510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION

DECISION SUMMARY

A. 510(k) Number:

k121156

B. Purpose for Submission:

Addition of 4 new native allergens to a cleared device

C. Analyte:

Four new native allergen-specific IgE analytes: Cow's milk whey, Chick pea, Cedar, Cupressus arizonica

D. Type of Test:

Fluoroenzymeimmunoassay, Quantitative

E. Applicant:

Phadia AB

F. Proprietary and Established Names:

ImmunoCAP Specific IgE

ImmunoCAP Allergen f236, Cow's milk whey

ImmunoCAP Allergen f309, Chick pea

ImmunoCAP Allergen t212, Cedar

ImmunoCAP Allergen t222, Cupressus arizonica

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, and Phadia 1000. 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):

   Prescription use only

4. Special instrument requirements:

   For use on the instruments Phadia 100, Phadia 250 and Phadia 1000.

I. Device Description:

The ImmunoCAP system is a fully integrated and automated system for the determination of specific IgE in human serum, sodium heparin plasma or EDTA plasma sample. It is comprised of general, test and method specific reagents for Phadia 100, Phadia 250 and Phadia 1000 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) covalently coupled to a support in a plastic housing.

J. Substantial Equivalence Information:

1. Predicate device name and Predicate K number

   UniCAP® Specific IgE Assay and UniCAP® Specific IgE Conjugate 100 and 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 is to be used in clinical laboratories.	Same

Similarities		
Item	Device	Predicate
Assay type	Quantitative	Same
Test method	Fluoroenzymeimmunoassay	Same
Sample volume	40 µL	Same
Process time	2 hours 30 minutes for Phadia 100. 1 hour 45 minutes for Phadia 250 and 1000	Same
Incubation temperature	37°C	Same
Detection antibody	β-Galactosidase-anti- human IgE (mouse monoclonal antibody) for all ImmunoCAP	Same
Number of calibrators	Six	Same

Differences		
Item	Device	Predicate
Modification	Addition of new allergens: f236, Cow's milk whey f309, Chick pea t212, Cedar t222, Cupressus arizonica	Not included
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 and Phadia 1000	UniCAP 100

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

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

CSLI 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.

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

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

L. Test Principle:

The allergen of interest, covalently coupled to the ImmunoCAP solid phase, 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 the developing agent. After stopping the reaction, the fluorescence of the eluate is measured. The higher the response value, the more specific IgE present in the specimen. To evaluate the rest results, the response 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 ± 25%) and a high range sample (≥0.7 kU_A/L), each 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

ImmunoCAP Allergen	Positive Sample	n	Mean (kU_A/L)	Between Days CV%	Within-run CV%	Total CV%
f236, Cow's milk whey	1	80	2.68	3.47	3.58	4.98
	2	80	0.34	4.65	3.16	5.62
f309, Chick pea	1	80	3.30	1.56	3.20	3.56
	2	80	0.36	1.58	3.93	4.23
t212, Cedar	1	80	2.15	4.47	5.47	7.07
	2	80	0.30	3.61	3.66	5.14
t222, Cupressus arizonica	1	80	2.17	7.58	10.91	13.28
	2	80	0.32	4.09	4.41	6.02

n = number of samples

Pooled CV% values for individual allergens

ImmunoCAP Allergen	N	Between Day CV%	Within-run CV%	Total CV%
f236, Cow's milk whey	2	4.10	3.37	5.31
f309, Chick pea	2	1.57	3.58	3.91
t212, Cedar	2	4.06	4.65	6.18
t222, Cupressus arizonica	2	6.09	8.32	10.31

