SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Hepatitis B e antigen Hepatitis B e antigen control material
Device Trade Name:	Elecsys® HBeAg Immunoassay Elecsys® PreciControl HBeAg
Device Procode:	LOM
Applicant's Name and Address:	Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250
Date(s) of Panel Recommendation:	None

Premarket Approval Application (PMA) Number: P130015

Date of FDA Notice of Approval: March 14, 2014

Expedited: Not Applicable

II. INDICATIONS FOR USE

1. Elecsys HBeAg Immunoassay

The Elecsys HBeAg immunoassay is intended for the *in vitro* qualitative determination of hepatitis B e antigen (HBeAg) in human serum or plasma (K_2-EDTA, lithium or sodium heparin, and sodium citrate) in adult patients with symptoms of hepatitis or at risk for hepatitis B virus (HBV) infection. The assay results, in conjunction with other serological and clinical information, may be used for the laboratory diagnosis of individuals with acute or chronic hepatitis B or recovery from hepatitis B infection.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on the MODULAR ANALYTICS E170 immunoassay analyzer.

2. Elecsys PreciControl HBeAg

Elecsys PreciControl HBeAg is used for quality control of the Elecsys HBeAg immunoassay on the MODULAR ANALYTICS E170 analyzer.

III. CONTRAINDICATIONS

None

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the labeling for the Elecsys HBeAg immunoassay and the Elecsys PreciControl HBeAg.

V. DEVICE DESCRIPTION

Principle of Device Methodology

Elecsys HBeAg Immunoassay

The Elecsys HBeAg immunoassay employs "ECLIA" technology and is a qualitative serologic, two step sandwich assay. Total duration of the assay is 18 minutes.

1. 1st incubation: HBe antigen from 35 µL sample, a biotinylated monoclonal HBeAg-specific antibody, and a monoclonal HBeAg-specific antibody labeled with a ruthenium complex[1] 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 reaction product of the sample with the signal of the cut-off value previously obtained by HBeAg calibration.

Elecsys PreciControl HBeAg

The Elecsys HBeAg immunoassay uses the Elecsys PreciControl HBeAg for quality control. The Elecsys PreciControl HBeAg contains control serum based on human serum in the negative concentration range (PC HBEAG1) and control serum based on buffer for the positive concentration range (PC HBEAG2). The controls are used for monitoring the performance of the Elecsys HBeAg immunoassay.

Kit Configurations and Components

Reagents

The Elecsys HBeAg immunoassay is composed of five reagents:

Component 1
Reagent M contains streptavidin-coated microparticles (beads) at a concentration of 0.72 mg/mL in 50 mmol/L HEPES (4-(2-Hydroxyethyl)-1-piperazine-ethanesulfonic acid) buffer with protein stabilizers (bovine), saccharose, detergent and preservatives.

[1] Tris (2,2' -bipyridyl)ruthenium(II)complex (Ru(bpy)3 2+)

Component 2
The R1 reagent contains biotinylated monoclonal anti-HBeAg antibody (mouse) (> 0.8 mg/L) in a TRIS buffer (pH 7.4) with protein stabilizers (bovine), detergent and preservatives.

Component 3
The R2 reagent contains monoclonal anti-HBeAg antibody (mouse, 0.3 mg/mL) labeled with ruthenium complex in a TRIS buffer (pH 7.4) with protein stabilizers (bovine), detergent and preservatives.

Component 4
Cal 1 is the negative calibrator and consists of buffered (pH 7.4) and preserved human serum matrix, negative for HBeAg.

Component 5
Cal 2 is the positive calibrator which consists of E. coli, rDNA derived HBeAg (≥3.5 PEI U/mL) (Paul-Ehrlich-Institute units) buffered with HEPES buffer, pH 7.4 and preserved in an aqueous solution.

Elecsys PreciControl HBeAg

The Elecsys PreciControl HBeAg contains two reagents:

Component 1
PreciControl 1 (PC HBEAG1) is the negative control in human serum and is negative for HBeAg.

Component 2
PreciControl 2 (PC HBEAG2) is the positive control which consists of HBeAg (E. coli, rDNA derived) approximately 2.5 PEI U/mL in HEPES buffer, pH 7.4 with stabilizers and preservatives.

Calibration

The Elecsys HBeAg Calibrator 1 and Calibrator 2 are used to calibrate the Elecsys HBeAg test kit. The presence or absence of HBeAg in the sample is determined by comparing the electrochemiluminescence signal in the reaction to the cut-off signal determined from an active Elecsys HBeAg 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 Elecsys HBeAg immunoassay. The result of a sample is given in the form of a cut-off index (COI) which is calculated by dividing the signal for the sample by the signal for the cut-off. The result interpretation is as follows:

COI< 0.9	Non-reactive (Negative)
0.9 ≤ COI < 1.1	Border (Borderline)

COI ≥ 1.1 Reactive (Positive)

Table 1: Clinical Interpretation of HBeAg Testing

Initial Elecsys HBeAg Immunoassay Result			
COI	**Result**	**Interpretation of Results**	**Retest Procedure**
< 0.9	Non-reactive	No HBeAg detected	No retest required
0.9 ≤ COI < 1.1	Border	Borderline zone (undetermined)	Retest in duplicate with the Elecsys HBeAg immunoassay
≥ 1.1	Reactive	HBeAg detected	Presumptive evidence of the presence of HBeAg. No retest required. Follow CDC recommendations for ancillary testing.

Table 2: Clinical Interpretation of Repeat HBeAg Testing

Final Elecsys HBeAg Immunoassay Result			
COI	**Result after Retest (COI)**	**Final Results**	**Interpretation of Results**
< 0.9	No retest required	NON-REACTIVE [a]	HBeAg not detected. Does not exclude the possibility of exposure to HBV.
0.9 ≤ COI < 1.1	At least 2/3 results < 1.0	NON-REACTIVE [a]	HBeAg not detected. Does not exclude the possibility of exposure to HBV.
	At least 2/3 results ≥ 1.0	REACTIVE	HBeAg detected.
≥ 1.1	No retest required	REACTIVE	HBeAg detected

[a] A negative HBeAg result can indicate that the patient is either susceptible to HBV infection due to no past exposure, is in the recovery phase with HBV, or is immune to HBV due to a resolved past infection or vaccination.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are several other alternatives for the correction of Hepatitis B infection. The patient's medical history and thorough physical 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. 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 HBeAg immunoassay and Elecsys PreciControl HBeAg are marketed in the following 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 HBeAg Immunoassay is Marketed

Argentina	India	Romania
Australia	Indonesia	Russian Federation
Austria	Italy	Singapore
Belgium	Japan	Slovakia
Brazil	Kenya	South Africa
Canada	Korea	Spain
China	Latvia	Sweden
Colombia	Lithuania	Switzerland
Czech Republic	Malaysia	Taiwan
Denmark	Mexico	Thailand
Ecuador	Netherlands	Turkey
Finland	New Zealand	Uganda
France	Panama	United Kingdom
Germany	Peru	Venezuela
Greece	Philippines	Vietnam
Hong Kong	Poland	
Hungary	Portugal	

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 HBV infection requires the evaluation of the patient's blood for HBsAg, Anti-HBs and hepatitis B core antibody. The HBeAg assay is not usually required in the initial diagnosis of HBV. However, it may be used as an aid in guiding antiviral therapy for chronically infected patients, in conjunction with HBV viral load test results. Seroconversion from HBeAg positive to HBeAg negative status usually predicts long term reduction in viral replication and may be used as a response marker to antiviral therapy. Therefore, repeatedly false negative or false positive results could potentially lead to incorrect treatment decisions.

