



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

I Background Information:

A 510(k) Number

K200279

B Applicant

Phadia AB

C Proprietary and Established Names

ImmunoCAP Allergen o215, Component nGal-alpha-1,3-Gal(alpha-Gal) Thyroglobulin, bovine

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
DHB	Class II	21 CFR 866.5750 - Radioallergosorbent (Rast) Immunological Test System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

Addition of an allergen to a cleared device

B Measurand:

Allergen-specific IgE for o215, Component nGal-alpha-1,3-Gal (alpha-Gal) Thyroglobulin, bovine

C Type of Test:

Fluoroenzymeimmunoassay, Quantitative

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

ImmunoCAP Allergen o215, Component nGal-alpha-1,3-Gal (alpha-Gal) Thyroglobulin, bovine, is intended for in vitro diagnostic use, in human serum or EDTA plasma, as an aid in the diagnosis of IgE mediated mammalian (red) meat hypersensitivity, due to alpha-Gal sensitization, and to be used in conjunction with other clinical findings. This test is not to be the sole criterion for diagnosing allergy to alpha-Gal. It is a quantitative test to be used in clinical laboratories. ImmunoCAP Allergen o215, alpha-Gal is to be used with the ImmunoCAP Specific IgE assay on the instrument Phadia 250.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

For use on the Phadia 250 instrument

IV Device/System Characteristics:

A Device Description:

The ImmunoCAP system is a fully integrated and automated system for the determination of specific IgE in human blood serum or plasma samples. It is comprised of general, and test- and method-specific reagents for Phadia 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.

B Principle of Operation:

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.

V Substantial Equivalence Information:

A Predicate Device Name(s):

UniCAP system, UniCAP Specific IgE Assay and UniCAP Specific IgE Conjugate 100 and Conjugate 400

B Predicate 510(k) Number(s):

K051218

C Comparison with Predicate(s):

Device & Predicate Device(s):	Device K200279	Predicate K051218
Device Trade Name	ImmunoCAP Allergen o215, alpha-Gal	UniCAP Specific IgE
General Device Characteristic Similarities		
Intended Use/ Indications for Use	ImmunoCAP Allergen o215, Component nGal-alpha-1,3-Gal (alpha-Gal) Thyroglobulin, bovine, is intended for in vitro diagnostic use, in human serum or EDTA plasma, as an aid in the diagnosis of IgE mediated mammalian (red) meat hypersensitivity, due to alpha-Gal sensitization, and to be used in conjunction with other clinical findings. This test is not to be the sole criterion for diagnosing allergy to alpha-Gal. It is a quantitative test to be used in clinical laboratories. ImmunoCAP Allergen o215, alpha-Gal is to be used with the ImmunoCAP Specific IgE assay on the instrument Phadia 250.	UniCAP 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
Type of Test	Quantitative	Same
Test Principle	Fluoroenzyme immunoassay	Same
Software	Phadia Information Data Manager or Phadia Prime	Same
Detection antibody	β-Galactosidase-anti-human IgE	Same

	(mouse monoclonal antibody)	
Number of calibrators	Six	Same
Sample Matrix	Human serum or plasma (EDTA)	Same
Sample volume	40 µL	Same
Analytical Sensitivity (LoD/LoQ)	0.1 kU _A /L	Same

VI Standards/Guidance Documents Referenced:

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

CLSI EP06-A, Evaluation of Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

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

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

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

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

1. Precision/Reproducibility:

i) Within-laboratory imprecision:

Imprecision of the ImmunoCAP Allergen o215, alpha-Gal was evaluated by testing three positive samples including a low-level sample near the medical decision point ($0.35 \pm 25\%$ 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 a Phadia 250 instrument. Between-run and within-run coefficients of variance (% CVs) were calculated for each sample separately. Results are shown in the table below:

Specimen	N	Mean (kU _A /L)	Within-Run (Repeatability)		Between-Run (Repeatability)		Total (Within-Laboratory)	
			SD	% CV	SD	% CV	SD	% CV
1	80	0.18	0.02	9.2	0.02	8.9	0.02	12.8
2	80	0.42	0.02	5.1	0.02	5.1	0.03	7.3
3	80	3.03	0.07	2.4	0.11	2.4	0.13	4.4

N=number of replicates

ii) Lot-to-lot imprecision:

Three different lots of ImmunoCAP Allergen o215, alpha-Gal were tested using three positive 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. The assay was performed according to the ImmunoCAP Specific IgE, Directions for Use, using a 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:

Lot	Sample Panel					
	Positive 1		Positive 2		Positive 3	
	Mean (kU _A /L)	CV (%)	Mean (kU _A /L)	CV (%)	Mean (kU _A /L)	CV (%)
1	0.42	6.3	2.52	3.2	16.5	3.4
2	0.39	7.3	2.60	3.4	17.0	1.7
3	0.38	9.0	2.54	3.0	16.4	3.4

