

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

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

k123275

**B. Purpose for Submission:**

Modification of the device components - Allergen i3 and i4

**C. Measurand:**

Common wasp venom (Yellow jacket) Allergen i3 and Paper wasp venom Allergen i4

**D. Type of Test:**

Fluoroenzymeimmunoassay, Quantitative

**E. Applicant:**

Phadia AB

**F. Proprietary and Established Names:**

ImmunoCAP Specific IgE

ImmunoCAP Allergen i3, Common wasp venom (Yellow jacket)

ImmunoCAP Allergen i4, Paper wasp venom

**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):

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(s) for use:

Same as Intended use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

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) sample. It is comprised of general, 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 with recombinant) covalently coupled to a support in a plastic housing.

**J. Substantial Equivalence Information:**

1. Predicate device name(s) and Predicate 510(k) number(s):

UniCAP<sup>®</sup> Specific IgE Assay and UniCAP<sup>®</sup> Specific IgE Conjugate 100 and 400

(k051218) and UniCAP Specific IgE Assay (k962274)

2. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	An <i>in vitro</i> quantitative assay for the measurement of allergen specific IgE in human serum or plasma (EDTA or Na-Heparin). It is intended for <i>in vitro</i> 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.	Same
Number of calibrators	Six	Same
Assay type	Quantitative	Same
Antibody	$\beta$ -Galactosidase-anti-IgE (mouse monoclonal antibody) for all ImmunoCAP	Same
Basic principle	Fluoroenzymeimmunoassay	Same
Sample volume	40 $\mu$ L	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 - for all Phadia instruments	Same

Differences		
Item	Device	Predicate
Form of allergens	Purified whole native allergen proteins with added recombinant proteins	Purified native allergen extracts
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

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

CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.

CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; 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.

EN 13640: 2002 Stability Testing of *in vitro* Diagnostic Reagents

**L. Test Principle:**

The allergen of interest, covalently coupled to ImmunoCAP, reacts with the specific IgE in the patient sample. After washing away non-specific 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:

a. *Precision/Reproducibility:*

i) *Within-Lot imprecision:*

Imprecision of the individual allergen components was evaluated by using two positive plasma samples, including a low range sample ( $0.35 \pm 25\%$ ) and a high range sample ( $\geq 0.7$  kU<sub>A</sub>/L). Each sample was tested in 4 replicates in 1 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 Phadia 250. Between-day and within-run coefficients of variance (%CV) were calculated for each component and each sample separately.

CV% values for individual allergen components

Allergen Component, Group	Sample	n	Mean (kU <sub>A</sub> /L)	Between-Day %CV	Within-Run %CV	Total %CV
Allergen i3	1	80	2.14	2.94	2.56	3.90
	2	80	0.34	3.09	2.37	3.90
Allergen i4	1	80	2.32	5.08	3.35	6.08
	2	80	0.35	4.51	1.64	4.80

ii) *Lot-to-lot imprecision:*

For each allergen, three different ImmunoCAP Allergen Component lots were tested using two positive plasma samples ( $0.35 \pm 25\%$  and  $\geq 0.7$  kU<sub>A</sub>/L) and one negative plasma sample ( $< 0.1$  kU<sub>A</sub>/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 Phadia 250. Mean concentration values, %CV and concentration quotients between lots were calculated for the positive samples.

Lot	Positive 1		Positive 2		Negative	Concentration Quotient		
	Mean (kU <sub>A</sub> /L)	CV (%)	Mean (kU <sub>A</sub> /L)	CV (%)	Mean (kU <sub>A</sub> /L)		Positive 1	Positive 2
ImmunoCAP Allergen i3, Common wasp venom (Yellow jacket)								
1	0.36	1.5	8.2	4.7	<0.1	lot1/lot2	1.0	0.97
2	0.36	2.4	8.4	4.4	<0.1	lot1/lot3	1.0	0.97
3	0.36	3.1	8.5	4.0	<0.1	lot2/lot3	1.0	1.00
ImmunoCAP Allergen i4, Paper wasp venom								
1	0.38	2.0	11.7	5.5	<0.1	lot1/lot2	1.00	0.97
2	0.38	1.2	12.0	6.3	<0.1	lot1/lot3	1.03	1.05
3	0.37	2.2	11.2	3.8	<0.1	lot2/lot3	1.03	1.07

b. *Linearity/assay reportable range:*

The linearity of the 2 individual allergens was assessed following the CLSI I/LA20-A2 guidelines. For each allergen component, three positive plasma samples were each diluted in negative plasma generating at least five 2-fold consecutive dilutions. Undiluted 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 instrument Phadia 250. For each product one lot of ImmunoCAP Allergen Component was used. ImmunoCAP Specific Total IgE working range is LoD to 100 kU<sub>A</sub>/L.