In such cases, there may be a safety concern for the patient. However, for chronic HBV patients who are on antiviral therapy, suppression of the HBV viral load is often used as an end point of treatment.

Incorrect results with the HBeAg assay (false positive or negative) do not pose a public health problem because the assay is used in conjunction with other HBV markers to provide information on the disease status of the patient.

IX. SUMMARY OF PRECLINICAL STUDIES

All non-clinical studies, except as noted for the reproducibility study, were performed at Roche Diagnostics Laboratories using the Elecsys HBeAg immunoassay and Elecsys PreciControl HBeAg on the MODULAR ANALYTICS E170 immunoassay analyzer.

Cut-Off Determination

Studies were performed to establish, verify and validate the cut-off value and the cut-off formula for the Elecsys HBeAg immunoassay. The aim of the studies was to define an appropriate cut-off value that would result in acceptable sensitivity and specificity for the Elecsys HBeAg immunoassay.

Establishment of the Cut-Off: The cut-off is calculated from the signals for the negative calibrator (Cal 1) and positive calibrator (Cal 2) according to the following algorithm:

Cut-Off (CO) = a x (Cal 1 – b x Cal 1) + c x (Cal 2 – b x Cal 1) + d

The constants in the formula (a, b, c and d) were determined based on studies on the E2010 analyzer. For the Elecsys HBeAg immunoassay on the Elecsys 2010, the values were set to, a = 1.5, b = 0.6, c = 0.04, and d = 0. Therefore, for the Elecsys HBeAg immunoassay on the Elecsys 2010, the algorithm was set as:

Cut-Off (CO) = 1.5 x (Cal 1 – 0.6 x Cal 1) + 0.04 x (Cal 2 – 0.6 x Cal 1)

Because of the similarities between the E2010 and the E170 analyzers, the same constants were transferable from the E2010 to the E170.

Verification: The transferability of the algorithm for the Elecsys HBeAg immunoassay on the Elecsys 2010 to the E170 MODULAR ANALYTICS was verified by measuring a total of 192 samples. These samples were characterized on the Elecsys 2010 for reference testing as negative and positive, respectively. Receiver Operator Curve (ROC) analysis was performed to verify the cut-off algorithm and to determine sensitivity and specificity for the Elecsys HBeAg immunoassay on the E170 MODULAR ANALYTICS. The external clinical studies serve as validation of the cut-off algorithm.

Cut-Off Sensitivity: The analytical performance of the Elecsys HBeAg immunoassay is calculated by reading the concentration at the cut-off from the master calibrator curve (standardized against "HBe Reference Antigen 82 (HBeAg), from the Paul-Ehrlich-Institute, Langen, Germany). The sensitivity at the assay cut-off was determined to be around 0.24 PEI U/mL.

Limit of Blank and Limit of Detection

The Limit of Blank (LoB) and Limit of Detection (LoD) of the Elecsys HBeAg immunoassay were determined in accordance with CLSI guideline EP17-A. Test results are reported in cut-off index (COI = sample signal/ cut-off value). The Limit of Blank was determined to be 0.092 and 0.123 COI with two lots and the Limit of Detection was determined to be 0.161 and 0.156 COI. The acceptance criteria were:

LoB < 0.5 COI
LoD < 0.9 COI

Endogenous Interferences

To evaluate the effect of elevated levels of hemoglobin, bilirubin, lipemia (intralipid), biotin, total protein, and human anti-mouse antibodies (HAMA) on the Elecsys HBeAg immunoassay, interferent experiments were performed as described below:

- Hemoglobin: 4 HBeAg samples (one negative, one high negative, one low positive, one positive) were spiked at different levels of hemolysate with an upper concentration of 1.76 g/dL hemoglobin and compared to hemolysate-free samples.
- Bilirubin: 4 HBeAg samples (one negative, one high negative, one low positive, one positive) were spiked at different levels of bilirubin with an upper concentration of 27.5 mg/dL bilirubin and compared to bilirubin-free samples.
- Lipemia: 4 HBeAg samples (one negative, one high negative, one low positive, one positive) were spiked at different levels of intralipid with an upper concentration of 1650 mg/dL intralipid and compared to intralipid-free samples.
- Biotin: 4 HBeAg samples (one negative, one high negative, one low positive, one positive) were spiked at different levels of biotin with an upper concentration of 44 ng/mL biotin and compared to biotin-free samples.
- Total Protein: 4 HBeAg samples (one negative, one high negative, one low positive, one positive) were spiked with different levels of total protein with an upper concentration of 13.2 g/dL total protein and compared to unspiked samples.
- HAMA: 3 HBeAg samples (one negative, one high negative, one low positive) were spiked with different levels of HAMA with an upper concentration of 805 ng/mL HAMA and compared to unspiked samples.

All calculations were based on COI. Samples were tested in duplicate. Percent mean recovery of COI values of samples spiked with interfering substance were calculated against the corresponding sample without the interfering substance.

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

Samples <1.0 COI: Recovery ± 0.2 COI

Samples ≥1.0 COI: Recovery 80-120% (COI)

For samples <1.0 COI, recovery ranged from 0.00 to 0.09 COI while the average percent recovery for samples ≥1.0 COI ranged from 91-106%.

The results of this study demonstrated that samples containing hemolysate up to 1.76 g/dL, bilirubin up to 27.5 mg/dL, intralipid up to 1500 mg/dL, biotin up to 44 ng/mL, total protein up to 13.2 g/dL and HAMA up to 805 ng/mL should test accurately with the Elecsys HBeAg immunoassay. The following levels for non-interference are claimed in the package insert.

- Hemoglobin 1.6 g/dL
- Bilirubin 25 mg/dL
- Intralipid (Lipemia) 1500 mg/dL
- Biotin 40 ng/mL
- Total protein 12 g/dL
- HAMA 805 ng/mL

Linearity

Linearity studies were not performed as this is a qualitative assay.

Matrix Effects

Studies were conducted to verify the types of blood collection tubes that can be used with the Elecsys HBeAg immunoassay. Samples were collected into matched serum and plasma collection tubes from 40 donors and assayed in triplicate on the MODULAR ANALYTICS E170 immunoassay analyzer. Forty matched pairs were collected in the evaluation of each blood collection tube:

- Serum Gel Separation Tubes SST
- Lithium heparin plasma
- Sodium heparin plasma
- K_2-EDTA plasma
- Sodium citrate plasma

Each matched pair was spiked with one of the following levels of HBeAg:

- Negative (targeted to < 0.5 COI)
- High negative (targeted to approximately 0.8 COI)
- Low Positive (targeted to approximately 1.2 COI) and
- Positive (targeted to > 1.5 COI)

The acceptance criterion for samples < 1.0 COI was recovery ± 0.2 COI relative to the matched serum. The acceptance criterion for samples ≥ 1.0 COI was recovery 80-120% (COI) relative to the matched serum. Statistical analysis must show no overall trend of bias > 15% per sample type.

Statistical evaluations were carried out to analyze the cut-off index 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 plasma collected using blood collection tubes containing the following anticoagulants:

- Lithium heparin
- Sodium heparin
- K_2-EDTA
- Sodium citrate

The studies also support the use of serum gel separator tubes.

Drug Interferences

A drug interference study was performed with the Elecsys HBeAg immunoassay on the MODULAR ANALYTICS E170 immunoassay analyzer with 21 common therapeutic drugs. Each drug was spiked into a negative, a low positive, and a positive serum sample.