2. Linearity:

The linearity of ImmunoCAP Allergen o215, alpha-Gal was assessed following CLSI guideline I/LA-20 3rd Edition. Three positive samples were each diluted to generate 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 on the Phadia 250 instrument according to the ImmunoCAP Specific IgE, Directions for Use using one lot of ImmunoCAP Allergen o215 reagent. Results of the replicates from all three samples were analyzed for linearity. Regression statistics comparing the observed results to expected results are presented below:

Sample	Concentration range tested (kU _A /L)	r ²	Slope (95% CI)	Intercept (95% CI)
1	0.11 – 14.9	1.00	1.00 (0.98 – 1.02)	-0.07 (-0.19 – 0.05)
2	0.16 – 23.8	1.00	1.00 (0.95 – 1.03)	-0.10 (-0.49 – 0.29)
3	0.15 – 47.3	1.00	0.99 (0.95 – 1.03)	-0.38 (-1.14 – 0.39)
4	0.13 – 86.3	1.00	1.04 (1.03 – 1.05)	-0.06 (-0.07 – -0.05)
Pooled	0.11 – 86.3	1.00	1.03 (1.03 – 1.04)	-0.05 (-0.06 – -0.04)

The claimed assay range of ImmunoCAP Allergen o215, alpha-Gal is 0.1 to 86.3 kU_A/L.

3. Analytical Specificity/Interference:

i) *Inhibition studies:*

Immunological specificity of ImmunoCAP Allergen o215, alpha-Gal was verified by performing competitive inhibition studies in accordance with I/LA-20 3rd Edition. A positive sample with known concentration of IgE specific to allergen component o215 (4.8 kU_A/L) was tested. Equal volumes of the positive sample and varying serial dilutions of the specific allergen component o215 in solution were premixed. The mixture was incubated in a sample tube at room temperature for two hours before being analyzed with ImmunoCAP Allergen o215, alpha-Gal 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 (in kU_A/L) were calculated. The inhibition values in % were calculated according to the formula below:

$$\left(1 - \left(\frac{r - b}{t - b}\right)\right) \times 100 = i\%$$

r = concentration [kU_A/L]

b = background concentration (100% inhibition) [kU_A/L]

t = total concentration (0% inhibition) [kU_A/L]

i = inhibition

Results from the dose-dependent inhibition study showed that >50% inhibition was achieved with the specific allergen component o215 in solution at a concentration of 6.5 pmol/μL.

To further evaluate the immunological specificity of ImmunoCAP Allergen o215, alpha-Gal, three allergens from different or unrelated allergen groups [nGal d 1 (ovomucoid), Pla l 1 (Plantain) and rPhl p 11 (grass pollen)] and one allergen from the same or related allergen group [cross-reactive carbohydrate determinants (CCD)] were tested at doses that are 10-fold or higher than the specific allergen component concentration that yielded

>50% inhibition. None of the allergens tested show any significant inhibition. The inhibition studies indicate that the ImmunoCAP Allergen o215 solid phase contains the immunologically relevant allergen component.

ii) *Interference:*

a) *Endogenous Substance Interference:*

Interferences were assessed by testing two positive samples and one negative sample. Each sample was spiked with the interfering substances or substance-specific diluents and analyzed in four replicates in one assay run using the Phadia 250 according to the ImmunoCAP Specific IgE, Directions for Use. The data demonstrated that ImmunoCAP Allergen o215, alpha-Gal was not adversely affected by high levels of the following substances up to the concentrations listed in the table below:

Interferent	No inhibition up to the tested concentrations
Bilirubin, unconjugated	327.0 µmol/L
Bilirubin, conjugated	363.0 µmol/L
Chyle	1610.0 FTU (Formazine Turbidity Units)
Hemoglobin	4.9 g/L
Rheumatoid factor	550 IU/mL

b) *Exogenous Substance Interference:*

Two literature references were provided supporting the claim 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.

4. Assay Reportable Range:

The claimed measuring range is from 0.1 to 86.3 kU_A/L.

5. 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:*

A real-time stability study was performed using three lots of ImmunoCAP Allergen o215, alpha-Gal in accordance with CLSI EP25-A to demonstrate shelf-life stability (from the date of manufacture). The stability data support 24 months unopened shelf-life stability.

The studies to determine the stability of the calibration curve calibrators, real-time, and on-board stability of ImmunoCAP IgE calibrator are described in K100999.

6. Detection Limit:

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were determined on the Phadia 250 in alignment with the CLSI guideline EP17-A2 using two lots of ImmunoCAP Allergen o215, alpha-Gal.

