

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

k101572

**B. Purpose for Submission:**

Addition of six new allergens to a cleared device

**C. Measurand:**

Six Allergen Specific IgE: Basil, Cacao, Oregano, Parsley, Pine Nut, and Vanilla

**D. Type of Test:**

Chemiluminescent Immunoassay, Quantitative

**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) Immunological Test System
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. The test results are to be used in conjunction with clinical findings and other laboratory tests.
2. Indication(s) for use:  
Same as Intended Use.
3. Special conditions for use statement(s):  
Prescription use 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 high and low adjustors (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) and 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), chemiluminescent substrate, probe wash, probe cleaning kit, disposable reaction tubes, barcoded 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) and 510(k) number(s):  
IMMULITE® 2000 3gAllergy™ Specific IgE (k013134)
2. Comparison with predicate:

<b>Similarities</b>		
Item	New Device	Predicate
Intended use	For <i>in vitro</i> 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. The test results are to be used in conjunction with clinical findings and other laboratory tests.	Same
Technology	Chemiluminescence	Same
Assay performance	Specific to allergen-specific IgE	Same
Calibrators	Low and high	Same
Controls	Specific human IgE, Specific anti-human IgE 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

<b>Differences</b>		
Item	New Device	Predicate
Types of Specific Allergens	6 specific allergens: Basil Cacao, Oregano, Parsley, Pine Nut, and Vanilla.	110 Specific Allergens: 8 Animal epithelia, 2 House dusts, 39 Food, 13 Grasses, 5 Insects, 5 Mites, 10 Molds, 1 Latex, 14 Trees, 13 Weeds

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

FDA Guidance – Radioallergosorbent Test (RAST) Methods for Allergen-Specific Immunoglobulin E (IgE) 510(k); Final Guidance  
 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

**L. Test Principle:**

The assay is a solid-phase, two-step, chemiluminescent 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 chemiluminescent 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:*

Precision of the assay was assessed by testing three positive samples and one negative control sample of each allergen (Basil, Cacao, Oregano, Parsley, Pine Nut, and Vanilla) in duplicate twice a day for 20 different days (n = 80). Additional positive sample was added for precision study on Basil, Oregano, and Pine Nut at the higher concentration levels. The acceptance criterion for the negative sample was the average dose level must be <0.10 kU/L; mean results for all negative samples were within the acceptance criterion. The 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 %CV for within-run and total precision ranged from 2.83% to 8.54% and 3.65% to 9.29%, respectively.

Allergen: Basil

Sample	Mean (kU/L)	Within Run		Total	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	0.318	0.014	4.49	0.020	6.34
Positive #2	0.776	0.047	6.09	0.051	6.55
Positive #3	2.537	0.133	5.23	0.202	7.94
Positive #4	2.130	0.089	4.17	0.127	5.95

Allergen: Cacao

Sample	Mean (kU/L)	Within Run		Total	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	0.519	0.019	3.56	0.023	4.43
Positive #2	1.370	0.040	2.89	0.073	5.32
Positive #3	17.327	0.788	4.55	0.939	5.42

Allergen: Oregano

Sample	Mean (kU/L)	Within Run		Total	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	0.283	0.014	4.94	0.016	5.58
Positive #2	0.824	0.025	3.06	0.050	6.09
Positive #3	0.474	0.017	3.52	0.023	4.80
Positive #4	2.430	0.074	3.04	0.121	4.97

Allergen: Parsley

Sample	Mean (kU/L)	Within Run		Total	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	0.374	0.017	4.60	0.021	5.70
Positive #2	0.909	0.032	3.49	0.033	3.65
Positive #3	3.658	0.121	3.31	0.168	4.58

Allergen: Pine Nut

Sample	Mean (kU/L)	Within Run		Total	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	0.639	0.030	4.71	0.031	4.90
Positive #2	0.885	0.036	4.07	0.043	4.82
Positive #3	1.421	0.065	4.56	0.085	5.95
Positive #4	5.580	0.476	8.54	0.518	9.29

Allergen: Vanilla

Sample	Mean (kU/L)	Within Run		Total	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	0.663	0.023	3.50	0.043	6.52
Positive #2	3.090	0.092	2.98	0.117	3.77
Positive #3	6.080	0.172	2.83	0.228	3.75

