510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k100999

B. Purpose for Submission:
Modification of the device (Modification of Allergen f20 Almond Components)

C. Measurand:
Allergen specific IgE

D. Type of Test:
Fluoroenzymeimmunoassay, Quantitative and Semi-quantitative

E. Applicant:
Phadia AB

F. Proprietary and Established Names:
ImmunoCAP Allergen f20, Almond

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 Assay is an *in vitro* quantitative assay for the measurement of allergen specific IgE in human serum or plasma. ImmunoCAP specific IgE is to be used with the instruments ImmunoCAP100$^{\varepsilon}$, ImmunoCAP 250 and ImmunoCAP 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, as well as physician office laboratories.
2. Indication(s) for use:
   Same as Intended Use.
3. Special conditions for use statement(s):
   Prescription use only
4. Special instrument requirements:
   ImmunoCAP Specific IgE is to be used with the instrument ImmunoCAP100$^{\varepsilon}$, ImmunoCAP 250 and ImmunoCAP 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 plasma. It is comprised of instrument ImmunoCAP 100$^{\varepsilon}$, ImmunoCAP 250 and ImmunoCAP 1000, test system modules (comprising general, test and method specific reagents), as well as instrument and data management software. The 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.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   ImmunoCAP Specific IgE
2. Predicate 510(k) number(s):
   k051218
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indications for Use	ImmunoCAP Specific IgE is 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, as well as physician office laboratories.	Same
Number of calibrators	Six	Same
Sample matrix	Serum and Plasma	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 ImmunoCAP 100$^{\varepsilon}$. 1 hour 45 minutes for ImmunoCAP 250 and 1000.	Same
Incubation temperature	37°C	Same

Differences		
Item	Device	Predicate
Modification	Modification the proportions of allergen (Almond) components	Absent this modification

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

CLSI I/LA20-A: Evaluation Methods and Analytical Performance Characteristics of Immunological Assays for Human Immunoglobulin E (IgE) Antibodies of Defined Allergen Specificities; Approved Guideline (1997) I/LA20-A.

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

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

Quantitation.

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 patients plasma/serum 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:

Since the updated ImmunoCAP allergen f20, Almond is a modified version of the currently cleared device, a limited precision study was performed. To demonstrate lot-to-lot reproducibility of the updated ImmunoCAP allergen f20, Almond, three lots of ImmunoCAP Allergen f20, Almond were tested using three positive and one negative control samples (stored human plasma). The samples were tested on ImmunoCAP 250. Mean concentration values, concentration quotients, and %CV were calculated. The concentration quotients between lots based on uptake of positive sample were between 0.88 and 1.18, the concentrations of the negative samples were < 0.03 kU_A/L, and all %CV were less than 6.2%. The results were within Phadia's assay specifications.

b. Linearity/assay reportable range:

The linearity of specific IgE of the updated ImmunoCAP Allergen f20, Almond was evaluated by testing three positive samples with concentration at 99 kU_A/L, 63.3 kU_A/L and 50.5 kU_A/L. Each sample was diluted five times in a 3-fold scheme. The undiluted and diluted samples were tested in four replicates in one run on ImmunoCAP 250. One lot of ImmunoCAP Allergen f20, Almond was used. The results were pooled for the regression analysis. The reagent showed dilution linearity for the claimed measuring range to specific IgE to the updated ImmunoCAP Allergen f20, Almond. The provided regression equation is as follows:

$y=1.00x + 0.01$ ($R^2 = 0.99$)

(95% CI for slope 0.98 to 1.02 and for intercept -0.075 to 0.095)

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

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).

The stability studies were performed to demonstrate 24 month stability (at recommended storage temperature of 2-8°C) of the updated ImmunoCAP

Allergen f20, Almond by an on-going real time stability study and accelerated stability study. The study was planned in accordance with CEN 13640. For real time stability study, three lots of the updated ImmunoCAP Allergen f20, Almond were stored at recommended storage temperature, 2-8°C. Two positive and one negative control samples (stored human plasma) were tested in duplicates in one assay at monthly intervals. The mean concentrations were calculated for the positive samples. For accelerated study, one lot of the updated ImmunoCAP Allergen f20, Almond was stored at 30°C for 8 weeks. The same lot stored at 2-8°C was used as reference. Two positive and one negative control samples (stored human plasma) were tested in duplicates in one assay run with the updated ImmunoCAP Allergen f20, Almond stored at 30°C after 4 weeks and 8 weeks. The mean concentrations were calculated for each sample at each test occasion. Concentration quotients were calculated for the positive samples as follows: the concentration for the lot stored at 30°C was divided with the sample concentration for the reference lot [(mean conc. 30°C)/(mean conc. 2-8°C)]. As results, the calculated concentration quotients of positive samples ranged from 1.01-1.06 for 4 weeks and 1.02-1.03 for 8 weeks, while the negative sample remained at <0.1 kU_A/L. The results from accelerated stability study meet Phadia's specification and demonstrate at least 24 months stability from the date of manufacture.

