jaundice | 0 | 2 | 2 |
| Liver Abscess | 0 | 1 | 1 |

| Category | Reference HBsAg Assay Non-Reactive | | Total |
|---|---|---|---|
| | Elecsys HBsAg II | | |
| | RX[a] | NR[b] | |
| Liver Lesion | 0 | 1 | 1 |
| Malignant Neoplasm of Liver and intrahepatic bile ducts | 0 | 1 | 1 |
| Abdominal pain/pelvic mass | 0 | 2 | 2 |
| **Vaccination (n=10)** | | | |
| Flu Vaccination | 0 | 10 | 10 |
| | | | |
| **Total** | 1 | 268 | 269 |

[a] RX = Reactive
[b] NR = Non-reactive

Two hundred and sixty eight (268) samples were found to be non-reactive (negative) with both the Elecsys HBsAg II assay and the FDA-approved HBsAg reference assay while one sample was found to be non-reactive with the reference assay and reactive with the Elecsys HBsAg II assay. The sample was confirmed reactive by the Elecsys HBsAg Confirmatory Test.

**Precision**
A precision study was conducted to evaluate repeatability and the intermediate precision of within-laboratory precision according to CLSI guideline EP5-A3.

***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 the two PreciControls (PC1 and PC 2) was tested in duplicate determinations in two runs per day by two operators for 12 days. The measurements were performed on one **cobas e** 601 analyzer, at one site, with one reagent lot, performing calibration spanning at least two calibration cycles on days 1, 5, 10, and 12. Repeatability (within-run) and within-laboratory precision were calculated according to EP5-A3. Repeatability precision ranged from 2.04 to 7.94 % CV. Within-laboratory precision ranged from 3.02 to 8.89 % CV as shown in the following table:

**Table 11: Internal Precision Data**

| | | Mean | Repeatability Within-Run | | Within-Laboratory Precision | |
|---|---|---|---|---|---|---|
| **Sample** | **n** | **(COI[a])** | **SD[b] (COI)** | **CV (%)** | **SD (COI)** | **CV (%)** |
| Negative serum | 96 | 0.264 | 0.021 | 7.94 | 0.024 | 8.89 |
| High negative serum | 96 | 0.899 | 0.029 | 3.18 | 0.036 | 3.95 |
| Low positive serum | 96 | 1.140 | 0.044 | 3.82 | 0.046 | 4.04 |
| Positive serum | 96 | 2.72 | 0.055 | 2.04 | 0.091 | 3.34 |
| PC 1 | 96 | 0.340 | 0.020 | 5.86 | 0.027 | 8.01 |
| PC 2 | 96 | 4.20 | 0.088 | 2.10 | 0.127 | 3.02 |

[a] COI - Cut-off index
[b] SD - Standard deviation

***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, or AC), PreciControls PC 1 and PC 2, five near cut-off human serum pools, and a moderately positive human serum pool tested in replicates of 3 in 2 runs/day for 5 days according to the CLSI documents EP15-A2 and EP5-A3. 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 12: Reproducibility (External Precision) Data**

| | | | Repeat-ability | | Between-Run | | Between-Day | | Between-Lot | | Between-Site | | Reproduc-ibility | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sample** | **N** | **Mean COI** | **SD COI** | **%CV** | **SD COI** | **%CV** | **SD COI** | **%CV** | **SD COI** | **%CV** | **SD COI** | **%CV** | **SD COI** | **%CV** |
| HS1, negative | 180 | 0.72 | 0.03 | 4.6 | 0.02 | 2.4 | 0.01 | 0.8 | 0.02 | 2.7 | 0.00 | 0.0 | 0.04 | 5.9 |
| HS2, negative | 180 | 0.83 | 0.04 | 4.4 | 0.02 | 1.8 | 0.01 | 1.3 | 0.02 | 2.4 | 0.00 | 0.0 | 0.05 | 5.5 |
| HS3, negative | 180 | 0.94 | 0.04 | 3.9 | 0.01 | 1.4 | 0.01 | 1.2 | 0.02 | 2.6 | 0.00 | 0.0 | 0.05 | 5.0 |
| HS4, low | 180 | 1.18 | 0.04 | 3.4 | 0.01 | 0.9 | 0.02 | 1.8 | 0.03 | 2.2 | 0.00 | 0.0 | 0.05 | 4.5 |
| HS5, low | 180 | 1.22 | 0.04 | 3.3 | 0.00 | 0.0 | 0.02 | 1.7 | 0.02 | 1.4 | 0.01 | 1.2 | 0.05 | 4.1 |
| HS6, positive | 180 | 1.68 | 0.05 | 2.9 | 0.03 | 1.5 | 0.03 | 1.5 | 0.05 | 3.0 | 0.06 | 3.8 | 0.10 | 6.0 |
| PreciControl HBSAG II 1 | 180 | 0.32 | 0.02 | 7.8 | 0.01 | 2.8 | 0.01 | 4.3 | 0.01 | 4.5 | 0.01 | 3.9 | 0.04 | 11.1 |
| PreciControl HBSAG II 2 | 180 | 4.22 | 0.10 | 2.3 | 0.07 | 1.6 | 0.04 | 0.9 | 0.06 | 1.3 | 0.05 | 1.2 | 0.14 | 3.4 |

**HBsAg Mutant Detection Study**

The purpose of this study was to prove the detection of Hepatitis B mutants with Elecsys HBsAg II. To determine the detection of Hepatitis B mutants, 20 recombinant mutants were tested with the Elecsys HBsAg II. The panel comprised defined mutations that affect the antigenic structure of HBsAg. The mutants contained epitope clusters within amino acids (aa)100-160, including the "a-determinant" region (aa 124-147).

