Literature Compendium

Cross-reactive Carbohydrate Determinants (CCDs) in Allergy Testing

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Introduction

In vitro allergy diagnostics is a laboratory-based method widely used to identify allergens that induce an immune response in patients with allergies. This technique is based on the measurement of allergen-specific antibodies, particularly immunoglobulin E (IgE), in the patient's blood sample.

The process begins with the collection of a blood sample from the patient, which is then sent to a laboratory for analysis. The sample is incubated with different allergens, and the levels of allergen-specific IgE antibodies are measured.

In vitro allergy diagnostics is a safe, noninvasive, and accurate method for identifying specific allergens that trigger an immune response. It is particularly useful for patients who cannot undergo skin prick testing or have a history of severe allergic reactions. However, it is essential to interpret the results of in vitro allergy testing in the context of a patient's medical history and symptoms, as false positives and cross-reactivity can occur due to the presence of cross-reactive carbohydrate determinants (CCDs) in some allergens.[1]

CCDs are carbohydrate structures present in many plant and insect allergens. These structures are not recognized by IgE antibodies specific to the allergen itself, but they can bind to IgE antibodies specific to CCDs. This can lead to false-positive results and clinical overestimation of a patient's sensitization to certain allergens.[2]

Therefore, it is important for laboratories performing in vitro allergy testing to include controls that can detect the presence of CCD-specific IgE antibodies and interpret the results with caution, particularly when testing for allergens that are known to contain CCDs.[2,3] Despite these challenges, in vitro allergy testing remains a valuable tool for diagnosing and managing allergies, and ongoing research is aimed at improving the accuracy and reliability of these tests.

CCDs in Allergy Testing Literature Compendium

This literature compendium presents a selection of published studies that offer a comprehensive insight into the historical evolution of CCDs in allergy diagnosis and management, tracing their discovery in the early 1980s to their recently uncovered involvement in solid-phase technologies.

Each article is presented as an abstract from the published paper followed by Siemens Healthineers interpretation of the significance of each work. We hope that these synopses encourage you to read each article in its entirety for a more complete understanding of these highly relevant works in the field.

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Glossary of Terms[1]

Allergen extract: The part of allergen sources that is soluble in water or other specific solvents. Allergen extracts from different sources and batches may vary, and the allergen contents can be both qualitatively and quantitatively different. Many proteins or other kinds of molecules without allergenic properties are contained in an allergen extract. The main problem is the presence of multiple allergens in the mixture, some of which may be clinically relevant and others irrelevant, causing cross-sensitization patterns in subjects with sensitization to common components.

Allergen source: The raw material from which the allergen extract is obtained, such as pollens, animal furs, or cultures of molds. Allergen sources vary from producers and over time. Significant batch-to-batch heterogeneity has also been observed. Thus, standardization of allergen sources, and of allergen extracts, is important.

Allergen: The molecule that expresses epitopes recognized by an sIgE.

Allergy: The presence of sensitization to one or more allergens and the presence of clinical symptoms that can be associated with that sensitization. Laboratory tests can only identify sensitization, not an allergy. The diagnosis of allergy is the responsibility of the allergist.

Component: See Allergen.

Cross-binding: The ability of IgE to bind to allergens with significant sequence homology.

Cross-reactivity: Allergy caused by an allergen to which an individual is sensitized via cross-binding to the allergen that caused the primary sensitization.

Cross-reactive carbohydrate determinants (CCDs): CCDs are protein-linked carbohydrate structures. CCDs with wide homogeneity to many allergens are considered pan-allergens. CCDs can be found only in natural allergens, not in recombinant molecules produced in *E. coli*. In patients sensitized to CCDs, sIgE tests on allergen extracts may show false-positive results.

Cross-sensitization: Sensitization caused not through primary exposure, but due to cross-binding of IgE to allergens with significant sequence homology. Cross-sensitization may be clinically irrelevant. If it causes symptoms, it may be referred to as cross-reactivity.

Molecular allergen: See Allergen.

Primary sensitization: Sensitization caused by the individual allergen itself rather than through cross-sensitization to a homologous allergen.

Recombinant allergen: Allergens produced through genetic engineering and often expressed in *E. coli*.

Recombinant component: see Recombinant allergen.

