

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

A. 510(k) Number:

k113841

B. Purpose for Submission:

Addition of 9 new allergens to a cleared device

C. Measurand:

Nine new allergen-specific IgE analytes: rEqu c 1 (Horse), rFel d 4 (Cat), rPen a 1 (Tropomyosin, Shrimp), rBer e 1 (Brazil nut), rPru p 1 (PR-10, Peach), rPru p 3 (LTP, Peach), rPru p 4 (Profilin, Peach), nArt v 1 (Mugwort), and nArt v 3 (LTP, Mugwort)

D. Type of Test:

Fluoroenzymeimmunoassay, Quantitative and Semi-quantitative

E. Applicant:

Phadia AB

F. Proprietary and Established Names:

ImmunoCAP Specific IgE

ImmunoCAP Allergen e227, Allergen Component rEqu c 1, Horse

ImmunoCAP Allergen e228, Allergen Component rFel d 4, Cat

ImmunoCAP Allergen f351, Allergen component rPen a 1, Tropomyosin, Shrimp

ImmunoCAP Allergen f354, Allergen component rBer e 1, Brazil nut

ImmunoCAP Allergen f419, Allergen component rPru p 1, PR-10, Peach

ImmunoCAP Allergen f420, Allergen component rPru p 3, LTP, Peach

ImmunoCAP Allergen f421, Allergen component rPru p 4, Profilin, Peach

ImmunoCAP Allergen w231, Allergen component nArt v 1, Mugwort

ImmunoCAP Allergen w233, Allergen component nArt v 3, LTP, Mugwort

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):

For prescription use only

4. Special instrument requirements:

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 blood serum or sodium heparin 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 or 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[®] Specific IgE Assay and UniCAP[®] Specific IgE Conjugate 100 and 400 (k051218), Allergen Immunocap[™] (k991048) 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. 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	β-Galactosidase-anti-IgE (mouse monoclonal antibody) for all ImmunoCAP	Same
Basic principle	Fluoroenzymeimmunoassay	Same
Sample volume	40 μL	Same
Process time	2 hours 30 minutes for Phadia 100.	Same
Incubation temperature	37°C	Same

Differences		
Item	Device	Predicate
Form of allergens	Recombinant proteins and purified whole native proteins	Purified native allergens
Allergens	Individual recombinant proteins: rEqu c 1 (Horse), rFel d 4 (Cat), rPen a 1 (Shrimp), rBer e 1 (Brazil nut), rPru p 1, rPru p 3 and rPru p 4 (Peach).	Not included

Differences		
Item	Device	Predicate
	Whole allergens comprising multiple proteins from purified native allergen source: nArt v 1 and nArt v 3 (Mugwort)	
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 Document Referenced (if applicable):

CLSI EP5-A2: 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-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

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 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 (≥ 0.7 kU_A/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 _A /L)	Between-Day %CV	Within-Run %CV	Total %CV
e227, rEqu c 1, Horse	1	80	2.05	2.00	3.07	3.66
	2	80	0.33	3.17	2.55	4.07
e228, rFel d 4, Cat	1	80	2.87	2.28	3.32	4.03
	2	80	0.33	2.44	3.31	4.11
f351, rPen a 1, Shrimp	1	80	1.20	4.03	3.10	5.09
	2	80	0.38	4.21	2.56	4.93
f354, rBer e 1, Brazil nut	1	80	2.64	3.12	3.71	4.84
	2	80	0.41	6.15	5.06	7.97
f419, rPru p 1, PR-10, Peach	1	80	0.39	2.22	1.53	2.69
	2	80	2.26	1.19	2.48	2.75
f420, rPru p 3, LTP, Peach	1	80	1.81	2.91	4.06	4.99
	2	80	0.38	3.71	3.65	5.21
f421, rPru p 4, Profilin, Peach	1	80	1.86	2.99	2.42	3.84
	2	80	0.34	2.61	2.87	3.88
w231, nArt v 1, Mugwort	1	80	0.85	3.05	3.84	4.90
	2	80	0.33	3.35	2.60	4.24
w233, nArt v 3, LTP, Mugwort	1	80	1.00	2.48	2.17	3.29
	2	79*	0.35	2.30	3.25	3.99

n = number of samples; *One missing result due to laboratory error.

