

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

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

k112523

**B. Purpose for Submission:**

Modification to device

**C. Measurand:**

Five Allergen Specific IgE: Native Bet v 1 (Birch Tree), Native Ole e 1 (Olive Tree), Native Art v 1 (Mugwort weed), Cat Serum Albumin (Fel d 2), and Dog Serum Albumin (Can f 3)

**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) 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:  
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):  
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) and 510(k) number(s):  
IMMULITE® 2000 3gAllergy™ Specific IgE, k013134
3. Comparison with predicates:

<b>Similarities</b>		
Item	New Device	Predicate Device
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 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

<b>Differences</b>		
Item	New Device	Predicate Device
Total number of Allergens	5	110
Types of Specific Allergens	5 Specific Inhalant Allergens: nBet v 1 (A89: Birch Tree), nOle e 1 (A482 : Olive Tree), nArt v 1 (A753: Mugwort weed), Cat Serum Albumin (E220), and Dog Serum Albumin (E221)	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-A2: Analytical Performance Characteristics and Clinical Utility of Immunological Assays for IgE Antibodies  
 CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Methods; Approved Guideline – Second Edition  
 CLSI EP17-A: Protocols for Determination of Limits of Detection and Limit of Quantitation; Approved Guideline

**L. Test Principle:**

The assay is a solid-phase, two-step, chemiluminiscent immunoassay that uses 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: nBet v 1 (A89 Birch Tree), nOle e 1 (A482 Olive Tree), nArt v 1 (A753 Mugwort weed), Cat Serum Albumin (E220), and Dog Serum Albumin (E221) in duplicate twice a day for 20 different days (n = 80).

The sponsor’s acceptance criterion for the negative sample was the average dose level must be <0.10 kU/L; all negative sample mean 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 2.21% to 6.23 % and 3.75% to 9.17%, respectively (see tables below).

Allergen: nBet v 1 (A89)

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	2.42	0.102	4.22	0.103	4.23
Positive #2	9.24	0.304	3.29	0.352	3.81
Positive #3	23.78	0.898	3.77	1.038	4.36
Positive #4	0.39	0.020	5.16	0.024	6.28
Positive #5	0.41	0.018	4.34	0.025	6.37

Allergen: nOle e 1 (A482)

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	0.50	0.027	5.46	0.030	5.98
Positive #2	1.91	0.119	6.23	0.134	7.03
Positive #3	17.34	0.686	3.95	1.013	5.85

Allergen: nArt v 1 (A753)

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	1.28	0.040	3.15	0.066	5.51
Positive #2	5.13	0.196	3.83	0.288	5.61
Positive #3	52.54	1.676	3.19	2.671	5.08
Positive #4	0.44	0.015	3.14	0.021	4.67
Positive #5	0.46	0.018	3.94	0.033	7.10

Allergen: Cat Serum Albumin (E220)

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	0.91	0.031	3.41	0.055	6.03
Positive #2	36.38	1.173	3.22	1.365	3.75
Positive #3	45.81	2.032	4.44	2.032	4.44
Positive #4	0.36	0.013	3.58	0.021	5.80
Positive #5	0.38	0.014	3.74	0.023	6.12

Allergen: Dog Serum Albumin (E221)

Sample	Mean (kU/L)	Intra-assay		Inter-assay	
		SD (kU/L)	%CV	SD (kU/L)	%CV
Positive #1	47.84	1.056	2.21	1.979	4.14
Positive #2	23.10	0.950	4.11	1.170	5.07
Positive #3	0.87	0.024	2.81	0.064	7.35
Positive #4	0.44	0.015	3.50	0.040	9.17
Positive #5	0.46	0.025	5.46	0.034	7.24

Lot to lot imprecision:

The three tested lots were analyzed for lot-to-lot precision using three positive and one negative samples. Within-run imprecision between lots ranged from 2.76% to 10.31% and total imprecision from 3.26% to 9.61%. All three lots

were within the sponsor's claimed acceptable criterion of  $\leq 20\%$  variability.

*b. Linearity/assay reportable range:*

Linearity studies: 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 to expected results are presented below:

Allergen	Regression Equation	Slope 95% CI	Intercept 95% CI	R
A89: nBet v 1	$y=0.994x -0.1429$	0.972 to 1.016	-0.0569 to 0.3428	0.999
A482: nOle e 1	$y=0.997x +0.0993$	0.974 to 1.020	-0.0234 to 0.1753	0.999
A753: nArt v 1	$y=1.004x -0.3830$	0.972 to 1.035	-0.1692 to 0.9352	0.998
E220: Cat Serum Albumin	$y=0.975x +0.0230$	0.928 to 1.023	-0.6815 to 0.7275	0.995
E221: Dog Serum Albumin	$y=0.999x -0.832$	0.986 to 1.012	-0.01906 to 0.0242	1.00

The IMMULITE 2000 Calibrator assay range: 0.1 - 100 kU/L.

