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Allergens

Validation study of a new chemiluminescent singleplex IgE assay in a set of Italian allergic rhinitis patients

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Abstract

Background: The measurement of specific IgE to allergenic extracts and molecules in patients with allergic rhinitis (AR) is crucial for a precise diagnosis and further immunotherapy. Companies providing in vitro diagnostic methods in allergology continuously strive for the optimization and modernization of such methods. A new generation of automated allergy tests based on chemiluminescence detection and paramagnetic microparticles is now available, with possible advantages in sample volume, cost-effectiveness and avoidance of sample-related interference.

Objectives: To test whether sIgE antibody levels obtained with a new singleplex chemiluminescent method have a good agreement with the corresponding results obtained with a “gold standard” test.

Methods: We tested sera from 368 AR patients. Specific IgE sera levels (kU/L) to a comprehensive panel of 15 allergen extracts and 6 molecules were tested with ImmunoCAP® (Thermo Fisher Scientific Inc, Phadia AB, Uppsala, Sweden) and NOVEOS™ (HYCOR® Biomedical, Garden Grove, CA, USA). We evaluated the qualitative and quantitative performance of the new NOVEOS system in matching the outcome of ImmunoCAP to each of the examined allergens.

Results: In relation to ImmunoCAP, the overall diagnostic sensitivity and specificity of sIgE tests with NOVEOS were 90.8% (95% CI = 88.6–92.7) and 96.2% (95% CI = 93.9–97.8), respectively. These values were higher when only molecules were considered (sensitivity = 98.7% [95% CI = 96.4%–99.7%]; specificity = 94.2% [95% CI = 88.4%–97.6%]) and lower when only extracts were considered (sensitivity = 87.6% [95% CI = 84.7%–90.2%]; specificity = 97% [95% CI = 94.4%–98.6%]). Spearman's correlation between the data set of both methods for a ≥ 0.1 kU/L cut-off was 0.84 ($p < .001$).

Conclusions: The new singleplex NOVEOS system presented good results for qualitative and quantitative comparisons when testing specific serum IgE antibodies against

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a range of 21 allergens. This novel immunoassay system using only 4 μ l of sample per test appears to be robust and reliable and can, therefore, be used as an aid in allergy diagnosis.

KEYWORDS

allergic rhinitis, IgE, immunoassay, interassay comparison, precision medicine, singleplex

1 | INTRODUCTION

Allergic rhinoconjunctivitis due to airborne allergens is the most prevalent immunological disease, with a particular impact among children and young adults.¹ Although it can be controlled with symptom-relieving drugs, specific allergy immunotherapy (AIT) is the only disease-modifying treatment with long-term effects currently available. Additionally, it allows for symptom control when pharmacotherapy fails.^{2,3} The efficacy of AIT is based on the precise recognition of the allergen, as well as the allergenic molecules that elicit IgE sensitization and trigger patient's symptoms.⁴⁻⁶ Although the prevailing diagnostic approach remains to be based on patients' clinical history combined with skin prick tests (SPT),⁷ there are a plethora of situations where more precise information is required. In certain locations, like Southern European countries, it is often difficult to identify the eliciting allergen as patients are frequently multi-sensitized to multiple allergen sources with overlapping exposure, and often cross-reactive.⁸

Component resolved diagnostics (CRD) using molecular level allergen components is a valuable tool that aids physicians in overcoming the diagnosis problem, as it allows one to identify the eliciting allergen and thus choose the most suitable agent for AIT.^{5,9} Although considered too complex and detailed by many doctors,¹⁰ the use of CRD to uncover the clinical relevance of IgE sensitization is mandatory when making a precise AIT prescription. In an allergic rhinitis clinical scenario, the doctor needs to establish a cause-effect relationship between exposure to the pollen recognized by the patient's IgE and the patient's symptoms¹¹ and precisely assess the degree of severity of the symptoms^{12,13} since AIT is recommended for patients with moderate-severe rhinitis.¹⁴

The ImmunoCAP® specific IgE assay, processed on Thermo Fisher (previously Phadia) equipment, has been widely adopted in Europe. It provides IgE tests against allergenic extracts or molecules (components)¹⁵ and is often employed in the validation of new sIgE assays,¹⁶ as well as comparison with other test systems.¹⁷⁻¹⁹ In the ImmunoCAP sIgE assay, the allergen is covalently coupled during manufacturing to a flexible hydrophilic cellulose solid phase in a reaction vessel.²⁰

The NOVEOS System and specific IgE assay from HYCOR are novel in that they offer, among other advantages, a significantly lower (1/10th) sample volume per test in comparison with ImmunoCAP, and a robust chemistry design of chemiluminescence, fluorescence and paramagnetic microparticles which contribute to good precision and accuracy.²¹ The assay design appears unaffected

by known sample-related interferences including biotin, IgG, IgG4 and cross-reactivity by anti-carbohydrate determinants (CCD) antibodies that react with other cellulose-based technologies.²²⁻²⁴

The aim of this study is to test, in comparison with ImmunoCAP, the analytical performance of the NOVEOS sIgE tests used in routine procedures.