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

Table 4: Drugs Tested with the Elecsys HBeAg Immunoassay

Compound	Concentration (mg/L) *
Acetyl cysteine	150
Ampicillin-Na	1,000
Ascorbic acid	300
Ca-Dobesilate	200
Cyclosporine	5
Cefoxitin	2,500
Heparin	5,000 U
Intralipid	10,000
Levodopa	20
Methyldopa+ 1.5	20
Metronidazole	200
Phenylbutazone	400
Tetracycline	50
Acetylsalicylic acid	1,000
Rifampicin	60
Acetaminophen	200
Ibuprofen	500
Theophylline	100
PEG interferon alpha 2a	0.036

Compound	Concentration (mg/L) *
Zidovudine	500
Acyclovir	600

*U for Heparin

Each drug was found to not cause interference at the claimed concentrations. Since these studies were performed *in vitro*, they do not assess the potential interference when the drug is metabolized *in vivo*.

Carryover Study

On the MODULAR ANALYTICS E170 immunoassay analyzer, the use of disposable tips for sample pipetting eliminates any risk of sample carry over by design. However, a study was performed to determine the extent of bead carryover and the associated residual risk for signal carry-over in the instrument's measuring cell caused by a high signal-generating sample.

An HBeAg negative sample was tested in triplicate using the Elecsys HBeAg immunoassay. Thereafter, a high signal generating sample, e.g. a Toxo-IgG sample which creates high signals (≥ 2 Million counts) when tested by the Elecsys Anti-Toxo-IgG assay, was tested, followed again by the HBeAg negative sample tested with the Elecsys HBeAg immunoassay. The negative sample was tested in triplicate. This test was repeated seven times, with seven different HBeAg negative samples.

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

All signal count values were within the acceptance criteria. These studies demonstrate that there is no measurable signal carry over with the Elecsys HBeAg immunoassay.

High Dose Hook Effect

A study was conducted to prove that a high dose hook effect will not lead to false negative results with the Elecsys HBeAg immunoassay. A high dose hook effect is present if increased concentrations of HBeAg result in a reduction in the signal and corresponding COI.

Three high titer positive samples were diluted in human HBeAg negative serum in at least 11 dilution steps to generate a dilution series that covers the range from negative to high positive COI values. The samples were measured in triplicate.

The acceptance criterion was that no false negative results should be caused by the high dose hook effect.

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At very high HBeAg concentrations, a high dose hook effect was observed; however, no negative results were observed. The concentrations at which the high dose hook effect

was observed were extremely high and exhibited COIs >1000. Such high concentrations are unlikely in native samples and, if present, may cause a lower positive value but do not cause a false negative result.

Stability Studies

Sample Stability

Three studies were performed to verify the stability of patient samples using the Elecsys HBeAg immunoassay. Serum and plasma (K_2-EDTA) samples were tested. The potential influence of storage of samples for 7 days at 2-8°C, at -20°C for 3 months, and 6 freeze/thaw cycles were evaluated. Samples tested were 3 negatives, 3 high negatives, 3 low positives and 3 positives, run in triplicate on the MODULAR ANALYTICS E170 analyzer.

For the storage at 2-8°C, samples were tested unstressed on day 0 (reference) and at 3, 5 and 7 days. For storage at -20°C, samples were tested unstressed on day 0 (reference) and at 2 weeks, and 1, 2, and 3 months. Freeze/thaw cycle samples were tested unstressed on day 0, then frozen, thawed and tested for the six freeze/thaw cycles.

Recovery after storage for each test was calculated based on the mean of COI. The acceptance criterion for samples with a COI ≥1.0 was a mean recovery of 80-120% COI when compared to the initial unstressed result. The acceptance criterion for samples with a COI <1.0 was a mean recovery of ±0.2 COI when compared to the initial unstressed result.

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 and can withstand 6 freeze/thaw cycles prior to testing by the Elecsys HBeAg immunoassay.

Elecsys HBeAg Reagent Stability

Reagent Real (Shelf Life) Time Stability

To assess the real-time stability of reagents, Elecsys HBeAg immunoassay kits were chosen from three production lots. 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 time intervals started with the production date and measurements were performed at least in the middle of the shelf life and one month after expiration. The stability studies included testing of the kits with internal control serum samples (ICS, n=10), negative sera (NS, n=20), interfering sera against ruthenylated antibody (AKRu, n=1) and streptavidin (SA, n=1), and the PreciControls (PC, n=2) with duplicate determinations for each sample.

Key stability parameters monitored for the Elecsys HBeAg immunoassay kits were cut-off sensitivity and results with the internal control samples. The data showed that the

assay reagents are stable for 25 months at 2-8°C. Shelf life for the HBeAg immunoassay kit is claimed for 24 months at 2-8°C.

Reagent Temperature Stress Stability

A temperature stress stability study was conducted to determine the effect of elevated temperature stress on the Elecsys HBeAg immunoassay reagents during transportation.

To assess the stability of the Elecsys HBeAg immunoassay reagents after temperature stress, the reagent kit was stressed for one week at 35°C. The stressed kit was then used to determine recoveries of four human sera samples (negative, high negative, low positive, positive) and the two PreciControls in duplicate determinations on the MODULAR ANALYTICS E170 analyzer. The percent recoveries of the samples were calculated relative to samples tested with an unstressed reagent kit (stored refrigerated at 2-8ºC).

The acceptance criterion for samples < 1.0 COI was a recovery ± 0.2 COI relative to the result with the unstressed kit. The acceptance criterion for samples ≥ 1.0 COI was recovery of 80-120% (COI) relative to the result with the unstressed kit.

The results from the temperature stress study verify the stability of the Elecsys HBeAg reagent for 1 week at 35ºC.

Reagent Stability after First Opening

A stability study was performed to determine the time period over which the Elecsys HBeAg kits can be kept at 2-8°C once opened. A new reagent pack from the kit was opened and calibrated on the MODULAR ANALYTICS E170 analyzer. Four human sera (negative, high negative, low positive, and positive) and the two PreciControls were tested with the opened reagents 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 compared to the results with unstressed reagents.

The acceptance criterion for samples < 1.0 COI was a recovery ± 0.2 COI relative to unstressed reagents. The acceptance criteria for samples ≥ 1.0 COI was recovery of 80-120 % (COI) relative to unstressed reagents.

Acceptance criteria were met for 8 weeks at 2-8°C after first opening. Reagent stability for 8 weeks at 2-8°C after first opening is claimed.

On-Board Stability - Open Reagent Pack

A stability study was performed to determine the time period in which the Elecsys HBeAg immunoassay reagents can be kept on-board the MODULAR ANALYTICS E170 analyzer once opened. An unstressed reagent pack was opened and calibrated. Four human sera samples and two PreciControls were tested with the unstressed reagent pack (stored at 2-8°C) and with the reagent pack stored on-board for 1, 2, 3, 4, 5, 6, 7, and 8 weeks. For each test time point, the calibration occurred seven days prior. Recovery for each sample was calculated based on COI.

The acceptance criterion for samples < 1.0 COI was a recovery ± 0.2 COI relative to the zero time (unstressed) results. The acceptance criterion for samples ≥ 1.0 COI was recovery of 80-120 % (COI) relative to the zero time (unstressed) results.

All acceptance criteria were met for each of the time points tested. On-board reagent stability of eight weeks for reagents stored on the E170 MODULAR ANALYTICS immunoassay analyzer is claimed.

