SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:
IgM antibodies to hepatitis B core antigen
IgM antibodies to hepatitis B core antigen control material

Device Trade Names:
Elecsys Anti-HBc IgM Immunoassay, Elecsys PreciControl Anti-HBc IgM for use on the cobas e 601 immunoassay Analyzer

Applicant's Name and Address:
Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46256 USA

Date(s) of Panel Recommendation:

None

Premarket Approval Application (PMA) Number:
P110022

Date of FDA Notice of Approval:
October 26, 2011

Expedited:
Not Applicable

II. INDICATIONS FOR USE

Elecsys Anti-HBc IgM Immunoassay

The Elecsys Anti-HBc IgM immunoassay is intended for the in vitro qualitative determination of IgM antibodies to hepatitis B core antigen (anti-HBc IgM) in human serum or plasma (potassium EDTA, lithium heparin, sodium heparin, sodium citrate) in adult patients with symptoms of hepatitis or who may be at risk for hepatitis B (HBV) infection. The presence of anti-HBc IgM, in conjunction with other laboratory results and clinical information, is indicative of acute or recent hepatitis B virus (HBV) infection. The Elecsys Anti-HBc IgM immunoassay's performance has not been established for the monitoring of HBV disease or therapy.

The electrochemiluminescence Immunoassay "ECLIA" is intended for use on the cobas e 601 immunoassay analyzer.

PreciControl Anti-HBc IgM

Elecsys PreciControl Anti-HBc IgM is used for quality control of the Elecsys Anti-HBc IgM Immunoassay on the cobas e 601 immunoassay analyzer.

III. CONTRAINDICATIONS

None.

IV. WARNINGS AND PRECAUTIONS

Warnings and precautions for the Elecsys Anti-HBc IgM Immunoassay and Elecsys PreciControl Anti-HBc IgM are stated in the respective product labeling.

V. DEVICE DESCRIPTION

The Elecsys Anti-HBc IgM Immunoassay employs electrochemiluminescence technology and is a qualitative serologic, two step assay with μ-capture test format. The assay is run on the cobas e 601 immunoassay analyzer. Total duration of the assay is 18 minutes.

1st incubation: Pretreatment of 10 μL of sample (automatically prediluted 1:400 with Elecsys Diluent Universal) with anti-Fdγ reagent to block specific IgG.

2nd incubation: Biotinylated monoclonal h-IgM-specific-antibodies, HBcAg labeled with a Tris(2,2'-bipyridyl) ruthenium (II) complex ($Ru(bpy)^{2/3+}$) and streptavidin-coated microparticles are added to the pretreated sample. Anti-HBc IgM antibodies present in the sample react with the ruthenium-labeled HBc antigen and the biotinylated anti-h-IgM to form a sandwich complex which becomes bound to the solid phase via interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

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 cutoff value previously obtained by anti-HBc IgM calibration.

PreciControl Anti-HBc IgM:

The Elecsys Anti-HBc IgM Immunoassay uses the Elecsys PreciControl Anti-HBc IgM for quality control. The controls, 1 (negative) and 2 (positive) contain human serum in the negative and positive concentration ranges for anti-HBc IgM. The controls are used for monitoring the accuracy of the Elecsys Anti-HBc IgM Immunoassay.

Kit Configuration and Components

The Elecsys Anti-HBc IgM 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. (4-(2-Hydroxyethyl)-1- piperazine-ethanesulfonic Acid) buffer

with protein stabilizers (bovine), saccharose, detergent and preservatives. Component 1 is provided ready-to-use and is stored at 2-8°C upright.

Component 2
The Rl reagent contains anti-human-Fdy-antibody (sheep) (0.1 mg/mL) in a phosphate buffer (pH 7.4) with protein stabilizer (bovine), detergent and preservative. The reagent is provided ready-to-use and is stored upright at 2-8°C.

Component 3
The R2 reagent contains biotinylated monoclonal anti-h-IgM antibody (mouse) (> 600 ng/mL) and ruthenylated HBc antigen (> 200 ng/mL) in 100 mmol/L phosphate buffer, pH 7.4, with protein stabilizers (bovine) and preservatives. The reagent is provided ready-to-use and is stored at 2-8°C upright.

Component 4
Cal 1 is the negative calibrator and consists of buffered (50 mmol/L HEPES) human serum which is negative for anti-HBc IgM antibodies and preservatives. It is provided ready-to-use and is stored at 2-8°C.

Component 5
Cal 2 is the positive calibrator which consists of buffered (50 mmol/L HEPES) human serum positive for anti-HBc IgM antibodies (> 100 PEI U/mL) and preservatives. It is provided ready-to-use and is stored at 2-8°C.

The PreciControl Anti-HBc IgM contains two reagents:

Component 1
PreciControl 1, is the negative control and consists of two reagents, buffered (50 mmol/L HEPES) human serum and preservatives. The reagent is provided ready-to-use and should be stored at 2-8°C.

Component 2
PreciControl 2, is the positive control which consists buffered (50 mmol/L HEPES) human serum negative for anti-HBc IgM antibodies and preserved human serum that is positive for anti-HBc IgM antibodies (> 130 PEI U/mL). The reagent is provided ready-to-use and should be stored at 2-8°C.

Calibrator

The Elecsys Calibrator 1 and Calibrator 2 are used to calibrate the Elecsys Anti-HBc IgM Immunoassay test kit. The presence or absence of anti-HBc IgM in the sample is determined by comparing the electrochemiluminescent signal in the reaction to the cut-off signal determined from an active Elecsys Anti-HBc IgM 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 Anti-HBc IgM Immunoassay. The result of a sample is given in the form of a cut-off index (COI—signal sample/signal cut-off) along with a result interpretation 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 Anti-HBc IgM Testing

Initial Elecsys Anti-HBc IgM Immunoassay			
COI	**Result**	**Interpretation of Results**	**Retest Procedure**
< 0.9	Non-reactive [a]	No IgM antibodies to HBc were detected	No retest required
0.9≤ COI <1.1	Border	Borderline zone (undetermined)	Retest in duplicate with the Elecsys Anti-HBc IgM Immunoassay
≥ 1.1	Reactive	IgM antibodies to HBc detected	Presumptive evidence of IgM antibodies to HBc. Follow CDC recommendations for supplemental testing

a) Note: A negative anti-HBc IgM result can indicate that the patient is either susceptible to HBV infection due to no past exposure, is chronically infected with HBV, or is immune to HBV due to a resolved past infection or vaccination.