2 | METHODS

2.1 | Study population

Consenting allergic rhinitis patients were consecutively recruited between 2016 and 2018 in the outpatient clinic of the Department of Pediatrics of "Sandro Pertini" Hospital in Rome and of the Allergy Unit, Istituto Dermatologico dell'Immacolata (IDI), Rome. All patients were tested for serum specific IgE antibodies against environmental allergens with routine tests (ImmunoCAP singleplex at Pertini Hospital and with ImmunoCAP ISAC at IDI). All IDI sera were re-tested with ImmunoCAP before inclusion in the present study. The present paper focuses on the comparison of the in vitro outcomes obtained by re-testing the study sera bank with the NOVEOS system and sIgE assay.

2.2 | Ethical approval

All participants and/or their parents or tutors gave their informed and written consent to the use of sera in scientific studies for the diagnosis and therapy of allergic diseases. The study design and procedures were approved by the local Ethical committees Comitato Etico Lazio 2 (#9871; 01/02/2016) for the Pertini Hospital and the Ethical Committee of IDI-IRCCS (#493/1; 30/05/2017).

2.3 | Sera selection

Serum specific IgE was measured in the samples of 368 patients, initially with ImmunoCAP® (Thermo Fisher Scientific Inc, Phadia AB)^{15,20,25} and subsequently with NOVEOS IgE test (HYCOR Biomedical), for the following allergens: bermuda grass (g2), timothy grass (g6), Phl p 1 (g205), ryegrass (g5), alder (t2), birch (t3), Bet v 1 (t215), cypress (t23), olive (t9), ragweed (w1), Amb a 1 (w230), mugwort (w6), Russian thistle (w11), *D. pteronyssinus* (d1), Der p 1 (d202),

Der p 2 (d203), *D. farinae* (d2), cat (e1), Fel d 1 (e94), dog (e5), horse (e3). Sera for comparison with the NOVEOS system were selected according to a 2:1 (pos:neg) ratio, on the basis of the ImmunoCAP® outcome, for each allergen extract or molecule. The testing events and sera sets used for different allergens were therefore different. In particular, 40 positive samples were selected from the available sera, with a randomization procedure targeted to obtain the whole range of sIgE levels between 0.35 kU/L and the highest value for that allergen. The negative sera were in contrast randomly selected, for each allergen, from the sera bank.

2.4 | NOVEOS IgE test

The NOVEOS test [Figure 1] is a chemiluminescence detection system operating in a solid phase of fluorescently labelled and

streptavidin-coated paramagnetic microparticles. The microparticles are first incubated with a biotinylated allergen that binds the streptavidin molecules. After an extensive wash, the bound microparticles are then incubated with patient serum containing allergen-specific IgE and the resulting bound complex is washed by aspirating unbound material from retained beads in the cuvette. They are subsequently incubated with an anti-IgE antibody conjugated to horseradish peroxidase and, after an incubation period, are washed to remove any unbound conjugate from bound material. The chemiluminescent signal is originated by adding a substrate solution. The concentration of allergen-specific IgE is directly proportional to the light intensity after correction (via fluorescence) for microparticle loss and is compared to an IgE reference curve traceable to World Health Organization (WHO) reference preparations (NIBSC 11/234). The sample volume used per test is 4 µl, and the time to first result is 104 min.