Elecsys HBeAg Calibrator Stability Studies

On-Board Stability - Open Calibrators

While the calibrators are placed on the rack during calibration, the maximum temperature they might be exposed to is assumed to be 32°C, which is the upper limit of the specification for the ambient temperature of the E170 MODULAR ANALYTICS immunoassay analyzer. Calibrators 1 and 2 consequently need to be stable for 2 hours of incubation at 32°C which is the maximum time taken for calibration.

A stability study was performed to determine the time period over which the Elecsys HBeAg calibrators can be kept open on-board the MODULAR ANALYTICS E170 analyzer.

According to the product specification, one calibrator set may be used only once. Unless the entire volume is necessary for calibration on the analyzer, aliquots of the ready-for-use calibrators may be transferred into empty snap-cap bottles (CalSet vials) and should be left on the MODULAR ANALYTICS E170 analyzer only during calibration (2 hours in total).

A pair of Elecsys HBeAg 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).

The acceptance criterion was 90-110% recovery of signal counts after 2 hours at 32°C relative to the unstressed calibrators.

Acceptance criteria were met and calibrators are labeled as stable open and on-board the MODULAR ANALYTICS E170 for 2 hours.

Calibrator Stability after First Opening

A stability study was performed to determine the time period over which the Elecsys HBeAg immunoassay calibrators can be kept at 2-8°C once opened.

A new calibrator reagent pack was opened and calibration was carried out with the newly opened calibrators. The same opened calibrators were then tested again in duplicates after 4 and 8 weeks storage at 2-8°C. Calibrator stability was determined by calculation

of the recovery of the calibrator signals (counts) of opened calibrators compared to the calibrator signals (counts) of newly opened (unstressed) calibrators.

The acceptance criterion was 90-110% recovery of signal counts relative to unstressed calibrators.

The acceptance criterion was met and the Elecsys HBeAg calibrators are labeled as stable for eight weeks after first opening when stored at 2-8°C.

Elecsys HBeAg Calibration Stability Studies

Calibration must be performed once per reagent lot using the Elecsys HBeAg Cal 1, Cal 2 and fresh reagents (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended after 1 month with the same reagent lot and after 7 days with the same reagent kit.

Stability studies were performed to verify the claim that a lot calibration can be used for one month (28 days) with multiple reagent packs of the same lot (Lot Calibration Study) and that calibration is stable for 7 days (On-Board Calibration Study).

Lot Calibration Study

An Elecsys HBeAg reagent lot was tested on three separate MODULAR ANALYTICS E170 measuring channels. This was to ensure that the lot calibration was stable on different measuring channels/cells. Four human serum samples (negative, high negative, low positive, and positive) and two levels of PreciControl HBeAg were tested in duplicate. Calibration was performed with unstressed reagent on Day 1 and the four serum samples were tested in duplicate. After 29 days, unstressed reagent of the same lot was used again to test the four serum samples using the initial Day 1 calibration to demonstrate stability of the initial calibration and stability of the control measurements.

The acceptance criterion for samples < 1.0 COI was a recovery ± 0.2 COI relative to the Day 1 results. The acceptance criterion for samples ≥ 1.0 COI was recovery of 80-120% (COI) relative to the Day 1 results.

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 Study

An Elecsys HBeAg reagent pack, was used unstressed (stored at 2-8°C) and after storage on-board at 20 ± 3°C for one week to simulate the stability of weekly calibration.
A new reagent pack was opened and calibrated. Four human serum samples (negative, high negative, low positive, and positive) and the PreciControls (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 initial calibration of unstressed reagents. Recovery for each sample (stressed/unstressed) was calculated based on COI.

The acceptance criterion for samples < 1.0 COI was a recovery ± 0.2 COI. The acceptance criteria for samples ≥ 1.0 COI was recovery 80-120% (COI).

The acceptance criteria were met. The studies confirm calibration stability for 7 days on the MODULAR ANALYTICS E170 when using the same reagent kit.

Elecsys PreciControl HBeAg Studies

PreciControl Real-Time (Shelf Life) Stability

Shelf life was determined by testing three production lots of PreciControl 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 tested in duplicate measurements and recoveries for PC 2 to target values were calculated.

Acceptance criteria were based on lot-specific target ranges for each control and lot number (e.g. COI ≤ 0.2 for PC1; Recovery 72-128% for PC2). The data showed that PreciControl HBeAg is stable for 22 months when tested on MODULAR ANALYTICS E170 analyzer. Shelf life for the PreciControl HBeAg is claimed for 21 months.

The product labeling will state that the PreciControl is stable, unopened at 2-8°C, up to the stated expiration date.

PreciControl Temperature Stress Stability

A temperature stress stability study was conducted to determine the effect of elevated temperature stress on the Elecsys PreciControl HBeAg during transportation. To assess the stability of the Elecsys PreciControl HBeAg after temperature stress, the cut-off indices of the PreciControls were assessed in duplicate before and after incubation of PreciControls for one week at 35°C. The percent recovery was calculated for Elecsys PreciControls HBeAg.

The acceptance criteria were recovery of ± 0.2 (COI) for PreciControl 1 and recovery for PreciControl 2 of 80-120% (COI) relative to the corresponding unstressed PreciControls.

All acceptance criteria were met, demonstrating the Elecsys PreciControl HBeAg is stable for 1 week at 35°C.

PreciControl Stability after First Opening

A stability study was performed to determine the time period over which the Elecsys PreciControl HBeAg can be kept at 2-8°C once opened.

A new PreciControl kit pack was opened and tested on day 0 (unstressed reference), 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 initial results with the unstressed PreciControl kit pack was calculated.

The acceptance criteria were recovery of ± 0.2 COI for PreciControl 1 and recovery for PreciControl 2 of 80-120% (COI) relative to the corresponding unstressed PreciControls.

All acceptance criteria were met, demonstrating that the Elecsys PreciControl HBeAg is stable after first opening when stored at 2-8°C for 8 weeks. The stability of the Elecsys PreciControls HBeAg after first opening at 2-8°C for 8 weeks is claimed.

On-Board Stability - Open PreciControls

Stability studies were performed to determine the time period over which the Elecsys PreciControl HBeAg can be kept open on-board the MODULAR ANALYTICS E170 immunoassay analyzer. The maximum temperature the controls might be exposed to is assumed to be 32°C (upper limit of the specification for the ambient temperature of the MODULAR ANALYTICS E170 immunoassay analyzer). PreciControls may be used for a maximum of seven quality control procedures and should be left on the MODULAR ANALYTICS E170 immunoassay analyzer only during quality control measurement.

A new HBeAg reagent pack and a new PreciControl kit pack were opened and tested together. The HBeAg reagent pack was then stored at 2-8°C and the opened PreciControls stored at 32°C. In six one-hour intervals, the stressed PreciControls were tested in duplicate with the same HBeAg reagent pack. Recovery was calculated based on counts (signal).

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

The acceptance criteria was met after 6 hours on-board the MODULAR ANALYTICS E170. Stability of Elecsys PreciControl HBeAg of up to 6 hours is claimed.

Antimicrobial Effectiveness Testing

Antimicrobial effectiveness testing (AET) was performed according to the United States Pharmacopoeia (USP) Chapter 51. Testing was performed with all reagents of the Elecsys HBeAg immunoassay and Elecsys PreciControl HBeAg kits.

Each reagent was tested with a panel of microorganisms as required by the protocol. After inoculation, samples were plated on the appropriate media at Day 0, Day 7, Day 14, and Day 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.

All reagents met the USP requirements for antimicrobial effectiveness testing.

Analytical Specificity

This study was conducted to evaluate the Elecsys HBeAg immunoassay on the MODULAR ANALYTICS E170 module for potential cross-reactivity with samples from

individuals with various medical conditions and with antigen from various bacteria and viruses. Results were compared to the reference HBeAg assay.

