

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY

I Background Information:

A 510(k) Number

K191510

B Applicant

Hycor Biomedical

C Proprietary and Established Names

Noveos Specific IgE (slgE), Capture Reagent D002, *Dermatophagoides farinae*

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:

New Device

B Measurand:

Allergen specific IgE to House Dust Mite D002, *Dermatophagoides farinae*

C Type of Test:

Chemiluminescent, Quantitative

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The NOVEOS Specific IgE Assay is an in vitro quantitative assay for the measurement of allergen specific IgE in human serum. NOVEOS Specific IgE Assay is to be used with the NOVEOS Immunoassay Analyzer. It is intended for use as an in vitro diagnostic aid in the clinical diagnosis of IgE mediated allergic disorders in conjunction with other clinical findings and is to be used in clinical laboratories.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

For use on the NOVEOS Immunoassay Analyzer

IV Device/System Characteristics:

A Device Description:

The NOVEOS specific IgE (sIgE) Assay consists of the following reagents:

- IgE Common Kit:
 - Diluent A (human serum albumin in buffer with preservative)
 - Conjugate IgE (horse radish peroxidase labeled mouse monoclonal anti-IgE antibody with preservative)
 - Substrate A
 - Substrate B
 - Fluo Beads (streptavidin coated magnetic particles with preservative)
- Capture Reagent Pack
 - House Dust Mite D002, *Dermatophagoides farinae*
- IgE Calibrator Set (six levels: 0.07, 0.35, 0.70, 3.5, 17.5, 100 kU/L)
- IgE Calibrator Antibody Pack (mouse monoclonal anti-human IgE with preservative)
- IgE Positive Control Pack (known to be >0.35 kU/L)
- IgE Negative Control Packs (known to be <0.35 kU/L)
- Others:
 - Probe Wash Pack (phosphate buffered citric acid solution with preservative)
 - Wash Buffer Concentrate Pack (10X phosphate buffer saline solution with preservative)
 - Cuvette Wash Pack (citric acid solution with preservative)

The NOVEOS Immunoassay Analyzer is a high-throughput, highly automated immunoassay platform that employs magnetic microbeads as the solid phase. The immunoassay reaction takes

place in individual reaction chambers in the reaction rotor, with other rotors containing patient samples and reagents. Liquids are moved by robotic pipettors. The reaction is quantitated by a combination of chemiluminescence and fluorescence measurements compared to a calibration curve, all performed by the analyzer with the NOVEOS software.

B Principle of Operation:

The NOVEOS specific IgE (sIgE) Assay is an immunometric, chemiluminescent assay for the quantitative determination of IgE of known specificity in human serum samples. Magnetic beads coated with streptavidin and fluorescent dye are incubated with a biotinylated allergen that binds to the streptavidin molecule on the surface of the beads. The beads are collected by use of a magnetic field to remove the liquid, and then undergo a wash step to remove unbound biotinylated allergen prior to incubation with the sample. If present in the sample, sIgE binds to the captured biotinylated allergen. After incubation, the beads are washed and subsequently incubated with an HRP-labeled anti-IgE monoclonal antibody (Conjugate IgE) to form an antibody-conjugate complex that is bound to the captured biotinylated allergen. After washing away the non-bound conjugate, a chemiluminescent substrate is added to the assay mixture resulting in light generation in proportion to the amount of antibody-conjugate that has been bound to the beads. The fluorescence is used to correct for any bead loss during the assay, and the (corrected) chemiluminescence is compared to a calibration curve to provide quantification of bound sIgE. The higher the value of chemiluminescent signal detected by the instrument, the higher the amount of sIgE detected in the sample tested.

V Substantial Equivalence Information:

A Predicate Device Name(s):

ImmunoCAP Specific IgE Assay and ImmunoCAP Specific IgE Conjugate 100 and Conjugate 400

B Predicate 510(k) Number(s):

K051218

C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>K191510</u>	<u>K051218</u>
Device Trade Name	NOVEOS sIgE Assay	ImmunoCAP Specific IgE Assay
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The NOVEOS Specific IgE is an <i>in vitro</i> quantitative assay for the measurement of allergen specific IgE in human serum. NOVEOS Specific IgE is to be used with the NOVEOS Immunoassay Analyzer. It is intended 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