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 ≥ 0.7 kU_A/L) 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 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 e227, Allergen Component rEqu c 1, Horse								
1	1.41	4.70	0.47	3.00	<0.1	lot1/lot2	1.18	1.24
2	1.20	2.80	0.38	2.80	<0.1	lot1/lot3	1.07	1.18
3	1.32	3.00	0.40	2.90	<0.1	lot2/lot3	0.91	0.95
ImmunoCAP Allergen e228, Allergen Component rFel d 4, Cat								
1	1.24	1.60	0.35	1.90	<0.1	lot1/lot2	1.00	1.02
2	1.22	1.80	0.35	1.50	<0.1	lot1/lot3	1.00	1.02
3	1.22	1.50	0.35	2.10	<0.1	lot2/lot3	1.00	1.00
ImmunoCAP Allergen f351, Allergen component rPen a 1, Tropomyosin Shrimp								
1	1.41	4.70	0.47	3.00	<0.1	lot1/lot2	1.18	1.24
2	1.20	2.80	0.38	2.80	<0.1	lot1/lot3	1.07	1.18
3	1.32	3.00	0.40	2.90	<0.1	lot2/lot3	0.91	0.95
ImmunoCAP Allergen f354, Allergen component rBer e 1, Brazil nut								
1	2.83	2.10	0.46	4.00	<0.1	lot1/lot2	1.06	1.15
2	2.66	2.10	0.40	3.30	<0.1	lot1/lot3	1.07	1.18
3	2,64	2.20	0.39	4.20	<0.1	lot2/lot3	1.01	1.03
ImmunoCAP Allergen f419, Allergen component rPru p 1, PR-10, Peach								
1	2.21	1.80	0.39	2.00	<0.1	lot1/lot2	0.97	0.98
2	2.27	2.00	0.40	2.10	<0.1	lot1/lot3	1.04	1.05
3	2.12	3.10	0.37	2.50	<0.1	lot2/lot3	1.07	1.08
ImmunoCAP Allergen f420, Allergen component rPru p 3, LTP, Peach								
1	1.58	2.10	0.30	2.20	<0.1	lot1/lot2	0.90	0.88
2	1.76	2.40	0.34	1.90	<0.1	lot1/lot3	0.91	0.91
3	1.74	2.50	0.33	1.70	<0.1	lot2/lot3	1.01	1.03
ImmunoCAP Allergen f421, Allergen component rPru p 4, Profilin, Peach								
1	1.31	3.10	0.28	6.20	<0.1	lot1/lot2	0.76	0.90
2	1.73	2.40	0.31	5.70	<0.1	lot1/lot3	0.72	0.90
3	1.83	1.50	0.31	1.80	<0.1	lot2/lot3	0.94	1.00
ImmunoCAP Allergen w231, Allergen component nArt v 1, Mugwort								
1	0.77	2.10	0.30	2.10	<0.1	lot1/lot2	0.95	0.94
2	0.81	1.80	0.32	2.20	<0.1	lot1/lot3	0.89	0.88
3	0.87	2.70	0.34	1.70	<0.1	lot2/lot3	0.93	0.94
ImmunoCAP Allergen w233, Allergen component nArt v 3, LTP, Mugwort								
1	1.09	2.50	0.40	2.30	<0.1	lot1/lot2	1.02	1.08
2	1.07	2.50	0.37	3.80	<0.1	lot1/lot3	1.10	1.21
3	0.99	2.70	0.33	3.40	<0.1	lot2/lot3	1.08	1.12