The recombinant mutants were diluted with individual HBsAg negative human serum to yield a low positive sample close to the cut-off. The measurements were done in single determinations. The panel was tested using the Elecsys HBsAg II assay on the **cobas e** 601 analyzer.

**Table 13: Results of Mutant Detection**

| Sample | Mutation | Sample COI |
|---|---|---|
| mutant_1 | F8L / R24K / N40R / G43R / L94S / M103I / 133A114 / M133T / | 5.81 |
| mutant_2 | T/A45S / C107R / M195I | 4.66 |
| mutant_3 | S132Y, P142S, und G145R | 7.12 |
| mutant_4 | T123N | 9.38 |
| mutant_5 | G145K | 2.28 |
| mutant_6 | D144G | 8.37 |
| mutant_7 | D144A | 6.58 |
| mutant_8 | G145R | 5.15 |
| mutant_9 | 122RA123 | 4.02 |
| mutant_10 | Q129P, F134R, P142L, D144E, G145K, S171F, L175S | 1.28 |
| mutant_11 | R122I | 1.59 |
| mutant_12 | M125T/ T127P / P142A/G145R | 2.77 |
| mutant_13 | T131I | 5.51 |
| mutant_14 | C147S | 2.48 |
| mutant_15 | K141E | 5.10 |
| mutant_16 | S143L | 1.43 |
| mutant_17 | P142L | 1.37 |
| mutant_18 | Y134S | 3.65 |
| mutant_19 | E164D | 6.60 |
| mutant_20 | I126S | 2.55 |

The Elecsys HBsAg II assay demonstrated the ability to detect (as reactive) the 20 HBsAg mutants that were tested.

**Spiking Study in Support of the Pediatric Claim**

Due to the low prevalence of HBV in the US pediatric population, a study comparing 33 spiked pediatric samples against one adult sample was performed with the Elecsys HBsAg II. The purpose of this study was to give evidence that the assay can be used for a pediatric population (age 2 to 21 years of age).

The level of spiking for the samples was in the range of 4 times the COI. All of the samples were tested in duplicate with the Elecsys HBsAg II assay before and after spiking.

The deviation from the adult to each pediatric sample was calculated in percentage as:

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

**Table 14: Results of the Spiking Study in Support of the Pediatric Claim**

| Sample | Age | Sample COI (Mean) | Recovery (%) |
|---|---|---|---|
| adult_001 | 59 | 4.27 | - |
| pediatric_001 | 20 | 4.42 | 104 |
| pediatric_002 | 19 | 4.38 | 103 |
| pediatric_003 | 19 | 4.32 | 101 |
| pediatric_004 | 19 | 4.09 | 96 |
| pediatric_005 | 19 | 4.3 | 101 |
| pediatric_006 | 19 | 4.22 | 99 |
| pediatric_007 | 3 | 4.65 | 109 |
| pediatric_008 | 6 | 4.38 | 103 |
| pediatric_009 | 6 | 4.36 | 102 |
| pediatric_010 | 6 | 4.07 | 95 |
| pediatric_011 | 9 | 4.26 | 100 |
| pediatric_012 | 9 | 4.23 | 99 |
| pediatric_013 | 9 | 4.39 | 103 |
| pediatric_014 | 11 | 4.42 | 104 |
| pediatric_015 | 12 | 4.17 | 98 |
| pediatric_016 | 12 | 4.35 | 102 |
| pediatric_017 | 12 | 4.54 | 106 |
| pediatric_018 | 12 | 4.21 | 99 |
| pediatric_019 | 12 | 4.26 | 100 |
| pediatric_020 | 13 | 4.51 | 106 |
| pediatric_021 | 13 | 4.13 | 97 |
| pediatric_022 | 13 | 4.31 | 101 |
| pediatric_023 | 14 | 4.63 | 109 |

| Sample | Age | Sample COI (Mean) | Recovery (%) |
|---|---|---|---|
| pediatric_024 | 14 | 4.43 | 104 |
| pediatric_025 | 14 | 4.34 | 102 |
| pediatric_026 | 15 | 4.07 | 95 |
| pediatric_027 | 15 | 4.15 | 97 |
| pediatric_028 | 16 | 4.45 | 104 |
| pediatric_029 | 16 | 4.45 | 104 |
| pediatric_030 | 16 | 4.25 | 100 |
| pediatric_031 | 17 | 4.31 | 101 |
| pediatric_032 | 17 | 4.32 | 101 |
| pediatric_033 | 17 | 4.36 | 102 |

All 33 pediatric samples presented recoveries between 95% and 109%. The results of all 33 pediatric samples recovered within 10 % of the adult sample and thus support use of the assay with the pediatric population.

