510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k083853

B. Purpose for Submission:
Modification to device

C. Measurand:
Allergen Specific IgE (Bayberry/ Sweet gale; Live Oak; Locust Tree; Privet; Red Mulberry; White Bald Cypress; Baccharis; Dog Fennel; Hormodendrum Hordei; Stemphylium Solani; American Cockroach)

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: refer to tables attached below.	Same

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

Class	kU/L	Reactivity for Individual/Component Allergen(s)
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.

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	
V	15.00– 24.99	Very High
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)

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:*
      For the intra-assay study, three positive samples and one negative control sample for each of the eleven allergens (Bayberry/ Sweet gale; Live Oak; Locust Tree; Privet; Red Mulberry; White Bald Cypress; Baccharis; Dog Fennel; Hormodendrum Hordei; Stemphylium Solani; American Cockroach) were analyzed 80 times (for each allergens) in one run. For the inter-assay study, the same samples were analyzed 80 times in 2 different runs. All negative sample results were within the acceptance criterion that the average dose must be <0.10 kU/L. The acceptance criterion for the positive samples is ≤15% for both intra-assay and inter-assay studies. The intra-assay and inter-assay %CV ranges were from 3.18% to 6.37% and 3.90% to 8.46%, respectively (see tables below).

Allergen: Bayberry/ Sweet gale

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	1.36	0.50	3.68	0.066	4.85
Positive #2	6.70	0.266	3.97	0.359	5.36
Positive #3	4.20	0.142	3.38	0.226	5.38

Allergen: Live Oak

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	8.70	0.277	3.18	0.432	4.97
Positive #2	1.90	0.091	4.79	0.118	6.21
Positive #3	5.81	0.202	3.48	0.308	5.30

Allergen: Locust Tree

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	2.18	0.071	3.26	0.085	3.90
Positive #2	4.46	0.172	3.86	0.197	4.42
Positive #3	8.40	0.364	4.33	0.407	4.85

Allergen: Privet

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	9.21	0.351	3.81	0.415	4.51
Positive #2	4.57	0.181	3.96	0.208	4.55

Positive #3	1.22	0.045	3.69	0.067	5.49

Allergen: Red Mulberry

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	8.83	0.323	3.66	0.458	5.19
Positive #2	4.38	0.178	4.06	0.238	5.43
Positive #3	1.79	0.093	5.20	0.108	6.03

Allergen: White Bald Cypress

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	7.83	0.460	5.87	0.539	6.88
Positive #2	2.79	0.157	5.63	0.157	5.63
Positive #3	6.16	0.309	5.02	0.377	6.12

Allergen: Baccharis

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	9.31	0.340	3.65	0.522	5.61
Positive #2	3.97	0.141	3.55	0.183	4.61
Positive #3	2.34	0.149	6.37	0.187	7.99

Allergen: Dog Fennel

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	12.19	0.431	3.54	0.549	4.50
Positive #2	6.82	0.246	3.61	0.334	4.90
Positive #3	2.78	0.113	4.06	0.124	4.46

Allergen: Hormodendrum Hordei

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	1.82	0.115	6.32	0.154	8.46
Positive #2	11.35	0.627	5.52	0.667	5.88
Positive #3	4.17	0.179	4.29	0.235	5.64

Allergen: Stemphylium Solani

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD	%CV	SD	%CV

		(kU/L)		(kU/L)	
Positive #1	6.24	0.244	3.91	0.297	4.76
Positive #2	8.55	0.290	3.40	0.358	4.19
Positive #3	1.77	0.051	2.88	0.083	4.69

Allergen: American Cockroach

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	3.37	0.170	5.04	0.192	5.70
Positive #2	1.96	0.090	4.59	0.120	6.12
Positive #3	2.37	0.110	4.64	0.142	5.99

Lot to lot reproducibility:
Three lots were analyzed using 3 positive samples on each of the eleven allergens were analyzed 240 times. The acceptable criterion is ≤20%. The lowest variability was 3% and highest variability was 18%. All three different lots for the eleven allergens had <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 observed to expected results are presented below.