	as an <i>in vitro</i> diagnostic aid in the clinical diagnosis of IgE mediated allergic disorders in conjunction with other clinical findings and is to be used in clinical laboratories.	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.
Assay Type	Quantitative	Same
Allergen	Whole extract of allergen material: House Dust Mite, <i>Dermatophagoides farinae</i>	Same
Traceability	World Health Organization (WHO) reference reagent serum Immunoglobulin E (IgE) 11/234	Same
Number of Calibrators	Six	Same
Reaction Temperature	37 °C	Same
General Device Characteristic Differences		
Specimen Type	Serum	Serum and Plasma (EDTA, Na-Heparin)
Solid Phase	Magnetic microparticles	Cellulose derivative
Instrument(s)	NOVEOS Immunoassay Analyzer	Phadia 100, Phadia 250/1000/2500/5000
Assay Principle	Chemiluminescent assay	Fluoroenzyme-immunoassay
Detection Antibody	HRP conjugated mouse anti-human IgE monoclonal antibody	β-Galactosidase conjugated mouse anti-human IgE monoclonal antibody
Sample Volume	4 µL	40 µL
Detection Limit	LoB: 0.03 kU/L LoD: 0.05 kU/L LoQ: 0.17 kU/L	LoB: 0.001 kU/L LoD: 0.02 kU/L LoQ: 0.10 kU/L
Assay Range	0.17 – 100 kU/L	0.10 – 100 kU/L
Assay Duration	1 hour and 45 minutes	Phadia 100: 2 hours and 30 minutes Phadia 250/1000/2500/5000: 1 hour and 45 minutes from entering the first sample

VI Standards/Guidance Documents Referenced:

- CLSI I/LA20-A3, Performance Characteristics, Quality Assurance, and Clinical Utility of Immunological Assays for Human Immunoglobulin E Antibodies and Defined Allergen Specificities, Approved Guideline–Third Edition
- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline–Third Edition.

- CLSI EP6-A, Evaluation of Linearity of Quantitative Measurement, Approved Guideline
- 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, Approved Guideline – Second Edition
- CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition
- CLSI EP15-A3, User Verification of Precision and Estimation of Bias; Approved Guideline - Third Edition
- CLSI EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third 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 NOVEOS sIgE Assay, D002 was evaluated by testing a panel of eight (three negative and five positive) serum samples with concentrations that cover the assay range. The samples were tested in two replicates per run, two runs per day for 20 days using one reagent lot on one NOVEOS Immunoassay Analyzers for a total of 80 replicates per sample. The standard deviation (SD) and %CV of the within-run, between-run, between-day, and total imprecision were calculated for each sample and results are summarized in the following table:

Sample	Mean (kU/L)	Within-Run		Between-Run		Between-Day		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
LoQ 04	0.17	0.01	3.2	0.01	4.5	0.01	6.3	0.01	8.4
LoQ 01	0.22	0.02	8.4	0.00	0.0	0.01	5.1	0.02	9.8
LoQ 03	0.22	0.02	7.9	0.01	3.4	0.01	5.1	0.02	10.0
PP02	0.47	0.02	4.2	0.01	2.6	0.02	3.6	0.03	6.1
PP03	2.11	0.06	3.0	0.06	2.6	0.08	3.8	0.12	5.5
PP04	15.31	0.49	3.2	0.75	4.9	0.33	2.2	0.96	6.3
PP08	28.93	1.15	4.0	1.35	4.7	1.24	4.3	2.16	7.5
PP10G	81.80	4.69	5.7	2.01	2.5	5.21	6.4	7.29	8.9

ii) Lot-to-lot imprecision:

The same panel of samples used in within-laboratory precision study was also tested on two additional different lots of the NOVEOS sIgE Assay, D002 for a total of three reagent lots. Each sample was tested in two replicated per run, two runs per day for 20

days. The lot-to-lot imprecision was evaluated based on a total of 240 measurements per sample. The results are summarized in the following table:

Sample	Mean (kU/L)	Within-Run		Between-Day		Between-Lot		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
LoQ 04	0.16	0.01	4.5	0.02	9.8	0.01	6.5	0.02	13.1
LoQ 01	0.20	0.01	7.2	0.01	3.8	0.02	8.2	0.02	11.6
LoQ 03	0.20	0.01	6.1	0.01	4.2	0.02	8.2	0.02	11.6
PP02	0.42	0.02	3.7	0.01	3.2	0.05	11.5	0.06	13.3
PP03	1.96	0.07	3.8	0.06	2.9	0.16	8.1	0.19	9.9
PP04	13.54	0.52	3.8	0.36	2.7	1.79	13.2	1.96	14.5
PP08	26.68	1.26	4.7	0.91	3.4	2.40	9.0	3.06	11.5
PP10G	73.67	4.41	6.0	3.17	4.3	7.21	9.8	9.40	12.8

iii) Site-to-site reproducibility:

A panel of four patient (one negative and three positive) serum samples and two controls (LYP and NOV) were assayed at three sites using the same lot of reagent. Each sample was tested in five replicates per run, one run per day for five days on one NOVEOS Immunoassay Analyzer at each site (for a total of 75 replicates per sample). The results are summarized in the following table:

Sample	Mean (kU/L)	Within-Run		Between-Day		Between-Site		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
PP41	0.21	0.01	4.8	0.01	4.8	0.01	4.8	0.02	9.6
PP42	0.38	0.02	5.3	0.01	2.6	0.01	2.6	0.03	7.9
PP43*	2.58	0.25	9.7	0.04	1.5	0.16	6.2	0.30	11.6
PP53	76.42	5.20	6.8	1.75	2.3	2.06	2.7	5.86	7.7
LYP	12.75	0.66	5.2	0.29	2.3	0.14	1.1	0.73	5.7
NOV	25.40	1.63	6.4	0.41	1.6	0.73	2.9	1.83	7.2
* includes an outlier with a value of 4.43 kU/L: without the outlier, the within-run, between-run, between site, and total %CV are 5.1%, 2.7%, 5.1% and 7.8%.									

2. Linearity:

Linearity of the assay was evaluated following CLSI guideline EP6-A by preparing three dilution series. Three positive serum samples (low, medium and high level) were each diluted in negative serum pool generating at least five consecutive dilutions. Each diluted sample was tested in replicates of five in one assay run using one reagent lot on one analyzer. Results of the replicates for all three samples were analyzed for linearity. Regression statistics comparing the observed results (y) to expected results (x) of all samples in the three dilution series are presented in the table below.

	Dilution Range (kU/L)	Regression Equation	Slope (95% CI)	Intercept (95% CI)	R²	% Bias
1	0.04–6.12	$y = 0.99x - 0.03$	0.96–1.03	–0.13–0.06	1.00	–9% - 2%
2	0.04–70.43	$y = 0.99x - 0.74$	0.99–1.04	–2.19–0.71	1.00	–10% - 0%
3	0.06–126.49	$y = 1.01x - 0.78$	0.99–1.02	–1.72–0.16	1.00	–13% - 0%
All	0.04–126.49	$y = 1.00x - 0.47$	0.99 – 1.01	–0.90–0.05	1.00	–13% - 2%

The data summarized in the table above support linearity throughout the claimed measuring range from 0.17 kU/L to 100 kU/L.

3. Analytical Specificity/Interference:

a) Inhibition study:

Immunological specificity of D002 (*Dermatophagoides farinae*) allergen was verified through competitive inhibition according to CLSI guideline I/LA20-A3. A positive sample with specific IgE concentration of 50.9 kU/L was used in the study. The D002 allergen solution (inhibitor) was diluted into the positive serum sample to achieve a starting concentration of 100 µg/mL. The D002 sample solution was serially diluted five times in two-fold increments and each diluted sample was evaluated for dose-dependent inhibition in five replicates with three lots of NOVEOS sIgE Assay, D002. The results of the dose-dependent inhibition study showed that >50% inhibition was achieved with D002 at a final inhibitor concentration of 0.39 µg/mL. The related allergen D070 (Storage mite), and the unrelated allergens, F001 (Egg white), G006 (Timothy grass) and W003 (Giant ragweed) were also tested as potential inhibitors at a final concentration of at least ten times the concentration of D002 at which >50% inhibition was achieved. All allergen inhibitors were spiked into the positive serum sample at a 1:1 dilution and evaluated with three lots of NOVEOS sIgE Assay, D002. The results of the single-dose inhibition studies using one related allergen (D070) and three unrelated allergens (F001, G006 and W003) did not show inhibition at a test concentration of 3.9 µg/mL for the related allergen and 1.25 mg/mL for the three unrelated allergens. The inhibition studies indicate that the NOVEOS sIgE Assay, D002 contains the immunologically relevant allergen.

b) Interference

(1) *Endogenous Substance Interference:*

The effect of the presence of elevated level of human albumin, hemoglobin, triglyceride, conjugated bilirubin, unconjugated bilirubin, and rheumatoid factor in serum samples was evaluated by testing five (one negative, four positives including one near 0.35 kU/L) serum samples spiked with varying levels of each interferent and analyzed in 12 replicates in one assay run with three lots of NOVEOS sIgE Assay, D002. The % recovery for each sample spiked with the potential interfering substance was calculated by comparing its result to that of the corresponding reference sample spiked with an equal volume of the solvent without the interfering substance. No interference was noted for samples containing human serum albumin up to 120 mg/mL, hemoglobin up to 200 mg/dL, triglyceride up to 3,000 mg/dL,

conjugated bilirubin up to 30 mg/dL, unconjugated bilirubin up to 20 mg/dL, and rheumatoid factor up to 513 IU/mL.