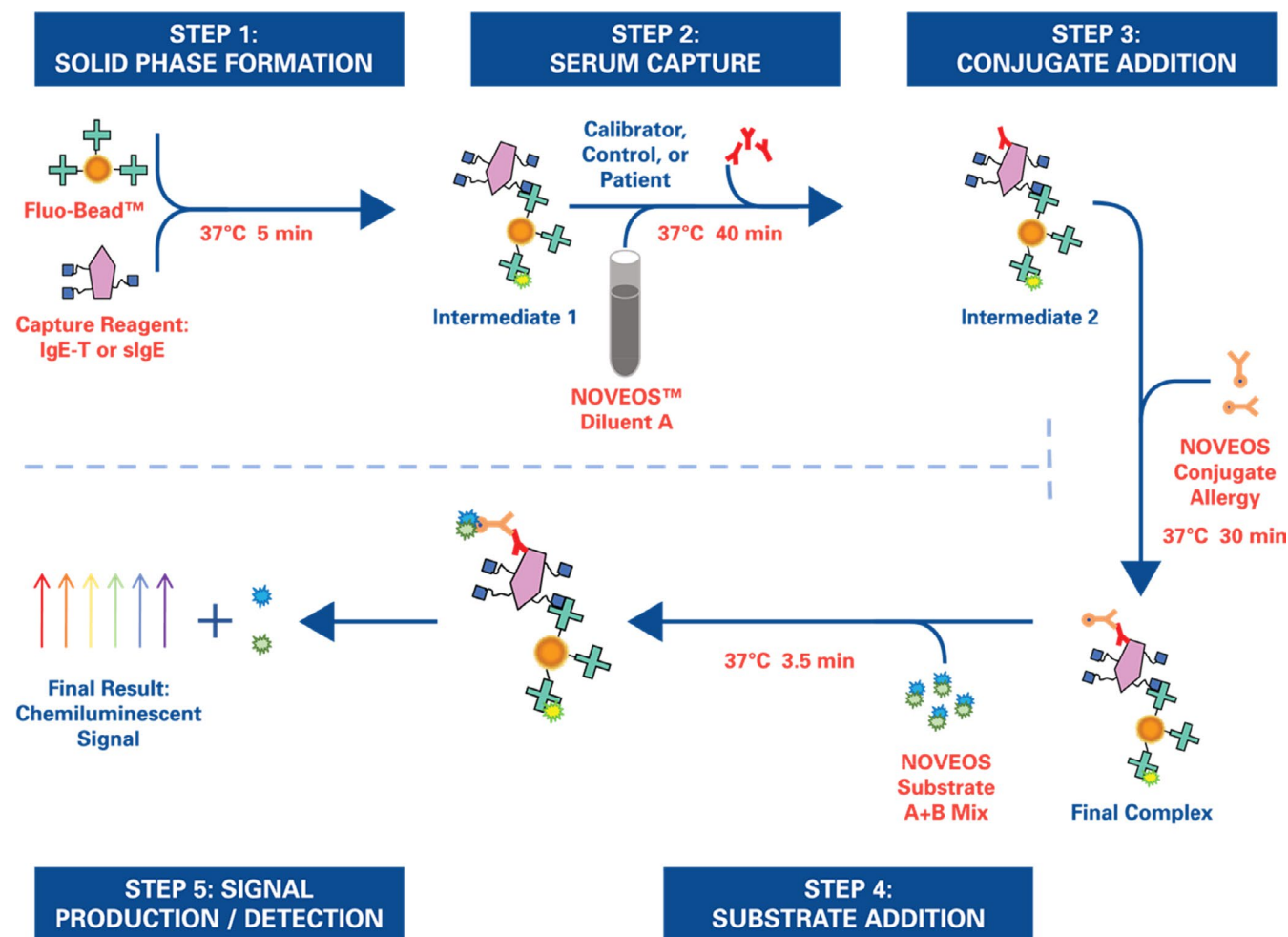


FIGURE 1 Fluorescently labelled and streptavidin-coated magnetic beads are incubated with a biotinylated allergen that bind to streptavidin on the surface of the beads. The allergen-coated beads are then incubated with patient serum containing allergen-specific IgE and, after an incubation period, are washed by aspirating unbound material from retained beads in the cuvette. The beads are subsequently incubated with an anti-IgE antibody conjugated to horseradish peroxidase and, after an incubation period, are washed to remove any unbound conjugate from bound material. The substrate solution is then added which generates a sustained chemiluminescence signal that is measured. The concentration of allergen-specific IgE is directly proportional to the light intensity after correction (via fluorescence) for any bead loss and is compared to an IgE reference curve traceable to World Health Organization (WHO) reference preparations

TABLE 1 Characteristics of the allergic rhinitis study population: gender, age and comorbidities of the 368 patients

	Total (n = 368)		Pertini (n = 208)		IDI (n = 160)	
	N	%	N	%	N	%
Male	179	48.6	121	58.2	58	36.3
Age (years) (mean ± SD)			19 ± 11.5		33 ± 15.6	
Asthma	89	24.2	66	31.7	23	14.4
Oral Allergic Syndrome	123	33.4	78	37.5	45	28.1
Urticaria/Angioedema	106	28.8	52	25.0	54	33.8
Atopic Dermatitis	91	24.7	53	25.5	38	23.8
Gastro-intestinal disorders	8	2.2	7	3.4	1	0.6
Anaphylaxis episode	21	5.7	15	7.2	6	3.8
Other	34	9.2	9	4.3	25	15.6

Abbreviation: Other, other allergic comorbidities.

2.5 | Statistical analysis

Age was summarized as mean and standard deviation (SD). Categorical data were summarized as numbers (*n*) and frequencies (%). Sensitivity, specificity, positive and negative predictive values were calculated with their confidence interval at 95% (95% CI). Accuracy, positive and negative likelihood ratios were also calculated in order to evaluate the diagnostic performance of NOVEOS in detecting IgE sensitization, compared to ImmunoCAP. Analysis was done considering each extract and molecule (component) separately and combined for a general appraisal. Bland-Altman plots were used to investigate the agreement between quantitative values of IgE detected with the two different methodologies (NOVEOS vs ImmunoCAP). They were applied using the log values of only positive samples (>0.1 kU/L). Mean difference (Bias), 95% CI, number of subjects under or over-limit of agreement (LOA), Lin's concordance index (Lin) and Spearman's correlation between the difference and average were reported. A *p*-value of <.05 was considered statistically significant. Statistical analyses were performed with Stata 16.0.

3 | RESULTS

3.1 | Study population and study design

Overall, 368 patients (208 in Pertini and 160 in IDI) (179 males; age 25 ± 15.2 years, ranging from 2 to 76 years of age) participated in this study (Table 1). Oral allergy syndrome (OAS) was the most frequent comorbidity, with a prevalence of 33.4% (123 patients), while asthma was diagnosed in 89 patients only (24.2%).