Sensitization: The presence of sIgE to one or more allergens in serum tests. In skin prick tests, sensitization is the presence of a cutaneous reaction in the presence of a given allergen. Sensitization cannot be considered an allergy.

sIgE: sIgE is the antibody secreted in sensitized patients and specific for a given allergen. The detection of these antibodies suggests that the patient is sensitized to the allergen. The presence of signs and symptoms compatible with the IgE profile allows the allergist to identify the patient as allergic.

Total IgE: Total IgE is the concentration of IgE circulating in the serum. It includes allergens that do not generally cause a severe or systemic reaction in sensitized patients, although exceptions are possible, and also includes profilins, polcalcins, PR-10, and CCDs.

Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom

Aalberse R, Koshte V, Clemens JGJ. Journal of Allergy and Clinical Immunology. 1981;68(5):356-364.

Objective

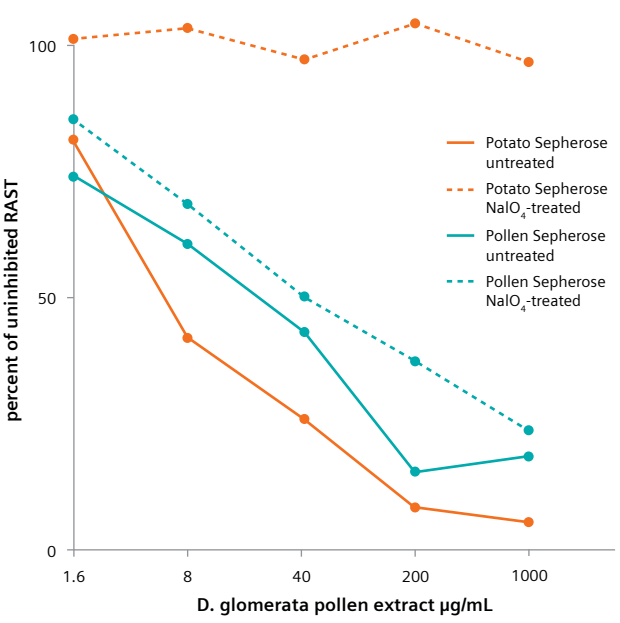
Elucidation of the phenomenon of multiple reactivity of individual sera to plant foods, pollen, and hymenopteran venoms

Methods

- To determine the presence of allergen specific IgE, RAST was performed on patients allergic to foods, pollen and/or Hymenoptera venoms.
- RAST inhibition assays for the determination of cross-reacting antigen were performed with potato or grass pollen extract.
- Periodate treatment to break down terminal sugar residues was performed with $NaIO_4$ at room temperature in the dark.

Results

- Serum P was positive in RAST with pollen from various grasses, weeds, and trees, house dust mite, dander from cats, dogs and rabbits, cow's milk, chicken egg white, mussel, rice, wheat, buckwheat flour, peanut, soybean, hazelnut, walnut, banana, strawberry, apple, pineapple, orange, ginger, potato, spinach, tobacco leaves, grass juice, buckwheat honey, honeybee venom, and vespid venoms.
- RAST inhibition results indicate complete immunological match of an allergen in grass pollen, buckwheat flour, potato tubers, and honeybee venom.
- The inhibiting capacity of potato or grass pollen extract was reduced by periodate treatment.



Authors' Conclusion

"We therefore conclude that the IgE-binding to potato or buckwheat is most likely caused by IgE antibodies that specifically bind to some ubiquitous antigen. The periodate susceptibility [...] lead us to believe that this antigen or determinant is most likely a carbohydrate."

Significance

- This early study provided information on multiple reactivity of patient sera to unrelated allergenic sources such as pollen, food, and Hymenoptera venoms.
- The study demonstrated the relevance of glycosylations on allergens and their nature as a confounding factor in in vitro allergy diagnostics.
- This study coined the term "cross-reactive carbohydrate determinant" and is the foundation of more than 30 years of ongoing research.