n = number of samples

ii) **Lot-to-lot imprecision:**

Three ImmunoCAP Allergen Component lots for each allergen were tested using two positive samples (0.35 ± 25% and ≥0.7 kU_A/l) and one negative 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 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_A/L)	CV (%)	Mean (kU_A/L)	CV (%)	Mean (kU_A/L)		Positive 1	Positive 2
ImmunoCAP Allergen f236, Allergen Component Cow's milk whey								
1	1.95	2.7	0.33	1.8	<0.1	lot1/lot2	1.04	1.05
2	1.87	3.8	0.32	1.2	<0.1	lot1/lot3	0.99	1.02
3	1.96	2.5	0.33	2.2	<0.1	lot2/lot3	0.96	0.97
ImmunoCAP Allergen f309, Allergen Component Chick pea								
1	1.89	5.8	0.27	3.6	<0.1	lot1/lot2	0.83	0.89
2	2.26	2.6	0.31	2.1	<0.1	lot1/lot3	0.84	0.93
3	2.23	2.7	0.29	5.4	<0.1	lot2/lot3	1.01	1.05
ImmunoCAP Allergen t212, Allergen Component Cedar								
1	3.23	3.7	0.32	2.7	<0.1	lot1/lot2	0.91	0.94
2	3.57	2.1	0.34	2.1	<0.1	lot1/lot3	0.86	0.94
3	3.75	4.0	0.34	1.7	<0.1	lot2/lot3	0.95	1.01

Lot	Positive 1		Positive 2		Negative	Concentration Quotient		
	Mean (kU_A/L)	CV (%)	Mean (kU_A/L)	CV (%)	Mean (kU_A/L)		Positive 1	Positive 2
ImmunoCAP Allergen t222, Allergen Component Cupressus arizonica								
1	1.90	2.0	0.40	2.6	<0.1	lot1/lot2	1.03	1.04
2	2.81	5.3	0.39	1.6	<0.1	lot1/lot3	0.84	1.01
3	3.45	3.7	0.40	3.4	<0.1	lot2/lot3	0.81	0.97

b. Linearity/assay reportable range:

The linearity of the four 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 to100 kU_A/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	Regression Equation	r^2	95% CI Slope	95% CI Intercept	Highest level tested (kU_A/L)
f236, Cow's milk whey	y = 0.96x + 0.09	1.00	0.95 – 0.97	0.08 – 0.10	85.61
f309, Chick pea	y = 0.95x + 0.07	1.00	0.94 – 0.95	0.06 – 0.08	52.51
t212, Cedar	y = 0.96x + 0.08	1.00	0.95 – 0.97	0.07 – 0.08	39.42
t222, Cupressus arizonica	y = 0.95x + 0.06	1.00	0.94 – 0.95	0.05 – 0.06	14.30

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 (from the date of manufacture

when stored at recommended temperature 2-8°C) of the ImmunoCAP Allergen f236 Cow's milk whey, f309 Chick pea, t212 Cedar and t222 Cupressus arizonica by an on-going real time stability study and accelerated stability study. For real-time stability study, three lots of ImmunoCAP Allergen Component were stored at recommended storage temperature 2-8°C. Two positive plasma samples and one negative plasma sample 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 were stored at 30°C and tested after 4 and 8 weeks using two positive plasma samples and one negative plasma sample. The same lots stored at 2-8°C were used as reference. 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 the 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 + $c_\beta \times$SD where SD, the standard deviation, was based on 20 determinations of 3 low positive samples, in total 60 determinations. The results are shown in the table below.

ImmunoCAP Allergen	LoB	LoD
f236, Cow's milk whey	0.032	0.043
f309, Chick pea	0.018	0.029
t212,Cedar	0.000	0.012
t222, Cupressus arizonica	0.05	0.067

d. Analytical specificity:

i) *Inhibition studies*:

Immunological specificity of the allergen components was verified through *competitive inhibition* studies in accordance with CLSI I/LA20-A2. The specific IgE concentration for the positive samples is shown in the table below.

ImmunoCAP Allergen	kU_A/L
f236, Cow's milk whey	9.3
f309, Chick pea	12.5
t212, Cedar	12.0
t222, Cupressus arizonica	9.2

The allergen solution was serially diluted with buffer to show an overall dose dependent inhibition. The unrelated allergen solutions are not further diluted. 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:

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

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 and the unrelated inhibitors indicate that the 4 new allergens contain the immunologically relevant allergen as shown below:

ImmunoCAP Allergen f236, Allergen Component Cow´s milk whey

The Cow´s milk whey allergen Inhibition study showed that ≥ 50% inhibition was achieved with the related inhibitor (Cow´s milk whey allergen) at a final inhibitor concentration of 480 ng/mL. The inhibition studies using four unrelated inhibitors, including three from unrelated groups (m36, e85, w204) and one from the related/same group (f83) did not show any significant inhibition at the highest inhibitor concentration of 1.4 mg/mL. The inhibition studies indicate that the ImmunoCAP Allergen f236, Cow´s milk whey solid phase contains the immunologically relevant allergen.