3.2 | Qualitative interassay comparison

Diagnostic overall performance criteria (sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, positive likelihood ratio (LR+), negative likelihood ratio (LR-)), that is, the outcomes of the NOVEOS sIgE compared to those of

ImmunoCAP, were rather straightforward (Table 2). NOVEOS predicted the results of ImmunoCAP sIgE with an overall (all 21 extracts and molecules) sensitivity of 90.8% (761/838), a specificity of 96.2% (404/420) and an accuracy of 92.5% (1165/1260). The positive and negative LR values were 23.8 and 0.10, respectively. The lowest and highest values of sensitivity were 57.5% for cypress allergen extract and 100% for Bet v 1, Phl p 1, Feld 1 and mugwort. The lowest and highest levels of specificity were 80% for Phl p 1 and 100% for a broad list of reagents, including grasses, cypress, mugwort, Amb a 1, *D. pteronyssinus*, Der p 2 and *D. farinae*. At the category level, the highest sensitivity and specificity values were observed for mite allergens (96.9% and 98.8%, respectively), the lowest sensitivity was trees (80.5%), while the lowest specificity was for grass and animals (95.0%). When comparing strictly extract-based assays with molecular component-based assays, no major difference was observed in the overall specificity (97.0% in extracts and 94.2% in molecular components), though molecular assays presented higher sensitivity (87.6% in extracts vs. 98.7% in molecular components).

3.3 | Quantitative interassay comparison

Spearman's correlation between positive (≥0.1 kU/L) data set of the NOVEOS and ImmunoCAP was 0.84 (*p* < .001) (Figure 2A), with 10 of 15 allergen extracts and 6/6 allergen molecules showing a correlation higher than 0.80 (Figure 3). Interestingly, the NOVEOS test seems to have a high binding capacity, as demonstrated by the values obtained in sera with very high IgE concentrations (Figure 2A). This is consistent with the manufacturer's claims that the use of approximately 35 million microparticles per test creates a vast binding surface. The Bland-Altman plot analysis, performed for the positive values using a ≥ 0.1 kU/L cut-off, also shows a good general quantitative correlation between the measurements obtained with the two systems, with Lin's index of 0.91, mean difference of 0.07, standard deviation of 0.33 and a Limit Of Agreement (LOA) from -0.6 to 0.7 (Figure 2B). Analysing separately each extract and molecular component, there is a very good agreement with little mean difference and no trend between difference and

TABLE 2 Diagnostic performance of NOVEOS method in identifying IgE sensitization, compared to ImmunoCAP method (cut-off > 0.35 kU/L)

