510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K220178

B Applicant

Beckman Coulter, Inc.

C Proprietary and Established Names

Total Immunoglobulin E (IgE)

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
DGC	Class II	21 CFR 866.5510 - Immunoglobulins A, G, M, D, And E Immunological Test System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

A new device

B Measurand:

Total immunoglobulin E (IgE)

C Type of Test:

Quantitative immunoturbidimetric assay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The IgE assay is intended for use in the quantitative determination of Total Immunoglobulin E (IgE) concentration in human serum and plasma (lithium heparin, sodium heparin, K2-EDTA, K3-EDTA) on Beckman Coulter AU/DxC AU clinical chemistry analyzers. The determination aids in the diagnosis of IgE-mediated allergic disorders in conjunction with other clinical findings. For in vitro diagnostic use only.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Beckman Coulter AU480, AU680, AU5800 and DxC 700 AU clinical chemistry analyzers.

IV Device/System Characteristics:

A Device Description:

The Total Immunoglobulin E (IgE) reagent kit is provided in a two-part ready-to-use liquid format designed for optimal performance on Beckman Coulter AU/DxC AU clinical chemistry analyzers.

Each IgE reagent kit contains the following:

- One (1) IgE R1 bottle that contains reaction buffer (41.5 mL).
- One (1) IgE R2 bottle that contains particle-bound mouse anti-IgE antibody (15.5 mL).
- One six-level IgE calibrator set (2 mL for each calibrator level; Level 1 – Level 6) to establish a multi-point calibration curve. The calibrators are matched to the IgE reagent lot and should not be interchanged.
- One (1) Value Assignment Sheet

Materials needed but not supplied:

- At least two levels of control material
- Deionized water

It is recommended that at least two levels of control material be utilized daily to establish quality control of the IgE reagent test system. In addition, these controls should be run with each new

calibration and each new lot of IgE reagent. Quality control materials can be purchased from Beckman Coulter or alternative suppliers.

B Principle of Operation:

The IgE assay is a fully automated assay that measures total IgE concentration in human serum and plasma by a turbidimetric immunoassay method. When a sample is mixed with R1 buffer and R2 antiserum solution, human IgE reacts specifically with anti-IgE antibody-coated particles to yield insoluble aggregates. Immune complexes formed in solution scatter light in proportion to their size, shape and concentration. Turbidimeters measure the reduction of incident light due to reflection, absorption or scatter. The decrease in intensity of light transmitted (increase in absorbance) through particles suspended in solution is as a result of complexes formed. The change in absorbance is measured at 800 nanometers and used by the system to calculate and express the IgE concentration (in IU/mL) based on the stored calibration curve.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Elecsys IgE II Immunoassay

B Predicate 510(k) Number(s):

K061970

C Comparison with Predicate(s):

Device & Predicate Device(s):	Candidate Device K220178	Predicate Device K061970
Device Trade Name	Total Immunoglobulin E (IgE)	Elecsys IgE II Immunoassay
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The IgE assay is intended for use in the quantitative determination of Total Immunoglobulin E (IgE) concentration in human serum and plasma (lithium heparin, sodium heparin, K2 EDTA, K3 EDTA) on Beckman Coulter AU/DxC AU clinical chemistry analyzers. The determination aids in the diagnosis of IgE-mediated allergic disorders in conjunction with other clinical findings. For in vitro diagnostic use only.	Immunoassay for the in vitro quantitative determination of immunoglobulin E in human serum and plasma. Determination of total IgE is useful as an aid in the diagnosis of allergic diseases.
Reagent Formulation	Liquid, ready-to-use	Same

Antibody:	Monoclonal, mouse anti-IgE	Same
Sample Types	Serum, plasma (heparin & EDTA)	Same
Storage	2–8 ºC	Same
General Device Characteristic Differences		
Operating Principle	Immunoturbidimetric	Electrochemiluminescence
Calibrator Scheme	6-level multipoint calibration curve	Barcoded master curve with two-point adjustment
Calibration Stability	14 days	7 days
Traceability/Standardization	WHO 3rd IRP 11/234	WHO 2nd IRP 75/502
Analytical Measuring Range	20−500 IU/mL	0.10−2500 IU/mL
Extended Measuring Interval (manual or auto-dilution)	500−1,000 IU/mL for 10-fold diluted samples	>2,500 up to 50,000 IU/mL for 20-fold diluted samples
Detection Capability	LoD: 15 IU/mL LoQ: 20 IU/mL	LoD: 0.100 IU/mL LoQ: 0.500 IU/mL

VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition
- CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
- CLSI EP07: Interference Testing in Clinical Chemistry; Approved Guideline – Third Edition
- CLSI EP09c: Measurement Procedure Comparison and Bias Estimation Using Patient Samples- Third Edition
- CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline Second Edition
- CLSI EP25-A: Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline
- CLSI EP28-A3c: Defining Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition
- CLSI EP37: Supplemental Tables for Interference Testing in Clinical Chemistry: Approved Guideline; First Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

All results met the manufacturer's pre-determined acceptance criteria.