*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 (15-25°C for 57 days at 1, 4, 8, 15, 30, and 60 day intervals; other assay kit components stored at recommended temperature 2-8°C). Testing was performed on two positive samples and one negative sample on three lots. The accelerated study supports a two year shelf-life stability claim.

*On-board/ open stability:*

Testing was performed on two positive samples and one negative sample for 91 days (at 1, 7, 14, 21, 29, 48, 60, 75 and 91 day intervals) for each individual specific allergens. Stability studies supports the 90 day stability claim.

*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.

*Sample stability:* Stress conditions representing the storage claims were set up at 3 and 7 days at 2-8°C and 6 months at -20°C. No significant variation to the reference samples that were run at day 0 was observed. The sample stability study supports the 7 days at 2-8°C and 6 months at -20°C stability claims.

*d. Detection limit:*

Limit of Blank (LoB): Three runs assaying the blank sample (zero calibrator)

were performed to estimate the LoB. One instrument was used to collect a total of 240 data points using 3 allergen lots over 20 days, at 2 runs per day, 2 replicates per run. The LoB was calculated using the formula  $LoB = (Mean + 1.65 * SD)$ . The Limit of Blank (highest value expected for a sample with no analyte), determined in accordance with CLSI EP17-A, is 0.03kU/L.

**Limit of Detection (LoD):**

A low level sample (0.35 – 0.69kU/L, Class I) was tested using 3 allergen lots over 20 days, at 2 runs per day, 2 replicates per run to estimate the LoD. Two instruments were used to collect a total of 480 data points. The LoD was calculated using the  $LoD = LoB_{Max} + (1.65 * SD_{LoD})$ . Limit of Detection (lowest detectable concentration), determined in accordance with CLSI EP-17-A is 0.10kU/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 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, 0.014 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 were 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 nBet v 1 (A89 Birch Tree), nOle e 1 (A482 Olive Tree), nArt v 1 (A753 Mugwort weed), Cat Serum Albumin (E220), and Dog Serum Albumin (E221) allergens.

Additional inhibition studies were conducted to show that the specific allergens are not cross-reacting to unrelated allergens. Testing was performed using one positive sample with three unrelated allergen extracts at 1 mg/mL. A negative sample was used to measure the background response. Results on the positive sample for the following specific allergens were below 11.92%: nBet v 1 (A89 Birch Tree), nOle e 1 (A482 Olive Tree), nArt v 1 (A753 Mugwort weed), Cat Serum Albumin (E220), and Dog Serum Albumin (E221).

IgE 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<sup>®</sup> 2000 3gAllergy Specific IgE assay for nBet v 1 (A89 Birch Tree), nOle e 1 (A482 Olive Tree), nArt v 1 (A753 Mugwort weed), Cat Serum Albumin (E220), and Dog Serum Albumin (E221) 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 total IgE <130 ng/mL or 54 IU/mL (2.4 ng = 1 IU). Testing was performed on 143 samples for nBet v 1 (A89 Birch Tree), 139 samples for nOle e 1 (A482 Olive Tree), 130 samples for nArt v 1 (A753 Mugwort weed), 143 samples for Cat Serum Albumin (E220), 138 samples for Dog Serum Albumin (E221).

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

<u>Allergen: nBet v 1 (A89)</u>		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	37	5	42
	negative	6	95	101
	Total	43	100	143

*Sensitivity: 86% (37/43) (95% CI: 76-68%)*

*Specificity: 95 % (95/100) (97% CI: 91-99%)*

<u>Allergen: nOle e 1 (A482)</u>		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	37	3	40
	negative	2	97	99
	Total	39	100	139

*Sensitivity: 95% (37/39) (95% CI: 88-102 %)*

*Specificity: 97 % (99/100) (95% CI: 45-100 %)*

Allergen: nArt v 1 (A753)		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	20	7	27
	negative	10	93	103
	Total	30	100	130

*Sensitivity: 67% (20/30) (95% CI: 50-84%)*

*Specificity: 93% (93/100) (95% CI: 88-100%)*

Allergen: Cat Serum Albumin (E220)		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	27	3	30
	negative	16	97	113
	Total	43	100	143

*Sensitivity: 63% (27/43) (95% CI: 48-77%)*

*Specificity: 97% (97/100) (95% CI: 94-100%)*

Allergen: Dog Serum Albumin (E221)		Clinical Diagnosis		
		Atopic	Non-atopic	Total
IMMULITE 2000	positive	27	3	30
	negative	11	97	108
	Total	38	100	138