	N	C+			C-		Sensitivity		Specificity		PPV		NPV		Accuracy	
		C+N+	C+N-	N+	C-N-	%	(95% CI) ^a	%	(95% CI) ^a	%	(95% CI) ^a	%	(95% CI) ^a	%	(95% CI) ^a	
Grass	240	153	7	4	76	95.6	91.2-98.2	95.0	87.7-98.6	97.5	93.6-99.3	91.5	83.4-96.5	95.4	19.1	0.05
Bermuda grass	60	35	5	0	20	87.5	73.2-95.8	100.0	83.2-100	100.0	90.0-100	80.0	59.3-93.2	91.7	∞	0.13
Timothy grass	60	39	1	0	20	97.5	86.8-99.9	100.0	83.2-100	100.0	91.0-100	95.2	76.2-99.9	98.3	∞	0.03
Phl p 1	60	40	0	4	16	100.0	91.2-100	80.0	56.3-94.3	90.9	78.3-97.5	100.0	79.4-100	93.3	5.0	0.00
Ryegrass	60	39	1	0	20	97.5	86.8-99.9	100.0	83.2-100	100.0	91.0-100	95.2	76.2-99.9	98.3	∞	0.03
Tree	300	160	39	4	96	80.4	74.2-85.7	96.0	90.1-98.9	97.6	93.9-99.3	71.1	62.7-78.6	85.3	20.1	0.20
Alder	60	34	6	1	19	85.0	70.2-94.3	95.0	75.1-99.9	97.1	85.1-99.9	76.0	54.9-90.6	88.3	17.0	0.16
Birch	60	30	10	1	19	75.0	58.8-87.3	95.0	75.1-99.9	96.8	83.3-99.9	65.5	45.7-82.1	81.7	15.0	0.26
Bet v 1	60	39	0	1	19	100.0	91.0-100	95.0	75.1-99.9	97.5	87.1-99.9	100.0	82.4-100	98.3	20.0	0.00
Cypress	60	23	17	0	20	57.5	40.9-73.0	100.0	83.2-100	100.0	85.2-100	54.1	36.9-70.5	71.7	∞	0.43
Olive	60	34	6	1	19	85.0	70.2-94.3	95.0	75.1-99.9	97.1	85.1-99.9	76.0	54.9-90.6	88.3	17.0	0.16
Weeds	240	152	7	3	77	95.6	91.1-98.2	96.3	89.4-99.2	98.1	94.4-99.6	91.7	83.6-96.6	95.4	25.5	0.05
Mugwort	60	40	0	0	20	100.0	91.2-100	100.0	83.2-100	100.0	91.2-100	100.0	83.2-100	100.0	∞	0.00
Ragweed	60	38	2	1	19	95.0	83.1-99.4	95.0	75.1-99.9	97.4	86.5-99.9	90.5	69.6-98.8	95.0	19.0	0.05
Amb a 1	60	39	1	0	20	97.5	86.8-99.9	100.0	83.2-100	100.0	91.0-100	95.2	76.2-99.9	98.3	∞	0.03
Russian thistle	60	35	4	2	18	89.7	75.8-97.1	90.0	68.3-98.8	94.6	81.8-99.3	81.8	59.7-94.8	89.8	9.0	0.11
Mites	240	155	5	1	79	96.9	92.9-99.0	98.8	93.2-100	99.4	96.5-100	94.0	86.7-98.0	97.5	77.5	0.03
<i>D. pteronyssinus</i>	60	38	2	0	20	95.0	83.1-99.4	100.0	83.2-100	100.0	90.7-100	90.9	70.8-98.9	96.7	∞	0.05
Der p 1	60	39	1	1	19	97.5	86.2-99.9	95.0	75.1-99.9	97.5	86.2-99.9	95.0	75.1-99.9	96.7	19.5	0.03
Der p 2	60	39	1	0	20	97.5	86.8-99.9	100.0	83.2-100	100.0	91.0-100	95.2	76.2-99.9	98.3	∞	0.03
<i>D. farinae</i>	60	39	1	0	20	97.5	86.8-99.9	100.0	83.2-100	100.0	91.0-100	95.2	76.2-99.9	98.3	∞	0.03
Animals	240	141	19	4	76	88.1	82.1-92.7	95.0	87.7-98.6	97.2	93.1-99.2	80.0	80.8-95.0	90.4	17.6	0.13
Cat	60	39	1	1	19	97.5	86.8-99.9	95.0	75.1-99.9	97.5	86.8-99.9	95.0	75.1-99.9	96.7	19.5	0.03
Fel d 1	60	40	0	1	19	100.0	91.2-100	95.0	75.1-99.9	97.6	87.1-99.9	100.0	82.4-100	98.3	20.0	0.00
Dog	60	27	13	1	19	67.5	50.9-81.4	95.0	75.1-99.9	96.4	81.7-99.1	59.4	40.6-76.3	76.7	13.5	0.34
Horse	60	35	5	1	19	87.5	73.2-95.8	95.0	75.1-99.9	97.2	85.5-99.9	79.2	57.8-92.9	90.0	17.5	0.13
Extracts	900	525	74	9	291	87.6	84.7-90.2	97.0	94.4-98.6	98.3	96.8-99.2	79.7	75.2-83.7	90.7	29.2	0.13
Molecules	360	236	3	7	113	98.7	96.4-99.7	94.2	88.4-97.6	97.1	94.2-98.8	97.4	92.6-99.5	96.9	16.9	0.01
ALL	1260	761	77	16	404	90.8	88.6-92.7	96.2	93.9-97.8	97.9	96.7-98.8	84.0	80.4-87.2	92.5	23.8	0.10

Abbreviations: C-, CAP negative; C+, CAP positive; LR-, negative likelihood ratio; LR+, positive likelihood ratio; N-, NOVEOS negative; N+, NOVEOS positive; (positivity cut-off value > 0.35 kU/L); NPV, negative predictive value; PPV, positive predictive value.

^aExact binomial confidence limits (95% CI).

