510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K173726

B. Purpose for Submission:

Addition of one new recombinant allergen to a cleared device

C. Measurand:

Allergen-specific IgE analyte: f447, rAra h 6, Peanut

D. Type of Test:

Fluoroenzymeimmunoassay, Quantitative

E. Applicant:

Phadia AB

F. Proprietary and Established Names:

ImmunoCAP Specific IgE

ImmunoCAP Allergen f447, Allergen component rAra h 6, Peanut

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, and test- 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 and numbers:

   UniCAP® system, UniCAP® Specific IgE Assay and UniCAP® Specific IgE Conjugate 100 and Conjugate 400 (K051218)

   UniCAP® Specific IgE Assay (K962274)

2. 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. 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 hrs 30 min. 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-containing reagent	Purified recombinant allergen component: rAra h 6, Peanut (f447)	Allergenic extract: Peanut (f13)
Sample matrix	Serum and plasma (EDTA or sodium heparin)	Serum and plasma (sodium heparin)
Laboratory settings	Clinical laboratories	Clinical laboratories and physician office laboratories
Instruments	Phadia 100, Phadia 250, Phadia 1000, Phadia 2500 and Phadia 5000	UniCAP 100
Built-in Software versions	Phadia 100 ISW: 3.08 Phadia 250 ISW: 3.10 Phadia 1000 ISW: 3.10 Phadia 2500 ISW: 1.45 Phadia 5000ISW: 1.45 Phadia Information Data Manager (IDM): 5.78 Phadia Prime: 2.2	Not available

K. Standard/Guidance Document Referenced:

CLSI EP05-A3: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.

CLSI EP07-A2: Interference testing in Clinical Chemistry; Approved Guideline – Second Edition.

CLSI EP17-A2: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline.

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

CLSI I/LA20-A3: Analytical Performance Characteristics and Clinical Utility of Immunological Assays for Human Immunoglobulin E (IgE) Antibodies and Defined Allergy Specificities; Approved Guidelines – Second Edition.

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 (if/when applicable):

1. Analytical performance:

   All results presented below met the manufacturer's pre-determined acceptance criteria.

   a. Precision/Reproducibility:

      i) Within-laboratory imprecision:

      Imprecision of the allergen component f447, rAra h 6, Peanut was evaluated by using five positive plasma samples, with the following concentrations of antigen-specific IgE: 0.13, 1.08, 17.93 and 51.06 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 a Phadia 250. Between-day and within-run coefficients of variance (%CV) were calculated for each sample separately. Results are shown in the table below:

Sample	N	Mean (kU_A/L)	Between-Day CV%	Within-run CV%	Total CV%
1	80	0.13	5.30	4.65	7.05
2	80	0.37	6.80	8.18	10.64
3	80	1.08	2.64	2.67	3.67
4	80	17.93	2.36	2.88	3.73
5	80	51.06	4.80	5.00	6.94

ii) Lot-to-lot imprecision:

Three different ImmunoCAP Allergen Component lots were tested using four positive plasma samples and one negative plasma sample (< 0.1 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 (lowest mean concentration/ highest mean concentration between lots) were calculated for the positive samples and are presented in the table below:

Lot	Sample									
	Positive 1		Positive 2		Positive 3		Positive 4		Negative	
	Mean (kU_A/L)	CV (%)	Mean (kU_A/L)	CV (%)	Mean (kU_A/L)	CV (%)	Mean (kU_A/L)	CV (%)	Mean (kU_A/L)	CV (%)
1	0.385	3.0	2.17	4.7	16.08	2.2	49.54	4.0	<0.1	--
2	0.388	1.5	2.17	2.7	16.53	2.0	48.95	3.4	<0.1	--
3	0.381	3.5	2.26	3.7	17.09	4.0	47.37	5.6	<0.1	--

Min, max mean concentration and concentration quotient for positive samples			
Sample	Min. Mean (kU_A/L)	Max. Mean (kU_A/L)	Concentration Quotient
Positive 1	0.381	0.388	1.02
Positive 2	2.17	2.26	1.04
Positive 3	16.08	17.09	1.06
Positive 4	48.95	49.54	1.05

b. Linearity/assay reportable range:

The linearity of the allergen component was assessed following CLSI guideline I/LA20-A2. Three positive plasma samples were each diluted in negative plasma 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 using one lot of ImmunoCAP Allergen Component. The range of the ImmunoCAP Specific IgE is LoD to 100 kU_A/L.

Results of the replicates from all 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
f447, rAra h 6, Peanut	y=1.00x + 0.06	1.00	0.98–1.03	0.01–0.11

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

i) *Traceability*:

The IgE calibrators are traceable (via an unbroken chain of calibrations) to the 2nd International Reference Preparation (IRP) 75/502 or 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:

Both an ongoing real-time stability study and an accelerated stability study were performed in accordance with CLSI EP25-A using three lots of ImmunoCAP Allergen f447, rAra h 6 (Peanut). The accelerated stability data support the manufacturer's claim of 24 month unopened shelf-life stability. The real-time stability study supports six month unopened shelf-life stability and is ongoing.