**Spiking Study in Support of the Pregnant Claim**

Due to the low prevalence of HBV in the US pregnant population and to ensure that HBsAg detection did not differ between samples from pregnant and non-pregnant women, 32 samples of pregnant women and 32 samples of non-pregnant women were spiked with analyte from a high positive HBsAg sample and tested with the Elecsys HBsAg II test. All specimens were tested with the Elecsys HBsAg II assay before and after spiking in duplicate.

The study included 8 high negative (near cut-off), 4 retest zone, and 20 low positive (near cut-off) samples for each group of pregnant and non-pregnant samples. For each pair of spiked and non-spiked samples, the value of the non-spiked sample was subtracted from the spiked sample. These corrected values obtained for the given concentrations were compared between samples of pregnant and non-pregnant women.

The percent deviation was calculated according to:

[Pregnant spiked COI – unspiked COI] / [Non-Pregnant spiked COI - unspiked COI] x 100%

The recoveries varied between 81% and 126% with the following distribution of the percent differences (X) from 100%:

| Distribution of Percent Differences | | |
|---|---|---|
| $X < 10\ \%$ | $10\ \% \leq X \leq 20\ \%$ | $X > 20\ \%$ |
| 81.2 % (26/32) | 12.5 % (4/32) | 6.3 % (2/32) |

**Seroconversion Sensitivity**

Seroconversion sensitivity of the Elecsys HBsAg II assay was evaluated by testing 14 commercially sourced seroconversion panels in comparison to an FDA approved HBsAg reference assay. In all panels, the Elecsys HBsAg II assay shows detection of seroconversion equal to the reference HBsAg assay except with one panel where the Elecsys HBsAg II assay detected seroconversion to a reactive status one draw later than the reference assay.

**Table 15: Results for Seroconversion Sensitivity**

| Days to Evidence of Seroconversion for Elecsys HBsAg II Compared to the Reference Assay | | | | | |
|---|---|---|---|---|---|
| | Elecsys HBsAg II | | Reference HBsAg Assay | | Difference in Days to Elecsys HBsAg II Reactivity |
| Panel ID | Non-Reactive | Reactive | Non-Reactive | Reactive | |
| 6272 | 74 | 94 | 74 | 94 | 0 |
| 6281 | 7 | 13 | 7 | 13 | 0 |
| 9092 | 37 | 42 | 37 | 42 | 0 |
| 11000 | 19 | 21 | 19 | 21 | 0 |
| PHM 906 | 0 | 137 | 0 | 137 | 0 |
| PHM 912 | 24 | 42 | 20 | 24 | + 18 (1 draw) |
| PHM 918 | 0 | 7 | 0 | 7 | 0 |
| PHM 924 | 0 | 23 | 0 | 23 | 0 |
| PHM 926 | 2 | 9 | 2 | 9 | 0 |
| PHM 927 | 0 | 4 | 0 | 4 | 0 |
| PHM 929 | 11 | 14 | 11 | 14 | 0 |
| PHM 930 | 0 | 3 | 0 | 3 | 0 |
| PHM 935B[a] | - | 128 | - | 128 | - |
| PHM 936[b] | - | 0 | - | 0 | - |

[a] Initial time point was positive (128 days). No panel member earlier than 128 days was included.
[b] Positive at day 0.

**Genotype Panel**
One commercially available HBsAg genotype panel, containing 20 unique specimens with the most common hepatitis B surface antigen genotypes (A through H) was tested with the Elecsys HBsAg II assay and the FDA approved reference assay to validate the performance of the assay. Surface antigens representing the most common genotypes were detected (20 out of 20) with HBsAg II and HBsAg Confirmatory assays.

## X. SUMMARY OF PRIMARY CLINICAL STUDY

The safety and effectiveness of the Elecsys HBsAg II was determined by a clinical trial consisting of the following studies:

**Clinical Study:**

### A. Study Design

Two sets of clinical specimens were tested in the clinical performance study.

i. Prospective Specimens: A total of 2389 prospective specimens comprising 2059 specimens from adult (non-pregnant) subjects, 202 specimens from pregnant women and 128 pediatric (< 22 years of age) specimens, were prospectively collected from individuals at increased risk (at-risk) of HBV infection due to lifestyle, behavior, occupation, disease state or known exposure event to hepatitis with and without signs and symptoms of hepatitis infection (non-pregnant adults, pregnant and pediatric subjects at increased risk for hepatitis).

ii. Retrospective Specimens: Retrospective specimens were obtained from commercial vendors and included 586 specimens from pregnant women in the US, 16 specimens from pregnant women outside the US, and 397 (supplemental) specimens from non-pregnant adults. The 586 specimens from pregnant women were from individuals with low or unknown risk whereas the 16 specimens from pregnant women outside the US were from individuals at increased risk for hepatitis. The 397 (supplemental) specimens from non-pregnant adults were selected from individuals from high hepatitis endemic areas, i.e, from individuals with a high prevalence of reactive HBsAg, HBeAg, anti-HBcAg IgM or diagnosed with acute or chronic hepatitis B).

The prospective specimens were obtained from subjects at increased risk for hepatitis and prospectively recruited at seven US clinical sites while the retrospective specimens were acquired from eight commercial vendors.