Table 2: Clinical Interpretation of Repeat Anti-HBc IgM Testing

Final Elecsys Anti-HBc IgM Immunoassay			
Initial Result COI	**Result after Retest (COI)**	**Final Results**	**Interpretation of Results**
< 0.9	No retest required	NON-REACTIVE [b]	IgM antibodies to HBc were not detected; does not exclude the possibility of exposure to HBV
0.9≤ COI <1.1	If 2 of the 3 results have a COI < 1.0	NON-REACTIVE	IgM antibodies to HBc were not detected; does not exclude the possibility of exposure to HBV
	If 2 of the 3 results have a COI ≥ 1.0	REACTIVE	Presumptive evidence of IgM antibodies to HBc. Follow CDC recommendations for supplemental testing.
≥ 1.1	No retest required	REACTIVE	Presumptive evidence of IgM antibodies to HBc. Follow CDC recommendations for supplemental testing.

b) Note: A negative anti-HBc IgM result can indicate that the patient is either susceptible to HBV infection due to no past exposure, or is immune to HBV due to a resolved past infection or vaccination.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are currently several FDA approved *in vitro* diagnostic tests for measuring antibodies (total with both IgG and IgM, and IgM only) to hepatitis B core antigen, which when used in conjunction with a patient's medical history, clinical examination, and other findings can be used for diagnostic purposes.

VII. MARKETING HISTORY

The Elecsys Anti-HBc IgM Immunoassay and PreciControl Anti-HBc IgM are currently marketed in Europe, Asia, and South America.

The device has not been withdrawn to date from the market in any country for reasons relating to safety and effectiveness of the device.

The following table provides the list of countries where the device is distributed:

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
Ecuador	Mexico	Thailand
Finland	Netherlands	Turkey
France	New Zealand	Uganda
Germany	Panama	Untied Kingdom
Greece	Peru	Venezuela
Hong Kong	Philippines	Hungary
Poland		

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Below is a list of the potential adverse effects (e.g., complications) associated with the use of the device. 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 Elecsys Anti-HBc IgM Immunoassay detects IgM antibodies to the hepatitis B core antigen. A false non-reactive result in a clinical setting can cause a physician to believe the patient's infection is not acute. Conversely, a false reactive result could lead the physician to believe the patient's infection is acute when it is chronic or the patient is not infected with Hepatitis B. This could lead to a misdiagnosis. To prevent these misdiagnoses, additional testing is often used, including the measurement of anti-hepatitis B e-antigen (Anti-HBe) and anti-hepatitis B surface antigen (Anti-HBsAg).

Incorrect results with an anti-HBc IgM Immunoassay (false positives or negatives) do not pose a public health problem because the assay alone does not provide information on chronic carrier status. Since chronic carriers are infectious for hepatitis B, they need to be tested periodically with an assay for hepatitis B surface antigen.

IX. SUMMARY OF PRECLINICAL STUDIES

All studies were performed at Roche Diagnostics Laboratories using the Elecsys Anti-HBc IgM Immunoassay and the Elecsys PreciControl Anti-HBc IgM on the cobas e 601 immunoassay analyzer.

A. Cutoff Determination

For the Elecsys Anti-HBc IgM Immunoassay, the cutoff is calculated from signal of the negative calibrator (Cal 1) and the positive calibrator (Cal 2) according to the following general formula:

$$(a) \times \text{Cal } 1 + (b) \times \text{Cal } 2 + c$$

The cut-off was established at about 45 PEI U/mL and verified with internal studies measuring a panel of 168 samples from hospitalized subjects, seroconversion samples, and banked negative samples. Receiver Operator Curve (ROC) analysis was done to verify the cut-off and determine sensitivity and specificity.

The analytical performance of the Elecsys Anti-HBc IgM Immunoassay is calculated by reading off the concentration at the cut-off from the master calibrator curve (standardized against "HBc-Reference Serum 84", Anti-HBc IgM of the Paul Ehrlich-Institute, Langen, Germany). For each production lot, the sensitivity at cut-off is determined by Quality Control during lot release. The acceptance criterion for the sensitivity at cut-off is 36-67 PEI U/mL.

B. Limit of Blank and Limit of Detection

The analytical sensitivity of the Elecsys Anti-HBc Immunoassay was evaluated by determining the Limit of Blank (LoB) and Limit of Detection (LoD) as described in CLSI guideline EP17-A. The limit of blank was determined to be 1.2450 and 1.9000 PEI U/mL with two lots, and the limit of detection was determined to be 2.2976 and 2.9976 U/mL. The analytical sensitivity is conservatively set at 3.0 PEI U/mL.

C. Endogenous Interference

To evaluate the effect of elevated levels of hemoglobin, bilirubin, lipids (intralipid), biotin and total protein on the Elecsys Anti-HBc IgM Immunoassay, interferent studies were performed, as described below:

- Hemolysis: 4 anti-HBc IgM samples (one negative, one high negative, one low positive, one positive) were spiked at different levels of hemolysate with an upper concentration of 2.27 g/dL hemoglobin and compared to hemolysate-free samples.

- Bilirubin: 4 anti-HBc IgM 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 anti-HBc IgM 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 anti-HBc IgM samples (one negative, one high negative, one low positive, one positive) were spiked at different levels of biotin with an upper concentration of 112 ng/mL biotin and compared to biotin-free samples.
- Total Protein: 4 anti-HBc IgM samples (one negative, one high negative, one low positive, one positive) were spiked with different levels of total protein with an upper concentration of 16 g/dL total protein and compared to total protein free samples.

All calculations were based on signal-to-cutoff ratio (COI). Samples were tested in duplicate. Percent mean recovery of COI values of samples spiked with interference substance were calculated against the respective sample without the interfering substance.

The acceptance criteria was mean recovery of 80 -120% COI for all samples when compared to the initial unspiked result.

The results of this study demonstrated that samples containing hemolysate up to 2 g/dL, bilirubin up to 27 mg/dL, lipids up to 1650 mg/dL, biotin up to 112 ng/mL, and total protein up to 16 g/dL should test accurately with the Elecsys Anti-HBc IgM Immunoassay. The labeled assay performance claims no interference at the following limits:

- Hemoglobin 2 g/dL
- Bilirubin 25 mg/dL
- Lipemia 1000 mg/dL
- Biotin 100 ng/mL
- Total protein 12 g/dL

D. Matrix Effects

Studies were conducted to verify the suitability of four types of blood collection tubes to be used with the Elecsys Anti-HBc IgM Immunoassay. Samples were collected into matched serum and plasma collection tubes from 40 donors and assayed in triplicate on the Elecsys cobas e 601 immunoassay analyzer. Forty matched pairs were collected in the evaluation of each: Serum/gel separator tubes, lithium heparin plasma, sodium heparin plasma, K_2-EDTA plasma and sodium citrate plasma. Samples were spiked with anti-HBc IgM antibodies. To cover the assay measuring range, the following samples were measured (in total 40 specimens):

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

The acceptance criteria for samples < 1.0 COI was a recovery ± 0.2 COI. The acceptance criteria for samples > 1.0 COI was recovery 80-120 % (COI). Statistical analysis must show no overall trend of bias > 15% per sample type.