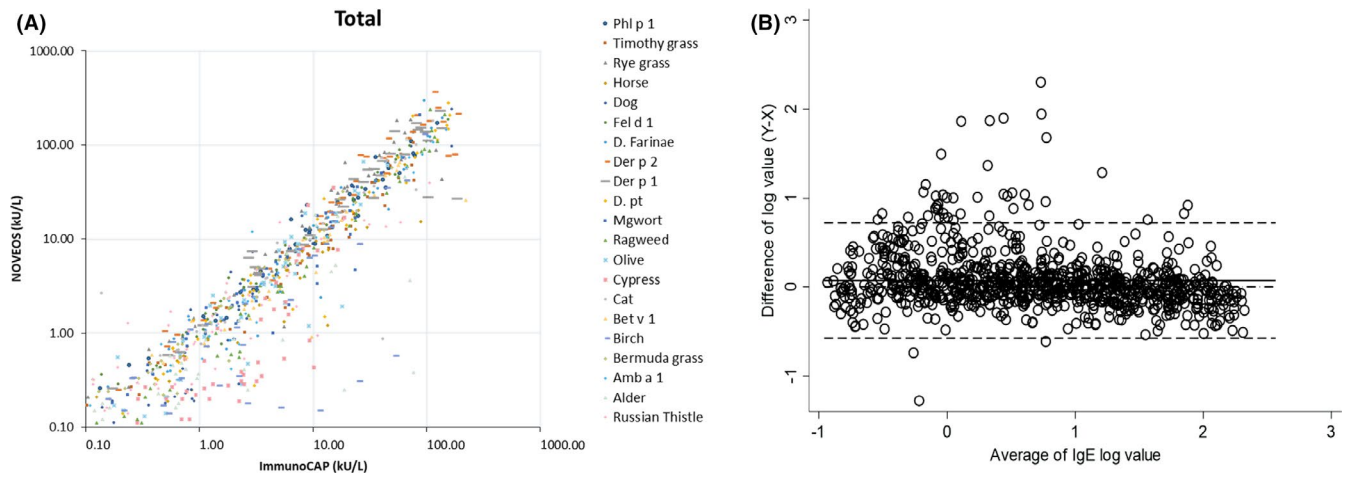


FIGURE 2 Correlation between the quantitative outcomes of NOVEOS vs ImmunoCAP IgE tests. (A) Scatter diagram; (B) Bland-Altman. In the Bland-Altman plot, the difference and the mean of the ImmunoCAP and the NOVEOS IgE log values (kU/L) are reported in the y- and x-axis, respectively. The continuous and the dashed lines parallel to the X-axis mark the population mean level and its bias, respectively, while the other two dash lines are the upper and lower limits of agreement, respectively

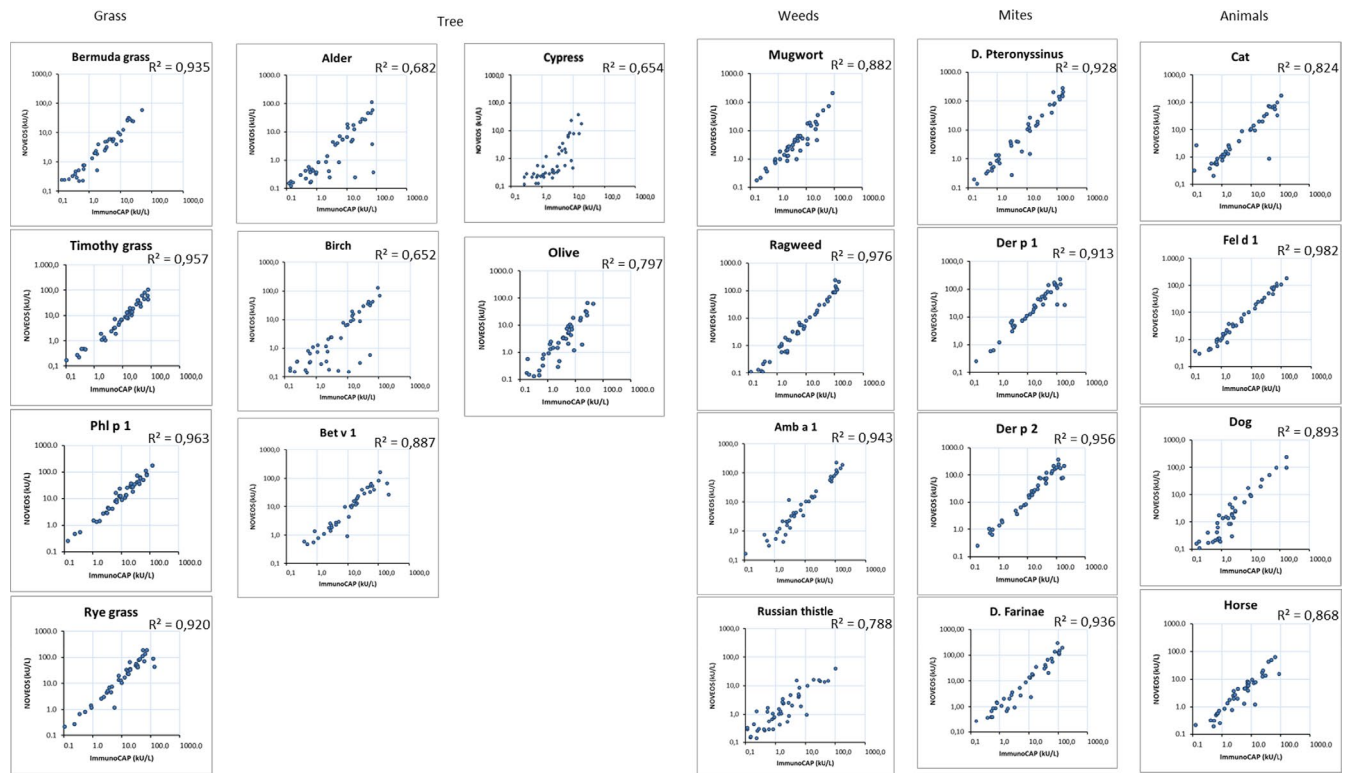


FIGURE 3 Scatter diagrams showing the relationship between quantitative IgE detection of allergen molecules through two different tests: NOVEOS (y-axis) vs ImmunoCAP (x-axis). The Spearman correlation coefficient (R^2) is also indicated

average for Bermuda grass, Phi p 1, Bet v 1, Mugwort, *D. pteronyssinus*, Der p 1, *D. farinae*, cat and Fel d 1. CAP has higher mean values than NOVEOS for alder, timothy grass, ragweed and horse. In birch and dog, the difference is higher for low values, while in Amb a 1 it is higher for low and median average values. Cypress shows two trends simultaneously; the difference first increases for low average values and then decreases for high average values. Olive

presents similar results as it has a positive difference for low average values and negative for high average values, but no difference in mean value. Russian thistle also has no difference in mean value, but positive and negative differences distributed throughout. Rye grass and Der p 2 have lower mean values for CAP than NOVEOS, with the last also showing a negative difference for high average values (Figure S1; Table 3).