ImmunoCAP Allergen f309, Allergen Component Chick pea

The Chick pea allergen Inhibition study showed that about 50% inhibition was achieved with the related inhibitor (Chick pea allergen) at a final inhibitor concentration of ~0.31 mg/mL. The inhibition studies using four unrelated inhibitors, including three from unrelated groups (m36, g6, d201) and one from the related/same group (f83) did not show any significant inhibition at the highest inhibitor concentration of 4.0 mg/mL. The inhibition studies indicate that the ImmunoCAP Allergen f309, Chick pea solid phase contains the immunologically relevant allergen.

ImmunoCAP Allergen t212, Allergen Component Cedar

The Cedar allergen Inhibition study showed that about 50 % inhibition was achieved with the related inhibitor (Cedar allergen) at a final inhibitor concentration of ~27 µg/mL. The inhibition studies using three unrelated inhibitors from unrelated groups (m36, w204, g6) did not show any significant inhibition at the highest inhibitor

concentration of 4.0 µg/mL. An unrelated allergen f222, Cupressus arizonica did show significant inhibition (>15%) at a final concentration of 2.7 mg/mL. In contrast, another unrelated allergen t3, birch from the related/same group did not show any significant inhibition at a final concentration of 2.7 mg/mL.

ImmunoCAP Allergen t222, Allergen Component Cupressus arizonica

The Cupressus arizonica allergen Inhibition study showed that about 50% inhibition was achieved at a final inhibitor concentration of ~ 8.7 µg/mL. The inhibition studies using three unrelated inhibitors from unrelated groups (m36, d74, w204) did not show any significant inhibition at the highest inhibitor concentration of 4.0 mg/mL. An unrelated allergen t212, Cedar from the related/same group did show significant inhibition (>15%) at a final concentration of 2.7 mg/mL. In contrast, another unrelated allergen t3, birch from the related/same group did not show any significant inhibition at a final concentration of 0.87 mg/mL.

ii) *Interference*:

a) Endogenous Substance Interference:

In order to show that icteric, hemolytic or lipemic samples do not adversely affects 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 affects 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 kUA/L. All results >0.1 kUA/L are interpreted as being analytically positive.

2. Comparison studies:

a. Method comparison with predicate device:

Refer to clinical studies.

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 nonatopic 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 all 4 individual allergen components 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. At least 30 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. 100 negative samples (<0.35 kU_A/L) from healthy non-atopic donors with no reported clinical reaction to the allergen were also tested.

ImmunoCAP Allergen f236, Cow's milk whey

		Clinical Diagnosis		
		Atopic	**Non-atopic**	**Total**
f236, Cow's milk whey	**Positive**	42	0	42
	Negative	0	100	100
	Total	42	100	142

Sensitivity =100% (95% CI: 91.6 – 100%)
Specificity =100% (95% CI: 96.4 – 100%)

ImmunoCAP Allergen f309, Chick pea

		Clinical Diagnosis		
		Atopic	**Non-atopic**	**Total**
f309, Chick pea	**Positive**	64	0	64
	Negative	0	100	100
	Total	64	100	164

Sensitivity =100% (95% CI: 94.4 – 100%)
Specificity =100% (95% CI: 96.4 – 100%)

ImmunoCAP Allergen t212, Cedar

		Clinical Diagnosis		
		Atopic	**Non-atopic**	**Total**
t212, Cedar	**Positive**	30	0	28
	Negative	0	100	100
	Total	30	100	128

Sensitivity =100% (95% CI: 87.7 – 100%)
Specificity =100% (95% CI: 96.4 – 100%)

ImmunoCAP Allergen t222, Cupressus arizonica

		Clinical Diagnosis		
		Atopic	**Non-atopic**	**Total**
t222, Cupressus arizonica	**Positive**	63	0	63
	Negative	0	100	100
	Total	63	100	163

Sensitivity =100% (95% CI: 94.3 – 100%)
Specificity =100% (95% CI: 96.4 – 100%)

All negative samples showed undetectable level (<0.1 kU_A/L) of allergen specific IgE. Studies described above were performed on the Phadia 1000 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 kU_A/L) for a specific allergen in a non-allergic person. The manufacturer recommends a cut-off of 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.