1. Precision/Reproducibility:

   Precision measurements were conducted in accordance with the CLSI guideline EP05-A3 using three lots of IgE reagent on a single DxC 700 AU analyzer, and one IgE reagent lot on

three DxC 700 AU analyzers. A panel consisting of three patient serum pools with levels of total IgE that cover the measuring range and two levels of human serum-based quality control material was assayed in duplicate, twice a day, for 20 days with one reagent lot (for a total of 80 replicates per sample) in random order. The results for repeatability (within-run) and total (within-laboratory) imprecision are summarized in the table below:

Sample*	Mean IgE (IU/mL)	Within-Run (Repeatability)		Between-Runs		Between-Days		Between-Lot		Between-Instrument		Total	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
Low Pool	70.4	2.1	3.0	0.4	0.6	0.9	1.3	2.2	3.1	1.5	2.1	2.3	3.3
Mid Pool	167.9	2.4	1.4	0.9	0.5	3.0	1.8	4.6	2.8	0.8	0.5	4.0	2.4
High Pool ^	413.6	3.9	0.9	0.0	0.0	4.2	1.0	12.1	2.9	5.6	1.4	5.7	1.4
Low Control	113.5	1.9	1.7	0.0	0.0	1.5	1.3	1.8	1.6	1.0	0.9	2.3	2.0
High Control	229.4	2.3	1.0	0.0	0.0	3.1	1.3	5.7	2.5	3.2	1.4	3.7	1.6

*Target IgE Levels (IU/mL) are 70 (Low Pool), 165 (Mid Pool), 400 (High Pool), 117 (Low Control) and 233 (High Control).
^Spiked with human IgE

2. Linearity:

Linearity of the Total Immunoglobulin E (IgE) assay was evaluated in accordance with the CLSI guideline EP06-A. A pool of human sera containing IgE concentration greater than the upper assay range limit (576 IU/mL) was combined with a pool of low IgE human sera (13 IU/mL) to create a 15-level dilution series spanning across the assay measuring range. Each dilution was tested in quadruplicate and in random order. The modelled non-linearity was not statistically significant and the assay was deemed linear. The observed values were graphed against the calculated values and a weighted linear regression was performed. The assay is linear from 13.2–575.5 IU/mL. The claimed linear assay range is from 20−500 IU/mL and the linear equation is $y= 1.01x + 0.63$ and $R^2=0.99$.

Automatic Dilution:

Samples exceeding the upper limit of linearity in the IgE concentration range 500–1000 IU/mL should be diluted with deionized water and repeated. The result is multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN. A study was performed to verify the automatic dilution settings on the DxC 700 AU Clinical Chemistry Analyzer. The accuracy and repeatability of automatic sample pre-dilution was assessed by comparing manual dilution with the instrument's 1:10 auto dilution function. To assess accuracy, three samples were prepared by diluting an aliquot of the World Health Organization (WHO) standard 11/234 with deionized water to achieve to achieve analyte levels spanning the claimed IgE extended measuring interval (EMI) of > 500 to 1,000 IU/mL. Test samples were assayed by auto-dilution in triplicate. For the repeatability assessment, portions of a single patient donor pool were spiked to three different analyte levels within the claimed IgE EMI. Twenty replicates of the neat test samples were programmed so that the instrument would perform the auto-dilution and re-run routine 20 times. For the manual dilution tests, the 20

replicates of a given test sample represented 20 offline (manual) dilution preparations. The results are summarized below:

Serum Concentration (IU/mL)	Manual Dilution		Auto-dilution		Bias Manual vs. Auto-Dilution
	SD (mg/dL)	%CV	SD (mg/dL)	%CV	
600	15.8	2.5	18.7	2.8	4.6
750	22.5	3.0	19.5	2.4	7.2
900	51.7	5.5	19.0	1.9	6.8

3. Analytical Specificity/Interference:

i. Endogenous Interference:

