510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k091289

B. Purpose for Submission:
Modification to device

C. Measurand:
Allergen Specific IgE (Sunflower Seed, Aspergillus niger, Cephalosporium acremonium, Poplar, Careless Weed, Saltbush)

D. Type of Test:
Quantitative, chemiluminiscent immunoassay

E. Applicant:
Siemens Healthcare Diagnostics, Inc.

F. Proprietary and Established Names:
IMMULITE® 2000 3gAllergy™ Specific IgE Assay kit

G. Regulatory Information:

1. Regulation section:
   21 CFR § 866.5750, Radioallergosorbent (RAST) test systems
2. Classification:
   Class II
3. Product code:
   DHB System, Test, Radioallergosorbent (RAST), Immunological
4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended use:
   For *in vitro* diagnostic use with the IMMULITE 2000 Analyzer – for the quantitative measurement of allergen-specific IgE in human serum, as an aid in the clinical diagnosis of IgE-mediated allergic disorders.
2. Indication(s) for use:
   Same as Intended use.
3. Special conditions for use statement(s):
   For prescription only.
4. Special instrument requirements:
   IMMULITE 2000 Analyzer (k970227)

I. Device Description:

Each device contains the following: 3gAllergy™ specific IgE bead pack (3 packs of 200 beads coated with anti-ligand); specific IgE reagent wedge: 30 mL alkaline phosphatase (bovine calf intestine) conjugated to monoclonal murine anti-human IgE antibody in a human/nonhuman serum buffer matrix (equally dispensed in 1 wedge with B & C chambers); specific IgE adjustors: low and high (2 vials, 2 mL each) of human IgE in a nonhuman serum matrix with preservative; specific IgE adjustor antibody: 2 tubes, 2.75 mL each) ready to use ligand-labeled polyclonal goat anti-human IgE antibody with preservative; specific IgE universal kit controls: (2 vials, 2 mL each) human IgE in a nonhuman sample matrix with preservative; specific IgE

control antibody: (2 tubes, 2.75 mL each) ready to use ligand-labeled polyclonal goat anti-human IgE antibody with preservative. Kit components supplied separately: 3gAllergy™ specific IgE sample diluent (concentrated ready to use 1 vial, 25 mL); chemiluminiscent substrate; probe wash; probe cleaning kit; disposable reaction tubes; bar coded allergen holder wedges serially coded 1-33; 34 -66; 67-99; allergen tube caps and tube septa.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   IMMULITE® 2000 3gAllergy™ Specific IgE
2. Predicate K number(s):
   k013134
3. Comparison with predicates:

Similarities		
Item	New Device	Predicate Device
Intended use	For *in vitro* diagnostic use with the IMMULITE 2000 Analyzer – for the quantitative measurement of allergen-specific IgE in human serum, as an aid in the clinical diagnosis of IgE-mediated allergic disorders.	Same
Technology	Chemiluminescence	Same
Assay performance	Assay to be specific to allergen-specific IgE	Same
Calibrators	Low and high	Same
Controls	Specific IgE and Antibody and Specific IgE Universal Controls	Same
Sample type	Serum	Same
Result Interpretation	Quantitative values in kU/L; Interpretation of class results for two scoring systems: Standard and Extended standard	Same

K. Standard/Guidance Document Referenced (if applicable):

Standard documents:
CLSI I/LA 20-A: Evaluation Methods and Analytical Performance Characteristics of Immunological Assays for Human Immunoglobulin E (IgE)
CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Methods; Approved Guideline – Second Edition
Guidance document:
FDA Guidance – Radioallergosorbent Test (RAST) Methods for Allergen-Specific Immunoglobulin E (IgE) 510(k); Final Guidance