(2) *Exogenous Substance Interference:*

The same protocol used in the endogenous substance interference study was used to evaluate potentially interfering exogenous substances. No interference was noted for samples containing biotin up to 1200 ng/mL, diphenhydramine at 19.6 µmol/L, methylprednisolone at 1000 ng/mL, omalizumab at 0.12 mg/mL and ranitidine at 6.0 mg/L.

c) *Cross-reactivity:*

The potential cross-reactivity of non-IgE (IgA, IgD, IgG, and IgM) to the NOVEOS sIgE Assay, D002 was evaluated by mixing serum samples spiked with the immunoglobulin stock solutions containing IgA, IgG, and IgM at 38.43 mg/mL and IgD at 7.72 mg/mL with immunoglobulin stripped serum to obtain a series of six two-fold serially diluted samples containing target levels of non-IgE within or above the physiological range. The control samples were prepared by serially diluting equal volumes of distilled water into stripped serum. No cross reactivity was noted up to 14.86 mg/mL for IgA, IgG and IgM, and up to 0.346 mg/mL for IgD.

4. Assay Reportable Range:

The claimed measuring range is from 0.17 kU/L to 100 kU/L.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a) *Traceability:*

The IgE calibrators are traceable to the World Health Organization (WHO) third International Standard 11/234 of Human Serum Immunoglobulin E.

b) *Kit Stability:*

Shelf life stability: Both an ongoing real-time stability study and an accelerated stability study were performed in accordance with CLSI EP25-A using three lots of NOVEOS sIgE Assay, D002. The accelerated stability data support the manufacturer's claim of 24-month unopened shelf-life stability for the Specific IgE Capture Reagent D002. All other reagents required to perform specific IgE testing on the NOVEOS system are not allergen specific and the shelf-life stability of these reagents has been previously established per K182479, with the exception of the Fluo Beads reagent which now has an extended shelf-life stability of up to 24 months based on the real-time stability data. The real-time stability study is on-going and available data supports eight months unopened shelf-life stability for NOVEOS sIgE Assay, D002 when stored at 2–8 °C per the manufacturer's instruction for use. The claimed shelf-life for individual assay components is listed in the table below:

		Shelf-life Stability (2–8 °C)
Specific IgE Capture Reagent D002*		24 months
IgE Common Kit	Diluent A	48 months
	Conjugate IgE	18 months
	Substrate A and B	24 months
	Fluo Beads	24 months
IgE Calibrator Set		24 months
Calibrator Antibody IgE		24 months
Controls	IgE Positive Control Pack	24 months
	IgE Negative Control Pack	48 months
Others	Wash Buffer Concentrate	48 months
	Cuvette Wash Pack	24 months
	Probe Wash Pack	24 months
*Results based on accelerated stability data		

On-board stability: A real-time stability study using three lots of NOVEOS sIgE Assay, D002 support the on-board stability claim 28 days for the Specific IgE Capture Reagent D002. The on-board stability for all the other assay components has been previously established per K182479 and are summarized in the table below.

		On-board Stability (2–8°C)
Specific IgE Capture Reagent D002		28 days
IgE Common Kit	Diluent A	14 days
	Conjugate IgE	14 days
	Substrate A and B	14 days
	Fluo Beads	14 days
IgE Calibrator Set		48 hours
Controls	IgE Positive Control Pack	48 hours
	IgE Negative Control Pack	48 hours
Others	1x Wash Buffer	28 days
	Cuvette Wash Pack	28 days
	Probe Wash Pack	Not applicable

6. Detection Limit:

The limit of blank (LoB), limit of detection (LoD), and limit of Quantitation (LoQ) of the NOVEOS sIgE Assay, D002 on the NOVEOS Immunoassay Analyzer were determined according to CLSI guideline EP17-A2.

LoB was determined by testing five IgE-free human serum samples in three replicates per run, one run per day, for six days on two analyzers using two lots of NOVEOS sIgE Assay, D002. The LoB was estimated as the 95th percentile of 90 measurements for each of the lots tested.

LoD was determined using five serum samples with low IgE concentrations. Each sample was tested in replicates of ten per run, one run per day, for six days on two analyzers using

two lots of NOVEOS sIgE Assay, D002. The LoD was determined for each lot based on 300 measurements as the mean concentration of the lowest sample with 95% or more of its replicates recovering above the LoB.