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

ImmunoCAP Allergen Component	Regression Equation	r <sup>2</sup>	95% CI Slope	95% CI Intercept	Highest Level tested (kU <sub>A</sub> /L)
i3	y = 0.971x + 0.048	0.998	0.962 – 0.979	0.040 – 0.056	70.46
i4	y = 0.983x + 0.040	0.999	0.977 – 0.989	0.034 – 0.045	66.05

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 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 EN 13640 (Stability Testing of *In Vitro* Diagnostic Reagents) to demonstrate 24 month unopened shelf-life stability (at recommended storage temperature of 2-8°C) of the ImmunoCAP Allergen i3 and i4 by an on-going real time stability study and accelerated stability study. For real time stability study, three lots of each ImmunoCAP Allergen Component i3 and i4 were stored at recommended storage temperature, 2-8°C. Two positive plasma samples (2.8 and 7.8 kU<sub>A</sub>/L) and one negative plasma sample (<0.1 kU<sub>A</sub>/L) were tested at different occasions according to the ImmunoCAP Specific IgE, Directions for Use, using Phadia 250. The study is ongoing. For accelerated study, three lots of ImmunoCAP Allergen Components i3 and i4 were stored at 30°C for 8 weeks. The same lot stored at 2-8°C was used as reference. They were tested after 4 and 8 weeks when stored in 30°C, using two positive plasma samples and one negative plasma sample. The results support the manufacture's claim of 24 months.

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 each allergen component on the Phadia 250 in alignment with CLSI EP17-A. The LoB was based on single determinations of 100 negative samples (blank samples) and was estimated as the 95% percentile of the distribution. LoD was calculated according to the equation:  $LoD = LoB + c\beta \cdot SD$  where SD, the standard deviation, was based on 20 determinations of 3 low positive samples, in total 60 determinations. LoQ for allergen specific IgE antibodies was determined to be 0.1 kUA/L according to CLSI EP 17-A (see k051218). The results are shown in the table below.

Allergen component	LoB	LoD
i3	0.028	0.035
i4	0.031	0.038

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 I/LA20-A2. The specific IgE concentration for the positive samples is shown in the table below.

Allergen component	kU <sub>A</sub> /L
i3	9.5
i4	8.0

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 1 hour before being analyzed with the corresponding ImmunoCAP Allergen Component on ImmunoCAP instrument according to the manufacturer's instructions. The testing was performed in duplicates in one assay run. Mean values were calculated.

The inhibition test was evaluated with inhibition values in %, calculated according to the formula below:

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

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 and the unrelated inhibitors indicate that the 2 modified allergens contain the immunologically relevant allergen as shown below:

ImmunoCAP Allergen i3, Common wasp venom (Yellow jacket)

The Allergen i3, Common wasp venom (Yellow jacket) Inhibition study showed that >50% inhibition was achieved with related inhibitor (i3 solution allergen) at a final inhibitor concentration of 10 µg/mL. The inhibition studies using six unrelated inhibitors, three from unrelated groups (f428 Hazel nut, g6 Timothy grass, and t3 Birch tree) and three from the related/same group (i206 Cockroach, i1 Honey bee venom and i205 Bumble bee venom) did not show any significant inhibition at 0.5 mg/mL. The inhibition studies indicate that the ImmunoCAP Allergen i3, Common wasp venom (Yellow jacket) solid phase contains the immunologically relevant allergen.

### ImmunoCAP Allergen i4 Paper wasp venom

The Allergen i4 Paper wasp venom Inhibition study showed that >50% inhibition was achieved with related inhibitor (i4 solution allergen) at a final inhibitor concentration of 0.075 mg/mL. The inhibition studies using six unrelated inhibitors, three from unrelated groups (f428 Hazel nut, g6 Timothy grass, and t3 Birch tree) and three from the related/same group (i206 Cockroach, i1 Honey bee venom and i205 Bumble bee venom) did not show any significant inhibition at a final inhibitor concentration of 0.2 mg/mL. The inhibition studies indicate that the ImmunoCAP Allergen i4, Paper wasp venom 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 in ImmunoCAP Specific IgE assay using representative allergens, 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 2 samples per allergen. The allergens tested were e228 rFel d 4 Cat, f351 rPen a 1 Tropomyosin Shrimp, f354 rBer e 1 Brazil nut, f420 rPru p 3 LTP Peach and w231 nArt v 1 Mugwort. The design of the studies was in general alignment with CLSI EP7-A2 Guideline. 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:*

All results >0.1 kUA/L are interpreted as being analytically positive.