L. Test Principle:

The assay is a solid-phase, two-step, chemiluminiscent immunoassay that exploits liquid phase kinetics in a bead format. The allergens are covalently bound to a soluble polymer/co-polymer matrix, which is labeled with a ligand. The assay specific

antibody is labeled with alkaline phosphatase. The use of an amino acid co-polymer amplifies the amount of allergen that the matrix can support. The chemiluminiscent detection system is a phosphatase ester of stabilized dioxatane. Cleavage of the phosphate ester by alkaline phosphatase results in the decomposition of dioxatane and the emission of photons, which are quantified by a luminometer.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility of the assay was assessed by testing three positive samples and one negative control sample of each allergen (Sunflower Seed, Aspergillus niger, Cephalosporium acremonium, Poplar, Careless Weed, Saltbush) in duplicate twice a day for 20 different days (n = 80).
The sponsor's criterion for the negative sample was the average dose must be <0.10 kU/L; all negative sample results were within the acceptance criterion. The sponsor's acceptance criterion for the positive samples was ≤15% CV for both within-run and total precision. Three allergen lots were tested for each allergen; representative data from one lot is shown below for the positive samples. The intra-assay and inter-assay %CV ranges were from 2.74% to 6.07% and 4.05% to 9.82%, respectively (see tables below).

Allergen: Sunflower Seed

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	0.56	0.034	6.07	0.055	9.82
Positive #2	1.26	0.047	3.73	0.067	5.32
Positive #3	10.42	0.457	4.39	0.648	6.22

Allergen: Aspergillus niger

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	1.45	0.061	4.21	0.102	7.03
Positive #2	4.36	0.210	4.82	0.407	9.33
Positive #3	2.67	0.103	3.86	0.220	8.24

Allergen: Cephalosporium acremonium

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	1.49	0.049	3.29	0.077	5.17
Positive #2	1.22	0.051	4.18	0.060	4.92
Positive #3	0.86	0.030	3.49	0.053	6.16

Allergen: Poplar

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	9.99	0.326	3.26	0.541	5.42
Positive #2	4.22	0.143	3.39	0.249	5.90
Positive #3	2.69	0.097	3.61	0.223	8.29

Allergen: Careless Weed

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	16.52	0.495	3.00	0.962	5.82
Positive #2	1.94	0.082	4.23	0.097	5.00
Positive #3	3.51	0.096	2.74	0.160	4.56

Allergen: Saltbush

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	2.63	0.078	2.97	0.108	4.11
Positive #2	0.79	0.027	3.42	0.032	4.05
Positive #3	4.72	0.174	3.69	0.236	5.00

Lot to lot reproducibility:
Each of the six allergens was tested as above with three positive samples using three different lots (n=240). Within run imprecision for the sample/allergen combinations ranged from 2.39% to 4.38%; total imprecision of the sample/allergen combinations ranged from 3.78% to 6.08%. All three lots for the six allergens were within the acceptable criterion of ≤ 20% variability.

b. Linearity/assay reportable range:

Linearity studies: For each allergen, two samples were diluted in 2-fold serial dilutions to 5 levels. The undiluted (neat) and diluted samples were tested with the specific allergen to demonstrate linearity at concentrations within the assay limits. Regression statistics for each allergen comparing the observed results to expected results are presented below.

Allergen	Regression Equation	N	Slope	95% CI	Intercept	95% CI
Sunflower Seed	Y= 0.999X - 0.023	12	0.999	0.986–1.012	-0.023	–0.059-0.014
Aspergillus niger	Y= 0.989X + 0.025	12	0.989	0.970–1.007	0.025	–0.032-0.083
Cephalosporium acremonium	Y= 0.961X + 0.059	8	0.961	0.939–0.983	0.059	0.045-0.073
Poplar	Y= 1.000X - 0.043	12	1.002	0.963–1.042	-0.043	-0.124-0.038
Careless Weed	Y= 1.013X - 0.040	12	1.013	0.990–1.036	-0.040	-0.227-0.147
Saltbush	Y= 1.000X – 0.030	12	1.002	0.989–1.015	0.030	-0.019-0.078

Assay working ranges: 0.1 – 100 kU/L.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Traceability: The calibrators and controls are traceable to the WHO 2nd IRP 75/502 reference standard for human IgE.

