510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K190315

B. Purpose for Submission:

Addition of three new recombinant allergens to a cleared device

C. Measurand:

Three Allergen-specific IgE analytes: e229, rCan f 4 (Dog); e230, rCan f 6 (Dog); and e231, rFel d 7 (Cat)

D. Type of Test:

Fluoroenzymeimmunoassay, Quantitative

E. Applicant:

Phadia AB

F. Proprietary and Established Names:

ImmunoCAP Specific IgE

- ImmunoCAP Allergen e229, Allergen component rCan f 4 (Dog)
- ImmunoCAP Allergen e230, Allergen component rCan f 6 (Dog)
- ImmunoCAP Allergen e231, Allergen component rFel d 7 (Cat)

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). 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. ImmunoCAP Specific IgE is to be used with instruments Phadia 100, Phadia 250, Phadia 1000, Phadia 2500 and Phadia 5000.

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 allergens (native or recombinant) covalently coupled to a support in a plastic housing.

J. Substantial Equivalence Information:

1. Predicate device name and number:

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

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 (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 is 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 250, Phadia 1000, Phadia 2500 and Phadia 5000: 1 hour 45 minutes from entering the first sample.	Same
Incubation temperature	37ºC	Same

Differences		
Item	Device	Predicate
Allergen-containing reagent	Purified recombinant allergen components: e229, rCan f 4 (Dog); e230, rCan f 6 (Dog); e231, rFel d 7 (Cat)	Allergenic extracts: Dog dander (e5); Cat dander (e1)
Sample matrix	Serum and plasma (EDTA or sodium heparin)	Serum and plasma (sodium heparin)
Laboratory settings	Clinical laboratories	Clinical laboratories and physician office

Differences		
Item	Device	Predicate
		laboratories
Instruments	Phadia 100, Phadia 250, Phadia 1000, Phadia 2500 and Phadia 5000	UniCAP 250

K. Standard/Guidance Document Referenced:

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

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

CLSI EP07 3rd Edition: Interference Testing in Clinical Chemistry.

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - 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. The fluorescence results are converted to 'kU_A/L' concentrations by the software with the use of a calibration curve.

M. Performance Characteristics:

1. Analytical performance:

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

a. Precision/Reproducibility:

i) Within-laboratory imprecision:
Imprecision of each ImmunoCAP Allergen Component was evaluated by testing five positive plasma samples with concentrations of antigen-specific IgEs spanning the analytical measuring range (AMR). Each sample was tested in four 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-run and within-run coefficients of variance (%CV) were calculated for each sample separately. Results are shown in the table below:

ImmunoCAP Allergen Component	Sample	Number of Test	Mean (kUA/)	Within-run CV (%)	Between-run CV (%)	Total CV (%)
e229, rCan f 4 (Dog)	1	80	0.18	8.61	4.67	9.79
	2	80	0.36	3.22	5.96	6.77
	3	80	1.17	2.67	2.17	3.44
	4	80	13.6	3.30	3.41	4.75
	5	80	73.87	4.40	6.18	7.59
e230, rCan f 6 (Dog)	1	80	0.17	3.32	4.68	5.74
	2	80	0.35	2.48	5.92	6.41
	3	80	1.28	2.02	2.53	3.24
	4	80	17.12	3.65	2.19	4.25
	5	80	59.74	3.98	4.41	5.94
e231, rFel d 7 (Cat)	1	80	0.16	2.24	5.45	5.89
	2	80	0.39	2.59	2.25	3.43
	3	80	1.35	1.65	2.84	3.29
	4	80	17.57	2.02	2.83	3.47
	5	80	76.93	3.29	6.96	7.70

ii) Lot-to-lot imprecision:

Three different lots of each ImmunoCAP Allergen Component: e229, rCan f 4 (Dog); e230, rCan f 6 (Dog); and e231, rFel d 7 (Cat), were tested using three or 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. Negative samples results were all below 0.1 kU_A/L and only positive results are included in the table below. Mean concentration values and %CV (lowest mean concentration/ highest mean concentration between lots) were calculated for the positive samples and are presented in the tables below:

ImmunoCAP Allergen Component e229, rCan f 4 (Dog)						
Lot	Sample Panel					
	Sample 1		Sample 2		Sample 3	
	Mean (kU_A/L)	CV (%)	Mean (kU_A/L)	CV (%)	Mean (kU_A/L)	CV (%)
1	0.27	4.0	3.49	2.6	13.2	3.0
2	0.28	4.2	3.46	3.5	13.1	3.1
3	0.29	1.8	3.53	2.1	14.2	2.5