Potential cross-reactivity due to various medical conditions: The Elecsys HBeAg immunoassay on the MODULAR ANALYTICS E170 module was used to test 278 samples from 22 potentially cross-reactive sub-groups. The comparison data to the comparator assay are presented in the following table:

Table 5: Comparison of Elecsys 170 HBeAg Immunoassay and the Comparator Assay Results for Subjects with Potentially Interfering Medical Conditions

Category Infection or Condition	Comparator Assay				Total
	Reactive		Non-Reactive		
	Elecsys HBeAg Immunoassay				
	RX	NR	RX	NR	
Autoimmune (AMA, ANA, SLE)	0	0	0	15	15

Cytomegalovirus (anti-CMV)	0	0	0	12	12
Epstein-Barr Virus (anti-EBV)	0	0	0	12	12
E. coli Infection	0	0	0	12	12
Flu Vaccination	0	0	0	10	10
Hepatitis A Virus (anti-HAV)	0	0	0	10	10
HAV Vaccination	0	0	0	10	10
HBV Vaccination	0	0	0	10	10
Hepatitis C Virus (anti-HCV)	0	0	0	12	12
Hepatitis D Virus (anti-HDV)	0	0	0	4	4
Hepatitis E Virus (anti-HEV)	0	0	0	12	12
Human Immunodeficiency Virus (anti-HIV-1)	0	0	0	12	12
Herpes Simplex Virus (anti-HSV)	0	0	0	12	12
HTLV I/II (anti-HTLV)	0	0	0	12	12
Non-Viral Liver Disease	0	0	0	40	40
Parvovirus B19 Infection	0	0	0	12	12
Pregnancy	0	0	0	12	12
Rheumatoid Factor	0	0	0	11	11
Rubella (anti-Rubella)	0	0	0	12	12
Syphilis	0	0	0	12	12
Toxoplasmosis (anti-Toxo)	0	0	0	12	12
Varicella Zoster Virus (anti-VZV)	0	0	0	12	12
Total	0	0	0	278	278

Potential cross-reactivity due to viral and bacterial materials: Two levels of antigens from cultures of three bacteria and four viruses were spiked into aliquots of two pools of HBeAg non-reactive and reactive sera. The purpose of this study was to determine if the bacterial and viral antigens present in the culture would interfere with the Elecsys HBeAg immunoassay.

The results of the Elecsys HBeAg testing of the blank control and spiked specimens are presented in the table below.

Table 6: Effects of Bacterial and Viral Antigens on the Elecsys HBeAg Immunoassay

Aliquot Culture Level	Serum Pool	
	HBeAg Non-Reactive (COI)	HBeAg Reactive (COI)
Blank, no Spike (Reference)	0.088	2.88
S. aureus at 900 cfu/mL	0.096	5.88
S. aureus at 9,000 cfu/mL	0.091	2.68
P. aeruginosa at 900 cfu/mL	0.088	2.92
P. aeruginosa at 9,000 cfu/mL	0.095	2.67
E. coli at 900 cfu/mL	0.092	2.85
E. coli at 9,000 cfu/mL	0.096	2.59
EBV at 0.9 ug/mL	0.094	2.99
EBV at 0.9 ng/mL	0.088	2.78
Cytomegalovirus at 0.9 ug/mL	0.090	3.75
Cytomegalovirus at 0.9 ng/mL	0.096	2.63
Rubella at 0.9 ug/mL	0.093	3.46
Rubella at 0.9 ng/mL	0.096	2.80
Varicella Zoster Virus at 0.9 ug/mL	0.094	3.16
Varicella Zoster Virus at 0.9 ng/mL	0.094	2.53

Precision

Precision measurements were conducted to evaluate repeatability and the intermediate precision of within-laboratory precision according to CLSI guideline EP5-A2.

Internal Precision

A seven member precision panel consisting of 5 human sera (two high negative, two low positive, one positive) and two PreciControls (one positive and one negative) was measured in duplicate determinations in two runs per day for 12 days. The measurements were performed on one MODULAR ANALYTICS E170 analyzer, at one site, with one reagent lot, performing weekly rack pack calibration, and spanning at least two calibration cycles. Precision values were calculated according to EP5-A2.

Table 7: Precision Studies

		Within-Run Precision		Within-Day Precision	
Sample	**Mean (COI)[a]**	**SD (COI)**	**CV (%)**	**SD (COI)**	**CV (%)**
HS1[b], high negative	0.886	0.040	4.5	0.000	0.000
HS2, high negative	0.913	0.038	4.2	0.000	0.000
HS3, low positive	1.15	0.041	3.6	0.000	0.000
HS4, low positive	1.13	0.049	4.3	0.000	0.000
HS5, positive	2.70	0.116	4.3	0.072	2.7
PC[c] HBeAg 1	0.091	0.004	4.5	0.003	3.3
PC HBeAg 2	19.0	0.210	1.1	0.143	0.75
		Between-Day Precision		**Total Precision**	
Sample	**Mean (COI)**	**SD (COI)**	**CV (%)**	**SD (COI)**	**CV (%)**
HS1, high negative	0.886	0.017	2.0	0.043	4.9
HS2, high negative	0.913	0.020	2.2	0.043	4.7
HS3, low positive	1.15	0.015	1.3	0.044	3.8
HS4, low positive	1.13	0.035	3.1	0.060	5.3
HS5, positive	2.70	0.000	0.0	0.137	5.1
PC HBeAg 1	0.091	0.002	2.7	0.006	6.2
PC HBeAg 2	19.0	0.336	1.8	0.421	2.2

[a] COI = cutoff index
[b] HS = human serum
[c] PC= PreciControl

Reproducibility (External Precision)

Precision results were collected on MODULAR ANALYTICS E170 modules at three external sites using three lots of reagents (two lots at each site: AB, BC, AC). PreciControls 1 and 2 and four near cut-off human serum pools (low negative HSP1, high negative HSP2, low positive HSP3, high positive HSP4) and moderately positive human serum pool (HSP5) were tested in replicates of 3 in 2 runs/day for 5 days according to CLSI EP15-A2 / EP5-A2. Data from all 3 reagent lots were combined to achieve SD and percent CV for repeatability (within-run), between-run, between-day, between-lot, between-site and reproducibility. The results are summarized in the following table:

Table 8: External Precision Data - Elecsys HBeAg Between-Site Reproducibility on the MODULAR ANALYTICS E170 Analyzer

		Repeatability		Between-run		Between-day	
Sample	**Mean COI**	**SD COI**	**CV %**	**SD COI**	**CV %**	**SD COI**	**CV %**
HS1	0.853	0.017	2.0	0.017	1.9	0.019	2.2
HS2	0.939	0.018	1.9	0.003	0.3	0.022	2.3
HS3	1.08	0.022	2.0	0.011	1.1	0.025	2.3
HS4	1.24	0.025	2.0	0.012	1.0	0.033	2.6
HS5	2.42	0.043	1.8	0.039	1.6	0.046	1.9
PC1[m]	0.099	0.007	7.4	0.007	7.0	0.000[n]	0.0
PC2	12.1	0.171	1.4	0.182	1.5	0.169	1.4