Each specimen was tested using the Elecsys HBsAg II assay and the Elecsys HBsAgConfirmatory Test at three clinical testing sites. Each specimen was also tested with the HBsAg comparator method at a reference laboratory. Agreement of

the Elecsys HBsAg II assay was assessed relative to a patient infected status algorithm.

The 2059 prospective specimens from the adult (non-pregnant) cohort at increased risk for hepatitis and the 397 retrospective adult (non-pregnant) supplemental samples were also tested with 6 FDA approved HBV reference assays, each detecting a unique serological marker (HBsAg, HBeAg, anti-HBc IgM, total anti-HBc, anti-HBs and anti-HBe). HBV classification was based on the reference marker patterns presented in Table 11**.**

**Table 16: Serological Classification by FDA-Approved HBV Panel**

| **HBV Classification** | **HBV Reference Markers** | | | | | |
|---|---|---|---|---|---|---|
| | **HBsAg** | **HBeAg** | **Anti-HBc IgM** | **Anti-HBc** | **Anti-HBe** | **Anti-HBs** |
| Acute | (+) | (+) | (+) | (+) | (-), (+) | (-) |
| Acute | (+) | (+) | (-), (+) | (-) | (-) | (-) |
| Acute | (+) | (-) | (-) | (-) | (-) | (-) |
| Acute | (+) | (+) | eq | (+) | (-), (+) | (-) |
| Acute | (+) | (-) | (+) | (+) | (-) | (-) |
| Acute | (+) | (-) | eq | (+) | (+) | (-) |
| Acute (late) | (+) | (-) | (+) | (+) | (+) | (-), (+) |
| Chronic | (+) | (+) | (+) | (+) | (+) | (+) |
| Chronic | (+) | (-) | (-) | (+) | (+) | (-), (+) |
| Chronic | (+) | (-) | (-) | (+) | eq | (-) |
| Chronic | (+) | (-) | (-) | (+) | (-) | (-), (+) |
| Chronic | (+) | (+) | (+) | (+) | (-) | (+) |
| Chronic | (+) | (+) | (-) | (+) | (-) | (-), (+) |
| Chronic | (+) | (+) | (-) | (+) | (+) | (-) |
| Early Recovery | (-) | (-) | (-) | (+) | (-), (+) | (-) |
| Early Recovery | (-) | (-) | (+) | (+) | (-) | (-), (+) |
| Early Recovery | (-) | (-) | (+) | (+) | (+) | (-), (+) |
| Recovery | (-) | (-) | (-) | (-), (+) | (+) | (+) |
| Recovery | (-) | (-) | (-) | (+) | (+) | eq |
| Recovered or Immune due to Natural Infection | (-) | (-) | (-) | (+) | (-) | (+), eq |
| HBV Vaccine Response | (-) | (-) | (-) | (-) | (-) | (+) |

| HBV Classification | HBV Reference Markers | | | | | |
|---|---|---|---|---|---|---|
| | **HBsAg** | **HBeAg** | **Anti-HBc IgM** | **Anti-HBc** | **Anti-HBe** | **Anti-HBs** |
| HBV Vaccination Response (?) | (-) | (-) | (-) | (-) | (-) | eq |
| Not previously infected | (-) | (-) | (-) | (-) | (-) | (-) |
| Not Interpretable | (-) | (+) | (-) | (+) | (-) | (+) |
| Not Interpretable | (-) | (-) | (-) | (-) | (+) | (-) |
| Not Interpretable | (-) | (+) | (-) | (+) | (+) | (-) |
| Not Interpretable | (-) | (+) | (-) | (-) | (-) | (-), eq, (+) |

eq:equivocal

***Prospective Specimens*:**

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; increased risk subjects with only information describing either the risks or the status of symptoms of hepatitis, but not both; 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.

## B. Study Population Demographics

The Elecsys HBsAg II clinical study population consisted of a total of 3,388 specimens from non-pregnant adults (n=2,456), pregnant women N=804), and non-pregnant pediatric (n=128) subjects; 2389 specimens were collected prospectively

and 999 specimens were retrospective. A demographic summary of the overall at risk specimen population by race, age and sex is provided in the following tables:

**Table 17: Demographic Summary of Clinical Populations by Sex**

| | Adult Non-Pregnant (n=2,456) | | | | Pregnant (n=804) | | | | | | Pediatric Non-Pregnant (n=128) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Increased Risk | | Supplemental | | Increased Risk | | Increased Risk* | | Low or Unknown Risk | | Increased Risk | |
| **Sex** | Prospective | | Retrospective | | Prospective | | Retrospective | | Retrospective | | Prospective | |
| | **n** | **%** | **n** | **%** | **n** | **%** | **n** | **%** | **n** | **%** | **N** | **%** |
| **Female** | 940 | 45.65 | 65 | 16.37 | 202 | 100.00 | 16 | 100.00 | 586 | 100 | 68 | 53.13 |
| **Male** | 1,119 | 54.35 | 331 | 83.38 | N/A | N/A | N/A | N/A | N/A | N/A | 60 | 46.88 |
| **Un-known** | N/A | N/A | 1 | 0.25 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| **Total** | 2,059 | 100.00 | 397 | 100.00 | 202 | 100.00 | 16 | 100.00 | 586 | 100.00 | 128 | 100.00 |