The LoB was determined by testing five blank samples in three runs with five replicates per run to yield a total of 75 replicates per lot for all samples. The LoB was estimated as the 95% percentile of the distribution and determined as 0 kU_A/L and 0.013 kU_A/L for the two lots of ImmunoCAP Allergen o215, alpha-Gal. The claimed LoB is 0.013 kU_A/L.

The LoD was determined by testing five low positive samples in three runs with five replicates to yield a total of 75 replicates per lot for all samples. The LoD was calculated according to the equation: $LoD = LoB + c\beta \cdot SDLoD$, where SDLoD is the pooled SD for the samples, and determined as 0.021 kU_A/L and 0.025 kU_A/L for the two lots of ImmunoCAP Allergen o215, alpha-Gal. The claimed LoD is 0.1 kU_A/L.

The LoQ was estimated as the mean value of the sample which fulfills the specification for the total within-laboratory imprecision $\leq 20\%$ CV. The LoQ was determined as 0.025 kU_A/L and 0.034 kU_A/L for the two lots of ImmunoCAP Allergen o215, alpha-Gal. The precision profile supports the LoQ claim of 0.1 kU_A/L which is the lower limit of the measuring range claimed for ImmunoCAP Allergen o215, alpha-Gal.

7. 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.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Refer to clinical studies.

2. Matrix Comparison:

To demonstrate that K₂-EDTA plasma samples yield results comparable with serum samples by the ImmunoCAP Allergen o215, alpha-Gal, a study was performed by using 40 serum/K₂-

EDTA plasma paired samples. None of the samples were contrived. Paired samples were each tested according to ImmunoCAP Specific IgE Directions for Use in one replicate in one assay run on one Phadia 250 instrument using one lot of ImmunoCAP Allergen o215, alpha-Gal. The Passing-Bablok regression analysis was performed, and the results are summarized in the following table:

Comparison	N*	Sample Range (kU _A /L)	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient (r)
K ₂ -EDTA plasma vs. serum	31	0.14–85.0	0.99 (0.97 – 1.00)	0.001 (-0.002 – 0.03)	1.00
* Excludes five samples with results below the measuring range for both matrices and four samples with results above the measuring range for both matrices.					

C Clinical Studies:

1. Clinical Sensitivity and Specificity:

Serum samples from 200 patients presenting to the University of Virginia Allergy Clinic with urticaria, anaphylaxis, or other symptoms following intake of red meat, were evaluated retrospectively (Study Group A: Allergic to red meat). Sixty allergic individuals without known alpha-Gal mediated red meat hypersensitivity were included as atopic controls in the study. Of these, 30 presented known allergies to foods, including animal-derived foods other than red meat (Study Group B: Atopic controls/ Other foods), and 30 were allergic to airborne allergens (Study Group C: Atopic controls/ Inhalants). Additionally, samples from 50 individuals, with no history of allergic disease and no evidence of atopic sensitization (Study Group D: Non-atopic controls), were analyzed. A summary of the demographic data per study group is presented in the table below.

	Study Group A (N=200)	Study Group B (N=30)	Study Group C (N=30)	Study Group D (N=50)
	Allergic to red meat	*Atopic controls/ Other foods	^Atopic controls/ Inhalants	Non-atopic controls
Gender				
Male/Female	87 / 113	18 / 12	11 / 19	25 / 25
Ethnicity				
Caucasian	189	2	12	0
African American	3	1	1	0
Asian	4	1	0	0
Hispanic	1	0	0	0
Other	1	1	1	0
Not recorded	2	25	16	50
Age at blood draw				
Years Median [Q1–Q3]	53.0 [43.0–64.0]	11.5 [7.2–21.8]	32.5 [19.8–46.2]	47.5 [39.2–54.8]
* Allergic to foods other than red meat ^ Allergic to inhalants but not red meat				

Allergy-related case history of the subjects in Study Groups A–C including history of food reactions, history of tick bites, doctor’s diagnosis of asthma and history of wheeze over the past 5 years are summarized in the table below.