ImmunoCAP Allergen Component e230, rCan f 6 (Dog)								
Lot	Sample Panel							
	Sample 1		Sample 2		Sample 3		Sample 4	
	Mean (kU_A/L)	CV (%)	Mean (kU_A/L)	CV (%)	Mean (kU_A/L)	CV (%)	Mean (kU_A/L)	CV (%)
1	0.345	2.2	2.14	1.5	17.79	2.3	59.48	3.1
2	0.331	1.8	2.16	3.1	17.71	2.4	59.36	3.1
3	0.361	1.9	2.29	1.8	18.98	3.2	63.62	3.6

ImmunoCAP Allergen Component e231, rFel d 7 (Cat)								
Lot	Sample Panel							
	Sample 1		Sample 2		Sample 3		Sample 4	
	Mean (kU_A/L)	CV (%)	Mean (kU_A/L)	CV (%)	Mean (kU_A/L)	CV (%)	Mean (kU_A/L)	CV (%)
1	0.290	1.9	2.68	1.8	18.8	1.9	78.8	3.6
2	0.285	4.4	2.66	1.1	18.7	1.1	79.3	5.0
3	0.299	2.0	2.71	1.5	18.8	2.1	80.8	1.7

b. Linearity/assay reportable range:

The linearity of each ImmunoCAP Allergen Component was assessed following CLSI guideline I/LA-20 3rd Edition. Three positive samples were each diluted in sample diluent generating at least six 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 each ImmunoCAP Allergen Component.

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 Component e229, rCan f 4 (Dog)						
Sample	r^2	Slope	Intercept	95% CI Slope	95% CI Intercept	Sample concentration range tested (kU_A/L)
1	0.99	1.01	−0.03	1.00–1.02	−0.04–0.02	0.18–46.71
2	1.00	1.01	0.01	1.00–1.01	0.00–0.02	0.10–26.41
3	1.00	0.99	0.01	0.98–1.00	0.00–0.02	0.10–96.58
Pooled	1.00	1.00	0.00	0.99–1.00	–0.01–0.01	0.10–96.58

ImmunoCAP Allergen Component e230, rCan f 6 (Dog)						
Sample	**r^2**	**Slope**	**Intercept**	**95% CI Slope**	**95% CI Intercept**	**Sample concentration range tested (kU_A/L)**
1	1.00	1.00	–0.01	1.00–1.01	–0.02–0.00	0.15–37.00
2	1.00	0.98	0.02	0.98–0.99	0.02–0.03	0.15–65.36
3	1.00	1.00	–0.01	0.99–1.01	–0.02–0.00	0.15–18.26
Pooled	1.00	0.99	0.00	0.99–1.00	0.00–0.01	0.15–65.36

ImmunoCAP Allergen Component e231, rFel d 7 (Cat)						
Sample	**r^2**	**Slope**	**Intercept**	**95% CI Slope**	**95% CI Intercept**	**Sample concentration range tested (kU_A/L)**
1	1.00	1.02	–0.02	1.01–1.03	–0.03–(–0.02)	0.19–12.38
2	1.00	1.01	–0.01	1.01–1.02	–0.01–0.00	0.15–20.96
3	1.00	1.01	0.00	1.00–1.01	–0.01–0.01	0.20–52.34
Pooled	1.00	1.01	–0.01	1.01–1.02	–0.01–(–0.01)	0.15–52.34

The claimed assay ranges claims are summerized in the table below:

ImmunoCAP Allergen Component	Claimed assay range (kU_A/L)
e229, rCan f 4 (Dog)	0.10–97.00
e230, rCan f 6 (Dog)	0.15–65.00
e231, rFel d 7 (Cat)	0.15–52.34

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*:

Calibration curve and onboard stability of the calibrators was previous reviewed in K100999.

Stability studies were performed in accordance with CLSI EP25-A using three lots of each ImmunoCAP Allergen component The accelerated stability data support the 24 month unopened shelf-life stability. The real-time stability study is ongoing and supports six month unopened shelf-life stability.

The studies to determine the stability of the calibration curve, real-time, and on-board stability of ImmunoCAP 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 each ImmunoCAP Allergen Component. 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 ≤20% 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)
e229, rCan f 4 (Dog)	0.001	0.018	0.034	0.088
e230, rCan f 6 (Dog)	0	0.013	0.021	0.063
e231, rFel d 7 (Cat)	0	0.006	0.010	0.031

e. Analytical specificity:

i) *Inhibition studies*:
Immunological specificity of each ImmunoCAP Allergen Component were verified through competitive inhibition. The studies were conducted in accordance with I/LA-20 3rd Edition. For each ImmunoCAP Allergen component, a positive sample was tested and the specific IgE concentration is shown in the table below:

ImmunoCAP Allergen Component	**kU_A/L**
e229, rCan f 4 (Dog)	2.99
e230, rCan f 6 (Dog)	3.32
e231, rFel d 7 (Cat)	3.07

For each ImmunoCAP Allergen component, three unrelated inhibitors (as listed in the Table below) were tested at a dose that is ten-fold or higher than the specific

allergen component concentration that yielded >50% inhibition.