		Between-lot		Between-site		Reproducibility	
Sample	**Mean COI**	**SD COI**	**CV %**	**SD COI**	**CV %**	**SD COI**	**CV %**
HS1	0.853	0.095	11.2	0.000*	0.0	0.100	11.7
HS2	0.939	0.086	9.2	0.000*	0.0	0.091	9.7
HS3	1.08	0.111	10.4	0.000*	0.0	0.117	10.9
HS4	1.24	0.105	8.4	0.000*	0.0	0.113	9.1
HS5	2.42	0.240	9.9	0.000*	0.0	0.251	10.4
PC1	0.099	0.017	16.7	0.000*	0.0	0.019	19.6
PC2	12.1	0.685	5.7	0.000*	0.0	0.748	6.2

[m] PC = PreciControl HBeAg

[n] or *: SD of 0.000 because the variance was below the stated significant figures

Precision and reproducibility of the Elecsys HBeAg immunoassay was acceptable from run to run, day to day, reagent lot to reagent lot and site to site. In the precision studies, repeatability ranged from 1.1 to 4.5 %CV and within-laboratory precision ranged from 2.2-6.2 %CV for the positive samples. The between-site reproducibility study demonstrated that SD values for samples with mean COI values <1.0 COI (negatives) ranged from 0.019 to 0.100 COI with a median of 0.091 COI. The %CV for samples with mean COI values >1.0 ranged from 6.2 to 10.9% with a median value of 9.8%.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The safety and effectiveness of the Elecsys HBeAg 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 HBeAg immunoassay on the MODULAR ANALYTICS E170 analyzer with specimens from patients at increased risk for infection with HBV. Agreement of the Elecsys HBeAg immunoassay was assessed relative to an FDA approved comparator HBeAg test and HBV disease classification. The clinical study included a prospective and a retrospective study as well as a study of seroconversion panels.

Prospective Study:

Inclusion criteria

The Symptomatic at Risk group was required to have clinical symptoms, laboratory data, or histological findings suggestive of hepatitis infection including jaundice, discoloration of urine or stool, non-specific GI symptoms such as nausea or vomiting, flu-like symptoms, elevated ALT, AST or bilirubin, cryoglobulinemia, lymphoma, autoimmune thyroiditis, renal disease, dermatologic conditions such as lichen planus or porphyria cutanea tarda, and histological evidence of liver disease (if available).

The Asymptomatic at Risk group contained individuals with medical risks, occupational risks, behavioral risks and sexual risks for HBV infection and this group was required to have no clinical symptoms of liver disease.

Exclusion criteria

Exclusion criteria consisted of the following: subjects younger than 21 years old; subjects who violated any of the inclusion criteria; subjects who were unable to understand and sign the informed consent; subjects who were unable to donate approximately twenty milliliters of blood.

Retrospective Study: Retrospective study specimens were obtained from commercial vendors.

Seroconversion Panels: The panels were obtained from commercial sources.

Specimen Classification

HBV classifications were determined based on test results for a HBV marker profile using FDA-approved tests for the detection of HBsAg, HBeAg, Anti-HBc IgM, Anti-HBc Total, Anti-HBe, and Anti-HBs. The specimens were assigned an HBV status based on the algorithm provided in the following table:

Table 9: Specimen Classification

Serological Classification by HBV Markers						
	HBsAg	HBeAg	Anti-HBc IgM	Anti-HBc	Anti-HBe	Anti-HBs
Acute	(+)	(+)	(+)	(+)	(-)	(-), eq
Acute	(+)	(+), (-)	(+)	(+)	(+), qns	(-)
Acute	(+)	(+)	(-)	(-)	(-)	(-)
Acute	(+)	(+)	eq	(+)	(+)	(-)
Acute	(+)	(-)	eq	(+)	(+)	(-)
Acute (late)	(+)	(-)	(+)	(+)	(+)	(+), eq
Chronic	(+) >6 mo.	(-)	(-)	(+)	(+) eq (-)	(-)
Chronic	(+) >6 mo.	(+)	eq	(+)	(-)	(-)
Chronic	(+)	(+)	(-)	(+)	(-)	(-),(+),eq
Chronic	(+)	(+)	(+)	(+)	(-),(+)	(+)
Chronic	(+)	(+)	(-)	(+)	(+)	(-)
Chronic	(+)	(-)	(-)	(+)	(+), eq	(-)
Early Recovery	(-)	(-)	(-)	(+)	(+), (-), eq, qns	(-)
Early Recovery	(-)	(-)	(+), eq	(+)	(+)	(+)
Early Recovery	(-)	(-)	eq	(+)	(+)	eq
Recovery	(-)	(-)	(-)	(+) (-)	(+)	(+)
Recovery	(-)	(-)	(-)	(+)	(+)	eq
Recovery	(-)	(-)	(-)	(+)	eq	(+)
Recovered or Immune due to Natural Infection	(-)	(-)	(-)	(+)	(-)	(+), eq
HBV Vaccine Response	(-)	(-)	(-)	(-)	(-)	(+)
HBV Vaccination Response (?)	(-)	(-)	(-)	(-)	(-)	eq
Not previously infected	(-), rr uncnf	(-)	(-)	(-)	(-)	(-)
Not Interpretable	qns	(-)	(-), (+)	(+)	(+)	(-)
Not Interpretable	qns	(-)	(-)	(-)	(-)	(-)
Not Interpretable	qns	(+)	(-)	(+)	(-)	(-)
Not Interpretable	(-)	(-)	(-)	qns	(-)	(+)
Not Interpretable	(-)	(-)	(-)	(-)	(+)	(-)
Not Interpretable	(-)	(-)	(-)	(-)	(-)	nd
Not Interpretable	(-)	(-)	(-)	(+)	qns	(+)
Not Interpretable	(-)	(-)	(-)	nd	(-)	(+), (-), qns
Not Interpretable	(-)	(-)	(-)	nd	(+)	(-)
Not Interpretable	(-)	(+)	(-)	(-)	(-)	(+), (-)
Not Interpretable	(+)	(+)	nd	(+)	(+), (-)	(-)

Key: **nd**, not done, **qns**, testing incomplete due to inadequate sample volume, **eq**, equivocal or indeterminate or borderline, **rr uncnf,** repeatedly reactive HBsAg with (-) confirmatory testing

B. Accountability of PMA Cohorts

The prospective study subjects were from populations representing subjects with signs and symptoms of hepatitis and asymptomatic subjects at increased risk for hepatitis. Prospective clinical specimens were obtained from multiple collection sites in the US including Miami, Florida; Los Angeles, CA; Newark, NJ and Atlanta, GA. A total of 1641 prospective subjects were recruited for the study and were divided into two groups, Asymptomatic at Risk (n=1120) and Symptomatic at Risk (n=521) populations. The sample collection was started in September 2008 and completed by December 2008.

Retrospective specimens (n=201) from subjects with a higher probability of HBV infection or reactive marker status were obtained for the retrospective study from commercial vendors.

Eleven seroconversion panels were obtained from commercial sources and tested.