Stability: Accelerated allergen stability testing (15-28ºC for 57 days; assay kits stored at recommended temperature 2-8ºC). The accelerated study supports a two year shelf-life stability claim.

d. *Detection limit:*
Limit of Blank (LoB): Three runs assaying the blank sample (zero calibrator) were performed to estimate the Limit of Blank (LoB). A total of three instruments were used per run. The maximum dose of the LoB was selected as the most conservative LoB: $LoB_{MAX} = 0.026$. The claimed LoB is 0.1 kU/L.

Limit of Detection (LoD): Five samples were used to estimate the Limit of Detection (LoD). Sixty replicates of each sample were assayed per run. A total of 2 runs were completed with testing performed on 2 different instruments. The LoD was calculated for each sample using the formula: $LoD = LoB_{MAX} + (1.65 * SD_{LOD})$. The claimed LoD is 0.1 kU/L

e. *Analytical specificity:*
Inhibition studies: Specificity of each allergen was verified through competitive inhibition testing using a single serum sample or a serum pool. A negative sample was used to measure the background response.
To initiate the inhibition experiment, 70 µL of undiluted and 4 levels of 5-fold serially diluted inhibitor extract were mixed with 250 µL of sample or pool. This mixture was incubated at room temperature (15-28°C) for 1 hour allowing the immunological reaction to occur. Each sample mixture containing the inhibitor extract and the appropriate controls was assayed with one lot of each allergen. The percent (%) inhibition was calculated according to the following formula:

$$\left[\frac{(\text{Response of pos. control}_{(\text{pos. sample} - \text{neg. sample})} - \text{sample response with inhibitor extract})}{(\text{Response of pos. control}_{(\text{pos. sample} - \text{neg. sample})})}\right] \text{X } 100$$

The inhibition study demonstrated that the allergens tested are inhibited by the relevant inhibitor extract in a concentration dependent fashion. Summary inhibition table is presented below.

Sunflower Seed			Aspergillus niger			Cephalosporium acremonium	
Inhibitor Concentration (mg/mL)	% Inhibition		Inhibitor Concentration (mg/mL)	% Inhibition		Inhibitor Concentration (mg/mL)	% Inhibition
5	96.89		5	95.01		5	81.53
1	94.53		1	94.01		1	83.33
0.2	46.77		0.2	90.46		0.2	80.18
0.04	19.15		0.04	80.93		0.04	43.69
0.008	6.59		0.008	65.67		0.008	34.68
			0.0016	37.87			

Poplar			Careless Weed			Saltbush	
Inhibitor Concentration (mg/mL)	% Inhibition		Inhibitor Concentration (mg/mL)	% Inhibition		Inhibitor Concentration (mg/mL)	% Inhibition
5	100.00		5	100.00		5	100.00
1	96.17		1	98.57		1	83.96
0.2	86.17		0.2	95.53		0.2	59.89
0.04	56.81		0.04	83.63		0.04	28.34
0.008	37.66		0.008	49.10		0.008	12.30

Cross-reactivity: The manufacturer states there is no detectable crossreactivity with human serum immunoglobulins IgG, IgA, IgM or IgD at normal physiological levels.

f. Assay cut-off:
Not applicable

2. Comparison studies:
   a. Method comparison with predicate device:
   Refer to Clinical studies
   b. Matrix comparison:
   Not applicable. Serum is the only matrix.
3. Clinical studies:
   a. Clinical Sensitivity and specificity
   Clinical performance of the IMMULITE® 2000 3gAllergy Specific IgE assay for Sunflower Seed, Aspergillus niger, Cephalosporium acremonium, Poplar, Careless Weed, Saltbush allergens was demonstrated by testing samples from non-atopic and atopic individuals. Atopic patients were selected from patients who had documented history of allergies to mold and/or pollen and/or skin prick tested. Information on the skin test allergen extracts (crude or purified) was not documented. Non-atopic patients were clinically known non-allergenic or total IgE <130 ng/mL or 54 IU/mL (2.4 ng = 1 IU). Testing was performed on 145 samples for Sunflower Seed; 144 samples for Aspergillus niger; 166 samples for Cephalosporium acremonium; 160 samples for Poplar; 155 samples for Careless Weed and 158 samples for Saltbush. Based on