Equal volumes of the positive sample and varying serial dilutions of the three specific allergen components were premixed. The mixture was incubated in a sample tube at room temperature for two hours before being analyzed with each ImmunoCAP Allergen Component 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 percent inhibition values are shown as 0% inhibition.

The following inhibitors for these three allergen components were tested and summarized as follows:

ImmunoCAP Allergen Component	Unrelated inhibitors
e229, rCan f 4 (Dog)	rMal d 3, rOle e 9, rPla 1 1 and rCan f 5
e230, rCan f 6 (Dog)	rCor a 14, rPla 1 1, rVes v 5 and rCan f 5
e231, rFel d 7 (Cat)	rCor a 14, rPla 1 1, rVes v 5 and rFel d 2

The e229, rCan f 4 (Dog) inhibition study showed that >50% inhibition was achieved with the related inhibitor (rCan f 4) at a final inhibitor concentration of 45 µg/mL. The inhibition studies using four unrelated inhibitors, including three unrelated inhibitors(rMal d 3, rOle e 9, rPla 1 1) and one from the related/same group (rCan f 5) did not show any significant inhibition. The inhibition studies indicate that the ImmunoCAP Allergen e220, rCan f 4 (Dog) solid phase contains the immunologically relevant allergen.

The e230, rCan f 6 (Dog) inhibition study showed that >50% inhibition was achieved with the related inhibitor (rCan f 6) at a final inhibitor concentration of 123 µg/mL. The inhibition studies using four unrelated inhibitors, including three from unrelated groups (rCor a 14, rPla 1 1, rVes v 5 and one from the related/same group (rCan f 5) did not show any significant inhibition. The inhibition studies indicate that the ImmunoCAP Allergen e230, rCan f 6 (Dog)

solid phase contains the immunologically relevant allergen.

The e231, rFel d 7 (Cat) inhibition study showed that >50% inhibition was achieved with the related inhibitor (rFel d 7) at a final inhibitor concentration of 27 µg/mL. The inhibition studies using four unrelated inhibitors, including three from unrelated groups (rCor a 14, rPla 1 1, rVes v 5) and one from the related/same group (rFel d 2) did not show any significant inhibition. The inhibition studies indicate that the ImmunoCAP Allergen e231, rFel d 7 (Cat) solid phase contains the immunologically relevant allergen.

ii) *Interference*:

a) Endogenous Substance Interference:

Interference from icteric, hemolytic or lipemic samples was tested. Interferents were spiked into two positive plasma samples and one negative plasma sample for each of the three new recombinant allergens 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. The results are summarized in the table below:

Interferant	**No inhibition up to concentration tested**
Bilirubin C	21.0 mg/dL
Bilirubin F	18.7 mg/dL
Hemoglobin	500 mg/dL
Chyle	1,760 FTU-Formazine Turbidity Units

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 that is determined as 0.1 kU_A/L. All results >0.1 kU_A/L are interpreted as being analytically positive.

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 each ImmunoCAP Allergen Component:rCan f 4, Dog (e229); rCan f 6, Dog (e230); and rFel d 7 (e231) 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 Components. A total of 30 to 37 clinical samples from individuals with a clinical history of allergy-like symptoms upon exposure to the allergens, as diagnosed by a physician, were tested 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 tables:

ImmunoCAP Allergen Component		**Clinical Diagnosis**		
		Atopic	**Non-atopic**	**Total**
rCan f 4, Dog (e229)	**Positive**	26	0	26
	Negative	7	100	107
	Total	33	100	133

Sensitivity = 78.8 % (26/33) 95% CI: 62.2%–89.3%
Specificity = 100% (100/100) 95% CI: 96.0%–100%

ImmunoCAP Allergen Component		Clinical Diagnosis		
		Atopic	**Non-atopic**	**Total**
rCan f 6, Dog (e230)	**Positive**	26	0	26
	Negative	4	100	104
	Total	30	100	130

Sensitivity = 86.7 % (26/30) 95% CI: 70.3%–94.6%
Specificity =100% (100/100) 95% CI: 96.3%–100%

ImmunoCAP Allergen Component		Clinical Diagnosis		
		Atopic	**Non-atopic**	**Total**
e231, rFel d 7 (Cat)	**Positive**	32	0	32
	Negative	5	100	105
	Total	37	100	137

Sensitivity = 86.5% (32/37) 95% CI:72.0%–94.1%
Specificity =100% (100/100) 95% CI: 96.3%–100%

Clinical 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 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.