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

d. *Detection limit:*

The Limit of Blank (LoB) and Limit of Detection (LoD) were determined on the Phadia 250 in alignment with CLSI guideline EP17-A2 using two lots of reagents. The LoB was based on determinations of five blank samples in three runs with five replicates per run and was estimated as the 95% percentile of the distribution.

LoD was calculated according to the equation: $LoD = LoB + c\beta \bullet SD_{LoD}$ where SD_{LoD}, is the pooled SD for each of five low positive samples measured in 15 replicates. Results from maximum LoD of the two lots were used in the calculation of LoQ. The precision profile provided supports the LoQ claim of 0.1 kU_A/L at ≤15% CV.

The results are shown in the table below.

ImmunoCAP Allergen Component	LoB (kU_A/L)	SD_{LoD}	LoD (kU_A/L)	LoQ (kU_A/L)
f447, rAra h 6 (Peanut)	0.001	0.01	0.02	0.10

e. Analytical specificity:

i) *Inhibition studies*:

Immunological specificity of the allergen component was verified through competitive inhibition. The studies were planned in accordance with CLSI I/LA20-A3. The specific IgE concentration for the positive sample is shown in the table below:

ImmunoCAP Allergen Component	kU_A/L
f447, rAra h 6 (Peanut)	2.8

The f447, rAra h 6 Peanut allergen solution (inhibitor) was serially diluted with buffer to show an overall dose dependent inhibition. The following unrelated inhibitors rFel d 2, Cat, rHev b 6.02, Latex and rVes v 5, Common wasp and related inhibitor rAra h 9 Peanut were tested at a dose that is ten-fold or higher than the f447, rAra h 6 Peanut concentration that yielded >50% inhibition. Equal volumes of the positive sample and varying serial dilutions of f447, rAra h 6 Peanut were premixed. The mixture was incubated in a sample tube at room temperature for two hours before being analyzed with ImmunoCAP Allergen f447 on the Phadia 250 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 with inhibition values in %, calculated 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 are shown as 0% inhibition.

The results of the inhibition with the allergen solution (inhibitor) and the unrelated and related inhibitors indicate that the new ImmunoCAP Allergen Component f447, rAra h 6, Peanut contains the immunologically relevant allergen as shown below:

The inhibition study showed that >50% inhibition was achieved with the inhibitor (rAra h 6 allergen) at a final inhibitor concentration of 0.93 mg/mL. The inhibition

studies using four unrelated inhibitors, including three from unrelated groups (rFel d 2, Cat; rHev b 6.02, Latex and rVes v 5, Common wasp) and one related inhibitor from the same allergen group (r Ara h 9, Peanut f427) did not show any significant inhibition. The inhibition studies indicate that the ImmunoCAP Allergen f447, rAra h 6 Peanut solid phase contains the immunologically relevant allergen.

ii) *Interference*:

a) Endogenous Substance Interference:

Interference from icteric, hemolytic or lipemic samples was tested using Bilirubin C [final concentration (fc) 19.8 mg/dL], Bilirubin F (19.1 mg/dL), Hemoglobin (510 mg/dL), Chyle (1,660 FTU-Formazine Turbidity Units) and Rheumatoid Factor (450 IU/mL). Interferents were spiked into two positive samples and one negative sample and analyzed in four replicates in one assay run using the Phadia 250. The results demonstrate that icteric, hemolytic or lipemic samples do not adversely affect the results in ImmunoCAP Specific IgE.

b) Exogenous Substance Interference:

Two literature references were provided supporting that commonly prescribed allergy medications do not interfere with ImmunoCAP Specific IgE. 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; 101:580-592.

f. Assay cut-off:

Limit of Quantitation for ImmunoCAP Specific IgE is 0.1 kU_A/L.

2. Comparison studies:

a. Method comparison with predicate device:

Refer to Clinical studies.

b. Matrix comparison:

Refer to K101251.

3. Clinical studies:

a. Clinical sensitivity and specificity:

The performance of the allergen component f447, rAra h 6, Peanut was compared to a clinical diagnosis of allergy. The objectives of this study were: *(i)* to show the linkage between specific IgE antibodies to ImmunoCAP Allergen Component and the corresponding extract based ImmunoCAP 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 individual ImmunoCAP Allergen Component. Thirty four 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. Non-atopic samples (<0.35 kU_A/L) from 100 healthy non-atopic donors were also tested. Clinical sensitivity and specificity in this sample cohort are summarized in the following table:

		Clinical Diagnosis		
		Atopic	**Non-atopic**	**Total**
f447, rAra h 6 (Peanut)	**Positive**	28	0	28
	Negative	6	100	106
	Total	34	100	134

Sensitivity = 82.4% (28/34) (95% CI: 65.5%–93.2%)
Specificity =100% (100/100) (95% CI: 96.4%–100%)

Studies described above were performed on the Phadia 1000 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 negative (< 0.35 kU_A/L) for a specific allergen in a non-atopic 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 Parts 801 and 809, as applicable.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.