510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k083314

B. Purpose for Submission:
Modification to device

C. Measurand:
Allergen Specific IgE (Red Maple, White Hickory, Red Cedar, Sweet Gum, Common Sagebrush, Wing Scale)

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(s):
   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

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

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)

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 six allergens (Red Maple, White Hickory, Red Cedar, Sweet Gum, Common Sagebrush, Wing Scale) 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. The acceptable criterion for the negative sample is for the average dose must be <0.10 kU/L. All negative sample results were within the acceptance criterion. The acceptable criterion for the positive samples is ≤15% for both intra-assay and inter-assay studies. The intra-assay CV ranges were from 3.04% to 5.24%. The inter-assay CV ranges were from 4.27% to 7.03% (see table below).

Allergen: Red Maple

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	2.17	0.073	3.36	0.117	5.39
Positive #2	3.19	0.096	3.01	0.162	5.08
Positive #3	12.06	0.452	3.75	0.634	5.26

Allergen: White Hickory

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	1.12	0.038	3.39	0.053	4.73
Positive #2	4.54	0.238	5.24	0.312	6.87
Positive #3	6.25	0.214	3.42	0.267	4.27

Allergen: Red Cedar

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	1.34	0.056	4.18	0.071	5.30
Positive #2	7.63	0.393	5.15	0.421	5.52
Positive #3	10.09	0.521	5.16	0.560	5.55

Allergen: Sweet Gum

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	2.70	0.082	3.04	0.139	5.15
Positive #2	3.00	0.115	3.83	0.154	5.13
Positive #3	7.97	0.332	4.17	0.502	6.30

Allergen: Common Sagebrush

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	1.42	0.049	3.45	0.069	4.86
Positive #2	4.14	0.191	4.61	0.291	7.03
Positive #3	10.47	0.425	4.06	0.558	5.33

Allergen: Wing Scale

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	1.56	0.056	3.59	0.072	4.62
Positive #2	5.20	0.246	4.73	0.267	5.13
Positive #3	9.81	0.324	3.30	0.424	4.32

Lot to lot reproducibility:
Three lots were analyzed using 3 positive samples on each of the six allergens were analyzed 240 times. The acceptable criterion is ≤20%. The lowest variability was 1% and highest variability was 2%. All three different lots for the six 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
Red Maple	Y= 1.00X + 0.06	12	0.999	0.961–1.037	0.059	–0.124-0.242
White Hickory	Y= 1.00X + 0.06	12	1.004	0.981–1.028	0.062	–0.122-0.246
Red Cedar	Y= 1.00X – 0.01	12	1.003	0.987–1.019	–0.007	–0.081-0.067
Sweet Gum	Y= 1.00X + 0.31	12	0.997	0.965–1.029	0.306	0.051-0.561
Common Sagebrush	Y= 1.00X + 0.62	12	0.999	0.970–1.028	0.615	–0.400-1.270
Wing scale	Y= 1.00X – 0.10	12	0.998	0.981–1.015	–0.098	–0.252-0.056

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 six allergens: 2 years.
Three 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 °C
T27 – Red Maple	110, 111, 112	76%
T41 – White Hickory	110, 111, 112	80%
T211 – Sweet Gum	110, 111, 112	85%
T219 – Red Cedar	110, 111, 112	79%
W43–Common Sagebrush	110, 111, 112	91%
W75 – Wing scale	114, 115, 116	80%

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 1 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 red maple, white hickory, red cedar, sweet gum, common sagebrush and wing

scale specific allergens. Summary inhibition table is presented below.

Red Maple			White Hickory			Red Cedar	
Inhibitor Concentration (mg/mL)	% Inhibition		Inhibitor Concentration (mg/mL)	% Inhibition		Inhibitor Concentration (mg/mL)	% Inhibition
5	100.00		5	100.00		5	97.59
1	99.04		1	100.00		1	93.78
0.2	96.83		0.2	97.87		0.2	83.63
0.04	79.15		0.04	89.03		0.04	70.98
0.008	0.00		0.008	27.33		0.008	69.28

Sweet Gum			Common Sagebrush			Wing scale	
Inhibitor Concentration (mg/mL)	% Inhibition		Inhibitor Concentration (mg/mL)	% Inhibition		Inhibitor Concentration (mg/mL)	% Inhibition
5	98.53		5	100.00		5	87.49
1	96.07		1	100.00		1	72.05
0.2	92.61		0.2	100.00		0.2	38.26
0.04	68.03		0.04	89.71		0.04	20.46
0.008	10.15		0.008	69.61		0.008	5.30

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
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 201 samples for Red Maple, White Hickory, Red Cedar; Sweet Gum; and 148 samples on Common Sagebrush and Wing Scale. Sensitivity and specificity, based on diagnosis of atopic status is shown in the tables below.

Allergen: Red Maple		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	36	8	44
	negative	12	145	157
	Total	48	153	201

		95% CI
Sensitivity	75% (36/48)	53-87%
Specificity	95% (145/153)	91-98%

Allergen: White Hickory		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	32	11	43
	negative	16	142	158
	Total	48	153	201

		95% CI
Sensitivity	67% (32/48)	53-80%
Specificity	93% (142/153)	89-97%

Allergen: Red Cedar		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	36	4	40
	negative	12	149	161
	Total	48	153	201

		95% CI
Sensitivity	75% 36/48)	63-87%
Specificity	97% (149/153)	95-100%

Allergen: Sweet Gum		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	31	9	40
	negative	17	144	161
	Total	48	153	201

		95% CI
Sensitivity	67% (31/48)	51-78%
Specificity	94% (144/153)	90=98%

Allergen: Common Sagebrush		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	33	3	36
	negative	15	97	112
	Total	48	100	148

		95% CI
Sensitivity	69% (33/48)	56-82%
Specificity	97% (97/100)	94-100%

Allergen: Wing Scale		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	28	2	30
	negative	20	98	118
	Total	48	100	148

		95% CI
Sensitivity	58% (28/48)	44-72%
Specificity	98% (98/100)	95-100%

c. Other clinical supportive data (when a. and b. are not applicable):
Not applicable.

4. Clinical cut-off**:**
Not applicable.
5. Expected values/Reference range:
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.