C. Study Population Demographics

The clinical study population for the Elecsys HBeAg immunoassay consisted of 1842 subjects. Of these subjects, 1641 were enrolled prospectively. In addition, 201 retrospective samples were also tested. A demographic summary of the overall adult specimen population by age, race and gender is provided in the following tables:

Table 10: Demographic Summary of Adult Clinical Population by Race

Ethnicity	**Asymptomatic**		**Symptomatic**		**Supplemental**		**Total**	
Race	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
American Indian/Alaska Native	7	0.62	3	0.58	0	0.00	10	0.54
Asian	2	0.18	4	0.77	10	4.98	16	0.87
African American/Black	645	57.54	237	45.49	7	3.48	889	48.24
Caucasian/White	438	39.16	274	52.59	8	3.98	720	39.12
Pacific Islander	2	0.18	2	0.38	0	0.00	4	0.22
Unknown	0	0.00	0	0.00	138	68.66	138	7.49
Other	26	2.32	1	0.19	38	18.91	65	3.53
Total	1120	100.00	521	100.00	201	100.01	1842	100.00

Table 11: Demographic Summary of Adult Clinical Population by Gender

Gender	**Asymptomatic**		**Symptomatic**		**Supplemental**		**Total**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**

Male	801	71.54	377	72.36	121	60.20	1299	70.54
Female	319	28.46	144	27.64	43	21.39	506	27.46
Unknown	0	0.00	0	0.00	37	18.41	37	2.01
Total	1120	100.00	521	100.00	201	100.00	1842	100.01

Table 12: Demographic Summary of Adult Specimen Population by Age

	Asymptomatic		**Symptomatic**		**Supplemental**		**Overall**	
Age Group	**n**	%	**n**	%	**n**	%	**n**	%
21-30	172	15.34	75	14.40	56	27.86	303	16.44
31-40	244	21.77	87	16.70	29	14.43	360	19.53
41-50	435	38.89	199	38.20	30	14.92	664	36.08
51-60	239	21.32	131	25.14	22	10.94	392	21.27
61-70	25	2.23	26	4.99	14	6.96	65	3.53
71-80	5	0.00	3	0.58	13	6.47	21	1.14
>80	-	-	-	-	1	0.50	1	0.05
Unknown	-	-	-	-	36	17.91	36	1.95
Total	1120	100	521	100	201	100	1842	100

Study Results:

Results of Method Comparison Studies

Comparison of Elecsys HBeAg Immunoassay on the 170 Modular Analytics analyzer to the Comparator Assay - Results by HBV Classification

The Elecsys HBeAg immunoassay was evaluated at three clinical laboratories to assess the performance of the assay in a testing environment which most closely resembles that of the final user. Results were compared to an HBeAg detection assay currently marketed in the US. The combined results from the three sites are shown in the following tables. The results from prospectively collected samples are summarized in Tables 13, 14, and 15. The results from retrospective samples are summarized in Table 16.

Table 13: Percent Agreement between Elecsys HBeAg (E170) and the HBeAg Comparator Assay for each Serological Classification: Asymptomatic Increased Risk Cohort

HBV Classification	Positive Percent Agreement % (n/N)	95% Score Confidence Interval	Negative Percent Agreement % (n/N)	95% Score Confidence Interval
Acute	100.00 (12/12)	75.75 to 100.00	100.00 (1/1)	20.65 to 100.00
Chronic	100.00 (21/21)	84.54 to 100.00	95.65 (22/23)	79.01 to 99.23
Early Recovery	N/A	N/A	100.00 (75/75)	95.13 to 100.00
Recovery	N/A	N/A	100.00 (172/172)	97.82 to 100.00
Recovered	N/A	N/A	100.00 (105/105)	96.47 to 100.00
HBV Vaccination	N/A	N/A	100.00 (219/219)	98.28 to100.00
Not Previously Infected	N/A	N/A	100.00 (483/483)	99.21 to 100.00
Not Interpretable	100.00 (3/3)	43.85 to 100.00	100.00 (6/6)	60.97 to 100.00
Total	100.00 (36/36)	90.36 to 100.00	99.91 (1083/1084)	99.48 to 99.98

N= result by comparator; n=result by Elecsys HBeAg immunoassay

Table 14: Percent Agreement between Elecsys HBeAg (E170) and the HBeAg Comparator Assay for each Serological Classification: Symptomatic Increased Risk Cohort

HBV Classification	Positive Percent Agreement % (n/N)	95% Score Confidence Interval	Negative Percent Agreement % (n/N)	95% Score Confidence Interval
Acute	100.00 (1/1)	20.65 to 100.00	N/A (0/0)	N/A
Chronic	100.00 (4/4)	51.01 to 100.00	100.00 (7/7)	64.57 to100.00
Early Recovery	N/A	N/A	100.00 (37/37)	90.59 to 100.00
Recovery	N/A	N/A	100.00 (71/71)	94.87 to 100.00
Recovered	N/A	N/A	100.00 (34/34)	89.85 to 100.00
HBV Vaccination	N/A	N/A	100.00 (136/136)	97.25 to100.00
Not Previously Infected	N/A	N/A	100.00 (227/227)	98.34 to 100.00
Not Interpretable	66.67 (2/3)	20.77 to 93.85	100.00 (1/1)	20.65 to 100.00
Total	87.50 (7/8)	52.91 to 97.76	100.0 (513/513)	99.26 to 100.00

Table 15: Percent Agreement between Elecsys HBeAg (E170) and the Comparator Assay for each Specimen Classification: Combined Asymptomatic and Symptomatic Increased Risk Cohorts

HBV Classification	Positive Percent Agreement % (n/N)	95% Score Confidence Interval	Negative Percent Agreement % (n/N)	95% Score Confidence Interval
Acute	100.00 (13/13)	77.19 to 100.00	100.00 (1/1)	20.65 to 100.00
Chronic	100.00 (25/25)	86.68 to 100.00	96.67 (29/30)	83.33 to 99.41
Early Recovery	N/A	N/A	100.00 (112/112)	96.68 to 100.00
Recovery	N/A	N/A	100.00 (243/243)	98.44 to 100.00
Recovered	N/A	N/A	100.00 (139/139)	97.31 to 100.00
HBV Vaccination	N/A	N/A	100.00 (355/355)	98.93 to100.00
Not Previously Infected	N/A	N/A	100.00 (710/710)	99.46 to 100.00
Not Interpretable	83.33 (5/6)	43.85 to 96.99	100.00 (7/7)	64.57 to 100.00
Total	97.73 (43/44)	88.19 to 99.60	99.94 (1596/1597)	99.65 to 99.99

Table 16: Percent Agreement between Elecsys HBeAg (E170) and the HBeAg Comparator Assay for each Serological Classification: Supplemental Cohort

HBV Classification	Positive Percent Agreement % (n/N)	95% Score Confidence Interval	Negative Percent Agreement % (n/N)	95% Score Confidence Interval
Acute	91.49 (43/47)	80.07 to 96.64	100.00 (22/22)	85.13 to100.00
Chronic	90.00 (18/20)	69.90 to 97.21	100.00 (13/13)	77.19 to100.00
Early Recovery	N/A	N/A	100.00 (11/11)	74.12 to 100.00
Recovery	N/A	N/A	100.00 (7/7)	64.57 to 100.00
Recovered	N/A	N/A	100.00 (4/4)	51.01 to 100.00
HBV Vaccination	N/A	N/A	100.00 (23/23)	85.69 to100.00
Not Previously Infected	N/A	N/A	100.00 (35/35)	90.11 to 100.00
Not Interpretable	75.00 (3/4)	30.6 to 95.44	100.00 (15/15)	79.61 to 100.00
Total	90.10 (64/71)	81.02 to 95.14	100.0 (130/130)	97.13 to 100.00

Results from Seroconversion Commercial Panels

Seroconversion sensitivity of the Elecsys HBeAg immunoassay was shown by testing eleven commercially-sourced seroconversion panels along with the comparator assay. The comparison of seroconversion timing for the Elecsys HBeAg immunoassay on the MODULAR ANALYTICS E170 module and for the comparator assay for each panel is presented in the following table.