Statistical evaluations were done to analyze the cutoff 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 separator tubes with the Elecsys Anti-HBc IgM Immunoassay.

E. Drug Interference

A drug interference study was performed with the Elecsys Anti-HBc IgM Immunoassay on the cobas e 601 immunoassay analyzer with 18 common therapeutic drugs. Each drug was spiked into a negative, a low positive, and a positive sample. Samples were tested in triplicate with the Elecsys Anti-HBc IgM Immunoassay and compared to an unspiked serum sample (reference) which was tested 9-fold. The spiked samples were evaluated at a concentration C1 ("x" times the maximum daily dosage). Mean COI, standard deviations and percent recoveries were calculated.

For acceptance, the mean of the drug-spiked sample had to be within the range of the mean COI value ± 3 SD or ± 10%. If 3 SD range is < ± 10%, the decision limit is ± 10%. Each drug was found to not cause interference at the claimed concentrations listed in the table below. Since these studies were performed *in vitro*, they do not assess the potential interference when metabolized *in vivo*.

Compound	Concentration
Acetyl cysteine	150 mg/L
Ampicillin-Na	1000 mg/L
Ascorbic acid	300 mg/L
Ca-Dobesilate	200 mg/L
Cyclosporine	5 mg/L
Cefoxitin	2500 mg/L

Compound	Concentration
Heparin	5000 U
Intralipid	10000 mg/L
Levodopa	20 mg/L
Methyldopa+ 1.5	20 mg/L
Metronidazole	200 mg/L
Phenylbutazone	400 mg/L
Tetracycline	50 mg/L
Acetylsalicyclic acid	1000 mg/L
Rifampicin	60 mg/L
Acetaminophen	200 mg/L
Ibuprofen	500 mg/L
Theophylline	100 mg/L

F. Denaturing Study – Specificity for the IgM Class Detection

The denaturing study was performed to verify the existence of anti-HBc IgM antibodies in positive samples. To verify the claim that anti-HBc IgM antibodies exist, upon addition of DDT (1,4-dithiothreitol) solution, positive samples should be recovered as negative, evidence that anti-HBc IgM antibodies are destroyed by denaturing. All positive samples switched from positive to negative findings after adding DDT solution, thereby validating the existence of anti-HBc IgM antibodies in positive serum samples.

G. Carryover Study

The cobas e 601 immunoassay analyzer uses disposable tips for sample pipetting and reagent pipetting which reduces or eliminates the risk of carryover. However, a study was performed to determine the extent of carryover in the instrument's measuring cell caused by a high signal-generating sample. First an anti-HBc IgM negative sample was tested in triplicate. Next a reactive anti-Toxo IgG sample (which creates a high signal for toxo IgG at $\geq$ 2 million counts) was tested with the Elecsys Anti-Toxo IgG assay. This was followed again by the respective anti-HBc IgM negative sample, tested in triplicate. This test was performed three times, with three different Anti-HBc IgM negative samples.

The deviation of the first signal value of the negative sample after the high-signal generating sample was compared to the median signal of the triplicate measurements

before testing the high-signal-generating sample. The observed deviation in counts recovery met acceptance criteria of within 75 – 125% relative to the median signal of the triplicate measurements. All signal counts values were within the acceptance criteria. These studies demonstrate there is no signal carryover with the Elecsys Anti-HBc IgM Immunoassay.

H. High Dose Hook Effect

The Elecsys Anti-HBc IgM Immunoassay is not influenced by the high dose hook effect phenomenon. Therefore this study was not performed.

The basis for the conclusion that the high dose hook effect is very unlikely is as follows:

- The Elecsys Anti-HBc IgM Immunoassay is not a sandwich format. It is a μ-capture assay format with Fdγ-components, selectively eliminating IgG.
- The concentration range of the analyte anti-HBc IgM is small (factor between detection limit and maximal concentration to be expected is approximately 1000 - 4000). Moreover, each sample is diluted at 1:400 with 10 μL of sample.
- The test procedure and concentration levels of test reactants ensure that in every concentration constellation, there is no depletion of marker molecules or a prevention of binding of the signal generating complex to the microparticles.

I. Stability Studies

1. Sample Stability

Three studies were performed to verify the stability of patient samples using the Elecsys Anti-HBc IgM Immunoassay. The potential influence of storage of serum and plasma (K_2-EDTA) samples for 7 days at 2-8°C, at -20°C, and 6 freeze/thaw cycles were evaluated. Serum (n=17) sample stability was evaluated at -20°C for 2 months (n=17). Tested were negative, high negative, low positive and positive samples run in triplicate on the cobas e 601 analyzer.

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

Recovery after storage for each test was calculated based on sample to cut-off (COI) index. The acceptance criteria for samples was a mean recovery of 80-120% (COI) when compared to the initial unstressed result.

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 Anti-HBc IgM Immunoassay.

2. Elecsys Anti-HBc IgM Reagent Stability

i. Reagent Real-Time Stability

To assess the real-time stability, whole kit samples 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 measured intervals started with the production date, at least in the middle of the shelf life and one month after expiration.

Samples tested were human internal control samples (ICS, n=10), negative sera (NS, n=5, tested at the beginning and at the end of real-time measurements), and PreciControl (PC, n=2). Key stability parameters monitored for the Elecsys Anti-HBc IgM Immunoassay were cut-off sensitivity and results of internal control samples.

These studies to characterize the stability of the Elecsys Anti-HBc IgM Immunoassay confirm a shelf life of 5 months when stored at 2-8 °C. The product will be labeled with a shelf life of 5 months and the labeling will state that the reagent is stable, unopened at 2-8°C up to the stated expiration date.

ii. Reagent Temperature Stress Stability

A reagent kit was stored for one week at 35°C. The stressed kit was then used to determine recoveries of 4 human serum samples (negative, high negative, low positive, and positive) and the two internal PreciControls, tested in duplicate with the Elecsys Anti-HBc IgM Immunoassay on the cobas e 601 analyzer.

The acceptance criteria 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 results from the temperature stress studies indicate stability of the Elecsys Anti-HBc IgM reagent for 1 week at 35°C.

iii. Reagent Stability After First Opening

Stability studies were performed to determine the time period over which the Elecsys Anti-HBc IgM kits can be stored at 2-8°C once opened. A new reagent pack was opened and calibrated on the cobas e 601 analyzer. Four human sera (negative, high negative, low positive, and positive) and the two PreciControls were tested with the opened reagent after 8 weeks at 2-8°C. The reagent pack stability was determined by calculating the recovery (COI) of PreciControls arid serum samples compared to the unstressed measurements.