*Non-US Subjects

**Table 18: Demographic Summary of Clinical Populations by Ethnicity**

| | Adult Non-Pregnant (n=2,456) | | | | Pregnant (n=804) | | | | | | Pediatric Non-Pregnant (n=128) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Increased Risk | | Supplemental | | Increased Risk | | Increased Risk* | | Low or Unknown Risk | | Increased Risk | |
| **Ethnicity** | Prospective | | Retrospective | | Prospective | | Retrospective | | Retrospective | | Prospective | |
| | **n** | **%** | **n** | **%** | **n** | **%** | **n** | **%** | **n** | **%** | **N** | **%** |
| **Hispanic / Latino** | 535 | 25.98 | 7 | 1.77 | 171 | 84.65 | 0 | 0 | 0 | 0 | 70 | 54.69 |
| **Not Hispanic / Latino** | 1517 | 73.68 | 29 | 7.30 | 31 | 15.35 | 0 | 0 | 0 | 0 | 57 | 44.53 |
| **Unknown** | 7 | 0.34 | 361 | 90.93 | 0 | 0 | 16 | 100 | 586 | 100.00 | 1 | 0.78 |
| **Total** | 2,059 | 100.00 | 397 | 100 | 202 | 100.00 | 16 | 100.00 | 586 | 100 | 128 | 100.00 |

*Non-US Subjects

**Table 19: Demographic Summary of Clinical Populations by Race**

| | Adult Non-Pregnant (n=2,456) | | | | Pregnant (n=804) | | | | | | Pediatric Non-Pregnant (n=128) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Increased Risk | | Supplemental | | Increased Risk | | Increased Risk* | | Low or Unknown Risk | | Increased Risk | |
| Race | Prospective | | Retrospective | | Prospective | | Retrospective | | Retrospective | | Prospective | |
| | n | % | n | % | n | % | n | % | n | % | N | % |
| AIAN[#] | 22 | 0.34 | 0 | 0 | 1 | 0.49 | 0 | 0 | 2 | 0.34 | 0 | 0 |
| Asian | 15 | 0.73 | 84 | 21.13 | 3 | 1.49 | 0 | 0 | 10 | 1.71 | 4 | 3.13 |
| African American/ Black | 1,020 | 49.54 | 182 | 45.84 | 15 | 7.43 | 16 | 100 | 316 | 53.92 | 32 | 25 |
| Caucasian/ White | 946 | 45.94 | 55 | 13.85 | 176 | 87.13 | 0 | 0 | 149 | 25.43 | 86 | 67.19 |
| NHOPI[&] | 4 | 0.19 | 0 | 0 | 1 | 0.49 | 0 | 0 | 0 | 0 | 1 | 0.78 |
| Unknown | 8 | 0.39 | 70 | 17.63 | 2 | 0.99 | 0 | 0 | 4 | 0.68 | 1 | 0.78 |
| Other | 44 | 2.14 | 6 | 1.51 | 4 | 1.98 | 0 | 0 | 105 | 17.92 | 4 | 3.12 |
| Total | 2,059 | 100.00 | 397 | 100.00 | 202 | 100.00 | 16 | 100.00 | 586 | 100.00 | 128 | 100.00 |

[#]AIAN: American Indian/ Alaska Native
[&]NHOPI: Native/ Pacific Islander
*Non-US Subjects

**Table 20: Distribution of Hepatitis B Disease States across Serologically Characterized Cohorts (Non-Pregnant Adult at Increased Risk (IR) for Hepatitis and Non-Pregnant Adult Supplemental).**

| HBV Classification | Adult IR | Supplemental |
|---|---|---|
| Acute | 7 | 74 |
| Chronic | 32 | 317 |
| Early Recovery | 198 | 2 |
| Not Interpretable | 9 | 0 |
| Not previously | 942 | 1 |
| Recovered | 245 | 2 |
| Recovery | 131 | 1 |
| Vaccination | 495 | 0 |
| Total | 2,059 | 397 |

The distribution of Elecsys HBsAg II results by age group and sex for the prospectively collected cohorts at increased risk of HBV infection is presented in the following table:

**Table 21: Elecsys HBsAg II Results by Age Range and Sex in the Prospectively Collected Population: Adult and Pediatric at Increased Risk for Hepatitis (Non-Pregnant and Pregnant), n=2,389.**