b. *Linearity/assay reportable range:*

The linearity of the 9 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_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 Component	Regression Equation	r ²	95% CI Slope	95% CI Intercept	Highest Level tested (kU _A /L)
E227, rEqu c 1	y = 1.00x - 0.03	1.00	0.99 – 1.01	-0.04 – (-0.02)	26.56
e228, rFel d 4	y = 0.99x	1.00	0.98 – 0.99	-0.01 – 0.01	60.97
F351, rPen a 1	y = 1.03x - 0.03	0.99	1.02 – 1.05	-0.04 – (-0.01)	71.02
F354, rBer e 1	y = 0.96x + 0.04	0.99	0.95 – 0.98	0.02 – 0.05	40.91
F419, rPru p 1	y = 1.05x - 0.08	1.00	1.04 – 1.07	-0.09 – (-0.07)	65.95
F420, rPru p 3	y = 0.97x + 0.02	1.00	0.96 – 0.98	0.02 – 0.03	61.32
F421, rPru p 4	y = 0.97x + 0.07	1.00	0.96 – 0.98	0.06 – 0.08	35.10
W231, nArt v 1	y = 1.01x	1.00	1.00 – 1.02	-0.01 – 0.01	80.66
w233 nArt v 3	y = 1.00x + 0.02	1.00	0.99 – 1.01	0.01 – 0.02	38.17

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 e227 rEqu c 1 (Horse), e228 rFel d 4 (Cat), f351 rPen a 1, Tropomyosin (Shrimp), f354 rBer e 1 (Brazil nut), f419 rPru p 1 (PR-10, Peach), f420 rPru p 3 (LTP, Peach), f421 rPru p 4 (Profilin, Peach), w231 nArt v 1 (Mugwort) and w233 nArt v 3 (LTP, Mugwort) 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 for 8 weeks or 4 weeks at 40°C. The same lots stored at 2-8°C were used as reference. They were tested after 4 and 8 weeks when stored in 30°C and after 2 and 4 weeks when stored in 40°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. The results are shown in the table below.

Allergen component	LoB	LoD
e227 rEqu c 1 (Horse)	0.017	0.027
e228 rFel d 4 (Cat)	0.031	0.043
f351 rPen a 1, Tropomyosin (Shrimp)	0.002	0.014
f354 rBer e 1 (Brazil nut)	0.045	0.070
f419 rPru p 1 (PR-10, Peach)	0.000	0.009
f420 rPru p 3 (LTP, Peach)	0.009	0.032
f421 rPru p 4 (Profilin, Peach)	0.015	0.026
w231 nArt v 1 (Mugwort)	0.035	0.045
w233 nArt v 3 (LTP, Mugwort)	0.002	0.010

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 _A /L
e227, rEqu c 1	4.5
e228, rFel d 4	3.6
f351, rPen a 1	8.5
f354, rBer e 1	4.0
f419, rPru p 1	11.1
f420, rPru p 3	5.1
f421, rPru p 4	10.3
w231, nArt v 1	2.9
w233, nArt v 3	17.4

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 9 new allergens contain the immunologically relevant allergen as shown below:

ImmunoCAP Allergen e227, Allergen Component rEqu c 1, Horse

The rEqu c 1, Horse allergen Inhibition study showed that about 50% inhibition was achieved with related inhibitor (rEqu c 1, Horse allergen) at a final inhibitor concentration of ~50 ng/mL. The inhibition studies using four unrelated inhibitors, including three from unrelated groups (birch, Timothy and Latex) and one from the related/same group (Cat) did not show any significant inhibition at 500 µg/mL inhibitor concentration. The inhibition studies indicate that the ImmunoCAP Allergen e227, rEqu c 1, Horse solid phase contains the immunologically relevant allergen.

ImmunoCAP Allergen e228, Allergen Component rFel d 4, Cat

The rFel d 4, Cat allergen Inhibition study showed that 50% inhibition was achieved with related inhibitor (rFel d 4, Cat allergen) at a final inhibitor concentration of ~27 ng/mL. The inhibition studies using four unrelated inhibitors, including three from unrelated groups (Birch, Bermuda Grass and Latex) and one from the related/same group (Dog) did not show any significant inhibition at 500 µg/mL inhibitor concentration. The inhibition studies indicate that the ImmunoCAP Allergen rFel d 4 (e228), Cat solid phase contains the immunologically relevant allergen.

ImmunoCAP Allergen f351, Allergen Component rPen a 1, Tropomyosin, Shrimp

The rPen a 1, Tropomyosin, Shrimp allergen Inhibition study showed that 50% inhibition was achieved with related inhibitor (rPen a 1, Tropomyosin, Shrimp allergen) at a final inhibitor concentration of ~50 ng/mL. The inhibition studies using four unrelated inhibitors, including three from unrelated groups (Cat, Latex and Timothy) and one from the related/same group (Soy) did not show any significant inhibition at 500 µg/mL inhibitor concentration. The inhibition studies indicate that the ImmunoCAP Allergen rPen a 1, Tropomyosin (f351), Shrimp solid phase contains the immunologically relevant allergen.