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

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

iv. On-Board Stability – Open Reagent Pack

Stability studies were performed to determine the time period in which the Elecsys Anti-HBc IgM Immunoassay can be kept on-board the cobas e 601 analyzer once opened. A new reagent rack-pack was opened, calibrated, and stored on-board for 8 weeks at 20°C ±3°C. Four human sera samples and two PreciControls were tested with the on-board reagent at week 1, 2, 3, 4, 5, 6, 7, and 8. Each week the reagent was checked with regard to stability of the weekly calibration. For each testing time point, the calibration occurred seven days prior. Recovery for each sample was calculated based on sample to cutoff index (COI).

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

All acceptance criteria were met for each of the 8 weeks tested. Reagent onboard stability is claimed for 8 weeks.

v. On-Board Stability – Open reagent Pack, On Board and Refrigerated

Stability studies were performed to determine the time period in which the opened Elecsys Anti-HBc IgM Immunoassay kits can be stored in the refrigerator and alternately on the cobas e 601 analyzer.

An Elecsys Anti-HBc IgM rack-pack was stored for 6 weeks in the refrigerator at 2 - 8°C and alternately on-board at 20°C±3°C (up to 56 hours in total) to simulate on-board stress. Each week the reagent was checked with regard to stability of the weekly calibration.

A new Elecsys Anti-HBc IgM reagent pack was opened and calibrated. Seven human sera samples and two PreciControls were tested in duplicates with the on-board reagent at week 1, 2, 3, 4, 5, and 6. For each testing time point the calibration occurred seven days prior. Recovery for each sample was calculated based on sample to cutoff index (COI).

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

Acceptance criteria were fulfilled.

3. Elecsys Anti-HBc IgM Calibrator Stability Studies

i. On-Board Stability—Open Calibrators

Stability studies were performed to determine the time period in which the Elecsys Anti-HBc IgM calibrators can be kept open on-board the cobas e 601 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 and should be left on the cobas e 601 analyzer only during calibration (2 hours in total). The maximum temperature the calibrators might be exposed to is

assumed to be 32°C (upper limit of the specification for the ambient temperature of the sample rotor disc of the cobas e 601 immunoassay analyzer). Calibrators 1 and 2 consequently need to be stable for 2 hours of incubation at 32 °C.

A pair of Elecsys Anti-HBc IgM 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 criterion was 90-110% recovery of signal counts after 2 hours at 32°C. Criteria were met and calibrators are labeled as stable open and on-board the cobas e 601 for 2 hours.

ii. Calibrator Stability after First Opening

Stability studies were performed to determine the time period in which the Elecsys Anti-HBc IgM Immunoassay calibrators can be kept at 2 - 8°C once opened.

A new reagent pack was opened and calibrated. The opened calibrators were then tested again in duplicate after 4, and 8 weeks stored at 2 - 8°C. Stability was determined by calculation of the recovery of the calibrator signals (counts) of opened calibrators compared to the unstressed calibrator signals (counts).

Acceptance criterion was 90-110% recovery of signal counts. The acceptance criteria were met and the Elecsys Anti-HBc IgM calibrators are labeled as stable for 8 weeks after first opening when stored at 2-8°C.

iii. Calibration Stability

Calibration must be performed once per reagent lot using the Anti-HBc IgM Cal 1, Cal 2 and fresh reagent (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).

iv. Lot Calibration Study

An Elecsys Anti-HBc IgM reagent lot was tested on three separate cobas e 601 instruments. Four human serum samples (negative, high negative, low positive, and positive) and two levels of PreciControl Anti-HBc IgM were tested. Every sample was measured in two-fold determination. Fresh reagent was calibrated on Day 1. On Day 29, fresh reagent of the same lot was run again using the calibration of Day 1 to demonstrate stability of the initial calibration, and stability of the control measurements.

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

v. Reagent Pack On-Board Calibration Study

An Elecsys Anti-HBc IgM reagent pack was stored for one week in the refrigerator at 2 - 8°C and alternately on-board at 20°C±3°C to simulate onboard stress. After 1 week, the reagent was checked with regard to stability of the weekly calibration.

A new reagent pack was opened and calibrated. Four human sera samples (negative, high negative, low positive, and positive) and the PreciControls (PC 1 and PC 2) were tested in duplicates with the on-board reagent at day 1, and after 1 week with weekly calibration. Recovery for each sample (stressed/unstressed) was calculated based on sample to cutoff index (COI).

The acceptance criteria 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 cobas e 601 when using the same Elecsys Anti-HBc IgM reagent kit.

4. PreciControl Anti-HBc IgM Studies

i. PreciControl Real-Time Stability

Shelf life was determined by testing three production lots of PreciControl kits stored at the recommended storage temperature of 2-8°C. The test measurement intervals started with the production date, and then followed with one intermediate time point, and one month past expiration. Acceptance criteria were based on lot specific target ranges for each control and lot number (e.g. COI=0-0.19 for PC1; COI=2.52-3.88 for PC2).

These studies to characterize the stability of the PreciControl Anti-HBc IgM confirm a shelf life of 8 months when stored at 2-8 °C. The product will be labeled with a shelf life of 8 months and the labeling will state that the reagent is stable, unopened at 2-8°C up to the stated expiration date.

ii. PreciControl Temperature Stress Stability

Temperature stress stability studies were conducted to determine the effect of elevated temperature stress on PreciControl Anti-HBc IgM during transportation. To assess the stability of the PreciControl Anti-HBc IgM reagent after temperature stress, the cutoff 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 Anti-HBc IgM.

Acceptance criteria was recovery of PreciControl 1 ≤ 0.13 (COI) and recovery of PreciControl 2 relative to unstressed at 80-120 % (COI).

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

iii. PreciControl Stability after First Opening

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

A new PreciControl reagent pack was opened and tested, then stored at 2-8 °C for 8 weeks. After 4 and 8 weeks, the stressed reagent was tested in duplicate. Recovery to unstressed PreciControl reagent pack, based on initial value, was calculated.

Acceptance criteria was recovery of PreciControl 1 ≤ 0.13 (COI) and recovery of PreciControl 2 relative to unstressed at 80-120 % (COI). All acceptance criteria were met, demonstrating that the Elecsys PreciControl Anti-HBc IgM is stable after first opening when stored at 2-8°C for 8 weeks.

iv. On-Board Stability - Open PreciControls

Stability studies were performed to determine the time period in which the Elecsys PreciControl Anti-HBc IgM can be kept open on-board the cobas e 601 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 cobas e 601 immunoassay analyzer). PreciControl Anti-HBc IgM may be used for a maximum of seven quality control procedures and should be left on the cobas e 601 immunoassay analyzer only during calibration.

A new Elecsys Anti-HBc IgM reagent pack and a new PreciControl Anti-HBc IgM reagent pack were opened and tested, the Elecsys Anti-HBc IgM reagent pack then stored at 2 - 8°C, the opened PreciControls stored at 32°C. In 6, one-hour intervals, the stressed PreciControls were tested in duplicate. Recovery was calculated based on counts (signal).