Lot-to-lot precision:

The three tested lots were analyzed for lot-to-lot precision using three positive and one negative sample. The lot-to-lot precisions were within the specified acceptable criterion of  $\leq 20\%CV$ .

b. *Linearity/assay reportable range:*

Assay range for the IMMULITE 2000 3gAllergy Universal Kit Total IgE is from 0.1 - 100 kU/L

For each allergen, two clinical 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 (y) to expected results (x) are presented below:

Allergen	Regression Equation	Slope 95% CI	Intercept 95% CI
Basil (F269)	$y=0.974x + 0.1141$	0.885 – 1.063	0.0227 – 0.2055
Cacao (F93)	$y=1.011x + 0.0444$	0.980 – 1.042	-0.0659 – 0.1547
Oregano (F283)	$y=0.966x + 0.0686$	0.870 – 1.062	0.0005 – 0.1368
Parsley (F86)	$y=1.002x + 0.0223$	0.969 – 1.036	-0.0483 – 0.0929
Pine Nut (F253)	$y=0.996x - 0.0099$	0.941 – 1.052	-0.0934 – 0.0736
Vanilla (F234)	$y=0.975x + 0.1233$	0.890 – 1.060	0.0321 – 0.2146

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

Stability:

*Allergen stability:* Accelerated allergen stability testing was done with two positive samples and one negative sample on three lots per allergen (15-25°C for 57 days; assay kits stored at recommended temperature 2-8°C). Tests were done at initiation (Day 1) and 5 time points thereafter (Day 4, 8, 15, 28 and 57). The accelerated study supports a two year shelf-life stability claim for the allergens tested (Basil, Cacao, Oregano, Parsley, Pine Nut, and Vanilla).

Real-time allergen stability was tested with two positive samples and one negative sample on three lots per allergen. Scheduled target dates tested were on days 30, 60, 180, 360, 540, 720 and 750. All samples were within the acceptance criteria.

*On-board/ Open-vial stability:* Two positive samples and one negative sample were tested per individual specific allergen lot on Basil, Cacao, Oregano, Parsley, Pine Nut, and Vanilla. Data points were collected on days 1, 7, 14, 21, 30, 45, 60, 75 and 91. The study supports the 2-week stability claim for on-board/open vial stability.

*Adjustment interval (calibration curve) stability:* Testing was performed at days 1 and 14 to validate the 2-week adjustment interval. The calibration curve stability study supports the 2-week stability claim.

- d. *Detection limit:*

Limit of Blank (LoB): To estimate the LoB, the blank sample (zero calibrator) was assayed for three runs on three instruments 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 LoD.

Sixty replicates of each sample were assayed per run for 2 runs 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 pool of sera. A negative sample was used to measure the background response.

To initiate the inhibition experiment, 70 µL of undiluted and 3-4 levels of 5-fold serially diluted inhibitor extract were mixed with 250 µL of sample or pool to achieve final inhibitor concentrations of 218.75, 43.75, 8.75, 1.75, 0.35, 0.08, 0.07, 0.02, 0.01, 0.003 µg/mL inhibitor. 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:

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

The inhibition plots demonstrate that the allergens tested are inhibited by the relevant inhibitor extract in a concentration-dependent fashion. Also, the target % inhibition of 50% for the highest inhibitor concentration tested was met. These results indicate specificity of Basil, Cacao, Oregano, Parsley, Pine Nut and Vanilla allergens.

Additional inhibition studies were conducted to show that the specific allergens are not cross-reacting to the unrelated allergens. Testing was performed using one positive sample with three unrelated allergen extracts. A negative sample was used to measure the background response. Results of inhibition against unrelated allergens with the positive sample for specific allergens Basil, Cacao, Oregano, Parsley, Pine Nut and Vanilla were within the sponsor's acceptance criteria.

