

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

A. 510(k) Number:

K150597

B. Purpose for Submission:

New device

C. Measurand:

Allergen specific IgE for rDer p 1

D. Type of Test:

Quantitative assay, automated immunofluorescence

E. Applicant:

Phadia US, Inc.

F. Proprietary and Established Names:

ImmunoCAP specific IgE
ImmunoCAP Allergen d202, Allergen component rDer p 1, House dust mite

G. Regulatory Information:

1. Regulation section:

21 CFR § 866.5750, Radioallergosorbent (RAST) immunological test system

2. Classification:

Class II (Assays)

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, 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:

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 serum, sodium heparin plasma or EDTA 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 are modular in concept and are available individually. The 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 consists of purified allergen (recombinant) covalently coupled to a support in a plastic housing.

J. Substantial Equivalence Information:

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

ImmunoCAP Allergen, component nDer p 1; K101251

2. A. Comparison with predicate:

| Similarities | | |
|---|---|-----------|
| Item | New Device | Predicate |
| Intended Use/Indications for Use | ImmunoCAP Specific IgE is an <i>in vitro</i> 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, Phadia 1000, Phadia 2500 and Phadia 5000. 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 |
| Sample Matrix | Human serum or plasma (EDTA or Na-Heparin) | Same |
| Assay system (Calibrators, Conjugate, Controls and Instruments) | ImmunoCAP Specific IgE run on Phadia 100, Phadia 250, Phadia 1000 or Phadia 2500 and Phadia 5000. | Same |
| Type of Test | Quantitative | Same |
| Assay test principle | Immunofluorescence | Same |
| Software | Phadia Information Data Manager | Same |
| Analytical sensitivity (LoD/LoQ) | 0.1 kU _A /L | Same |

| Differences | | |
|-------------------|-------------------------------|--|
| Item | New Device | Predicate |
| Allergen material | Recombinant, purified protein | Native, purified protein from allergen extract |

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

- CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Procedures, Second Edition.
- CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second edition.
- CLSI EP25-A: Evaluation of Stability of *in vitro* Diagnostic Reagents.
- 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.
- ISO 14971 Second edition 2007, Medical devices – application of risk management to medical devices.

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 is 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 allergen component was evaluated by using two positive plasma samples including a low range sample ($0.35 \pm 25\%$ kU_A/L) and a high range sample (≥ 0.7 kU_A/L), each 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 Phadia 250 instrument. Between-day and within-run coefficients of variance (%CV) were calculated. Results of the study are presented below and %CV values were all within the acceptance criteria (< 15%):

| ImmunoCAP Allergen | Sample | N | Mean (kU _A /L) | Between-run %CV | Within-run %CV | Total %CV |
|----------------------------------|--------|----|---------------------------|-----------------|----------------|-----------|
| d202, rDer p 1 (House Dust mite) | 1 | 80 | 0.29 | 3.26 | 3.10 | 4.50 |
| | 2 | 80 | 2.38 | 3.32 | 2.95 | 4.44 |

| ImmunoCAP Allergen | %CV for pooled samples | | | |
|----------------------------------|------------------------|-----------------|----------------|-----------|
| | Number of sample pool | Between-run %CV | Within-run %CV | Total %CV |
| d202, rDer p 1 (House Dust mite) | 2 | 3.29 | 3.03 | 4.47 |

ii. Lot-to-lot Imprecision

Three ImmunoCAP Allergen Component lots were tested using two positive samples ($0.35 \pm 25\%$ and $\geq 0.7 \text{ kU}_A/\text{L}$) and one negative sample ($< 0.1 \text{ kU}_A/\text{L}$). Each lot of samples was 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 instrument. Mean concentration values, %CV and concentration quotients between lots were calculated for the positive samples. The results are all within the acceptance criteria ($< 12\%$).

| 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 |
| 1 | 1.97 | 4.7 | 0.31 | 4.1 | < 0.1 | lot1/ lot 2 | 1.00 | 1.00 |
| 2 | 1.97 | 2.9 | 0.31 | 2.7 | < 0.1 | lot 1/ lot 3 | 0.96 | 0.99 |
| 3 | 2.06 | 2.9 | 0.32 | 4.7 | < 0.1 | lot 2/ lot 3 | 0.95 | 0.99 |

b. *Linearity/assay reportable range:*

The linearity was assessed following the CLSI I/LA20-A2 guidelines. For this study, three positive plasma samples were each diluted in negative plasma generating at least five 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 Phadia 250 instrument. One lot of ImmunoCAP Allergen Component was used. ImmunoCAP Specific IgE working range is LoD to 100 kU_A/L.

Results of the replicates from all three samples were pooled and analyzed for linearity. Regression statistics 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) |
|---------------------------------|---------------------|----------------|--------------|------------------|---|
| d202, rDer p 1, House Dust Mite | $y=1.00x+(-0.024)$ | 1.00 | 0.99–1.01 | -0.03–(-0.016) | 75.6 |

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 from World Health Organization (WHO).

ii. Kit Stability

Real-time and Accelerated stability: The stability studies were performed in accordance with CLSI EP25-A: *Evaluation of Stability of In Vitro Diagnostic Reagents*. Accelerated stability studies demonstrated 24 months unopened shelf-life stability of d202, rDer p 1 dust mite allergen. For real-time stability study, three lots of ImmunoCAP Allergen Component d202 were stored at recommended storage temperature, 2–8°C. Two positive plasma samples (0.8 and 1.2 kU_A/L) and one negative plasma sample (<0.1 kU_A/L) were tested at different occasions according to the ImmunoCAP Specific IgE, Directions for Use, using Phadia 250 instrument. The study is ongoing.