Allergen	Regression Equation	N	Slope	95% CI	Intercept	95% CI
Bayberry/ Sweet gale	Y= 1.01X + 0.03	12	1.009	0.988–1.031	-0.029	–0.180-0.123
Live Oak	Y= 1.00X + 0.07	12	0.997	0.979–1.015	0.068	–0.122-0.158
Locust Tree	Y= 0.99X – 0.004	12	0.994	0.968–1.020	–0.004	–0.103-0.096
Privet	Y= 0.99X + 0.09	12	0.992	0.952–1.031	0.092	-0.102-0.285
Red Mulberry	Y= 1.00X + 0.12	12	1.004	0.975–1.033	0.116	-0.113-0.346
White Bald Cypress	Y= 1.01X + 0.14	12	1.006	0.985–1.028	0.135	-0.082-0.353
Baccharis	Y= 1.00X + 0.11	12	0.999	0.979–1.019	-0.114	-0.358-0.131
Dog Fennel	Y= 1.00X + 0.14	12	1.000	1.000–1.000	0.138	-0.018-0.294
Hormodendrum Hordei	Y= 1.01X + 0.01	12	1.007	0.987–1.026	0.011	-0.101-0.122
Stemphylium Solani	Y= 0.99X + 0.16	12	0.994	0.966–1.021	0.158	–0.018-0.334
American Cockroach	Y= 1.00X – 0.05	12	0.998	0.979–1.017	0.049	0.006-0.091

Assay working ranges: 0.1 – 100 kU/L.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
The calibrators and controls are traceable to the WHO 2nd IRP 75/502 reference standard.
Stability studies:
Expiration date claim for the eleven allergens: 2 years (storage at 2-8º C)

Two positive samples and one negative sample were tested on three lots per allergen. Acceptance criteria for the accelerated stability study were as follows: Positive sample: no more than 30% loss; Negative sample: remained negative (<0.10 kU/L). Results were as follows:

ALLERGEN ID	LOT # TESTED	AVG %RECOVERY AT 57 ^{o}C
T218 - Bayberry/ Sweet gale	110, 111, 112	91%
T103 - Live Oak	113, 114, 115	94%
T208 - Locust Tree	113, 114, 115	98%
T210 - Privet	110, 111, 112	94%
T71 - Red Mulberry	110, 111, 112	91%
T37 - White Bald Cypress	111, 112, 113	94%
W67 - Baccharis	110, 111, 112	92%
W46 - Dog Fennel	112, 113, 114	94%
M45 Hormodendrum Hordei	110, 111, 112	102%
M88 - Stemphylium Solani	110, 111, 112	98%
I206 - American Cockroach	110, 111, 112	91%

d. *Detection limit:*
Analytical sensitivity: 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 pool of sera. A negative sample was used to measure the background response.
To initiate the inhibition experiment, 70µL 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:

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

The inhibition study demonstrated that the allergens tested are inhibited by the relevant inhibitor extract in a concentration dependent fashion. Also, the target % inhibition of 50% was met. These results indicate specificity of the Bayberry/ Sweet gale; Live Oak; Locust Tree; Privet; Red Mulberry; White Bald Cypress; Baccharis; Dog Fennel; Hormodendrum Hordei; Stemphylium Solani; American Cockroach specific allergens. Summary inhibition table is presented below.

Bayberry/ Sweet gale			Live Oak			Locust Tree	
Inhibitor Concentration (mg/mL)	% Inhibition		Inhibitor Concentration (mg/mL)	% Inhibition		Inhibitor Concentration (mg/mL)	% Inhibition
5	88.24		5	94.24		5	90.20
1	67.32		1	92.18		1	83.66
0.2	48.69		0.2	89.05		0.2	67.97
0.04	2.61		0.04	62.62		0.04	16.34
0.008	0.00		0.008	37.67		0.008	0.00

Privet			Red Mulberry			White Bald Cypress	
Inhibitor Concentration (mg/mL)	% Inhibition		Inhibitor Concentration (mg/mL)	% Inhibition		Inhibitor Concentration (mg/mL)	% Inhibition
5	96.70		5	100.0		5	100.00
1	95.50		1	100.0		1	96.30
0.2	93.85		0.2	91.88		0.2	88.26
0.04	91.17		0.04	76.88		0.04	81.51
0.008	83.03		0.008	23.75		0.008	61.41