Acceptance criterion was 90-110% recovery of signal counts. All acceptance criteria were met after 6 hours on-board the cobas e 601. Stability of PreciControl Anti-HBc IgM of up to 6 hours is claimed.

J. Antimicrobial Effectiveness Testing

Antimicrobial effectiveness testing (AET) has been performed according to United States Pharmacopoeia (USP) chapter <51>. Testing was performed with all reagents of Elecsys Anti-HBc IgM and PreciControl Anti-HBc IgM.

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

After inoculation, samples were plated on appropriate media at Day 0, Day 7, Day 14, and Day 28. To pass USP criteria, the bacterial concentration has to be reduced to 10% of the original inoculum by day 7, < 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.

Preservation of all reagents tested has been sufficient to pass USP.

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 product insert. No microbial outgrowth has been observed in components stored at elevated temperatures, relative to recommended 2 – 8° C storage, in previous accelerated stability studies.

K. 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 six member precision panel consisting of 4 human sera (one positive, one negative, one low positive (approximately 95 %), and one high negative (approximately 95 %)) and two PreciControl Anti-HBc IgM (one positive and one negative) was measured in duplicate determinations in two runs per day for 12 days. The measurements were performed on one Elecsys cobas e 601 analyzer, at one site, with one reagent lot performing weekly calibration, spanning at least two calibration cycles. Repeatability and within-laboratory precision was calculated according to EP5-A2.

Table 3: Repeatability

Sample	Mean (COI)	SD (COI)	CV (%)	n
Negative serum	0.069	0.00099	**1.44**	48
High negative serum	0.942	0.025	**2.64**	48
Low positive serum	1.17	0.032	**2.72**	48
Positive serum	2.48	0.077	**3.10**	48
PC 1	0.071	0.00099	**1.40**	48
PC 2	3.43	0.098	**2.84**	48

Table 4: Within-Laboratory Precision

Sample	Mean (COI)	SD (COI)	CV (%)	n
Negative serum	0.069	0.001	**2.10**	48
High negative serum	0.942	0.032	**3.44**	48
Low positive serum	1.17	0.040	**3.42**	48
Positive serum	2.48	0.171	**6.90**	48
PC 1	0.071	0.001	**1.92**	48
PC 2	3.43	0.121	**3.54**	48

External Precision

Precision was further evaluated incorporating between-run, between-day, between-lot and between-site variation. This study was done following CLSI EP5-A2 and CLSI EP15-A2 using three cobas e 601 instruments at three sites using three lots of reagent, with two lots at each site in an AB, BC, AC manner. The panel used consisted of PreciControl Anti-HBc IgM 1 and 2, three human serum pools near the cut-off and one moderately reactive human serum pool. Pools were tested in replicates of 3 in 2 runs per day for 5 days according to CLSI EP15-A2/EP 5-A2. The analysis of data was based on guidance from the CLSI documents EP5-A2 and EP15-A2. Data from all three reagent lots were combined to achieve SD and percent CV for repeatability, intermediate precision, between-day, between-lot, between-site and reproducibility. Results are summarized below.

Table 5: External Precision Data

			Repeatability		Between Run		Between Day		Between Lot		Between Site		Reproducibility	
Sample	N	Mean COI	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
HSP 01	180	0.824	0.035	4.2	0.029	3.5	0.000[c]	0.0	0.064	7.8	0.000[c]	0.0	0.079	9.5
HSP 02	180	1.053	0.052	4.9	0.000[c]	0.0	0.018	1.7	0.078	7.4	0.000[c]	0.0	0.095	9.0
HSP 03	180	1.525	0.061	4.0	0.000[c]	0.0	0.032	2.1	0.100	6.5	0.000[c]	0.0	0.121	8.0
HSP 04	180	1.218	0.049	4.0	0.014	1.1	0.015	1.3	0.098	8.0	0.000[c]	0.0	0.112	9.2
PC 1	180	0.092	0.002	2.1	0.000[c]	0.0	0.001	1.6	0.018	19.9	0.000[c]	0.0	0.018	20.1
PC 2	180	3.229	0.100	3.1	0.079	2.4	0.005	0.2	0.273	8.5	0.000[c]	0.0	0.301	9.3

c) SD of zero due to variance contributed by particular component was below stated significant figure

L. Analytical Specificity

A study was conducted to evaluate the Elecsys Anti-HBc IgM Immunoassay for potential cross-reactivity for specimens from individuals with medical conditions unrelated to hepatitis B infection. The study was performed by testing 256 samples from subjects with 22 various medical conditions that could potentially interfere with hepatitis B core IgM antibody testing. All specimens in the study were tested with the Elecsys Anti-HBc IgM and the reference assay. Of this group, 98 were white (38.3%), 36 black (14.1%), 3 Asian (1.2%), 1 Native American (0.4%), 23 other (9.0%) and 96 unknown (37.5%).

In this cohort, 256 samples (100%) were found to be non-reactive with both the reference and Elecsys Anti-HBc IgM Immunoassays.

The study demonstrated a negative percent agreement between Elecsys and the reference Anti-HBc IgM assays of 100.00% (256/256) with a 95th percentile confidence interval of 98.57-100.0%. The overall negative and positive percent agreement was 100% (256/256).

Table 6: Analytical Specificity in Subjects with Other Known Conditions

Category	ORTHO VITROS ECi HBc M Assay				Total
	Reactive		Non-Reactive		
	Elecsys Anti-HBc IgM Immunoassay on the cobas e 601				
	RX[a]	NR[b]	RX	NR	
Autoimmune (AMA, ANA, SLE)	0	0	0	12	12
Cytomegalovirus (anti-CMV)	0	0	0	11	11
Epstein-Barr Virus (anti-EBV)	0	0	0	9	9
E. coli infection	0	0	0	9	9
Flu Vaccination	0	0	0	10	10
Hepatitis A Virus (anti-HAV total)	0	0	0	9	9
HAV Vaccination	0	0	0	9	9
HBV Vaccination	0	0	0	7	7
Hepatitis C Virus (anti-HCV)	0	0	0	11	11
Hepatitis D Virus (anti-HDV)	0	0	0	12	12
Hepatitis E Virus (anti-HEV)	0	0	0	10	10
Human Immunodeficiency Virus (anti-HIV-1)	0	0	0	12	12
Herpes Simplex Virus (anti-HSV)	0	0	0	12	12
HTLV I / II	0	0	0	12	12
Non-Viral Liver Disease	0	0	0	33	33
Parvovirus B19 Infection	0	0	0	11	11
Pregnancy	0	0	0	11	11
Rheumatoid factor	0	0	0	14	14
Rubella (anti-Rubella)	0	0	0	9	9
Syphilis	0	0	0	10	10
Toxoplasmosis (anti-Toxo)	0	0	0	14	14
Varicella Zoster (anti-VZV)	0	0	0	9	9
Total	0	0	0	256	256

a) Reactive b) Non-Reactive

M. Seroconversion Analysis

Seroconversion sensitivity of the Elecsys Anti-HBc IgM Immunoassay was assessed by testing seven commercially sourced seroconversion panels in comparison to that of a reference anti-HBc IgM assay. The sources of the panels were ZeptoMetrix (ZPT), SeraCare/bbi (SC/bbi), and BioRad. Demographics and medical history on most of these subjects were not supplied.