Accelerated stability was assessed with three lots of ImmunoCAP Allergen Component d202 stored at 30°C and tested after four and eight 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 manufacturer's claim of 24 months.

The stability of the calibration curve, real time and on-board stability of ImmunoCAP Specific IgE Calibrators are detailed in K100999.

d. *Detection limit:*

The Limit of Blank (LoB) and the Limit of Detection (LoD) were determined for d202, rDer p 1 allergen component on the Phadia 250 instrument according to CLSI EP17-A guideline. The LoB was based on single determinations of 100 negative samples (blank samples) and was estimated as the 95th 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 three low positive samples, in total 60 determinations (20 determinations of three low positive samples). The results are shown in the table below.

| ImmunoCAP Allergen | LoB (kU_A/L) | LoD (kU_A/L) |
|---------------------------|-------------------------------|-------------------------------|
| d202, rDer p 1 | 0.0210 | 0.0340 |

e. *Analytical specificity:*

i. Inhibition studies

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

| ImmunoCAP Allergen | (kU _A /L) |
|--------------------|----------------------|
| d202, rDer p 1 | 9.4 |

The allergen solution was serially diluted with buffer to show an overall dose dependent inhibition. The unrelated allergen solutions were 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 one hour before being analyzed with the corresponding ImmunoCAP Allergen Component on Phadia 250 instrument according to the manufacturer's instructions. The testing was performed in duplicate in one assay run and 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

The results of the inhibition with the allergen solution and the unrelated inhibitors indicate that ImmunoCAP Allergen d202; Allergen component rDer p 1, House dust mite contains the immunologically relevant allergen as shown below:

The House dust mite d202, rDer p 1 allergen inhibition study showed that $\geq 85\%$ inhibition was achieved with the related inhibitor (rDer p1 allergen) at a final inhibitor concentration of 40 $\mu\text{g/mL}$. The inhibition studies using three unrelated inhibitors, including three from unrelated groups (rPla l 1, Plantain; rPla a 1, London Plane; and rFel d 2, Cat) and one from the related/same group (rDer p 2, House dust mite) did not show any significant inhibition at the highest inhibitor concentration of 0.4 mg/mL. The inhibition studies indicate that the ImmunoCAP Allergen d202, rDer p1, House dust mite solid phase contains the immunologically relevant allergen.

ii. Interference

a. *Endogenous Substance Interference*

In order to demonstrate that icteric, hemolytic or lipemic samples do not adversely affect the results in ImmunoCAP Specific IgE assays using representative allergens, bilirubin C [final concentration (fc) 20 mg/dL], bilirubin F (fc 19 mg/dL), hemoglobin (fc 489 mg/dL) and chyle (1,440 Formazine Turbidity Units) were spiked and analyzed in duplicate in one assay run using Phadia 250. The results demonstrate that icteric, hemolytic or lipemic samples do not adversely affect the results in ImmunoCAP Specific IgE assays.

b. *Exogenous Substance Interference*

Two literature references were provided to support 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 0.10 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:*

Demonstration that different sample matrices (heparin plasma, EDTA plasma and serum) are interchangeable for representative ImmunoCAP Allergen Components was provided in the clearance for K101251. Serum, sodium heparin plasma, and EDTA plasma samples were collected from four patients with known clinical history of specific allergies and four non-atopic 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 two replicates in one assay run using Phadia 250 instrument. 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 ImmunoCAP allergen d202 components, rDer p 1 and nDer p 1, was compared. The objective of this study was (i) to demonstrate that the rDer p 1 Allergen Component d202 performs as well as the currently cleared product using samples with clinical information of house dust mite allergy, 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 rDer p1 component of ImmunoCAP Allergen d202. At least 35 clinical serum samples (atopic) 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. A total of 100 negative (non-atopic) samples ($< 0.35 \text{ kU}_A/\text{L}$) from healthy non-atopic donors with no reported clinical reaction to the allergen were also tested. The assay was performed according to ImmunoCAP Specific IgE Directions for Use using Phadia 250 instrument. Results are presented in the table below:

| | | Clinical Diagnosis | | |
|--|----------|--------------------|------------|-------|
| | | Atopic | Non-atopic | Total |
| Allergen d202, rDer p 1 (House Dust mite) | Positive | 28 | 0 | 28 |
| | Negative | 7 | 100 | 107 |
| | Total | 35 | 100 | 135 |

Sensitivity = 80% (95% CI: 63.1–91.6)

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

All negative samples showed an undetectable level ($< 0.35 \text{ kU}_A/\text{L}$) of allergen specific IgE. The assay was performed according to ImmunoCAP Specific IgE Directions for Use using Phadia 250.

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 ($< 0.35 \text{ kU}_A/\text{L}$) 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.