LoQ was determined as functional sensitivity using a set of five samples with low IgE concentrations. Each sample was tested in replicates of five per run, two run per day, for five days with two reagent lots on two instruments for a total of 50 data points per sample per reagent lot. The LoQ is defined as the mean value of the sample which fulfills the specification for the total within-laboratory imprecision (<20%CV).

The claimed LoB, LoD, and LoQ are based on the highest value obtained from the lots tested and are summarized in the table below.

	LoB	LoD	LoQ
NOVEOS sIgE Assay, D001	0.03 kU/L	0.05 kU/L	0.17 kU/L

7. Assay Cut-Off:

See clinical cut-off

8. Accuracy (Instrument):

Not applicable

9. Carry-Over:

Evaluation of instrument carryover was conducted using one lot of reagent of NOVEOS sIgE, D002 on three NOVEOS Immunoassay Analyzers. The carryover assessment is based on five cycle counts per instrument. Pooled high-level patient samples with sIgE concentration of 88.80 kU/L were aliquoted into five tubes, and the pooled low-patient samples with sIgE concentration of 0.27 kU/L and aliquoted into 25 tubes. Each cycle consists of one high sample test followed by five low sample tests against D002 capture reagent. Low Sample 1 following High Sample is denoted as “possibly affected” which has the highest chance of potential carryover thus leading to signal change > 0.10 kU/L. Low Samples 3–5 is denoted as “very probably unaffected” and therefore should not have elevated kU/L signal. Carryover assessment is done by measuring the change (Δ) of [mean concentration of Low Sample 1] and [mean concentration of Low Samples 3–5]. The results are summarized in the table below:

NOVEOS Immunoassay Analyzer	Mean Conc. of Low Sample 1 (kU/L)	Mean Conc. of Low Sample 3-5 (kU/L)	Δ Carryover (kU/L)
1	0.28	0.27	0.01
2	0.28	0.27	0.01
3	0.30	0.27	0.03

B Comparison Studies:

1. Method Comparison with Predicate Device:

A total of 234 serum samples spanning the measuring range were tested with the NOVEOS sIgE Assay, D002 (candidate) and the ImmunoCAP Specific IgE, ImmunoCAP Allergen d2, House dust mite (predicate). Agreement between the candidate and predicate devices was evaluated using a clinical cut-off value of 0.35 kU/L. The results are summarized in the following table:

		ImmunoCAP sIgE, Allergen d2		
		Positive	Negative	Total
NOVEOS™ sIgE Assay, D002	Positive	96	0	96
	Negative	6	132	138
	Total	102	132	234

Positive Agreement: 94.1% (96/102) (95% CI: 87.8–97.3%)

Negative Agreement: 100.0% (132/132) (95% CI: 97.2–100.0%)

Total Agreement: 97.4% (218/234) (95% CI: 94.5–98.8%)

2. Matrix Comparison:

Not Applicable

C Clinical Studies:

1. Clinical Sensitivity and Specificity:

To demonstrate the clinical performance of the NOVEOS sIgE Assay, D002, test results of the clinical samples from 75 atopic and 112 healthy, non-atopic donors evaluated in the method comparison study were compared to a clinical diagnosis of allergy. The atopic donors with a clinical history of allergy-like symptoms were identified as positive by skin prick testing (SPT) and the non-atopic donors with no reported allergy. The clinical sensitivity and specificity in this study cohort are summarized in the following table:

		Clinical Diagnosis		
		Atopic	Non-atopic	Total
NOVEOS sIgE Assay, D002	Positive	58	0	58
	Negative	17	112	129
	Total	75	112	187

Sensitivity: 77.3% (58/75) (95% CI: 66.2%–86.2%)

Specificity: 100.0% (112/112) (95% CI: 97.8%–100.0%)

2. Other Clinical Supportive Data:

Not applicable

D Clinical Cut-Off:

The clinical cut-off of the assay is 0.35 kU/L. The interpretation of results of the assay uses the following classification system:

Class	Concentration (kU/L)	Interpretation
0	<0.35	Negative
I	0.35 to <0.70	Positive with increasing sIgE concentration
II	0.70 to <3.50	
III	0.35 to <17.50	
IV	17.50 to <50.00	
V	50.00 to <100.00	
VI	≥100	

E Expected Values/Reference Range:

The expected value is negative (< 0.35 kU/L) for a specific allergen in a non-atopic person. Each laboratory should establish its own expected value.

The expected value/reference range of NOVEOS sIgE, D002 in the normal population was evaluated by testing samples from 120 apparently healthy subjects in clinical study. All samples were below <0.35 kU/L.

F Other Supportive Instrument Performance Characteristics Data:

Not applicable

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.