Interference studies were peformed in accordance with the CLSI guidelines EP07 and EP37. For the endogenous interference studies, five levels of each interferent were evaluated at three IgE levels spanning the measuring range, including an IgE concentration near the upper reference interval limit to represent a medical decision level (MDL). Test samples were prepared from a human serum pool of known IgE concentration that was diluted to achieve the desired total IgE levels using Ig-depleted human serum. Each test sample was spiked with known quantities of potentially interfering substances and analyzed in five replicates, in one assay run, with a single lot of the IgE reagent and a single DxC 700 AU analyzer. The recovery was calculated by comparing to control samples spiked with the same volume of diluents. The sponsor defines significant interference as ±10.0 IU/mL for recovered values ≤100 IU/mL and ±10% for values >100 IU/mL. No significant interference was demonstrated with the following substances up to the levels indicated: hemoglobin (1g/dL), unconjugated bilirubin (60 mg/dL), Intralipid (1 g/dL), and rheumatoid factor (250 IU/mL). Human anti-mouse antibody (HAMA) was not evaluated in the study. A limitation statement was added to the package insert stating that patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain HAMA and may show either falsely elevated or depressed values when tested.

ii. Exogenous Interference:

The potential interference of 21 commonly used drugs, including those used for allergy treatment, was evaluated by testing each potential drug interferent at approximately three times the peak therapeutic concentrations and comparing the observed difference to the allowable bias specification. Test samples were prepared from pooled patient serum adjusted with human IgE or IgE-depleted serum to achieve a total IgE concentration of 165 IU/mL. To ensure that the test sample matrices were diluted as little as possible, interferent stock solutions were prepared in the appropriate solvents to achieve drug concentrations at least 20 times the maximum dose. Paired test and control samples were assayed in five replicates, each using a single lot of the IgE reagent and a single DxC 700 AU analyzer, and mean values were used to evaluate the observed bias. No significant interference was demonstrated with the common drugs at the indicated test concentrations listed in the table below. The drug omalizumab (Xolair), a synthetic anti-

IgE antibody, was not included in the study given its known interference with immunoassay based IgE methodologies[1]. A limitation statement was added to the package insert stating that '*falsely decreased results may occur in patients being treated with omalizumab*'.

Drugs	Test Concentration	IgE Mean Recovery (IU/mL)		Mean Bias (IU/mL)	Mean %Bias*
		Control Sample (no drug)	Test Sample (drug-spiked)		
Acetaminophen	15.6 mg/dL	157.9	158.0	0.1	0.1
Acetylcysteine	15 mg/dL	158.5	158.0	-0.5	-0.3
Acetylsalicylic Acid	3 mg/dL	159.8	158.6	-1.3	-0.8
Ampicillin-Na	7.5 mg/dL	156.4	155.9	-0.5	-0.3
Cefoxitin	660 mg/dL	159.8	157.4	-2.5	-1.6
Cetirizine	0.435 mg/dL	158.3	159.7	1.4	0.9
Cyclosporine	0.18 mg/dL	159.8	159.6	-0.2	-0.1
Diphenhydramine	0.0774 mg/dL	156.1	156.9	0.8	0.5
Doxycycline	1.8 mg/dL	158.9	159.5	0.6	0.4
Fexofenadine	0.116 mg/dL	162.6	162.1	-0.5	-0.3
Heparin	330 units/dL	160.6	158.8	-1.8	-1.1
Ibuprofen	21.9 mg/dL	160.7	160.0	-0.7	-0.4
Levodopa	0.75 mg/dL	159.6	158.0	-1.7	-1.0
Methyldopa	2.25 mg/dL	160.7	160.0	-0.7	-0.4
Metronidazole	12.3 mg/dL	156.9	157.5	0.7	0.4
Mometasone	0.000045 mg/dL	162.5	159.9	-2.6	-1.6
Phenylbutazone	32.1 mg/dL	160.0	160.1	0.1	0.1
Prednisolone	0.12 mg/dL	159.3	157.5	-1.8	-1.1
Rifampicin	4.8 mg/dL	157.2	158.3	1.2	0.7
Salicylic Acid	2.86 mg/dL	156.4	157.7	1.3	0.9
Theophylline	6 mg/dL	161.8	162.6	0.8	0.5
*Allowable bias criterion for no significant interference: ±10%					

iii. Cross-reactivity:

Antibody cross-reactivity studies were performed externally by the supplier of the monoclonal antibody component (R2) of the IgE Reagent. The cross-reactivity data from two material lots of human IgE monoclonal antibody provided on the Certificate of Analysis showed no cross-reactivities with human IgA, IgD, IgG, or IgM were detected.

