510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K163370

B. Purpose for Submission:

Modification of the device (Modification of Allergen w1, Common ragweed)

C. Measurand:

Allergen-specific IgE analyte: Common ragweed

D. Type of Test:

Fluoroenzymeimmunoassay, Quantitative

E. Applicant:

Phadia AB

F. Proprietary and Established Names:

ImmunoCAP Specific IgE
ImmunoCAP Allergen w1, Common ragweed

G. Regulatory Information:

1. Regulation section:

   21 CFR § 866.5770, 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:

   ImmunoCAP Specific IgE is an in vitro quantitative assay for the measurement of allergen specific IgE in human serum or plasma (EDTA or Na-Heparin). ImmunoCAP Specific IgE is to be used with instruments Phadia 100, Phadia 250, Phadia 1000, Phadia 2500 and Phadia 5000. It is intended for in vitro diagnostic use as an aid in the clinical diagnosis of IgE mediated allergic disorders in conjunction with other clinical findings, and is to be used in clinical laboratories.

2. Indication for use:

   Same as intended use.

3. Special conditions for use statement:

   For prescription use only.

4. Special instrument requirements:

   For use on the instruments Phadia 100, Phadia 250, Phadia 1000, Phadia 2500 and Phadia 5000

I. Device Description:

The ImmunoCAP system is a fully integrated and automated system for the determination of specific IgE in human blood serum or plasma (EDTA or Na-Heparin) samples. It is comprised of general reagents, test reagents and method-specific reagents for Phadia 100, Phadia 250, Phadia 1000, Phadia 2500 and Phadia 5000 test system modules, as well as instrument and data management software.

The general ImmunoCAP reagents include ImmunoCAP Specific IgE Conjugate, ImmunoCAP Specific IgE Curve Control, ImmunoCAP Specific IgE Calibrators, Specific IgE anti-IgE ImmunoCAP, Allergen ImmunoCAP carriers, ImmunoCAP development solution and stop solution. The method-specific reagents consist of individual purified allergen (native or recombinant) covalently coupled to a support in a plastic housing.

J. Substantial Equivalence Information:

1. Predicate device names:

   K962274 and K051218

2. Predicate 510(k) numbers:

ImmunoCAP Specific IgE Assay and ImmunoCAP Specific IgE Conjugate 100 and Conjugate 400 (K051218)

ImmunoCAP Specific IgE Assay (K962274)

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	An *in vitro* quantitative assay for the measurement of allergen specific IgE in human serum or plasma (EDTA or Na-Heparin). It is intended for *in vitro* diagnostic use as an aid in the clinical diagnosis of IgE mediated allergic disorders in conjunction with other clinical findings and to be used in clinical laboratories.	Same
Assay type	Quantitative	Same
Basic principle	Fluoroenzymeimmunoassay	Same
Detection antibody	β-Galactosidase-anti-human IgE (mouse monoclonal antibody)	Same
Sample volume	40 µL	Same
Number of calibrators	Six	Same
Process time	Phadia 100: 2 hours 30 minutes Phadia 250, Phadia 1000, Phadia 2500 and Phadia 5000: 1 hour 45 minutes from entering the first sample.	Same
Incubation temperature	37°C	Same

Differences		
Item	Device	Predicate
Allergen	Whole extract of allergen raw material: ragweed pollen with naturally increased amounts of the main allergen component *Amb a 1.*	Whole extract of allergen raw material: ragweed pollen
Sample matrix	Serum and plasma (EDTA or sodium heparin)	Serum and plasma (EDTA and heparin)
Laboratory settings	Clinical laboratories	Clinical laboratories and physician office laboratories.

Differences		
Item	Device	Predicate
Instruments	Phadia 100, Phadia 250, Phadia 1000, Phadia 2500 and Phadia 5000	UniCAP 100
Built-in Software versions	Phadia 100: v3.08 Phadia 250: v3.00 Phadia 1000: v3.0 Phadia 2500: v1.45 Phadia 5000: v1.45 Phadia Information Data Manager (IDM): v5.78 Phadia Prime data management: v2.1	Not available

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

CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition.

CLSI EP17-A2: Evaluation of detection capability for Clinical Laboratory measurement procedures; Approved Guideline – second edition.

CLSI EP25-A: Evaluation of stability of in vitro diagnostic reagents; Approved Guideline.

CLSI I/LA20-A2: Analytical Performance Characteristics and Clinical Utility of Immunological Assays for Human Immunoglobulin E (IgE) Antibodies and Defined Allergy Specificities; Approved Guidelines – Second Edition.

ISO 14971 – Risk management to medical device

L. Test Principle:

The allergen of interest, covalently coupled to ImmunoCAP solid phase, reacts with the specific IgE in the patient sample. After washing away nonspecific IgE, enzyme-labeled antibodies against IgE are added to form a complex. After incubation, unbound enzyme-anti-IgE is washed away and the bound complex is then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate is measured. The higher the response value, the more specific IgE is present in the specimen. To evaluate the test results, the responses for the patient samples are transformed to concentrations with the use of a calibration curve.