The comparison of the seroconversion detection between the assays in terms of days is summarized in the table below.

Table 7: Results for Days of Evidence of anti-HBc IgM Seroconversion for the Elecsys Anti-HBc IgM Compared to the Reference Assay

Panel ID	Reference anti-HBc IgM		Elecsys Anti-HBc IgM		Difference in days to Elecsys Anti-HBc IgM reactivity (Reference - Elecsys)
	NR	RX	NR	RX	
6278	37	41	37	41	0
6281	41	43	41	43	0
9092	82	89	82	89	0
9093	42	49	56	74	25 (2 draws)
PHM933	16	144	16	144	0
PHM934	14	84	14	84	0
PHM935(M2)	66	68	50	68	0

The Elecsys Anti-HBc IgM Immunoassay was reactive in the same bleed as the reference assay in 6 of the 7 panels tested. The Elecsys Anti-HBc IgM Immunoassay was reactive 2 draws later than the reference assay in 1 panel, wherein both draws yielded near cutoff testing in both assays before conversion.

X. SUMMARY OF PRIMARY CLINICAL STUDY

To evaluate the Elecsys Anti-HBc IgM Immunoassay's ability to detect IgM antibodies to HBc in a group of individuals that would normally be tested in a clinical situation, a multi-center prospective study was conducted to evaluate the clinical performance on the cobas e 601 analyzer.

The study population included individuals with specific risks or history associated with hepatitis B infection. Medical/clinical risks included a history of transplants or blood transfusions and/or clotting factors, HIV infection or other immunodeficiency diseases, hemodialysis patients, prenatal exposure to HBV, a family history of any hepatitis, or living in high endemic areas for

hepatitis. Occupational risks included healthcare workers, tattoo artists, morticians and individuals with a history of incarceration. Sexual risks included individuals with multiple sex partners, individuals with a history of sexual contact with partners with STDs (including HIV), male-on-male sex partners and commercial sex workers. Behavioral risks included IV drug use, cocaine users who ingested the drug through shared straws, and individuals with tattoo or body piercings.

A total of 1,581 prospective subjects were recruited for the diagnostic accuracy analysis and were divided into two groups, Asymptomatic at Risk (n=1337, 85%) and Symptomatic at Risk (n=244, 15%) populations. The Asymptomatic at Risk population was required to have no clinical symptoms of liver disease. The Symptomatic at Risk group was required to have clinical symptoms, laboratory data, or histological findings suggestive for 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 plannus or porphyria cutanea tarda, and histological evidence of liver disease (if available). The signs and symptoms population must also belong to one of the listed at-risk groups for HBV.

The 1581 prospective specimens were collected from 3 collection sites located in California (49.6 %), Florida (19.9 %), and Georgia (30.5 %). Of the 1581 at risk subjects, 388 (24.5 %) were female and 1193 (75.5 %) were male. Overall, 73.7% of patients were black, 0.70% Native American, 0.90% Asian, 24.3% white (Hispanic and non-Hispanic), 0.10% Pacific Islander with 0.25% defined as other and 0.05% classified as unknown. Ages for both cohorts ranged from 21 to 75 with a median of 45.8 years.

Table 8: Demographic Summary of Race of Subject by Gender and Age in the Clinical Cohort (Asymptomatic and Symptomatic)

		African American/ Black	American Indian/ Alaska Native	Asian	Caucasian /White	Other	Pacific Islander	Unknown	
Age Group (Years)	Gender								Total
21 to 30	Female	16	0	1	17	0	0	0	34
	Male	48	0	4	55	0	1	0	108
31 to 40	Female	46	0	0	23	1	0	0	70
	Male	104	0	1	72	0	1	0	178
41 to 50	Female	131	3	2	27	0	0	0	163
	Male	403	4	2	98	2	0	1	510
51 to 60	Female	81	0	1	18	0	0	0	100
	Male	296	4	2	57	0	0	0	359
61 to 70	Female	13	0	0	6	1	0	0	20
	Male	25	0	1	11	0	0	0	37
71 to 80	Female	1	0	0	0	0	0	0	1
	Male	1	0	0	0	0	0	0	1
Total	Female	288	3	4	91	2	0	0	388
	Male	877	8	10	293	2	2	1	1193
Total	All	1165	11	14	384	4	2	1	1581

Testing of the specimens was performed at 3 clinical testing sites located in St. Louis, MO, Ft. Lauderdale, FL and Boston, MA.

To supplement the clinical study, a total of 148 samples from subjects with increased risk for hepatitis due to living in areas endemic for hepatitis or who were potential candidates for acute disease or reactive anti-HBc IgM status were tested with the Elecsys Anti-HBc IgM Immunoassay on the cobas e 601 analyzer and by the reference methods.

Specimen Classification:

Tests from an HBV marker assay panel were run on each clinical specimen to determine a single time-point serological diagnosis of HBV disease status. The Elecsys HBsAg, HBsAg Confirmatory, and Anti-HBs assays on the Elecsys 2010 or MODULAR ANALYTICS E170 served as FDA approved reference assays. Further reference testing was conducted with ORTHO VITROS ECi AHBC (anti-HBc) and ECi HBc M (anti-HBc IgM) assays, the Abbott Architect CORE-M (Anti-HBc IgM) and ADVIA Bayer Centaur aHBcM (anti-HBc IgM) assays, and the DiaSorin ETI-AB-EBK Plus (anti-HBe) and ETI-EBK Plus (HBeAg) assays. These assays were performed according to the manufacturer's instructions. The interpretation algorithm for the various HBV classifications based on the serological profiles is presented in the following table:

Serological classification by FDA-approved HBV panel						
	HBsAg	HBeAg	Anti-HBc IgM	anti-HBc	Anti-HBe	Anti-HBs
Acute	(+)	(+)	(-)	(-)	(-)	(-)
Acute	(+)	(-)	(-)	(-)	(-)	(-)
Acute	(+)	(+)	(+)	(+)	(-)	(-)
Acute	(+)	(-)	(+)	(+)	(+)	(-)
Acute	(+)	(-)	(+)	(+)	(-)	(-)
Acute (late)	(+)	(-)	(+)	(+)	(+)	(+) or eq
Chronic	(+) > 6 mo.					
Chronic	(+) > 6 mo.	(-)	(-)	(+)	(+)	(-)
Chronic	(+) > 6 mo.	(+)	(-)	(+)	(-)	(-)
Chronic	(+)	(+)	(-)	(+)	(-)	(-), (+), eq
Chronic	(+)	(-)	(-)	(+)	(+)	(-), (+)
Chronic	(+)	(-)	(-)	(+)	(+)	(+)
Chronic	(+)	(+)	(-)	(+)	(+)	(-)
Chronic	(+)	(+)	(+)	(+)	(+)	(+)
Early recovery	(-)	(-)	(+)	(+)	(+) or (-)	(-)
Early recovery	(-)	(-)	(+)	(+)	(+)	(+)
Early recovery	(-)	(-)	(-)	(+)	(+), eq, (-)	(-)
Recovery	(-), rr uncnf	(-)	(-)	(+)	(+)	(+) or eq
Recovery	(-)	(-)	(-)	(+)	eq	(+)
Recovery	(-)	(-)	(-)	(-)	(+)	(+)

Serological classification by FDA-approved HBV panel						
	HBsAg	HBeAg	Anti-HBc IgM	anti-HBc	Anti-HBe	Anti-HBs
Recovered or immune due to natural infection	(-)	(-)	(-)	(+)	(-)	(+) or eq
HBV vaccine response	(-), rr uncnf	(-)	(-)	(-)	(-)	(+)
HBV vaccination response (?)	(-)	(-)	(-)	(-)	(-)	eq
Not previously infected	(-)	(-)	(-)	(-)	(-)	(-)
Not previously infected	rr uncnf	(-)	(-)	(-)	(-)	(-)
Not interpretable	rr uncnf	(+)	(+)	(+)	(-)	(-)
Not interpretable	(-)	(-)	(-)	nd	(+)	(-)
Not interpretable	(-)	(-)	(-)	(-)	(+) or eq	(-)
Not interpretable	(-)	(-)	(-)	(-)	eq	(+)
Not interpretable	(-)	(+)	(-)	(-)	(-)	(+) or (-)
Not interpretable	qns	(-)	(-)	(-)	(-)	(-)
Not interpretable	rr uncnf	(+)	(-)	(+)	(-)	(-)
Not interpretable	(+)	(-)	(-)	(-)	(-)	(+)
nd = not done or blank						
eq = equivocal or indeterminate or borderline						
rr uncnf = repeatedly reactive HBsAg with (-) confirmatory testing						
qns = incomplete or unconfirmed HBsAg						

Sample Classification Results

Table 9 presents the HBV classifications reported among the 1581 increased risk population specimens based on the combined available serological reference test results for HBV markers as per the interpretation scheme. These totals are broken down by cohort in Tables 10 and 11. Endemic subjects (n = 148) are presented in table 12. Elecsys Anti-HBc IgM results were not used in this classification. Those doing the categorization were blinded to those values.

Table 9: HBV Classification by Serology in Increased Risk Clinical Population

HBV Classification	Total	Percent
Acute	9	0.57
Chronic	45	2.85
Early Recovery	129	8.16
Recovery	291	18.41
Recovered	120	7.59
HBV Vaccination	338	21.38
Not Previously Infected	630	39.85
Not Interpretable	19	1.20
Total	1581	100.0

Table 10: HBV Classification by Serology in Asymptomatic Increased Risk Cohort

HBV Classification	Total	Percent
Acute	7	0.52
Chronic	34	2.54
Early Recovery	101	7.55
Recovery	257	19.22
Recovered	95	7.11
HBV Vaccination	293	21.91
Not Previously Infected	537	40.16
Not Interpretable	13	0.97
Total	1337	100.00

Table 11: HBV Classification by Serology in Symptomatic Increased Risk Cohort

HBV Classification	Total	Percent
Acute	2	0.82
Chronic	11	4.51
Early Recovery	28	11.48
Recovery	34	13.93
Recovered	25	10.25
HBV Vaccination	45	18.44
Not Previously Infected	93	38.11
Not Interpretable	6	2.46
Total	244	100.00

Table 12: HBV Classification by Serology in Endemic/Acute/Reactive Clinical Cohort

HBV Classification	Total	Percent
Acute	45	30.41
Chronic	96	64.68
Early Recovery	4	2.70
HBV Vaccination	1	0.68
Not Previously Infected	2	1.35
Total	148	100.0

Results by Specimen Classification

Samples were then run on the cobas e 601 analyzer using the Elecsys Anti-HBc IgM Immunoassay, and on the reference analyzer using the reference anti-HBc IgM assay. The reference method value for samples found to be reactive by either method was determined by consensus of results from the reference method and 2 additional FDA approved anti-HBc IgM assays (2 out of 3).

Tables 14 and 16 below show the results of the two assays in comparison to the subject classification, while Tables 15 and 17 show the positive and negative agreement.

Table 14: Comparison of Elecsys Anti-HBc IgM on cobas e 601 to the Reference Assay Results by HBV Classification in the Asymptomatic At Risk Cohort

HBV Classification	2 of 3 anti-HBc IgM Assays Algorithm				Total
	+		-		
	Elecsys Anti-HBc IgM Test Results				
	+	-	+	-	
Acute	4	0	0	3	7
Chronic	0	0	0	34	34
Early Recovery	6	0	0	95	101
Recovery	0	0	0	257	257
Recovered	0	0	0	95	95
HBV Vaccination	0	0	0	293	293
Not Previously Infected	0	0	0	537	537
Not Interpretable	0	0	0	13	13
Total	10	0	0	1327	1337

The following table summarizes the percent agreement between the Elecsys Anti-HBc IgM and the reference assay for each specimen classification, and provides the upper and lower 95% exact confidence bounds. The percent positive agreement among the Asymptomatic Increased Risk subjects was 100.00 % (10/10) and the percent negative agreement was 100.00 % (1327/1327).

Table 15: Percent Agreement between Elecsys Anti-HBc IgM and anti-HBc Reference Assay for each Specimen Classification: Asymptomatic at Risk Population

HBV Classification	Positive Percent Agreement	95% Exact Confidence Interval	Negative Percent Agreement	95% Exact Confidence Interval
Acute	**100.00** (4/4)	39.76 to 100.00	**100.00** (3/3)	29.24 to 100.00
Chronic	**na**	na	**100.00** (34/34)	89.72 to 100.00
Early Recovery	**100.00** (6/6)	54.07 to 100.00	**100.00** (95/95)	96.19 to 100.00
Recovery	**na**	na	**100.00** (257/257)	98.57 to 100.00
Recovered	**na**	na	**100.00** (95/95)	96.19 to 100.00
HBV Vaccination	**na**	na	**100.00** (293/293)	98.75 to 100.00
Not Previously Infected	**na**	na	**100.00** (537/537)	99.32 to 100.00
Not Interpretable	**na**	na	**100.00** (13/13)	75.29 to 100.00
Total	**100.00** (10/10)	69.15 to 100.00	**100.00** (1327/1327)	99.72 to 100.00

The following table summarizes the results of the two assays in comparison to the HBV classification in the symptomatic at risk cohort. In this group, 1 sample was negative by the reference assay and positive by the Elecsys Anti-HBc IgM Immunoassay.