[1] Hamilton RG. Accuracy of Food and Drug Administration-cleared IgE antibody assays in the presence of anti-IgE (omalizumab). J Allergy Clin Immunol. 2006;117(4):759-766

4. Assay Reportable Range:

The assay reportable range is 20 – 500 IU/mL. Samples exceeding the upper limit of linearity in the IgE concentration range 500– 1000 IU/mL should be diluted with deionized water and repeated. The result is multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

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

i) Traceability and value assignment:

The Total Immunoglobulin E (IgE) calibrators are prepared from stabilized normal human plasma spiked with purified human IgE. Through the value assignment process, the human IgE in the calibrators is traceable to the WHO 3rd International Reference Preparation for human serum Immunoglobulin E (IgE), 11/234.

ii) Kit stability:

Closed kit stability− A real-time stability study was performed in accordance with the CLSI guideline EP25 using three lots of IgE reagent with paired IgE calibrators on a single DxC 700 AU analyzer to evaluate assay performance for reagent kits stored under normal conditions (2–8°C). As a worst-case scenario, one of these test lots was pre-stressed to environmental test conditions that simulated potential winter and summer transport profiles and compared to the performance of unstressed (control) product through the duration of the stability period. Testing was performed with three levels of human serum-based control materials at multiple time points throughout the claimed stability period and at least one month past the expiration date. Results support the 24-month shelf-lfe stability claim for the Total Immunoglobulin E (IgE) reagent kit.

In-use reagent stability− A real-time stability study was performed to support the in-use stability claims for the IgE kit components and the IgE assay calibration interval. For the reagent in-use study, opened IgE reagents (R1 and R2) were stored on-board the analyzer in the reagent storage compartment (2–8ºC), and a new set of IgE calibrators was opened and used for each calibration event. For the calibrator in-use study, the reagent was removed from the analyzer after use on each test day (capped and stored in the refrigerator at 2–8ºC), and one set of calibrators was opened on Day 0, used throughout the study period, and stored at 2–8ºC. The mean recovery at each time point was used to calculate the bias versus the Day 0 mean recovery. Results supports the stability of *(i)* the IgE reagents (R1 and R2) after first opening for 28 days at 2−8ºC, *(ii)* the calibrator bottle after first opening for 45 days at 2−8ºC and *(iii)* the calibration interval of 14 days.

iii) Sample stability and storage:

Sample stability studies were initiated on the day of specimen collection using one lot of candidate IgE reagent on a single DxC 700 AU analyzer. Three patient pools with total IgE levels spanning the claimed assay range were aliquoted and stored under each temperature condition. Two test runs were performed with fresh samples to establish a

time-zero (T_0) mean recovery value for all temperatures. Aliquots of the serum and plasma samples were assayed in quadruplicate at each time point of each storage temperature. The final time point exceeded the claimed in-use duration claim by at least one day. Results for each sample type and temperature condition support the sample stability claims summarized in the table below.

Sample Type	Storage Conditions		
	Room Temperature (+15ºC to +25ºC)	**Refrigerator (+2ºC to +8ºC)**	**Freezer (-20ºC to -15ºC)**
Serum	8 hours	7 days	60 days; freeze/thaw once
Plasma	8 hours	7 days	60 days; freeze/thaw once

6. Detection Limit:

The Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) studies were conducted in accordance with the CLSI guideline EP17-A2 using the Classical Approach. The studies evaluated two lots of IgE reagents on one DxC 700 AU analyzer.

The LoB was determined with four different immunoglobulin (Ig)-depleted human serum samples used as the individual blank samples. Each blank sample was tested in five replicates per sample, one run per day for three days to obtain a total of 60 replicates. The LoB was estimated as the 95th percentile of the measurements for each of the lots tested and determined to be 5.9 IU/mL and 7.5 IU/mL for the two IgE reagent lots. The LoB claim that corresponds to the highest observed values between the two IgE reagent lots is 7.5 IU/mL.

The LoD and LoQ were determined using four native patient pools diluted with Ig-depleted serum to achieve the low analyte levels. The target analyte concentration was approximately 14 IU/mL for the LoD test samples and approximately 19 IU/mL for the LoQ test samples. Each LoD sample was tested in five replicates per sample, one run per day for three days to obtain a total of 60 replicates. The LoD was calculated as the LoB + 1.645 x SD of the replicates for the low-level samples and determined as 13.8 IU/mL and 12.5 IU/mL for the two IgE reagent lots. The LoD claim that corresponds to the highest observed values between the two IgE reagent lots is 13.8 IU/mL.

Each LoQ sample was tested in three replicates per sample, one run per day for three days to obtain a total of 36 replicates. The LoQ, defined as the mean IgE value of the sample which fulfills the specification of 20 IU/mL that corresponds to the IgE concentration that can be quantitatively determined with ≤ 35% CV, was estimated as 19.6 IU/mL with 11.9% CV and 17.8 IU/mL with 7.8% CV for the two lots of reagents. The LoQ claim that corresponds to the highest observed values between the two IgE reagent lots is 19.6 IU/mL which is the lower limit of the measuring range claimed for the assay.