M. Performance Characteristics:

1. Analytical performance:

All analytical performance met the manufacturer's pre-determined acceptance criteria.

a. Precision/Reproducibility:

i) Comparison with predicate:

Imprecision of this updated device was evaluated by using four positive samples with the following concentration ranges: 0.1–0.2 kU_A/L, ≤1.5 kU_A/L, 10-20 kU_A/L, and ≥50 kU_A/L). Each sample was tested in 4 replicates in one assay run per day for a total of 20 operating days (a total of 80 replicates per sample). The assay was performed according to the ImmunoCAP Specific IgE Directions for Use using the Phadia 250 instrument. Between-day and within-run coefficients of variance (%CVs) were calculated for each sample separately. Results of CV% are shown below:

Sample	N	Mean (kU_A/L)	CV% Within-run	CV% Between-days	CV% Total
1	80	0.13	5.90	7.08	9.22
2	80	0.57	4.92	3.72	6.17
3	80	16.06	4.34	6.84	8.10
4	80	70.76	5.37	10.59	11.88

ii) Lot-to-lot imprecision:

Three lots of the updated device containing different ImmunoCAP Allergen lots were tested using three positive samples at concentration levels of 0.57 kU_A/L, 16.06 kU_A/L, and 70.76 kU_A/L and one negative sample at 0.13 kU_A/L. For each lot, the samples were tested in 12 replicates in one assay run. Each lot represented a different preparation of the allergen from routine production. The assay was performed according to the ImmunoCAP Specific IgE Directions for Use, using the Phadia 250 instrument. Mean concentration values, %CV, and concentration quotients between lots were calculated and the results are shown below.

Lot	Positive 1		Positive 2		Negative	Concentration Quotient		
	Mean (kU_A/L)	CV (%)	Mean (kU_A/L)	CV (%)	Mean (kU_A/L)	Lot to Lot	Positive 1	Positive 2
1	2.08	1.98	0.31	3.35	<0.1	lot1/lot2	1.12	1.19
	1.86	2.21	0.26	2.56	<0.1	lot1/lot3	1.16	1.11
3	1.80	2.23	0.28	3.88	<0.1	lot2/lot3	1.03	0.93

b. *Linearity/assay reportable range:*

The linearity of this updated allergen was assessed following the CLSI guideline I/LA20-A2 guideline. For this study, three positive samples were each diluted in sample diluent generating at least five 2-fold consecutive dilutions. Undiluted samples were tested in 12 replicates and diluted samples were tested in four replicates in one assay run. The assay was performed according to the ImmunoCAP Specific IgE Directions for Use using the Phadia 250 instrument. For this updated product, one lot of ImmunoCAP Allergen Component was used.

For this allergen, results of the replicates for the three samples were analyzed for linearity. Regression statistics comparing the observed results to expected results are presented below:

ImmunoCAP Allergen	Regression Equation	r^2	95% CI Slope	95% CI Intercept	Highest level tested
Sample 1	y=1.00x + 0.04	0.9990	0.968–1.031	-0.011–0.089	12.53
Sample 2	y=0.98x + 0.07	0.9993	0.961–1.004	0.027–0.117	47.56
Sample 3	y=0.99x + 0.02	0.9994	0.971–1.011	-0.031–0.068	92.15

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

i) *Traceability*:

The IgE calibrators are traceable to the equivalent 3rd International Standard 11/234 of Human Serum Immunoglobulin E from World Health Organization (WHO).

ii) *Kit Stability*:

Real-time and accelerated stability: The stability studies were performed in accordance with CLSI EP25-A (Stability Testing of *In Vitro* Diagnostic Reagents). The results demonstrated 24-month unopened shelf-life stability from the date of manufacture when stored at the recommended storage temperature of 2–8°C. using three lots of ImmunoCAP Allergen w1 (Common ragweed). Both an ongoing real-time stability study and an accelerated stability study were performed. The accelerated stability data supports the manufacturer's claim of 24 months, while available real-time stability data supports 7 months stability.

The stability of the calibration curve, real-time, and on-board stability of ImmunoCAP Specific IgE calibrator are detailed in K100999.

d. Detection limit:

The Limit of Blank (LoB) and Limit of Detection (LoD) were determined for this updated allergen on the Phadia 250 following the CLSI guideline EP17-A2.

LoB was determined by testing five blank plasma samples with five replicates in three runs and was estimated as the 95% percentile of the n = 75 negative results. The maximum LoB of two lots was used in the calculation of the LoD according to the equation*: LoD = LoB + cβ* x *SD* where SD is the pooled SD of measuerments for each of the five low positive samples (0.135, 0.159, 0.158, 0.186 and 0.209 kU_A/L), in total n = 75 measurements. Claimed LoB is 0.027 kU_A/L and LoD is 0.058 kU_A/L.

e. Analytical specificity:

i) *Inhibition studies*:

Immunological specificity of the allergen components was verified through competitive inhibition. The studies were planned in accordance with CLSI guideline I/LA20-A2. The specific IgE concentration for the positive sample is 6.2 kU_A/L.