Table 1. Comparison between the crossreactivity pattern of sera from two patients (P and W).*

	Serum P	Serum W
Grass pollen	33	28
Buckwheat honey	29	35
Peanut	28	34
Oats	27	32
Coffee	27	33
Potato	25	36
Spinach	25	33
Mussel	22	30
Wheat	21	20
HBV	20	44
Pectin	19	16
Tragacanth	19	18
Vespid venom	16	26
Carob	7	1

**A direct RAST assay was used (25 µL of serum per test). The results, which were obtained in a single assay, are expressed as percentage bound radioactivity after blank correction.*

Peanut allergy diagnosis in the context of grass pollen sensitization for 125 patients: Roles of peanut and cross-reactive carbohydrate determinants specific IgE

Guilloux., Morisset M, Codreanu F, Parisot L, Moneret-Vautrin DA. International Archives of Allergy and Immunology. 2009;149(2):91-7.

Objective

The performances of two in vitro methods were evaluated for peanut sIgE measurement in patients allergic to grass pollen with or without subsequent allergy to peanuts. The correlation between clinically irrelevant peanut sIgE and the presence of CCD sIgE was investigated.

Methods

- In vitro measurement of peanut sIgE was performed using the Pharmacia IMMUNOCAP system radioimmunoassay and the IMMULITE® 2000 3gAllergy™ system.
- Discrepancies between in vitro results and peanut allergy diagnosis were evaluated by measurement of CCD sIgE using bromelain and ascorbic acid oxydase (AAO).
- A total of 125 subjects were recruited and selected according to their clinical history and sensitization to peanuts and/or pollens.
  - Group Ia: 29 peanut allergy patients without symptoms related to pollen and with negative SPT to grass pollen.
  - Group Ib: 29 peanut allergy patients with positive SPT to grass pollen.
  - Group IIa: 26 patients not sensitized to any pollen.
  - Group IIb: 41 patients with hay fever and positive SPT results to grass pollen.
- Peanut allergy was assessed with positive SPT results to peanuts and either a positive double-blind placebo-controlled food challenge (DBPCFC) or a positive labial challenge to peanuts.

Results

- IMMULITE 3gAllergy and PHADIA IMMUNOCAP both demonstrated 100% sensitivity in Peanut allergic patients.
- IMMULITE 3gAllergy and PHADIA IMMUNOCAP both demonstrated 96% specificity in non-peanut allergic patients who were not sensitized to any pollen (IIa).
- IMMULITE 3gAllergy demonstrated significantly greater specificity than PHADIA IMMUNOCAP in non-peanut allergic patients who were sensitized to pollen (IIb).
- PHADIA IMMUNOCAP produced 2x as many false positive results as IMMULITE 3gAllergy (22 vs. 11).
- The specificity for IMMULITE 3gAllergy was 73%, significantly higher than the 46% specificity for PHADIA IMMUNOCAP ($p = 0.02$)
- Anti-CCD IgE was >0.35 kU/L in 86% of the 22 patients with clinically irrelevant positive peanut results by PHADIA IMMUNOCAP and in 100% of the 11 patients by IMMULITE 3gAllergy.

Authors' Conclusion

"Taking all groups into account, IMMULITE 3gAllergy had a significantly better specificity (82% vs. 66%), better PPV (82% vs. 71%) and a more positive likelihood ratio (5.6 vs. 2.9) than the PHADIA IMMUNOCAP system."

Significance

- This is the first study taking CCDs into account while comparing the performance of IMMULITE 2000 3gAllergy to a solid-phase system.
- The study showed high diagnostic accuracy of IMMULITE 3gAllergy for patients with CCD sensitizations.
- The authors linked false-positive results on the solid-phase platform to CCD sensitization.

Table 1. A summary of skin tests, food challenges and peanut butter sIgE measurements performed in this study.

Patient group	Atopic	Peanut SPT, mm		DBPCFC threshold of reactivity, mg		IMMUNOCAP peanut sIgE kU/L		Immulite peanut sIgE kU/L	
		Median	Range	Median	Range	Median	Range	Median	Range
Ia (n = 29)	22	8	5–16	100	4.4–7000	92	1.4 to >100	>100	1.1 to >100
Ib (n = 29)	29	10	0.5–17	265	4.4–7000	49	3.3 to >100	>100	3.1 to >100
IIa (n = 26)	11	0	0–0	n.d.	n.d.	<0.35	<0.35–0.35	<0.10	<0.10–0.91
IIb (n = 41)	41	0	0–1.5	n.d.	n.d.	0.68	<0.35–53	0.11	<0.10–14

Ia = Peanut-allergic patients without symptoms related to pollen; Ib = peanut-allergic patients with grass pollen sensitization; IIa = control patients not sensitized to any pollen; IIb = control patients with hay fever; n.d. = not determined.