3.4 | Discordant sera

Overall, 93 out of 1260 (7%) comparisons in 75 of the 368 patients had a discordant outcome (CAP-/NOV+, $n = 16$, CAP+/NOV-, $n = 77$) (Table 2). However, most of these discordant IgE values (64/93, 69%) fall in the low discordancy level group (≤ 1 kU/L). Within the 64 cases, 8 occurred in the CAP-/NOV+, and 56 in the CAP+/NOV- group. Out of the remaining 29 discordances, 24 fell in the medium discordancy group (1–3.5 kU/L; 7 CAP-/NOV+, 17 CAP+/NOV-), and 5 in the high discordancy group (>3.5 kU/L; 1 CAP-/NOV+ for horse allergen, 4 CAP+/NOV-, of which 3 for birch and 1 for alder). In particular, the three highly discordant results for the birch extract (strong positivity with ImmunoCAP but negative to NOVEOS) were observed in three birch pollen sensitized patients with high levels of

IgE antibodies to Bet v 4 (polcalcin) and no IgE antibodies to Bet v 1 (PR.10) or Bet v 2 (profilin).

4 | DISCUSSION

In a large sera bank of Italian allergic patients, we extensively examined the performance of new, singleplex, allergen-specific IgE assay NOVEOS in comparison with an older standard test (ImmunoCAP). Our study, based on 1260 comparisons on 21 allergen extracts and molecules, demonstrated that NOVEOS predicts the outcome of ImmunoCAP with a 91% sensitivity and a 96% specificity. The obtained results, combined with high analytical reliability and other characteristics (eg low serum volume, fast turnover time), suggest

TABLE 3 Summary of quantitative data; Lin's CCC and Bland-Altman methods

	N	Bias ^a	# over limit ^b	# under limit ^c	SD	SE	CI (95%)	Lin ^d	Spr ^e
Grass									
Bermuda grass	42	-0.01	2	0	0.18	0.06	-0.07 to 0.4	0.97	-0.07
Timothy grass	42	0.1	1	1	0.16	0.02	0.05 to 0.15	0.97	-0.14
Phl p 1	42	-0.09	0	2	0.13	0.02	-0.13 to -0.05	0.97	0.02
Ryegrass	41	-0.17	2	0	0.22	0.03	-0.24 to -0.10	0.93	-0.24
Tree									
Alder	45	0.29	3	0	0.51	0.08	0.14 to 0.44	0.78	-0.09
Birch	45	0.32	4	0	0.54	0.08	0.15 to 0.48	0.75	-0.07
Bet v 1	40	13.00	2	0	0.24	0.04	0.05 to 0.21	0.93	-0.11
Cypress	42	0.5	1	2	0.4	0.06	0.37 to 0.62	0.57	-0.34
Olive	42	0.09	4	0	0.34	0.05	-0.01 to 0.20	0.87	-0.48
Weeds									
Mugwort	42	0.06	3	0	0.23	0.04	-0.01 to 0.13	0.93	-0.16
Ragweed	45	0.15	2	1	0.18	0.03	0.1 to 0.21	0.97	-0.63
Amb a 1	46	0.45	1	1	0.28	0.04	0.37 to 0.54	0.71	0.43
Russian thistle	48	0.07	1	1	0.34	0.05	-0.02 to 0.17	0.87	0.3
Mites									
<i>D. pteronyssinus</i>	42	0.05	2	0	0.27	0.04	-0.03 to 0.14	0.96	-0.37
Der p 1	40	-0.1	2	0	0.22	0.04	-0.17 to -0.03	0.95	-0.25
Der p 2	40	-0.18	2	0	0.18	0.03	-0.23 to -0.12	0.96	-0.35
<i>D. farinae</i>	40	-0.02	2	1	0.23	0.04	-0.09 to 0.06	0.97	-0.18
Animals									
Cat	42	-0.01	1	1	0.36	0.06	-0.12 to 0.10	0.91	-0.17
Fel d 1	42	-0.09	1	2	0.12	0.02	-0.13 to -0.05	0.98	-0.25
Dog	40	0.12	1	0	0.3	0.05	0.02 to 0.21	0.92	-0.51
Horse	40	0.16	2	0	0.25	0.04	0.08 to 0.24	0.9	-0.04
Total	883	0.07	41	3	0.33	0.01	0.05 to 0.09	0.91	-0.26

^aBias, in Bland-Altman, calculated as the mean of the difference of values. obtained with the two methods.

^bNumber of cases over the limit in Bland-Altman.

^cNumber of cases under the limit in Bland-Altman.

^dLin's concordance correlation coefficient.

^eSpearman correlation between difference and average (r).

that the NOVEOS test may be effectively used as an aid in *in vitro* diagnosis of IgE-mediated allergic diseases.