Baccharis			Dog Fennel			Hormodendrum Hordei	
Inhibitor Concentration (mg/mL)	% Inhibition		Inhibitor Concentration (mg/mL)	% Inhibition		Inhibitor Concentration (mg/mL)	% Inhibition
5	100.0		5	96.40		5	89.23
1	100.0		1	95.47		1	72.48
0.2	100.0		0.2	91.61		0.2	62.76
0.04	91.04		0.04	39.28		0.04	46.14
0.008	75.62		0.008	22.50		0.008	17.33

Stemphylium Solani			American Cockroach	
Inhibitor Concentration (mg/mL)	% Inhibition		Inhibitor Concentration (mg/mL)	% Inhibition
5	98.43		5	67.95
1	92.50		1	71.04
0.2	73.01		0.2	54.83
0.04	24.96		0.04	34.94
0.008	3.30		0.008	10.23

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:*

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Refer to Clinical studies
3. Clinical studies:
   a. Clinical Sensitivity and specificity

Clinical performance of the allergens was demonstrated by testing samples from non-atopic individuals and atopic patients with case histories of suspected clinical reactions to the specific allergen or allergy group in the IMMULITE® 2000 3gAllergy Specific IgE assay and comparing results to accompanying clinical information. Testing was performed on 146 samples for Bayberry/ Sweet gale; 222 samples for Live Oak; 148 samples for Locust Tree; 205 samples for Privet; 148 samples for Red Mulberry; 207 samples for White Bald Cypress; 170 samples for Baccharis; 170 samples for Dog Fennel; 204 samples for Hormodendrum Hordei; 137 samples for Stemphylium Solani; 200 samples for American Cockroach. Sensitivity and specificity, based on diagnosis of atopic status is shown in the tables below.

Allergen: Bayberry/ Sweet gale		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	25	3	28
	negative	21	97	118
	Total	46	100	146

		95% CI
Sensitivity	54% (25/46)	40-69%
Specificity	97% (97/100)	94-100%

Allergen: Live Oak		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	24	1	25
	negative	45	152	197
	Total	69	153	222

		95% CI
Sensitivity	35% (24/69)	24-46%
Specificity	99% (152/153)	98-101%

Allergen: Locust Tree		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	24	4	28
	negative	19	101	120
	Total	43	105	148

		95% CI
Sensitivity	56% (24/43)	41-71%

Specificity	96% (101/105)	93-100%

Allergen: Privet		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	15	14	29
	negative	37	139	176
	Total	52	153	205

		95% CI
Sensitivity	29% (15/52)	17-41%
Specificity	91% (139/153)	86-95%

Allergen: Red Mulberry		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	21	0	21
	negative	25	102	127
	Total	46	102	148

		95% CI
Sensitivity	46% (21/46)	31-60%
Specificity	100% (102/102)	100-100%

Allergen: White Bald Cypress		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	22	4	26
	negative	32	149	181
	Total	54	153	207

		95% CI
Sensitivity	41% (22/54)	28-54%
Specificity	97% (149/153)	95-100%

Allergen: Baccharis		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	34	2	36
	negative	36	98	134
	Total	70	100	170

		95% CI
Sensitivity	49% (34/70)	37-60%
Specificity	98% (98/100)	95-100%

Allergen: Dog Fennel		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	33	3	36
	negative	37	97	134
	Total	70	100	170

		95% CI
Sensitivity	47% (33/70)	35-59%
Specificity	97% (97/100)	94-100%

Allergen: Hormodendrum Hordei		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	16	0	16
	negative	35	153	188
	Total	51	153	204

		95% CI
Sensitivity	31% (16/51)	19-44%
Specificity	100% (153/153)	100-100%

Allergen: Stemphylium Solani		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	25	3	28
	negative	10	99	109
	Total	35	102	137

		95% CI
Sensitivity	71% (25/35)	56-86%
Specificity	97% (99/102)	94-100%

Allergen: American Cockroach		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	18	2	20
	negative	29	151	180
	Total	47	153	200

		95% CI
Sensitivity	38% (18/47)	24-52%
Specificity	99% (151/153)	97-100%

    c. Other clinical supportive data (when a. and b. are not applicable):
       Not applicable.
4. <u>Clinical cut-off</u>**:**
   Not applicable.
5. <u>Expected values/Reference range:</u>
   Not detected.

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.