Table 16: Comparison of Elecsys Anti-HBc IgM on the cobas e 601 to the Reference Assay Results by HBV Classification in the Symptomatic At Risk Cohort

HBV Classification	2 of 3 anti-HBc IgM Assays Outcome				Total
	+		-		
	Elecsys Anti-HBc IgM Test Results				
	+	-	+	-	
Acute	1	0	0	1	2
Chronic	0	0	0	11	11
Early Recovery	2	0	0	26	28
Recovery	0	0	1	33	34
Recovered	0	0	0	25	25
HBV Vaccination	0	0	0	45	45
Not Previously Infected	0	0	0	93	93
Not Interpretable	1	0	0	5	6
Total	4	0	1	239	244

The following table summarizes the percent agreement between the Elecsys Anti-HBc IgM Immunoassay and the reference assay for each specimen classification, and provides the upper and lower 95% exact confidence bounds. As shown, among the symptomatic at risk subjects, the percent positive agreement among the Symptomatic Increased Risk subjects was 100.00 % (4/4) and the percent negative agreement was 99.58 % (239/240).

Table 17: Percent Agreement between Elecsys Anti-HBc IgM and anti-HBc Reference Assay for each Specimen Classification: Symptomatic At Risk

HBV Classification	Positive Percent Agreement	95% Exact Confidence Interval	Negative Percent Agreement	95% Exact Confidence Interval
Acute	**100.00** (1/1)	2.50 to 100.00	**100.00** (1/1)	2.50 to 100.00
Chronic	**na**	na	**100.00** (11/11)	71.51 to 100.00
Early Recovery	**100.00** (2/2)	15.81 to 100.00	**100.00** (26/26)	86.77 to 100.00
Recovery	**na**	na	**97.06** (33/34)	84.67 to 99.93
Recovered	**na**	na	**100.00** (25/25)	86.28 to 100.00
HBV Vaccination	**na**	na	**100.00** (45/45)	92.13 to 100.00
Not Previously Infected	**na**	na	**100.00** (93/93)	96.11 to 100.00
Not Interpretable	**100.00** (1/1)	2.50 to 100.00	**100.00** (5/5)	47.82 to 100.00
Total	**100.00** (4/4)	39.76 to 100.00	**99.58** (239/240)	97.70 to 99.99

Table 18 summarizes the results of the two assays in comparison to the HBV classification in the endemic/acute/reactive cohort. In this group, 2 samples (1.4%) were negative by the reference assay and positive by the Elecsys Anti-HBc IgM Immunoassay. Additionally, 3 samples (2.0%) were positive by the reference assay and negative by the Elecsys Anti-HBc IgM Immunoassay.

Table 18: Comparison of Elecsys Anti-HBc IgM on the cobas e 601 analyzer to 2 of 3 anti-HBc IgM Assays Outcome by HBV Classification: Endemic/Acute/Reactive Cohort

	2 of 3 anti-HBc IgM Assays Outcome				
HBV Classification*	+		-		**Total**
	Elecsys Anti-HBc IgM Test Results				
	+	-	+	-	
Acute	29 [b]	3 [a]	0	13	45
Chronic	1	0	1 [c]	94	96
Early Recovery	4	0	0	0	4
HBV Vaccination	0	0	0	1	1
Not Previously Infected	0	0	0	2	2
Total	34	3	1	110	148

Table 19 summarizes the percent agreement between the Elecsys Anti-HBc IgM Immunoassay and the reference assay for each specimen classification, and provides the upper and lower 95% exact confidence bounds. As shown, among the endemic/acute/reactive subjects, the positive and negative percent agreements were 91.67% (33/36) and 98.21% (110/112), respectively.

Table 19: Percent Agreement between Elecsys Anti-HBc IgM and anti-HBc Reference Assay for each Specimen Classification: Endemic/Acute/Reactive Cohort

HBV Classification	Positive Percent Agreement	95% Exact Confidence Interval	Negative Percent Agreement	95% Exact Confidence Interval
Acute	**90.63** (29/32)	74.98 to 98.02	**100.00** (13/13)	75.29 to 100.00
Chronic	**100.00** (1/1)	2.50 to 100.00	**98.95** (94/95)	94.27 to 99.97
Early Recovery	**100.00** (4/4)	39.76 to 100.00	**na**	na
HBV Vaccination	**na**	na	**100.00** (1/1)	2.50 to 100.00
Not Previously Infected	**na**	na	**100.00** (2/2)	15.81 to 100.00
Total	**91.89** (34/37)	78.09 to 98.30	**99.10** (110/111)	95.08 to 99.98

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 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. Safety Conclusions

The adverse effects of the device are based on data collected in a clinical studies conducted to support PMA approval as described above. As a diagnostic test, the Elecsys Anti-HBc IgM Immunoassay for use on the cobas e 601 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 – Potential Adverse Effects of the Device on Health. There were no adverse effects of the device reported while the study was conducted.

B. Effectiveness Conclusions

- The Elecsys Anti-HBc IgM Immunoassay performance is acceptable when testing in serum, 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). They can also withstand 6 freeze/thaw cycles.
- The Elecsys Anti-HBc IgM reagent is stable up to the stated expiration date when stored at 2-8°C. It 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 cobas e 601 for 4 weeks.
- The Elecsys Anti-HBc IgM calibrators are stable on-board the cobas e 601 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 PreciControl Anti-HBc IgM is stable up to the stated expiration date when stored at 2-8°C. It can withstand stress at 35°C for one week. It is stable for 8 weeks after opening when stored at 2-8°C, and is stable on-board the cobas e 601 for 6 hours.
- The preservative systems that the Elecsys Anti-HBc IgM Immunoassay reagents and PreciControls contain have been shown to meet USP Chapter 51.

- The Elecsys Anti-HBc IgM Immunoassay demonstrated precision estimates (CVs) of < 5% for repeatability, < 2% between-run, < 2% between-day, < 9% between-lot, and < 9% for reproducibility.

C. **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 Anti-HBc IgM Immunoassay, when compared to reference clinical laboratory procedures, has a similar ability to detect the presence of IgM antibodies to hepatitis B core antigen in specimens from individuals infected with HBV (state of infection or associated disease not determined). The rate of false positivity and false negativity are within acceptable limits compared to the reference 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 of HBV.

XIII. CDRH DECISION

CDRH issued an approval order on October 26, 2011. The final conditions of approval can be found in the approval order.

The applicant's manufacturing facilities were 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.