In addition, the stability of calibration curve, real time and on-board stability of ImmunoCAP Specific IgE calibrator were evaluated. The results support Phadia's claims: 1) the ImmunoCAP Specific IgE calibration curve is valid for 28 days; 2) the shelf life of ImmunoCAP Specific IgE Calibrators (0, 0.35, 0.7, 3.5, 17.5, and 100 kU_A/L) is 24 months; 3) the on-board stability for ImmunoCAP Specific IgE Calibrators is 28 days during 24 months shelf life.

d. Detection limit:

Limit of Blank (LoB): In order to demonstrate LoB for the updated ImmunoCAP Allergen f20, Almond, data from comparison study was provided for calculating LoB. In this study, 111 negative samples (N=111) were tested on ImmunoCAP 250. LoB was estimated as the 95% percentile of the distribution. LoB = 0.0195 kU_A/L.

Limit of Detection (LoD): 20 samples with low concentration of analyte were tested in 2 replicates (N=40, f=20). SD was determined and LoD was calculated according to the equation: LoD = LoB + $c_\beta \times$SD while c_β = 1.645/[1-1/(4×f)]. LoD = 0.06 kU_A/L. The claimed LoD is 0.1 kU_A/L.

e. Analytical specificity:

The inhibition study was designed to verify the immunological specificity of almond allergen solution as bound to the updated ImmunoCAP allergen f20, Almond. The study was planned in accordance with CLSI I/LA20-A. In order to establish the theoretical response level for 100% inhibition, (100%), 100 µl of negative sample was premixed with 100 µl of buffer. This is done to mimic the state where all IgE antibodies are bound by the added soluble inhibitor (allergen) and thus leaving no available IgE antibodies that may be

bound to the ImmunoCAP Allergen f20, Almond solid phase.

To establish the maximum response level for 0% inhibition, ("0%"), 100 µl of positive sample was premixed with 100 µl buffer. To show an overall dose dependent inhibition, 100 µl of positive sample was premixed with varying dilutions of allergen solution (inhibitor). The allergen solution was serially diluted with buffer. The initial allergen solution dilution factor is dependent on the concentration of the allergen solution at hand. Subsequent dilutions were then adjusted to the 1/10-dilution sequence. The unrelated allergen solutions were not further diluted. Only concentrated solutions are used for the inhibition studies. The mixture was incubated in a sample tube at room temperature for 1 hour before being analyzed with ImmunoCAP Allergen f20, Almond on ImmunoCAP 250 instrument according to the manufacturer's instructions. The testing was performed in duplicates in one assay run on ImmunoCAP 250. Mean value was calculated. The inhibition (%) at inhibitor dilution factor (x) was calculated according to the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Response}_{(0\%\ \text{inhibition})} - \text{Response}_{(x)}}{\text{Response}_{(0\%\ \text{inhibition})} - \text{Response}_{(100\%\ \text{inhibition})}} \times 100$$

The results are shown below.

Inhibitor	**Dilution factor (1/x, w/v)**	**Response (RU)**	**Inhibition (%)**
100%	-	34	100
0%	-	1376	0
Almond	15000	1068	23
	1500	849	39
	150	580	59
	15	349	76
	1.5	260	83
	Undiluted	234	85
Shrimp allergen	10	1138	13
Dog allergen	10	1328	4
Cat allergen	10	1397	0

The results indicated that the binding of specific IgE to the ImmunoCAP Allergen f20, Almond is inhibited in an overall dose dependant fashion by increasing amounts of the specific allergen extract. Three unrelated allergens show no significant inhibition.

f. Assay cut-off:
Refer to k051218

2. Comparison studies:
   a. Method comparison with predicate device:
   Refer to Clinical Studies
   b. Matrix comparison:
   Not applicable.

3. Clinical studies:
   a. *Clinical Sensitivity/Clinical Specificity:*
      To demonstrate that a higher proportion of almond positive patients are detected with the updated ImmunoCAP Allergen f20, Almond and no elevated values of specific IgE on samples from healthy, non-atopic donors with no reported clinical reaction to the allergen, 46 clinical and 111 negative samples were tested with the updated and the currently cleared ImmunoCAP Allergen f20, Almond. The clinical sample was defined as a sample from an individual with a clinical history of allergy-like symptoms upon exposure to the specific allergen, as diagnosed by a physician. Negative samples were collected from healthy non-atopic donors. The samples were tested in duplicates as one occasion using ImmunoCAP Specific IgE Assay on ImmunoCAP 250. One lot of updated and one lot of currently cleared ImmunoCAP Allergen f20, Almond were used in the study. The results for updated ImmunoCAP Allergen f20, Almond are shown in the table below.

ImmunoCAP Allergen f20, Almond	Samples		
	Clinical	Negative	Total
Positive ImmunoCAP result ($\geq$0.1 kU_A/L)	42	0	42
Negative ImmunoCAP result (<0.1 kU_A/L)	4	111	115
Total	46	111	157

Sensitivity: 91% (42/46)
Specificity: 100% (111/111)

By using relevant clinical samples, the updated ImmunoCAP Allergen f20, Almond showed increased sensitivity [91% (42/46)] compared to the currently cleared product [63% (29/46)]. All the negative samples (N=111) showed undetectable level (<0.1 kU_A/L) of allergen specific IgE with the updated ImmunoCAP Allergen f20, Almond.

   b. *Other clinical supportive data:*
      Not applicable
4. Clinical cut-off**:**
   Refer to k051218
5. Expected values/Reference range:
   0.35 kU_A/L is the recommended cut off. 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.