

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

A. 510(k) Number:

k121463

B. Purpose for Submission:

Modification of the device (Modification of Allergen k82 Latex Components)

C. Measurand:

Allergen specific IgE

D. Type of Test:

Fluoroenzymeimmunoassay, Quantitative

E. Applicant:

Phadia AB

F. Proprietary and Established Names:

ImmunoCAP Allergen k82, Latex

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, 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 (k051218/A002)
Phadia 2500, Phadia 5000 (k051218/A003)

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, Phadia 1000, Phadia 2500 and Phadia 5000 test system modules, 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. The method specific reagents consist of individual purified allergen covalently coupled to a solid phase in a plastic housing.

J. Substantial Equivalence Information:

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

ImmunoCAP Allergen k82, Latex (k972068)

2. Comparison with predicate:

Similarities		
Item	New 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
Assay type	Quantitative	Same
Basic principle	Fluoroenzyme immunoassay	Same
Detection Antibody	β -Galactosidase-anti-IgE (mouse monoclonal antibody) for all ImmunoCAP	Same
Substrate	MUG (4-Methylumbelliferyl- β -D-galactoside)	Same
Number of calibrators	Six (0, 0.35, 0.7, 3.5, 17.5, and 100 kU _A /L)	Same
Controls	Two	Same
Sample volume	40 μ L	Same
Process time	2 hours 30 minutes for Phadia 100. 1 hour 45 minutes for Phadia 250, 1000, 2500 and 5000.	Same
Incubation temperature	37°C	Same

Differences		
Item	New Device	Predicate
Modification of Allergen	Adding recombinant protein Hev b 5 component to native extract of allergen	Native extract of allergen

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.

CEN 13640: 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 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 test 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 allergen 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. The results are summarized in the following tables:

Sample	n	Mean (kU _A /L)	Between Days (CV%)	Within Run (CV%)	Total (CV%)
1	80	2.35	1.25	2.49	2.79
2	80	0.30	2.63	3.19	4.14

ii. *Lot-to-Lot Imprecision:*

Three different lots of the updated ImmunoCAP Allergen k82 from routine production were tested using two positive samples ($0.35 \pm 25\%$ 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. 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. The acceptance criteria for lot-to-lot precision include:

- Positive sample quotient of mean concentration: 0.7 to 1.3
- Negative sample mean concentration: < 0.1 kU/l
- CV: < 12% for sample concentrations \geq 0.1 kU/l

The results for lot-to-lot precision study were within company's assay specifications and are summarized in the following table:

Lot	Positive 1		Positive 2		Negative
	Mean (kU _A /L)	CV (%)	Mean (kU _A /L)	CV (%)	Mean (kU _A /L)
1	2.35	1.8	0.27	2.3	<0.1
2	2.37	2.3	0.30	3.7	<0.1
3	2.30	2.7	0.28	4.8	<0.1
Quotient Lot1/Lot2	0.99		0.92		
Quotient Lot1/Lot3	1.02		0.95		
Quotient Lot2/Lot3	1.03		1.04		

b. Linearity/assay reportable range:

The linearity was assessed following the CLSI/LA20-A2 guidelines using one lot of the updated ImmunoCAP Allergen k82, Latex. The assay was performed according to the ImmunoCAP Specific IgE, Directions for Use using instrument Phadia 250. The working range for ImmunoCAP Specific Total IgE is LoD to 100 kU_A/L.

Three positive samples were each diluted in negative sample generating at least five 2-fold consecutive dilutions (the highest level tested was 147.54 kU_A/L). Undiluted and diluted samples were tested in four replicates in one assay run. The results of the replicates from all three samples for each level were pooled. The observed values were graphed against the expected values and linear regression was performed. The provided regression equation is as follows:

$$y=0.98x + 0.01 (R^2 = 0.99)$$

(95% CI for slope 0.97 to 0.98 and for intercept 0.00 to 0.03)

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

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).

Stability:

The real time stability study was performed to demonstrate 24-month stability of the updated ImmunoCAP Allergen k82, Latex at recommended storage temperature of 2-8°C. The study was planned in accordance with CEN 13640 (Stability Testing of In Vitro Diagnostic Reagents). Three lots of the updated ImmunoCAP Allergen k82, Latex were stored at recommended storage temperature, 2-8°C. The assay was performed according to the ImmunoCAP Specific IgE, Directions for Use, using Phadia 250. Two positive and one negative control samples (stored human plasma) were tested in duplicates in one assay at different time intervals (0, 3, 6, 12, 24, 25 months). The mean concentrations were calculated for each sample at each test occasion. The results were compared to the results of the same samples tested at time 0. The results support the manufacture's claim of 24 months stability at 2-8°C.

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 the updated ImmunoCAP Allergen k82, Latex 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	LoB (kU _A /L)	LoD (kU _A /L)
k82, Latex	0.041	0.051

e. Analytical specificity:

Immunological specificity of latex allergen solution as bound to the updated ImmunoCAP Allergen k82, Latex was verified through competitive inhibition. The study was planned in accordance with CLSI I/LA20-A2.

The allergen solution was serially diluted with buffer to show an overall dose dependent inhibition. A positive sample with the specific IgE concentration at 10.1 kU_A/L was used in the study. Equal volumes of the 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 ImmunoCAP Allergen k82, Latex on Phadia 250 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 inhibitions study showed that about 50% inhibition was achieved with related inhibitor (k82, Latex) at a final inhibitor concentration of ~3.4 µg/mL. The inhibition studies using four unrelated inhibitors, including three from unrelated groups (Aspergillus terreus, Storage mite, and Sunflower) and one from the related/same group (k84, Sunflower seed) did not show any significant inhibition at 3.4 mg/mL inhibitor concentration. The inhibition studies indicate that the ImmunoCAP Allergen k82, Latex solid phase contains the immunologically relevant allergen.

f. Assay cut-off:

Limit of Quantitation for ImmunoCAP Specific IgE is 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 k113841

3. Clinical studies:

a. Clinical Sensitivity and Clinical Specificity:

To demonstrate that the updated ImmunoCAP Allergen k82, Latex performs

at least the same as the currently cleared device with no detectable levels of specific IgE in samples from healthy, non-atopic donors. Seventy-five (75) clinical and 100 negative samples were tested with the updated and the currently cleared ImmunoCAP Allergen k82, Latex. The clinical samples were defined as samples from individuals 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 singlicate using ImmunoCAP Specific IgE Assay on Phadia 250. One lot of the updated and one lot of currently cleared ImmunoCAP Allergen k82, Latex were used in the study. The results for the updated ImmunoCAP Allergen k82, Latex are shown in the table below:

Updated ImmunoCAP Allergen k82, Latex	Samples		
	Clinical	Non-atopic	Total
Positive ImmunoCAP result (≥ 0.1 kU _A /L)	75	0	75
Negative ImmunoCAP result (< 0.1 kU _A /L)	0	100	100
Total	75	100	175

Sensitivity: 100% (95% CI: 95.2 – 100.0%)

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

By using relevant clinical samples, the updated ImmunoCAP Allergen k82, Latex showed higher sensitivity [100% (75/75)] compared to the currently cleared product [96% (72/75)]. No specific IgE antibodies to the updated ImmunoCAP Allergen k82, Latex were detected with samples from healthy, non-atopic donors.

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.