Cross-reactivity: The manufacturer states there is no detectable cross reactivity 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:*  
Not applicable.
  - 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 Basil, Cacao, Oregano, Parsley, Pine Nut and Vanilla allergens was demonstrated by testing samples from non-atopic and atopic individuals. Atopic patients were selected from patients who had clinical documentation of allergy to specific allergen(s) or allergen group of interest and/or positive skin prick/ scratch test to specific allergen(s) of interest evaluated as 2+ or greater. Information on the skin test allergen extracts (crude or purified) was not documented. Non-atopic patients were clinically known non-allergenic or had total IgE <130 ng/mL or 54 IU/mL (2.4 ng = 1 IU). Testing was performed on 142 samples for Basil, 130 samples for Cacao, 142 samples for Oregano, 142 samples for Parsley, 130 samples for Pine Nut, and 142 samples for Vanilla.

Sensitivity and specificity of the new device, based on diagnosis of atopic status, are shown in the tables below:

<u>Allergen: Basil</u>		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	Positive	17	0	17
	Negative	25	100	125
	Total	42	100	142

*Sensitivity: 40% (17/42) (95% CI: 26-55%)*

*Specificity: 100% (100/100) (95% CI: 100-100%)*

<u>Allergen: Cacao</u>		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	Positive	19	0	19
	Negative	11	100	111
	Total	30	100	130

*Sensitivity: 63% (19/30) (95% CI: 46-81%)*

*Specificity: 100% (100/100) (95% CI: 100-100%)*

<u>Allergen: Oregano</u>		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	Positive	17	0	17
	Negative	25	100	125
	Total	42	100	142

*Sensitivity: 40% (17/42) (95% CI: 26-55%)*

*Specificity: 100% (100/100) (95% CI: 100-100%)*

<u>Allergen: Parsley</u>		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	Positive	31	0	31
	Negative	11	100	111
	Total	42	100	142

*Sensitivity: 74% (31/42) (95% CI: 61-87%)*

*Specificity: 100% (100/100) (95% CI: 100-100%)*

<u>Allergen: Pine Nut</u>		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	Positive	16	0	16
	Negative	14	100	114
	Total	30	100	130

*Sensitivity: 53% (16/30) (95% CI: 35-71%)*

*Specificity: 100% (100/100) (95% CI: 100-100%)*

<u>Allergen: Vanilla</u>		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	Positive	16	0	16
	Negative	26	100	126
	Total	42	100	142

*Sensitivity: 38% (16/42) (95% CI: 23-53%)*

*Specificity: 100% (100/100) (95% CI: 100-100%)*

Literature support was provided on allergens with low prevalence and % sensitivity as shown below:

Allergen (Specific Allergen/% Clinical Sen.)	Literature(s)	General Allergy Prevalence	Clinical Sensitivity
Basil (F269/40%)	<ol style="list-style-type: none"> <li>Golec M, et al., Immunologic reactivity to work-related airborne allergens in people occupationally exposed to dust from herbs. <i>Ann Agric Environ Med.</i> 2004;11(1):121-7.</li> <li>Dutkiewicz J, et al. Response of herb processing workers to work-related airborne allergens. <i>Ann</i></li> </ol>	<p>Frequency Among Herb Workers: 2.9 – 5.8%</p> <p>Based on Sage (3/148) and Chamomile (6/148)</p>	<p>Sage (also Lamiaceae Family) 2.9 - 41.2%<sup>2</sup></p> <p>Mint (also Lamiaceae Family) 8.8 – 50.0 %<sup>2</sup></p>