Table 17: Results for Days to Evidence of HBeAg Seroconversion for the Elecsys HBeAg Immunoassay Compared to the Comparator Assay

	Comparator HBeAg Assay		Roche Elecsys HBeAg		Difference in Days to Elecsys Reactivity (Comparator-Elecsys)	Disease Status
Panel ID	**NR**	**RX**	**NR**	**RX**		
6278	12	16	12	16	0	Acute
6281	22	33	N/A [1]	N/A [1]	N/A [1]	Acute to Early Recovery
6282	21	26	21	26	0	Acute
6284	61	64	61	64	0	Acute
9072	108	128	128	135	-7 (1 draw)	Early Acute
11015	43	70	43	70	0	Potentially Acute or Chronic
11024	43	49	49	54	-5 (1 draw)	Chronic
PHM933	16	144	16	144	0	Acute
PHM934	10	14	10	14	0	Acute
PHM935A	30	35	30	35	0	Acute
PHM935B	203	189	203	189	0	Early Recovery
6278	12	16	12	16	0	Acute
6281	22	33	N/A [1]	N/A [1]	N/A [1]	Acute to Early Recovery
6282	21	26	21	26	0	Acute
6284	61	64	61	64	0	Acute
9072	108	128	128	135	-7 (1 draw)	Early Acute
11015	43	70	43	70	0	Potentially Acute or Chronic
11024	43	49	49	54	-5 (1 draw)	Chronic
PHM933	16	144	16	144	0	Acute
PHM934	10	14	10	14	0	Acute

	Comparator HBeAg Assay		Roche Elecsys HBeAg		Difference in Days to Elecsys Reactivity (Comparator-Elecsys)	Disease Status
Panel ID	**NR**	**RX**	**NR**	**RX**		
PHM935A	30	35	30	35	0	Acute
PHM935B [2]	203	128	203	128	0	Early Recovery

[1] Panel 6281 did not convert for the Elecsys HBeAg immunoassay and remained NR for the complete panel.
[2] No initial negative samples were available for this panel.

D. Safety and Effectiveness Results of the Clinical Studies

1. Safety Results

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

   The HBV classification of the prospective population showed eight clinical categories of patient populations. In the pivotal prospective study, the reactive (positive) percent agreement between the Elecsys HBeAg immunoassay and the comparator assay was 97.7 % (43/44) with a 95% confidence interval (CI) of 88.2% to 99.6% and the non-reactive (negative) percent agreement was 99.9 % (1596/1597) with a 95 % CI of 99.7% to 99.99%. These values are within the range of the acceptance criteria of a point estimate of 95% or higher with the lower bound of the two-sided 95% CI 90% or higher.

   In the retrospective study, the positive percent agreement was 90.10% (64/71) with a 95% CI of 81.02% to 95.14%. The negative percent agreement was 100.00% (130/130) with a 95% CI of 97.13% to 100.00%. These results for retrospective specimens are in line with the results presented by other manufacturers of FDA approved HBeAg detection assays.

Testing of eleven seroconversion panels generated data that reflected the sensitivity of the Elecsys HBeAg immunoassay. Results for the Elecsys HBeAg immunoassay were equivalent to the results for the comparator assay in eight of the eleven seroconversion panels. Seroconversion as detected by the Elecsys HBeAg immunoassay was one draw later than the comparator assay in two panels. Seroconversion was not detected by the Elecsys HBeAg immunoassay in one panel while the comparator assay was briefly reactive. Overall, the sensitivity of the Elecsys HBeAg immunoassay is comparable to the comparator device.

The specificity of the Elecsys HBeAg immunoassay when tested for potential cross-reactivity with samples from individuals with various medical conditions and with antigen from various bacteria and viruses showed no reactivity. Thus indicating low risk of false positive results by the test device.

Overall, the clinical studies showed the effectiveness of the Elecsys HBeAg immunoassay in detecting accurately the presence of the HBeAg marker for hepatitis B 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 six 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)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the FDA Microbiology 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 HBeAg immunoassay performance is acceptable when testing in serum from gel separator tubes and lithium heparin, sodium heparin, K_2-EDTA and sodium citrate plasma.
- 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) or frozen for 3 months (-20°C). The samples can also withstand 6 freeze/thaw cycles.
- The claimed reagent stability information was adequately substantiated by analytical study results. The real-time stability information provided supports a shelf life of 24 months when stored at 2-8°C.
- The Elecsys HBeAg reagents can withstand stress at 35°C for one week. It is stable for 8 weeks after opening when stored at 2-8°C. It is stable on-board the - MODULAR ANALYTICS E170 for 8 weeks.
- The Elecsys HBeAg calibrators are stable on-board the MODULAR ANALYTICS E170 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 HBeAg can withstand stress at 35°C for one week. The PreciControls are stable for 8 weeks after opening when stored at 2-8°C, and are stable on-board the MODULAR ANALYTICS E170 for 6 hours.
- The preservative systems that the Elecsys HBeAg immunoassay reagents and PreciControls contain have been shown to meet USP Chapter 51 criteria.
- The Elecsys HBeAg immunoassay demonstrated precision estimates that met internal specifications for repeatability and laboratory precision. The repeatability ranged from 1.1 to 4.5% CV and within-laboratory precision ranged from 2.2-6.2% CV for the positive samples.
- Reproducibility of the Elecsys HBeAg immunoassay was acceptable from run to run, day to day, reagent lot to reagent lot and site to site. The between-site reproducibility study showed %CV for samples with COI values >1.0 (positives) from 6.2 to 10.9% with a median value of 9.8%.
- The clinical performance was evaluated in an ethnically diverse population representative of the intended use population and of different HBV infected groups. The positive and negative percent agreement values obtained for the Elecsys HBeAg immunoassay relative to an FDA approved comparator assay 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 HBeAg immunoassay for use on the MODULAR ANALYTICS E170 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 above under Potential Adverse Effects of the Device on Health. 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 HBeAg is an important component of hepatitis B serology panels. Although not directly needed for the diagnosis of hepatitis B infection, it is a marker of viral replication and infectivity in the setting of chronic hepatitis. Seroconversion from HBeAg positive to negative status occurs during resolution of acute hepatitis B infection, and prolonged detection of HBeAg in serum is one marker of chronic hepatitis.

Seroconversion from HBeAg positive to HBeAg negative status (with seroconversion to anti-HBeAg status) during chronic hepatitis is used as indicative of entry into an inactive carrier state (in most instances) with less hepatic inflammation and a better prognosis and is also used as a marker of treatment response to drug therapy. HBeAg is less useful as a marker of inflammation in vertically-acquired infection.

The risks from use of the Elecsys HBeAg immunoassay are false positive and false negative results. In both instances, risks are mitigated since HBeAg status would always be interpreted in conjunction with other serological markers, hepatitis B DNA measurements, and serum markers of hepatic inflammation.

A false positive result may suggest more active liver disease than is truly present; however, this would be mitigated by inconsistent serum transaminases and/or inconsistent other markers such as anti-HBeAg status or DNA levels.

A false negative result for a patient chronic hepatitis may be more problematic as conceivably treatment could be withheld in this setting; however, current guidelines include other markers in the consideration of treatment. In most cases of chronic hepatitis, seromarkers would be obtained and it is likely that errors would be detected.

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 hepatitis B e antigen in human serum or plasma by the Elecsys HBeAg 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 submitted clinical studies have shown that the Elecsys HBeAg immunoassay, when compared to reference clinical laboratory procedures, has a similar ability to detect the presence of HBe antigen in specimens from individuals infected with HBV. The rate of false positivity and false negativity are within acceptable limits compared to the comparator assay. It has been shown that the device has no demonstrable cross-reactivity with the majority of viruses or organisms that may cause clinical hepatitis. 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 March 14, 2014. 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 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.