ImmunoCAP Allergen f354, Allergen Component rBer e 1, Brazil nut

The rBer e 1, Brazil nut allergen Inhibition study showed that 50% inhibition was achieved with related inhibitor (rBer e 1, Brazil nut allergen) at a final inhibitor concentration of ~50 ng/mL. The inhibition studies using four unrelated inhibitors, including three from unrelated groups (Cat, Timothy and Birch) and one from the related/same group (Peanut) did not show any significant inhibition at 500 µg/mL inhibitor concentration. The inhibition studies indicate that the ImmunoCAP Allergen rBer e 1 (f354), Brazil nut solid phase contains the immunologically relevant allergen.

ImmunoCAP Allergen f419, Allergen Component rPru p 1, PR-10, Peach

The rPru p 1, PR-10, Peach allergen Inhibition study showed that 50% inhibition was achieved with related inhibitor (rPru p 1, PR-10, Peach allergen) at a final inhibitor concentration of ~5 µg/mL. The inhibition studies using four unrelated inhibitors, including three from unrelated groups (Dog, Birch and Timothy) and one from the related/same group (Peach) did not show any significant inhibition at 500 µg/mL inhibitor concentration. The inhibition studies indicate that the ImmunoCAP Allergen rPru p 1 (f419) Peach solid phase contains the immunologically relevant allergen.

ImmunoCAP Allergen f420, Allergen Component rPru p 3, LTP, Peach

The rPru p 3, LTP, Peach allergen Inhibition study showed that 50% inhibition was achieved with related inhibitor (rPru p 3, LTP, Peach allergen) at a final inhibitor concentration of ~5 µg/mL. The inhibition studies using four unrelated inhibitors, including three from unrelated groups (Dog, Birch and Timothy) and

one from the related/same group (Shrimp) did not show any significant inhibition at 500 µg/mL inhibitor concentration. The inhibition studies indicate that the ImmunoCAP Allergen rPru p 3, LTP (f420) Peach solid phase contains the immunologically relevant allergen.

ImmunoCAP Allergen f421, Allergen Component rPru p 4, Profilin, Peach

The rPru p 4, Profilin, Peach allergen Inhibition study showed that 50% inhibition was achieved with related inhibitor (rPru p 4, Profilin, Peach allergen) at a final inhibitor concentration of ~50 ng/mL. The inhibition studies using four unrelated inhibitors, including three from unrelated groups (Cat, Latex and Timothy) and one from the related/same group (Shrimp) did not show any significant inhibition at 500 µg/mL inhibitor concentration. The inhibition studies indicate that the ImmunoCAP Allergen rPru p 4, Profilin (f421) Peach solid phase contains the immunologically relevant allergen.

ImmunoCAP Allergen w231, Allergen Component nArt v 1, Mugwort

The nArt v 1, Mugwort allergen Inhibition study showed that 50% inhibition was achieved with related inhibitor (nArt v 1, Mugwort allergen) at a final inhibitor concentration of ~1.7 µg/mL. The inhibition studies using four unrelated inhibitors, including three from unrelated groups (Timothy, Birch and Latex) and one from the same/related group (Mugwort nArt v 3) did not show any significant inhibition at 360 to 500 µg/mL inhibitor concentration. The inhibition studies indicate that the ImmunoCAP Allergen nArt v 1 (w231) Mugwort solid phase contains the immunologically relevant allergen.

ImmunoCAP Allergen w233, Allergen Component nArt v 3, LTP, Mugwort

The nArt v 3, LTP, Mugwort allergen Inhibition study showed that 50% inhibition was achieved with related inhibitor (nArt v 3, LTP, Mugwort allergen) at a final inhibitor concentration of ~3.6 µg/mL. The inhibition studies using four unrelated inhibitors, including three from unrelated groups (Peach, Dog and Timothy) and one from the related/same group (Mugwort nArt v 1) did not show any significant inhibition at 170 to 500 µg/mL inhibitor concentration. The inhibition studies indicate that the ImmunoCAP Allergen nArt v 3, LTP (w233), Mugwort 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:

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 9 individual allergen components was compared to a clinical

diagnosis of allergy. The objective of this study was, (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 were also tested.