Inhibition of cross-reactive carbohydrate determinants (CCDs) enhances the selectivity of in vitro allergy diagnosis

Aberer W, Holzweber F, Hemmer W, Koch L, Bokanovic D, Fellner W, Altmann F. Allergologie Select. 2017;1(2):141-9.

Objective
A CCD inhibitor was tested as a generally applicable solution for false-positive tests due to CCDs in in vitro allergy diagnostics.

Methods
- CCD sensitized patients were recruited out of clinical routine.
- sIgE was tested on three different, commercially available allergy diagnostic systems before and after incubation with CCD inhibitor.
- The CCD inhibitor used was a MUXF^3 carrying glycopeptide coupled to human serum albumin.

Results
- Of approximately 6000 serum samples tested, 22% showed IgE antibodies to CCDs.
- In the age group of 10- to 20-year-old people, 35% were affected by anti-CCD IgE.
- In a random sample of 43 patients, complete inhibition of CCDs as well as specific allergens was achieved in 41.9%.
- In 51.2% of the patients the inhibition was partial and failed in 3 patients.
- IgE titer to recombinant Api m 1 measured on a cellulose-based, solid-phase platform was reduced after the patient sera was incubated with CCD inhibitor (see Hemmer et al. 2018).

Authors' Conclusion
"The present study does clearly show that the CCD problem is, quantitatively speaking, the prime cause of discrepant allergy reports."

Significance
- The study demonstrated the relevance of CCD sensitization and its role as a confounding factor in allergy diagnostics.
- The authors described, for the first time, CCD-related false-positive IgE results on a solid-phase platform when tested with recombinant allergens.
- This study was the basis for subsequent research investigating the role of remnant CCDs on solid phases used in in vitro allergy diagnostics.

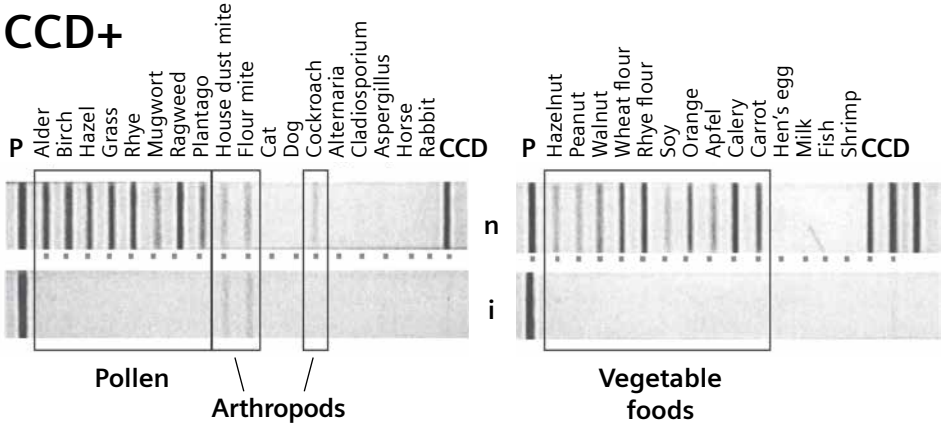


Table 1. Results for patient "m 46", investigated with the multi-allergen strip (MW), IMMUNOCAP, and IMMUNOCAP ISAC without (n) and with (i) CCD inhibition. A check mark (✓) indicates anticipated inhibition or non-inhibition that was rated correct (☑). Questionable results are marked by the (☒) sign.

Allergen source	MW n	MW I	CAP n	CAP I		Component	ISAC n	ISAC I	
	U/mL	U/mL	U/mL	U/mL			ISU-E	ISU-E	
Bee venom	—	—	28.5	1.1	☒	—	—	—	—
rApi m1	—	—	1.63	0.31	↗	rApi m1	0	0	↗
Wasp venom	—	—	28.5	18.3	☑	rPol d5	1.0	1.5	→
rVes v1 / v5	—	—	11.8 / 48.7	9.7 / 44.6	→	rVes v5	6.2	8.9	→

IMMUNOCAP cellulose displays cross-reactive carbohydrate determinant (CCD) epitopes and can cause false-positive test results in patients with high anti-CCD IgE antibody levels

Hemmer W, Altmann F, Holzweber F, Gruber C, Wantke F, Wöhrl S. Journal of Allergy and Clinical Immunology. 2018;141(1):372-81.e3.