7. Assay Cut-Off:

Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparions study was performed in accordance with the CLSI guideline EP09c. The study evaluated 136 fresh neat serum samples spanning the analytical measuring range of the Total Immunoglobulin E (IgE) assay. Each sample was assayed using a single lot of candidate IgE reagent and a single lot of calibrator on a single DxC 700 AU analyzer with one test replicate per sample cup and two sample cups per test run. The second cup served only as a backup in the event of a sampling error with the first replicate. Quality control materials were included to qualify each test run. Results based on Weighted Deming regression analysis are summarized in table below.

Comparison	Sample Range (IU/mL)	N	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient (r)
Beckman Coulter IgE Assay vs. Roche Elecsys IgE II Assay	24.1 – 491.2	136	0.97 (0.95 – 0.98)	1.01 (-1.03 – 3.03)	0.996
N = Number of samples tested					

2. Matrix Comparison:

To demonstrate that sodium (Na)-Heparin, lithium (Li)-Heparin, K_2-EDTA and K_3-EDTA plasma samples yield results comparable with serum samples tested by the Total Immunoglobulin E (IgE) assay, a study was performed by using freshly drawn matched samples from apparently healthy adult volunteer donors, where five specimen tubes were drawn from each donor: one serum tube and one tube of each type of anticoagulant. Four (~7%) of the 57 donor sets were spiked with purified human IgE to ensure adequate test sample distribution across the claimed measuring range. Paired samples were run in duplicates with one IgE reagent lot on a single DxC 700 AU analyzer and analyzed using only the first replicate result. Two of the 57 donor sets recovered outside of the IgE analytical range for all sample types and were removed from the final data set. The Weighted Deming regression analysis was performed, and the results are summarized in the following table:

Comparison	Sample Range (IU/mL)	N	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient (r)
Na-Heparin plasma vs. serum	23.6 – 474.4	55	0.99 (0.98 – 1.00)	0.0 (-1.02 – 1.02)	0.999
Li- Heparin plasma vs. serum	21.9 – 464.6	55	0.99 (0.98 – 1.00)	-0.47 (-1.57 – 0.62)	0.999
K_2-EDTA plasma vs. serum	22.7 – 457.5	55	0.99 (0.97 – 1.01)	-1.73 (-3.39 – -0.11)	0.997
K_3-EDTA plasma vs. serum	21.2 – 444.7	53*	0.96 (0.95 – 0.98)	-2.4 (-3.67 – -1.06)	0.998
N = Number of samples tested * Two K3 EDTA samples were confirmed to have been mishandled and excluded from the analysis					

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable

2. Clinical Specificity:

   Not applicable

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

   Not applicable

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

A reference interval study was performed in accordance with the CLSI guideline EP28-A3c using two sets of serum samples collected from an adult non-atopic donor population (N=169). The first set of 119 serum samples were collected from a population of apparently healthy, non-atopic, male and female adults (> 18 years of age) in Carlsbad, California. Another set of 50 serum samples was obtained from donor volunteers (adult, non-atopic) in Chaska, Minnesota. All test samples were from individuals that stated no known allergies and listed no allergy medications on the Donor Information form. Testing was performed using a single lot of IgE reagent on a DxC 700 AU analyzer, one test replicate per sample. Three levels of control material were included to qualify each test run. The reference interval was determined by a non-parametric method based on the central 95% of the data distribution, using 90% confidence

intervals for the upper and lower limits (2.5th and 97.5th percentiles, respectively). Results established a total IgE reference interval of 2.6 to 699.9 IU/mL for a non-atopic, asymptomatic adult population.

IgE reference intervals are significantly influenced by age, sex, geographic location, microflora of the gastrointestinal tract, diet of the population, as well as environmental factors such as climate change[1]. As such, the Sponsor chose to present a range from a more recent literature citation[2] in conjunction with the recommendation that each clinical laboratory should verify the transferability of the expected values to its own population, and if necessary, determine its own reference interval according to good laboratory practice.

Age	Levels (kIU/L)*
0 − <7 years	<25 – 440
7 − <19 years	<25 – 450
20 − 60 years	0 – 160
*Equivalent to IU/mL	

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

[2] CLSI I/LA20, Analytical Performance Characteristics, Quality Assurance, and Clinical Utility of Immunological Assays for Human Immunoglobulin E Antibodies of Defined Allergen Specificities, 3rd Edition, October 2016, pp. 23; 27.