## 2. Comparison studies:

### a. *Method comparison with predicate device:*

Allergen i3, Common wasp venom (Yellow jacket)

The following table summarizes the results of comparison study of native allergen extract to the modified allergen with the added recombinant component.

		<i>i3, Common Wasp Venom Native Extract</i>		
		Positive	Negative	Total
<i>i3</i> Modified Common Wasp Venom	Positive	50	1	51
	Negative	0	100	100
	Total	50	101	151

Positive percent agreement: 100% (50/50) (95% CI: 92.9-100%)

Negative percent agreement: 99% (100/101) (95% CI: 94.6-100%)

Overall percent agreement: 99.3% (150/151) (95% CI: 96.4-100%)

Allergen *i4*, Paper wasp venom

The following table summarizes the results of comparison study of native allergen extract to the modified allergen with the added recombinant component.

		<i>i4, Paper Wasp Venom Native Extract</i>		
		Positive	Negative	Total
<i>i4</i> , Modified Paper Wasp Venom	Positive	51	0	51
	Negative	0	100	100
	Total	51	100	151

Positive percent agreement: 100% (51/51) (95% CI: 93.0-100%)

Negative percent agreement: 100% (100/100) (95% CI: 96.4-100%)

Overall percent agreement: 100% (100/100) (95% CI: 97.6 -100%)

*b. Matrix comparison:*

The "Proof of Principle" study that different matrix samples (heparin plasma, EDTA plasma and serum) are interchangeable for ImmunoCAP Allergen Components was provided in k101251. Serum, sodium heparin plasma, and EDTA plasma samples were collected from four patients with clinical history of known specific allergies and four non-atopic patients. The samples contained specific IgE antibodies for one or more of the allergen components tested. All sample matrices (heparin plasma, EDTA plasma and serum) from each patient were tested with ImmunoCAP Allergen Components in 2 replicates in one assay run. Mean concentration values for each

sample matrix were calculated. Mean logarithmic ratios for 17 results were -0.022 (Plasma heparin/Serum) and 0.054 (Plasma EDTA/Serum). The results from the study show that samples of different matrices (heparin plasma, EDTA plasma and serum) are interchangeable for ImmunoCAP Allergen Components.

### 3. Clinical studies:

#### a. *Clinical Sensitivity and specificity:*

The performance of the two modified individual allergen components was compared to a clinical diagnosis of allergy. The clinical studies were performed using thirty-one (31) atopic samples for ImmunoCAP Allergen *i3*, Common wasp venom (Yellow jacket) and thirty (30) atopic samples for ImmunoCAP Allergen *i4*, Paper wasp venom. The clinical serum samples in these studies were from individuals with a clinical history of allergy-like symptoms upon exposure to the allergen, as diagnosed by a physician. Information about clinical symptoms and manifestations was available for all clinical samples. 100 negative samples used in these studies were from healthy non-atopic donors.

#### ImmunoCAP Allergen *i3*

		Atopic	Non-atopic	Total
Allergen <i>i3</i> , Common wasp venom (Yellow jacket)	Positive	31	0	30
	Negative	0	100	100
	Total	31	100	130

Sensitivity = 100% (31/31) (95% CI: 88.8-100%)

Specificity = 100% (100/101) (95% CI: 96.4-100%)

#### ImmunoCAP Allergen *i4*

		Atopic	Non-atopic	Total
Allergen <i>i4</i> , Paper wasp venom	Positive	30	0	30
	Negative	0	100	100
	Total	30	100	130

Sensitivity = 100% (30/30) (95% CI: 88.4-100%)

Specificity = 100% (100/100) (95% CI: 96.4-100%)

All negative samples showed undetectable level of allergen specific IgE. Studies described above were performed on the Phadia 250 instrument system.

#### b. *Other clinical supportive data (when a. is not applicable):*

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

The expected value is negative ( $< 0.35 \text{ kU}_A/\text{L}$ ) for a specific allergen in a non-allergic person. The manufacturer recommends a cut-off of  $0.35 \text{ kU}_A/\text{L}$ . Each laboratory should establish its own expected range of values.

**N. Instrument Name:**

Phadia 100, Phadia 250, Phadia 1000, Phadia 2500 and Phadia 5000 instrument system.

**O. System Descriptions:**

1. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes  X  or No

**P. ~~Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:~~**

Refer to k101251 for assay Precision study of ImmunoCAP Allergen Components on Phadia 100, Phadia 250 and Phadia 1000.

**Q. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**R. Conclusion:**

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