Objective

The study investigated the potential role of the IMMUNOCAP cellulose matrix (solid phase) as the origin of the observed carbohydrate directed reactivity (see Aberer et al. 2017).

Methods

- 52 CCD positive sera with varying levels of anti-CCD antibodies (bromelain, 1.01-59.6 kUA/L) were tested on a blank IMMUNOCAP (SA-CAP-1).
- Fifteen of the CCD-positive sera were also tested on SA-CAP-1 after serum preincubation with a CCD blocker.
- Blocking of anti-CCD IgE was done by preincubating sera with a commercially available semisynthetic CCD inhibitor made up of purified MUXF^3 glycopeptides.
- Ten sera with anti-CCD IgE (14.0–52.5 kUA/L) were tested on a panel of 4 recombinant allergens, rBet v 2, rPru p 3, rFel d 1, and rAra h 2, with and without prior CCD inhibition.
- Seven CCD positive patients with a history of anaphylaxis after a Hymenoptera sting and 1 CCD-positive control subject were tested by using IMMUNOCAP with whole venoms and the recombinant major venom allergens rApi m 1, rVes v 1, and rVes v 5 before and after serum inhibition with the CCD blocker.

Results

- Of 52 CCD-positive sera tested, 35 (67%) bound with a score of greater than 0.35 kUA/L to SA-CAP-1 (0.41-4.22 kUA/L).
- Of 10 selected CCD-positive sera tested on a panel of 4 recombinant allergens, 8 bound with a score of greater than 0.35 kUA/L to at least 1 of the components (0.36–1.63 kUA/L).
- Antibody binding to the nonculprit venom for patients with venom allergy was inhibited by MUXF^3-HSA by 75% to 95% in all sera.
- 4 of 5 had positive results to rApi m 1 (0.45–1.63 kUA/L), and 1 of 5 had a borderline result. Reactivity with rApi m 1 was strongly inhibited by MUXF^3-HSA, whereas binding to rVes v 1 and 5 was not.

Authors' Conclusion

"In conclusion, we showed in this study that the allergen carrying cellulose matrix of the IMMUNOCAP contains small amounts of residual CCDs sufficient to cause nonspecific background binding in sera with high levels of anti-CCD IgE antibodies."

Significance

- The study demonstrated the presence of remnant CCDs on cellulose-based solid phases in commercially available allergy diagnostic platforms.
- This publication assessed the impact and magnitude of CCD-related false-positive results on the diagnosis of Hymenoptera venom allergy.