ImmunoCAP Allergen e227, Allergen component rEqu c 1, Horse

		Clinical Diagnosis to Horse or furry animals		
		Atopic	Non-atopic	Total
e227, rEqu c 1	Positive	30	0	30
	Negative	0	100	100
	Total	30	100	130

Sensitivity =100% (95% CI: 88.4 – 100.0%)

Specificity =100% (95% CI: 96.4 – 100.0%)

ImmunoCAP Allergen e228, Allergen component rFel d 4, Cat

		Clinical Diagnosis to Cat or furry animals		
		Atopic	Non-atopic	Total
e228, rFel d 4	Positive	30	0	30
	Negative	0	100	100
	Total	30	100	130

Sensitivity =100% (95% CI: 88.4 – 100.0%)

Specificity =100% (95% CI: 96.4 – 100.0%)

ImmunoCAP Allergen f351, Allergen Component rPen a 1, Shrimp

		Clinical Diagnosis to Shrimp		
		Atopic	Non-atopic	Total
f351, rPen a 1	Positive	40	0	40
	Negative	0	100	100
	Total	40	100	140

Sensitivity =100% (95% CI: 91.2 – 100.0%)

Specificity =100% (95% CI: 96.4 – 100.0%)

ImmunoCAP Allergen f354, Allergen component rBer e 1, Brazil nut

		Clinical Diagnosis to Brazil nut or nuts		
		Atopic	Non-atopic	Total
f354, rBer e 1	Positive	34	0	34
	Negative	0	100	100
	Total	34	100	134

Sensitivity = 100% (95% CI: 89.7 – 100.0%)

Specificity = 100% (95% CI: 96.4 – 100.0%)

ImmunoCAP Allergen f419, Allergen component rPru p 1 PR-10, Peach

		Clinical Diagnosis to Peach		
		Atopic	Non-atopic	Total
f419, rPru p 1	Positive	34	0	34
	Negative	0	100	100
	Total	34	100	134

Sensitivity = 100% (95% CI: 89.7 – 100.0%)

Specificity = 100% (95% CI: 96.4 – 100.0%)

ImmunoCAP Allergen f420, Allergen component rPru p 3 LTP, Peach

		Clinical Diagnosis to Peach		
		Atopic	Non-atopic	Total
f420, rPru p 3	Positive	124	0	124
	Negative	0	100	100
	Total	124	100	224

Sensitivity = 100% (95% CI: 97.1 – 100.0%)

Specificity = 100% (95% CI: 96.4 – 100.0%)

ImmunoCAP Allergen f421, Allergen component rPru p 4 Profilin, Peach

		Clinical Diagnosis to Peach or Fruit		
		Atopic	Non-atopic	Total
f421, rPru p 4	Positive	33	0	33
	Negative	0	100	100
	Total	33	100	133

Sensitivity = 100% (95% CI: 89.4 – 100.0%)

Specificity = 100% (95% CI: 96.4 – 100.0%)

ImmunoCAP Allergen w231, Allergen component nArt v 1, Mugwort

		Clinical Diagnosis to Mugwort		
		Atopic	Non-atopic	Total
w231, nArt v 1	Positive	32	0	32
	Negative	0	100	100
	Total	32	100	132

Sensitivity = 100% (95% CI: 89.1 – 100.0%)
Specificity =100% (95% CI: 96.4 – 100.0%)

ImmunoCAP Allergen w233, Allergen component nArt v 3, LTP, Mugwort

		Clinical Diagnosis to Mugwort		
		Atopic	Non-atopic	Total
w233, nArt v 3	Positive	32	0	32
	Negative	0	100	100
	Total	32	100	132

Sensitivity = 100% (95% CI: 89.1 – 100.0%)
Specificity =100% (95% CI: 96.4 – 100.0%)

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. Instrument Name:

Phadia 100, Phadia 250 and Phadia 1000 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.