4.1 | Qualitative performance

The overall high sensitivity of NOVEOS sIgE tests in predicting a positive outcome of ImmunoCAP sIgE tests was accompanied by an even higher specificity across all the 21 reagents (15 extracts and 6 molecules). Molecules had higher sensitivity than extracts, while the inverse occurred for the specificity outcome, with molecules presenting a specificity of 94%. This was not surprising since the recombinant allergen molecules have a defined purity and biological activity.²⁶ In a preliminary study,²¹ we had found that the NOVEOS test is a robust one, as it achieved good results in all the required parameters of the clinical validation performed at 2 sites. Concerning repeatability, it yielded a coefficient of variation of 2.6%–7.6%, and within-lab precision ranged from 3% to 11.9%. Limits of detection–limit of blank (LoB) ranged within 0.01–0.03 kU/L, and limit of detection (LoD) 0.03–0.08 kU/L. No significant deviation from linearity was observed for the tests done, as the slope ranged from 0.97 to 1.11. Interference was tested with methylprednisolone, diphenhydramine, omalizumab, biotin and ranitidine, and the obtained IgE recovery values were within the accepted variation. A comparison between the new systems' calibrators and the World Health Organization ones indicated a linear correlation with a high correlation coefficient of $r = 0.994$ and $r = 0.997$ for the 2 sites.²¹

4.2 | Discordant sera

Within the discordant results, the highest proportion of the sera (69% of the cases) has low levels of IgE (≤ 1 kU/L). This difference can be considered as a normal, unavoidable consequence of result variability of sera whose sIgE concentrations fall around the cut-point selected for positivity (0.35 kU/L). The intrinsic variability of both tests (NOVEOS and ImmunoCAP) in this area critical for a dichotomic (POS vs NEG) evaluation of the test is the most likely explanation for the “apparent” discordance. By contrast, a true, highly discordant outcome was obtained in some sera with positive ImmunoCAP for birch extract, but negative NOVEOS result. Our results lead us to speculate that the extract of birch pollen used in the ImmunoCAP, but not the one used in the NOVEOS contains high amounts of polyclonal Bet v 4. We also speculate that other discordant outcomes may be explained by specific, relevant differences in the molecular composition of the solid phase of the allergens used in the two tests. On the other hand, these differences, although important for individual cases, seem to be limited to isolated sera samples and did not affect the overall accuracy of the NOVEOS assays in comparison with the ImmunoCAP assays. The exception in this scenario is the 8 results that have a moderate discordance level for the cypress allergen, with CAP providing a higher result than NOVEOS.

4.3 | Quantitative performance

The results of our qualitative analysis, based on a positive-negative outcome, were confirmed by the quantitative analyses, showing a high level of correlation, either global or across all the 21 reagents examined. The observation that NOVEOS sIgE values could exceed 100 kU/L is of interest. We speculate that this outcome is a sign of a high binding capacity of the NOVEOS assay design due to the vast surface area of about 35 million microparticles per test. Accordingly, this conclusion has been already achieved by a previous study of ours, focusing on the intrinsic analytical properties of the NOVEOS test.²⁶

4.4 | Study limitations

We acknowledge a few limitations of our study. First, as the population samples of children and adults were investigated in two hospitals in Rome, the generalizability of our results may be limited, and our conclusions require to be confirmed by studies in sera from patients living in other world areas. Further studies to investigate the diagnostic performance of NOVEOS in different European and extra-European countries are already in progress. Second, we were not able to test all samples with the 21 allergens due to sera volume limitations. Third, we have not investigated the interference of IgE to carbohydrate determinants (CCD) in the diagnostic performance of NOVEOS, for example by means of inhibition studies with CCD blocking reagents. However, the NOVEOS test has been proven to be scarcely influenced by CCD interference as this method does not use a cellulose-based solid phase.²⁴ Fourth, our study is limited to a set of 15 extracts and 6 molecular components, which is rather small considering hundreds of allergens (many of which are rare) may be used in hospital-based clinical laboratories in Europe. For example, we could not test IgE to pellitory, a very relevant pollen in Italy. Nevertheless, the allergens in this our study cover the vast majority of diagnostic needs of the European patient population affected by allergic rhinoconjunctivitis and asthma.

5 | CONCLUSIONS

In a large sera bank of Italian allergic patients, NOVEOS sIgE predicted the qualitative and quantitative outcomes with high precision for most of the 15 whole allergen (extracts) and 6 molecular components for airborne allergens tests used in this study. Although additional studies are necessary to demonstrate the economic and operational benefits, this study suggests the NOVEOS system is a viable alternative in assessing sIgE levels to these allergens in routine laboratory testing. Our study also suggests room for further improvement of the NOVEOS test by including pellitory in the allergen portfolio, spiking for Bet v 4 the birch extract and improving sensitivity of the cypress extract.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTION

EP and DB carried out the experiments and data collection. VV, GM, ES, IS and ST recruited the patients. VP performed the statistical analysis. SD, CS and PMM conceived the study and assisted in data interpretation. All authors reviewed, edited and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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