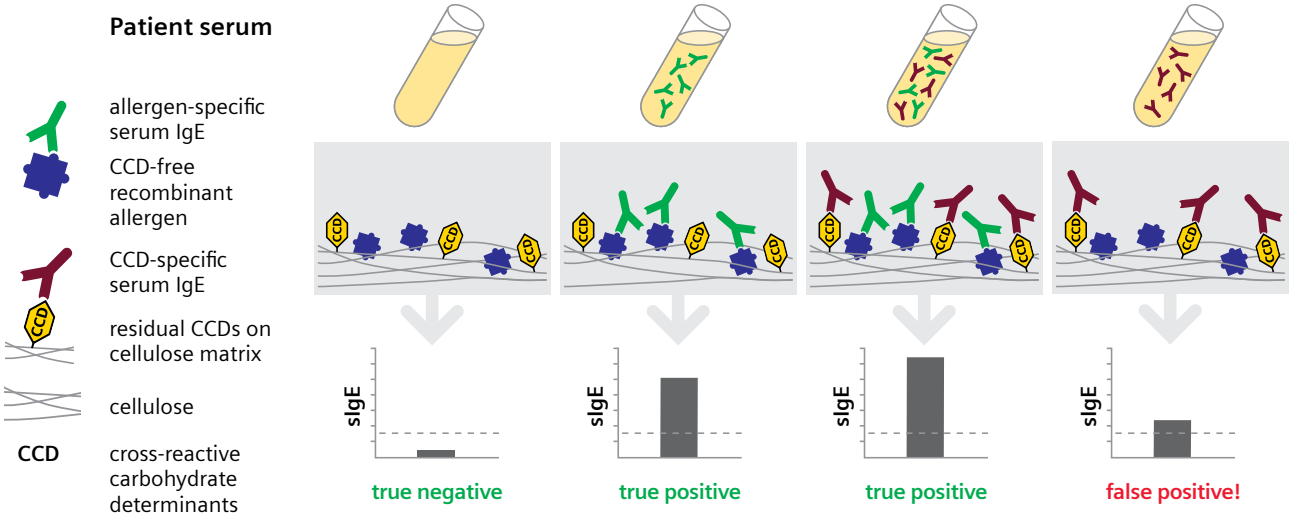


Table 1. IgE reactivity with recombinant allergens tested by using IMMUNOCAP singleplex before and after inhibition with MUXF³-HSA in 2 patients with high anti-CCD IgE levels.

	IMMUNOCAP (kU/L)	
	Before CCD inhibition	After CCD inhibition
Case 1		
Bromelain/MUXF³-CCD	44.60	0.38
Almond	49.00	0.24
rAra h 1 (peanut 7S)	0.42	0.04
rAra h 2 (peanut 2S)	1.63	0.00
rAra h 3 (peanut 11S)	1.16	0.01
rAra h 9 (peanut LTP)	0.53	0.00
rCor a 8 (hazelnut LTP)	0.58	0.00
rBet v 1	0.94	0.02
rBet v 2	1.12	0.04
Case 2		
Bromelain/MUXF³-CCD	24.60	1.17
Vespula species venom	34.00	17.40
rVes v 1	11.80	9.72
rVes v 5	48.70	47.6
Honeybee venom	27.60	1.25
rApi m 1	1.63	0.11
Birch pollen	19.60	0.11
rBet v 1	0.75	0.03
rBet v 2	0.74	0.04
rBet v 4	1.00	0.00
Grass pollen	25.00	1.11
rPhl p 1	0.42	0.04
rPhl p 7	0.47	0.02
rPhl p 12	0.88	0.05
Mugwort pollen	21.60	0.21
nArt v 1	1.28	0.02
Ragweed pollen	25.7	0.49
nAmb a 1	1.94	0.04

Table 2. IgE binding of 10 CCD-positive sera with bromelain, allergen-free streptavidin-CAP (SA-CAP-1), before (–CCD) and after (+CCD) CCD inhibition.

Patient number	Bromelain (kU/L)	SA-CAP-1 (kU/L)	
		–CCD	+CCD
1	44.6	4.22	0.04
2	52.5	ND	ND
3	40.1	2.44	0.05
4	32.1	1.33	0.09
5	24.6	2.55	0.04
6	17.2	1.79	0.01
7	16.7	2.36	0.02
8	14.0	1.24	0.01
9	40.9	0.71	0.01
10	15.4	0.89	0.06

ND: not determined.

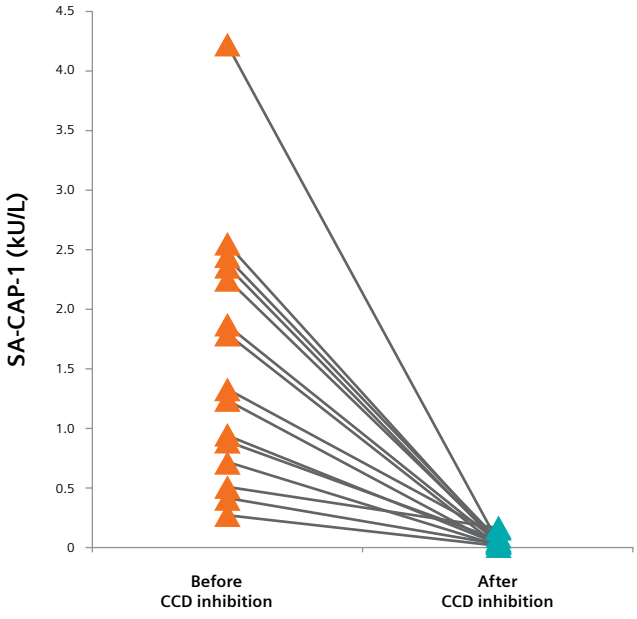


Fig 1. IgE binding to allergen-free IMMUNOCAP's coupled with streptavidin (SA-CAP-1) in 15 CCD-positive sera before and after serum inhibition with a CCD inhibitor (MUXF³-HSA).

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