*Sensitivity: 71% (27/38) (95% CI: 57-85%)*

*Specificity: 97% (97/100) (95% CI: 94-100%)*

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

Allergen (Specific Allergen/% Clinical Sen.)	Literature(s)	Prevalence	Clinical Sensitivity
nArt v 1 (A753): 67%	1. Spieksma FT, Charpin H, Nolard N, Stix E. City spore concentrations in the European Economic Community (EEC). IV. Summer weed pollen (Rumex, Plantago, Chenopodiaceae, Artemisia, 1976 and 1977. Clin Allergy. 1980 May;10(3):319-29.	Among those suffering from pollinosis	Compositae family: 15.1% (198/1311) <sup>2</sup>
	2. Gioulekas D, Papkosta D, Damialis A, Spieksma F, Giouleka P, Patakas D. Allergenic pollen records (15 years) and sensitization in patients with respiratory allergy in Thessaloniki,	Artemisia vulgaris (mugwort),	

Allergen (Specific Allergen/% Clinical Sen.)	Literature(s)	Prevalence	Clinical Sensitivity
	<p>Greece. Allergy. 2004. Feb;59(2):174-84.</p> <p>3. Scala E, Alessandri C, Bernardi ML, Ferrara R, Palazzo P, Pomponi D, et al. Cross-sectional survey on immunoglobulin E reactivity in 23077 subjects using an allergenic molecule-based microarray detection system. Clin Exp Allergy. 2010;40(6):911-21.</p>	<p>Artemisia annua (common wormwood): 10-14%<sup>1</sup></p>	<p>nArt v 1: 4.09% (671/16408)</p>
Dog Serum Albumin, Can f 3 (E221)/ 56%	<p>1. Spitzauer S, Pandjaitan B, Soregi G, Muhl S, Ebner C, Kraft D, et al. IgE cross-reactivities against albumins in patients allergic to animals. J Allergy Clin Immunol. 1995 Dec;96(5):951-9.</p> <p>2. Spitzauer S, Schweiger C, Sperr W, Pandjaitan B, Valent P, Muhl S, et al. Molecular characterization of dog albumin as a cross-reactive allergen. J Allergy Clin Immunol. 1994 Mar; 93:614-27.</p> <p>3. Spitzauer S, Schweiger C, Anrather J, Ebner C, Scheiner O, Kraft D. Characterisation of dog allergens by means of immunoblotting. Int Arch Allergy Immunol. 1993;100:60-7.</p> <p>4. Lindgren S, Belin L, Dreborg S, Einarsson R, Phlman I. Breed-specific dog-dandruff allergens. J Allergy Clin Immunol. 1988;82(2):196-204.</p> <p>5. Mattson L, Lundgren T, Everberg H, Larsson H, Lidholm J. Prostatic kallikrein: A new major dog allergen. J Allergy Clin Immunol. 2009;123:362-8.</p>	<p>Among those positive to animal allergens.</p> <p>Animal albumins: 30%<sup>1</sup></p> <p>Among those positive to dog hair/dander.</p> <p>Dog Serum Albumin (Can f 3): 35%<sup>2</sup></p> <p>Among those positive to dog hair, dander, saliva, serum, liver, and/or salivary glands:</p> <p>Dog Serum Albumin (Can f 3): 12-50%<sup>3</sup></p>	<p>Dog Serum Albumin (Can f 3): 16-40%<sup>4-5</sup></p>
Cat Serum Albumin, Fel d 2/ 63%	<p>1. Spitzauer S, Pandjaitan B, Soregi G, Muhl S, Ebner C, Kraft D, et al. IgE cross-reactivities against albumins in patients allergic to animals. J Allergy Clin Immunol. 1995 Dec;96(5):951-9.</p> <p>2. Hilger C, Kohlen M, Grigioni F, Lehnert C, Hentges F. Allergic cross-reactions between cat and pig serum albumin. Study at the protein and DNA levels. Allergy. 1997;52:179-87.</p> <p>3. van Ree R, van Leeuwen A, Bulder I, Bond J, Aalberse R. Purified natural and recombinant</p>	<p>Among those positive to animal allergens.</p> <p>Animal albumins: 30%<sup>1</sup></p> <p>Among those</p>	<p>Cat Serum Albumin: 17-22%<sup>3-4</sup></p>

Allergen (Specific Allergen/% Clinical Sen.)	Literature(s)	Prevalence	Clinical Sensitivity
	Fel d 1 and cat albumin in in vitro diagnostics for cat allergy. J Allergy Clin Immunol. 1999;104:1223-30.	positive to cat.	
	4. Cabanas R, Lopez-Serrano M, Carreira J, Ventas P, Polo F, Caballero M, et al. Importance of albumin in cross-reactivity among cat, dog and horse allergens. J Investig Allergol Clin Immunol. 2000;10(2):71-7.	Cat Serum Albumin (Fel d 2): 14-23% <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.