prevalence of mold allergies, % sensitivity ranged from 20-50% except for *S. cerevisiae* (invertase) with 80% sensitivity (Horner et al Allergy and Asthma Proceedings Nov-Dec 2008). Sensitivity and specificity of the new device, based on diagnosis of atopic status, are shown in the tables below:

Allergen: Sunflower Seed		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	25	8	33
	negative	20	92	112
	Total	45	100	145

Sensitivity: 56% (25/45) (95% CI: 41-70%)
Specificity: 92% (92/100) (95% CI: 87-97%)

Allergen: Aspergillus niger		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	18	2	20
	negative	24	100	124
	Total	42	102	144

Sensitivity: 43% (18/42) (95% CI: 28-58%)
Specificity: 98% (100/102) (95% CI: 95-101%)

Allergen: Cephalosporium acremonium		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	13	10	23
	negative	29	114	143
	Total	42	120	166

Sensitivity: 31% (13/42) (95% CI: 17-45%)
Specificity: 95% (114/120) (95% CI: 91-100%)

Allergen: Poplar		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	28	5	33
	negative	26	101	127
	Total	54	106	160

Sensitivity: 52% (28/54) (95% CI: 39-65%)
Specificity: 95% (101/106) (95% CI: 91-99%)

Allergen: Careless Weed		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	29	3	32
	negative	25	98	123
	Total	54	101	155

Sensitivity: 54% (29/54) (95% CI: 40-67%)
Specificity: 97% (98/101) (95% CI: 94-100%)

Allergen: Saltbush		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	22	0	22
	negative	30	106	136
	Total	52	106	158

Sensitivity: 42% (22/52) (95% CI:29-56%)
Specificity: 100% (106/106) (95% CI: 100-100%)

b. Other clinical supportive data (when a. is not applicable):
Not applicable.

4. Clinical cut-off**:**
Not applicable.

5. Expected values/Reference range:
Not detected.
Refer to the Hoffman's 'Standard' and 'Extended Standard' classification system utilizing Class 0 to Class IV cut-offs (see Tables I and II below).

Table I: The Standard classification system utilizes the following class cutoffs:

Class	kU/L	Reactivity for Individual/Component Allergen(s)
0*	< 0.10	Absent or ND†
	0.10 – 0.34	Very Low
I	0.35 – 0.69	Low
II	0.70 – 3.49	Moderate
III	3.50 – 17.49	High
IV	17.5 – 52.49	Very High
V	52.5 – 99.99	
VI	≥ 100	

* Class 0 in the standard system signifies: not detectable by second-generation assays.
† ND: not detectable by IMMULITE 2000 3gAllergy.

Table II: The Extended standard classification system utilizes the following class cutoffs.

Class	kU/L	Reactivity for Individual/Component Allergen(s)
0	< 0.10	Absent or ND†
0/1	0.10 – 0.24	Very Low
I	0.25 – 0.39	Low
II	0.40 – 1.29	Moderate
III	1.30 – 3.89	High
IV	3.90–14.99	Very High
V	15.00– 24.99	
VI	≥ 25	

† ND: not detectable by IMMULITE 2000 3gAllergy

The choice of classification systems can be made by the user within the IMMULITE 2000 operational software.
Reference: Hoffman, DR. Comparison of methods of performing the Radioallergosorbent test: Phadebas, Fadal-Nalebuff and Hoffman protocols. Ann Allergy. 1980 Dec; 45(6)

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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