| Elecsys HBsAg II | | | | | |
|---|---|---|---|---|---|
| **Age years** | **Sex** | **Positive n (%)** | **Indeterminate n (%)** | **Non-Reactive n (%)** | **Total** |
| 2-11 | Female | 0 (0.00) | 0 (0.00) | 11 (100.00) | 11 |
| | Male | 0 (0.00) | 0 (0.00) | 13 (100.00) | 13 |
| 12-21 | Female | 0 (0.00) | 0 (0.00) | 116 (100.00) | 116 |
| | Male | 0 (0.00) | 0 (0.00) | 47 (100.00) | 47 |
| 22-29 | Female | 1 (0.42) | 0 (0.00) | 237 (99.58) | 238 |
| | Male | 2 (1.85) | 0 (0.00) | 106 (98.15) | 108 |
| 30-39 | Female | 2 (0.85) | 0 (0.00) | 232 (99.15) | 234 |
| | Male | 5 (2.81) | 0 (0.00) | 173 (97.19) | 178 |
| 40-49 | Female | 4 (1.53) | 1 (0.38) | 256 (98.08) | 261 |
| | Male | 14 (4.14) | 0 (0.00) | 324 (95.86) | 338 |
| 50-59 | Female | 5 (1.93) | 0 (0.00) | 254 (98.07) | 259 |
| | Male | 8 (2.11) | 0 (0.00) | 372 (97.89) | 380 |
| 60-69 | Female | 2 (2.56) | 0 (0.00) | 76 (97.44) | 78 |
| | Male | 0 (0.00) | 0 (0.00) | 107 (100.00) | 107 |
| 70-79 | Female | 0 (0.00) | 0 (0.00) | 10 (100.00) | 10 |
| | Male | 0 (0.00) | 0 (0.00) | 8 (100.00) | 8 |
| >=80 | Female | 0 (0.00) | 0 (0.00) | 3 (100.00) | 3 |
| | Male | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0 |
| Totals | Female | 14 (1.16) | 1 (0.08) | 1,195 (98.76) | 1,210 |
| | Male | 29 (2.46) | 0 (0.00) | 1,150 (97.54) | 1,179 |
| All | All | 43 (1.80) | 1 (0.04) | 2,345 (98.16) | 2,389 |

**Study Results:**

**Results of Method Comparison Studies**

The Elecsys HBsAg II was evaluated at six clinical sites located at St. Louis, MO, Miami, FL, Fort Lauderdale, FL, South Bend, IN, and Louisville, KY.

The negative percent agreement and positive percent agreement results for the prospectively collected non-pregnant adults at increased risk are presented in Table 22.

**Table 22: Percent Agreement between Elecsys HBsAg II and Comparator HBsAg Final Interpretation by HBV Disease Classification: Non-Pregnant Adult at Increased Risk Cohort (n=2,059)**

| HBV Classification | Positive Percent Agreement (%) | 95% Confidence Interval | Negative Percent Agreement (%) | 95% Confidence Interval |
|---|---|---|---|---|
| **Acute** | 100.00 (7/7) | 64.60 - 100.00 | N/A | N/A |
| **Chronic** | 100.00 (32/32) | 89.30 -100.00 | N/A | N/A |
| **Early** | N/A | N/A | 99.50 (197/198) | 97.20 - 99.99 |
| **Recovery** | N/A | N/A | 100.00 (131/131) | 97.20 - 100.00 |
| **Recovered** | N/A | N/A | 99.60 (244/245) | 97.80 - 99.99 |
| **HBV** | N/A | N/A | 99.80 (494/495) | 98.90 - 99.99 |
| **Not Previously** | N/A | N/A | 99.80 (940/942) | 99.20 - 99.97 |
| **Not Interpretable** | N/A | N/A | 100.00 (9/9) | 70.10 - 100.00 |
| **Total** | 100.00 (39/39) | 90.97 - 100.00 | 99.75 (2,015/2,020) | 99.42 - 99.92 |

N/A Not Applicable

The positive percent agreement between the Elecsys HBsAg II assay results and the comparator HBsAg assay final interpretation for the prospectively collected, non-pregnant adult at increased risk population was 100% (39/39) with a 95% confidence interval of 90.97% to 100% while the negative percent agreement was 99.75% (2,015/2,020) with a two-sided 95% confidence interval of 99.42% to 99.92%.

The negative percent agreement and positive percent agreement results for the non-pregnant adult supplemental specimens are presented in Table 23.

**Table 23: Percent Agreement between Elecsys HBsAg II and Comparator HBsAg Final Interpretation by HBV Disease Classification: Supplemental Cohort (n=397)**

| HBV Classification | Positive Percent Agreement (%) | 95% CI | Negative Percent Agreement (%) | 95% CI |
|---|---|---|---|---|
| **Acute** | 100.00 (74/74) | 95.06- 100.00 | N/A (0/0) | N/A |
| **Chronic** | 99.05 (314/317) | 97.26 - 99.70 | N/A (0/0) | N/A |
| **Early Recovery** | N/A | N/A | 50.00 (1/2) | 9.45 - 90.55 |
| **Recovery** | N/A | N/A | 100.00 (1/1) | 20.65 - 100.00 |
| **Recovered** | N/A | N/A | 100.00 (2/2) | 34.24 - 100.00 |
| **Not Previously Infected** | N/A | N/A | 100.00 (1/1) | 20.65 - 100.00 |
| **Total** | 99.23 (388/391) | 97.77 - 99.74 | 83.33 (5/6) | 43.65 - 97.00 |

N/A Not Applicable

The positive percent agreement between the Elecsys HBsAg II assay results and the comparator HBsAg assay final interpretation for the supplemental adult population combined was 99.23% (388/391) with a 95% confidence interval of 97.77% to 99.74% while the negative percent agreement was 83.33% (5/6) with a 95% confidence interval of 43.65 to 97.00%.