	Study Group A (N=200)	Study Group B (N=30)	Study Group C (N=30)
	Allergic to red meat	*Atopic controls/ Other foods	^Atopic controls/ Inhalants
History of wheeze 5 years			
Yes/No/Unknown	59 / 139 / 2	10 / 5 / 15	11 / 11 / 8
Doctor's diagnosis of Asthma			
Yes/No/Unknown	33 / 166 / 1	8 / 7 / 15	13 / 17 / 0
History of food reaction			
Yes/No/Unknown [#]	39 / 159 / 2	30 / 0 / 0	13 / 17 / 0
History of tick or chigger bites			
Yes/No/Unknown	197 / 2 / 1	1 / 4 / 25	11 / 3 / 16
Total IgE levels (kU_A/L)			
Median [Q1–Q3]	146 [66–265]	516 [175–1039]	144 [54–263]
* Allergic to foods other than red meat ^ Allergic to inhalants but not red meat # Excluding reactions to red meat			

The reactions to red meat and dairy products or other foods in Study Group A is summarized in the table below:

Clinical Features	Positive Cases (Positive Rate)
Gastro intestinal (GI) symptoms to red meat	125/9 (63%)
Skin symptoms to red meat	184 (92%)
Anaphylaxis to red meat	111 (56%)
Reactions to dairy products	16 (8%)
Reactions to other food(s)	39 (20%)

Among the patients with a red meat hypersensitivity (Group A), 97.5% (195/200) were found to be sensitized to alpha-Gal as measured by ImmunoCAP Allergen o215, alpha-Gal. Consistent with the ubiquitous presence of alpha-Gal in non-primate mammals, more than 75% of these patients also had specific IgE to other animal allergens tested: 98% were sensitized to beef (f27), 78% to cat dander (e1), 86% to dog dander (e5), 94% to pork (f26) and 93% to cow’s milk (f2). Consistent with the absence of alpha-Gal in non-mammalian animals, only 5% and 18% were sensitized to chicken (f83) and egg white (f1), respectively. Among the atopic controls (Study Groups B and C), 16.7% were sensitized to alpha-Gal. None of the non-atopic controls in Study Group D showed sensitization either to alpha-Gal or beef.

In total, 195 of 200 patients with a red meat hypersensitivity had specific IgE antibodies to alpha-Gal, corresponding to a clinical sensitivity of 97.5%. Of the 110 control subjects (60 atopic and 50 non-atopic), 10 (five food allergic subjects and five inhalant allergic subjects) were

sensitized to alpha-Gal. Clinical performance, sensitivity (Se) and specificity (Sp) was calculated based on the detection limit of 0.1 kU_A/L. The results are summarized in the following table:

		Clinical Diagnosis of red meat hypersensitivity		
		Present	Absent	Total
ImmunoCAP Allergen Component o215	Positive ≥ 0.1 kU _A /L	195	10	205
	Negative < 0.1 kU _A /L	5	100	105
	Total	200	110	310
Sensitivity = 97.5% (195/200) 95% CI: 94.3 – 99.2%				
Specificity = 90.9% (100/110) 95% CI: 83.9 – 95.6%				

The performance of samples from atopic controls and non-atopic subjects is summarized below:

Study Groups	N Total	N Negative (< 0.1 kU _A /L)	Specificity (%) (95% CI)
All atopic controls (Study Groups B and C)	60	50	83.3 (72.0 – 90.7)
All atopic controls (Study Group B)	30	25	83.3 (66.4 – 92.7)
All atopic controls (Study Group C)	30	25	83.3 (66.4 – 92.7)
Non-atopic controls (Study Group D)	50	0	100 (92.9 – 100)
Total number of samples	200		
N=Number			

The specificity obtained from the atopic controls (83.3%) agrees with previous random cohort studies indicating that 15% or more of the general U.S. population is sensitized to alpha-Gal ¹⁻³.

D Clinical Cut-Off:

Not applicable

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- ¹ Kennedy JL, Stallings AP, Platts-Mills TA, Oliveira WM, Workman L, James HR, Tripathi A, Lane CJ, Matos L, Heymann PW, Commins SP. 2013. Galactose-alpha-1,3-galactose and delayed anaphylaxis, angioedema, and urticaria in children. *Pediatrics* 131: e1545-52
 - ² Chung CH, Mirakhur B, Chan E, Le QT, Berlin J, Morse M, Murphy BA, Satinover SM, Hosen J, Mauro D, Slebos RJ, Zhou Q, Gold D, Hatley T, Hicklin DJ, Platts-Mills TA. 2008. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. *The New England journal of medicine* 358: 1109-17
 - ³ Commins SP, Kelly LA, Ronmark E, James HR, Pochan SL, Peters EJ, Lundback B, Nganga LW, Cooper PJ, Hoskins JM, Eapen SS, Matos LA, McBride DC, Heymann PW, Woodfolk JA, Perzanowski MS, Platts-Mills TA. 2012. Galactose-alpha-1,3-galactose-specific IgE is associated with anaphylaxis but not asthma. *Am J Respir Crit Care Med* 185: 723-30

E Expected Values/Reference Range:

The expected value determined in healthy, non-sensitized, individuals is $< 0.1 \text{ kU}_A/\text{L}$. Each laboratory is recommended to establish its own expected range of values.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.