The allergen solution was serially diluted with buffer to show an overall dose-dependent inhibition. Equal volumes of a positive sample and varying dilutions of allergen solution (inhibitor) were premixed. The mixture was incubated in a sample tube at room temperature for two hours before being analyzed on the Phadia 250 instrument according to the ImmunoCAP Specific IgE Directions for Use. The testing was performed in duplicate in one assay run. Mean values were calculated.

The inhibition test was evaluated by calculating percent inhibition according to the formula below:

$$\left(1-\left(\frac{r-b}{t-b}\right)\right)\times 100 = i\%$$

r = response [RU]
b = background response (100% inhibition) [RU]
t = total response (0% inhibition) [RU]
i = inhibition

Any negative inhibition %-values were reported as 0% inhibition.

The results of the inhibition with the allergen solution and the unrelated inhibitors indicated that the updated ImmunoCAP Allergen contains the immunologically relevant allergen as described below:

The w1 Common ragweed allergen inhibition study showed that >50% inhibition was achieved with the related inhibitor at a final inhibitor concentration of 25 µg/mL.

The inhibition studies using three unrelated inhibitors (Timothy grass, g6;

Cottonwood, t14 and Sesame seed, f10) and one from the related/same group (Goosefoot, w10) did not show any significant inhibition at an inhibitor concentration of 1 mg/mL.

Results from the inhibition studies indicated that the ImmunoCAP Allergen w1, Common weed solid phase contains the immunologically relevant allergen.

ii) *Interference*:

a) Endogenous Substance Interference:

In order to show that icteric, hemolytic or lipemic samples do not adversely affect the results of the ImmunoCAP Specific IgE assay using the updated ragweed allergen, Bilirubin C [final concentration (fc) 20 mg/dL], Bilirubin F (fc 19 mg/dL), Hemoglobin (fc 489 mg/dL) and Chyle (fc 1,440 Formazine Turbidity Units) were spiked into two samples and were analyzed in duplicates in one assay run using Phadia 250. The results demonstrate that icteric, hemolytic or lipemic samples do not adversely affect the results in ImmunoCAP Specific IgE as has been shown in previous submissions, e.g., K113841.

b) Exogenous Substance Interference:

Two literature references were provided supporting that commonly prescribed "allergy medications" do not interfere with ImmunoCAP Specific IgE assays. The references included *(i)* Robert G. Hamilton, Accuracy of US Food and Drug Administration-cleared IgE antibody assays in the presence of anti-IgE (omalizumab), *J. Allergy Clin. Immunol*. 2006; 759-766, and *(ii)* Linda Cox et. al., Pearls and pitfalls of allergy diagnostic testing: report from the American College of Allergy, Asthma and Immunology/American Academy of Allergy, Asthma and Immunology Specific IgE Test Task Force, *Annals of Allergy, Asthma & Immunology*, 2008:580-592. Refer to K150854.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

In order to show that different sample matrices (heparin plasma, EDTA plasma and serum) are interchangeable for this allergen, serum, Na-heparin-plasma, and EDTA-plasma samples were collected from patients with clinical history of known specific allergies and four non-atopic patients. The samples contained specific IgE antibodies

for the allergen tested. All sample matrices (Na-heparin plasma, EDTA plasma and serum) from each patient were tested with this allergen in two replicates in one assay run. The results from the study demonstrate that samples of different matrices (Na-heparin plasma, EDTA plasma and serum) are interchangeable for allergen as has been shown in previous submissions, e.g. K101251.

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

The performance of the updated allergen was compared to a clinical diagnosis of allergy. The objectives of this study were: *(i)* to show the linkage between specific IgE antibodies to the allergen using clinical samples, and *(ii)* to demonstrate that samples from healthy, non-atopic donors with no reported clinical reaction to the allergen have undetectable or very low levels of specific IgE to the allergen. A total of 50 clinical serum samples from individuals with a clinical history of allergy-like symptoms upon exposure to the allergen as diagnosed by a physician were used in the study. Information about clinical symptoms and manifestations was available for all clinical samples. Negative samples (<0.35 kU_A/L) from 100 healthy non-atopic donors were also tested.

		Clinical Diagnosis		
		Atopic	**Non-atopic**	**Total**
Allergen, w1, (Common ragweed)	**Positive**	47	0	47
	Negative	3	112	115
	Total	50	112	162

Sensitivity =94.0% (47/50) (95% CI: 83.5–98.7%)
Specificity =100% (112/112) (95% CI: 96.8–100.0%)

All negative samples showed an undetectable level (< 0.35 kU_A/L) of allergen specific IgE. Studies described above were performed on the Phadia 250 instrument system.

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

Not applicable

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

The expected value is literature based and negative (< 0.35 kU_A/L) for a non-allergic person. The manufacturer recommends a cut-off at 0.35 kU_A/L. Each laboratory should establish its own expected range of values.

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.