The table below summarizes the overall agreement between the Elecsys HBsAg II assay on the **cobas e** 601 analyzer and the reference HBsAg assay for the pediatric cohort (n=128). Percent agreement (positive PPA and negative NPA) with their respective confidence limits are listed.

**Table 24: Concordance Table for the Pediatric at Increased Risk Cohort (non-pregnant) Tested with the Elecsys HBsAg II on the cobas e 601 Analyzer and the Reference HBsAg Assay, n=128**

| **Elecsys HBsAg II** | **Reference HBsAg Assay** | | |
|---|---|---|---|
| | **Reactive** | **Non-Reactive** | **Total** |
| **Reactive** | 0 | 0 | 0 |
| **Non-reactive** | 0 | 128 | 128 |
| **Total** | 0 | 128 | 128 |
| **PPA** | N/A (0/0) | | |
| **95% CI** | N/A | | |
| **NPA** | 100.00% (128/128) | | |
| **95% CI** | 97.09 - 100 | | |

N/A Not Applicable

The table below summarizes the overall agreement between the Elecsys HBsAg II assay on the **cobas e** 601 analyzer and the reference HBsAg assay for the specimens from pregnant women (n=804). Percent agreement (positive PPA and negative NPA) with their respective confidence limits are listed.

**Table 25: Concordance Table for the Pregnant Cohort tested with the Elecsys HBsAg II on the cobas e 601 Analyzer and the Reference HBsAg Assay, n=804**

| Elecsys HBsAg II | Reference HBsAg Assay | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Pregnant IR (US) | | Pregnant IR (non-US) | | Pregnant Low or Unknown Risk | | Total | |
| | Reactive | Non-Reactive | Reactive | Non-Reactive | Reactive | Non-Reactive | Reactive | Non-Reactive |
| **Reactive** | 0 | 0 | 13 | 0 | 5 | 1 | 18 | 1 |
| **Negative** | 0 | 202 | 0 | 3 | 1 | 579 | 1 | 784 |
| **Total** | 0 | 202 | 13 | 3 | 6 | 580 | 19 | 785 |
| **PPA** | N/A (0/0) | | 100.00 (13/13) | | 83.33 (5/6) | | 94.74 (18/19) | |
| **95% CI** | N/A | | 77.19 - 100.00 | | 43.65 - 96.99 | | 75.36 - 99.07 | |
| **NPA** | 100.00 (202/202) | | 100.00 (3/3) | | 99.83 (579/580) | | 99.87 (784/785) | |
| **95% CI** | 98.13 - 100.00 | | 43.85- 100.00 | | 99.03 - 99.97 | | 99.28 - 99.98 | |

N/A: Not Applicable

Specimens from pregnant subjects collected in the US were also analyzed by trimester. Comparison of Elecsys HBsAg II assay results to the reference results by trimester for the specimens is shown in the following tables.

**Table 26: Pregnant at Increased Risk (Prospective Specimens)**

| | First trimester | | Second trimester | | Third trimester | | Total |
|---|---|---|---|---|---|---|---|
| | Reference HBsAg Assay | | | | | | |
| **Elecsy HBsAg II** | **Reactive** | **Non-Reactive** | **Reactive** | **Non-Reactive** | **Reactive** | **Non-Reactive** | |
| **Reactive** | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Non-reactive** | 0 | 62 | 0 | 66 | 0 | 74 | 202 |
| **Total** | 0 | 62 | 0 | 66 | 0 | 74 | 202 |

**Table 27: Pregnant at Low or Unknown Risk (Retrospective Specimens)**

| | First trimester | | Second trimester | | Third trimester | | Total |
|---|---|---|---|---|---|---|---|
| | Reference HBsAg assay | | | | | | |
| **Elecsy HBsAg II** | **Reactive** | **Non-Reactive** | **Reactive** | **Non-Reactive** | **Reactive** | **Non-Reactive** | |
| **Reactive** | 2 | 0 | 2 | 1 | 1 | 0 | 6 |
| **Non-reactive** | 0 | 196 | 0 | 192 | 1 | 191 | 580 |
| **Total** | 2 | 196 | 2 | 193 | 2 | 191 | 586 |

## C. Safety and Effectiveness Results of the Clinical Studies

1. Safety Results

   As an *in vitro* diagnostic test, the Elecsys HBsAg II 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 HBsAg II were comparable to current commercially available, FDA approved assays.

   The clinical study with prospectively collected specimens from the non-pregnant adult at increased risk clinical population resulted in a positive percent agreement between the Elecsys HBsAg II and the reference assay of 100% (39/39) with a 95% confidence interval of 90.97-100%. The negative percent agreement between the Elecsys HBsAg II and the reference assay in non-pregnant adult at increased risk clinical population was 99.75% (2,015/2,020) with a 95% confidence interval of 99.42-99.92%.

   The clinical study with retrospective specimens from the non-pregnant adult supplemental cohort resulted in a positive percent agreement between the Elecsys HBsAg II assay and the reference assay of 99.23% (388/391) with a 95% confidence interval of 97.77-99.74%. The negative percent agreement between the Elecsys HBsAg II assay and the reference assay in non-pregnant adult supplemental cohort was 83.33% (5/6) with a 95% confidence interval of 43.65-97.00%.