Allergen (Specific Allergen/% Clinical Sen.)	Literature(s)	General Allergy Prevalence	Clinical Sensitivity
	Agric Environ Med. 2001;8(2):275-83.	(also Lamiaceae Family) <sup>1</sup>	
Cacao (F93/63%)	<ol style="list-style-type: none"> <li>Osterballe M, et al. The prevalence of food hypersensitivity in an unselected population of children and adults. <i>Pediatr Allergy Immunol.</i> 2005 Nov;16(7):567-73.</li> <li>Pereira B, et al. Prevalence of sensitization to food allergens, reported adverse reaction to foods, food avoidance, and food hypersensitivity among teenagers. <i>J Allergy Clin Immunol.</i> 2005 Oct;116(4):884-92</li> <li>Eriksson NE. Food sensitivity reported by patients with asthma and hay fever. A relationship between food sensitivity and birch pollen-allergy and between food sensitivity and acetylsalicylic acid intolerance. <i>Allergy.</i> 1978;33(4):189-96.</li> <li>Rokaite R, et al. Role of the skin patch test in diagnosing food allergy in children with atopic dermatitis. <i>Medicina (Kaunas).</i> 2004; 40(11):1081-7.</li> </ol>	<p>Chocolate: 7-12%<sup>1</sup> 23%<sup>2</sup></p> <p>*Cacao is the key ingredient in chocolate.</p>	<p>Chocolate: 2.3%<sup>3</sup></p> <p>* Cacao is the main ingredient in chocolate.</p> <p>Cacao &amp; Potato 16.7%<sup>4</sup> (in children)</p>
Oregano  Lamiaceae (Labiatae) Family (F283/40%)	<ol style="list-style-type: none"> <li>Golec M, et al. Immunologic reactivity to work-related airborne allergens in people occupationally exposed to dust from herbs. <i>Ann Agric Environ Med.</i> 2004;11(1):121-7.</li> <li>Dutkiewicz J, et al. Response of herb processing workers to work-related airborne allergens. <i>Ann</i></li> </ol>	<p>Frequency Among Herb Workers: 2.9 – 5.8%</p> <p>Based on Sage (3/148) and Chamomile (6/148)</p>	<p>Sage (also Lamiaceae Family): 2.9 - 41.2%<sup>2</sup></p> <p>Mint (also Lamiaceae Family): 8.8 – 50.0 %<sup>2</sup></p>

Allergen (Specific Allergen/% Clinical Sen.)	Literature(s)	General Allergy Prevalence	Clinical Sensitivity
	Agric Environ Med. 2001;8(2):275-83.	(also Lamiaceae Family) <sup>1</sup>	
Parsley  (Apiaceae Family) (F86/74%)	1. Niinimäki A, et al. Skin prick tests and <i>in vitro</i> immunoassays with native spices and spice extracts. Ann Allergy Asthma Immunol. 1995 Sep;75(3):280-6. 2. Moneret-Vautrin DA, et al. Food allergy and IgE sensitization caused by spices: CICBAA data (based on 589 cases of food allergy). Allergy Immunol (Paris). 2002Apr;34(4):135-40.	Allergy to Apiaceae spices: 13% <sup>1</sup> (117 out of 922 atopic patients)	Apiaceae Family Global rate of sensitization  23-32% <sup>2</sup>  (43/188 in adults) (26/81 in children)
Pine Nut (F253/53%)	1. Goetz D, et al. Cross-reactivity among edible nuts: double immunodiffusion, crossed immunoelectrophoresis, and human specific IgE serologic surveys. Ann Allergy Asthma Immunology 2005;95:45-52. 2. Maloney J, et al. The use of serum-specific IgE measurements for diagnosis of peanut, tree nut, and seed allergy. Journal Allergy Clinical Immunology 2008;122(1):145-151	Tree Nut Allergy 0.5 - 1.0 % In the US population *Pine Nut Allergy Less Common <sup>1</sup>  Pine Nut 4.6% (Among 324 patients with suspected peanut or tree nut allergies) <sup>2</sup>	Pine Nut 4 - 35% (Among patients with suspected peanut or tree nut allergies) <sup>2</sup>
Vanilla (F234/38%)	1. Rancé, F., et al. Food hypersensitivity in children: Clinical aspects and distribution of allergens. Pediatric Allergy and Immunology. 1999;10: 33–38. 2. Rancé F, et al. Prevalence and main characteristics of schoolchildren diagnosed with food allergies in France. Clinical & Experimental Allergy 2005;35(2):167-172. 3. Schöll I, et al. Allergenic Potency of Spices: Hot, Medium Hot, or Very Hot. Int Arch Allergy Immunol 2004;135:247-261.	Food Hypersensitivity: 1.4% (in general population) <sup>1</sup>  Food Allergies 6.7% (182 out of 2716) <sup>2</sup>  Spices 2% (among population with food allergies) <sup>3</sup>	Vanilla <1% (4 out of 544) <sup>1</sup>  Vanilla 0.4 % (1 out of 182) <sup>2</sup>

- 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 <sup>†</sup>
	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.

<sup>†</sup> 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 <sup>†</sup>
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	

<sup>†</sup> 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.