The clinical study with prospectively collected specimens from the non-pregnant pediatric at increased risk population resulted in a negative percent agreement between the Elecsys HBsAg II assay and the reference assay of 100% (128/128) with a 95% confidence interval of 97.09-100%. No HBsAg reactive result was observed for this population.

The clinical study in the US with prospectively collected specimens from the pregnant population at increased risk resulted in a negative percent agreement between the Elecsys HBsAg II assay and the reference assay of 100% (202/202) with a 95% confidence interval of 98.13-100%. No HBsAg reactive result was observed for this population. However, 16 specimens from pregnant subjects outside the US were acquired in an attempt to obtain HBsAg reactive results from pregnant subjects. The positive percent agreements between the Elecsys HBsAg II assay and the reference assay with the non-US increased risk pregnant population was 100.00% (13/13) with a 95% confidence interval of 77.19 to 100.00%. The negative percent agreement between the Elecsys HBsAg II assay and the reference assay in the non-US increased risk pregnant population was 100.00% (3/3) with a 95% confidence interval of 43.85 to 100.00%.

The clinical study in the US with retrospective specimens from the low or unknown risk pregnant population resulted in a positive percent agreement between the Elecsys HBsAg II assay and the reference assay of 83.33% (5/6) with a 95% confidence interval of 43.65-96.99%. The negative percent agreement between the Elecsys HBsAg II assay and the reference assay with these specimens was 99.83% (579/580) with a 95% confidence interval of 99.03 to 99.97%.

Testing of 14 seroconversion panels generated data that reflected the sensitivity of the Elecsys HBsAg II assay. Results from the Elecsys HBsAg II assay were equivalent to the results of the reference assay in 13 of the 14 seroconversion panels. In one seroconversion panel, the Elecsys HBsAg II assay detected seroconversion to a reactive status one draw later than the reference assay.

The specificity of the Elecsys HBsAg assay 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 HBsAg II assay in detecting accurately the presence of viral Hepatitis B surface antigen.

3. Subgroup Analyses
   See Tables 22 to 27 above.

4. Pediatric Extrapolation
   In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

### D. 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 fifteen 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 performance of the Elecsys HBsAg II is acceptable when testing human serum and plasma (sodium heparin, lithium heparin, $K_2$-EDTA and sodium citrate) samples.
- Samples are stable when refrigerated for 14 days at 2-8°C; 6 days at 20-25°C; and 6 months at -20°C. The samples may also be frozen/thawed up to 6 cycles.
- Elecsys HBsAg II reagents are stable up to the expiration date when stored unopened at 2-8°C; 6 weeks after opening when stored at 2-8°C; and 4 weeks on the **cobas e** 601 immunoassay analyzer.
- Elecsys HBsAg II calibrators are stable up to the stated expiration date when stored unopened at 2-8°C; 8 weeks after opening when stored at 2-8°C; and are to be used once on the **cobas e** 601 immunoassay analyzer.
- PreciControl HBsAg II is stable up to the stated expiration date when stored unopened at 2-8°C; 8 weeks after opening when stored at 2-8°C; and up to 5 hours at 20-25°C on the **cobas e** 601 immunoanalyzer.
- The preservatives used in the Elecsys HBsAg II, Elecsys HBsAg Confirmatory Test and PreciControl HBsAg II have been shown to meet USP Chapter 51 criteria.
- Elecsys HBsAg II precision performance meets all internal specifications for repeatability and intermediate precision.
- There are no issues with endogenous interferents at the physiological levels or with commonly administered medications.
- Elecsys HBsAg II clinical performance was evaluated in an ethnically diverse population representative of the intended use population and demonstrates effective positive and negative agreement values relative to an FDA-approved reference assay for HBsAg.

**B. Safety Conclusions**

The adverse effects of the device are based on data collected in the clinical studies conducted to support Premarket Approval Application approval as described in the above studies. As a diagnostic test, Elecsys HBsAg II for use on the **cobas e** 601 immunoassay analyzer involves the removal of blood from an individual for testing purposes. The test, therefore, presents risk, but no more safety hazard to an individual than any other test where blood is drawn.

False positive and false negative results were discussed in Section **VIII** above under Potential Adverse Effects of the Device on Health. There were no adverse effects of the device reported while this study was conducted.

**C. Benefit-Risk Conclusions**

The probable benefits of the device are based on data collected in the clinical studies conducted to support PMA approval as described above. Detection of Hepatitis B virus surface antigen is an important component of hepatitis B diagnosis. Although it cannot be used by itself to determine the state of infection (acutely or chronically infected with HBV), a positive Hepatitis B surface antigen result is followed by additional HBV DNA testing.

The risks from use of the Elecsys HBsAg II assay are false positive and false negative results. In both cases, risks are mitigated since HBV status would always be interpreted in conjunction with HBV DNA, 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.

Patient Perspectives:
This submission did not include specific information on patient perspectives for this device.

In conclusion, given the available information above, the data support that for the qualitative detection of Hepatitis B surface antigen in human serum or plasma by the Elecsys HBsAg 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 HBV infected patients.

## XIII. CDRH DECISION

CDRH issued an approval order on December 23, 2016. The final conditions of approval can be found in the approval order.

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.