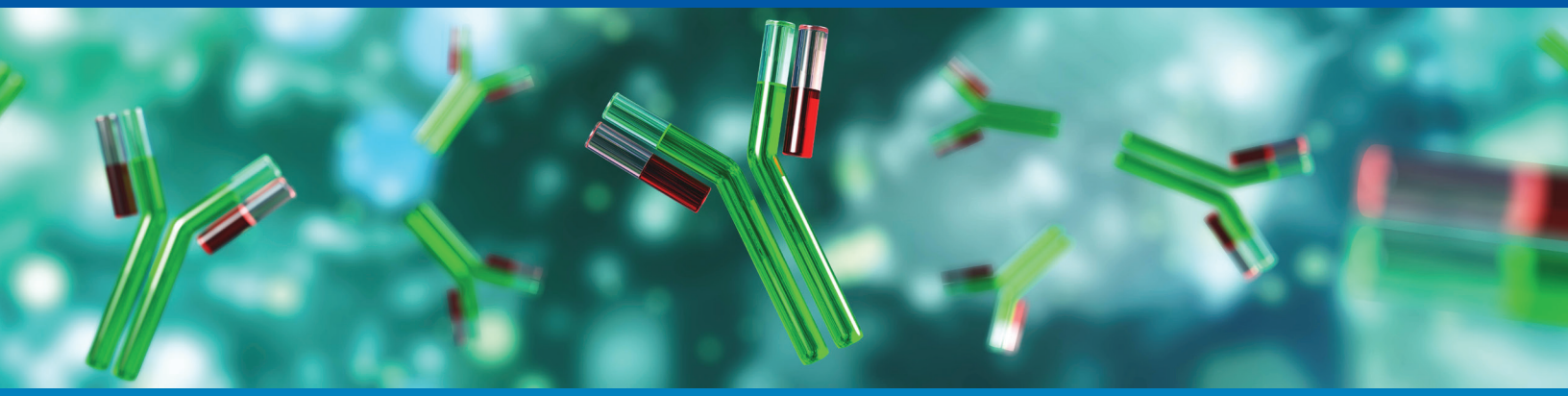

CCD Interference in Allergy Testing:
Why Laboratorians Should Worry

Eric Whitters, Ph.D.

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EXECUTIVE SUMMARY

False positive results due to cross-reactive carbohydrate determinants (CCD) have been observed in allergy blood testing since the early 1980's. The positive *in vitro* tests caused by CCDs give the impression that a patient is sensitized to a given allergen even though the patient does not have clinical symptoms. When the cross-reactivity was discovered, researchers concluded that this phenomenon was innate to the proteins themselves because carbohydrates, bound to the allergen, produced the aberrant patient result. Laboratorians, therefore, accepted that false positive results would occur as a result of the protein chemistry used in blood testing for specific IgE measurement.

More recent studies have demonstrated that the false positive results may not be, in many cases, associated with the allergen at all; rather, the result may be a procedural artifact where patient IgE binds indiscreetly to the carbohydrate solid phase of the market leading technology. Laboratorians using this method need to exercise caution since they could be reporting out results that are positive strictly because of the technology's underlying design rather than anything associated with immunochemistry.

"Cellulose used as a solid-phase allergen carrier can contain varying amounts of cross-reactive carbohydrate determinants (CCDs) sufficient to cause false-positive test results up to 2 kU_A/L."[1]

– Hemmer W, et al., 2018

This paper provides a technical overview of the CCD phenomenon and is intended to help laboratories understand the potential of false positive results in *in vitro* allergy testing when using a carbohydrate containing solid phase.

INTRODUCTION

The terms CCD or CCDs describe protein-linked carbohydrate structures responsible for cross-reactivity of sera from allergic patients toward a wide range of seemingly unrelated allergens. The incidence of anti-CCD interference in allergy immunoassays began in the early 1980's where laboratories began reporting cross-reactivity between plant and insect allergens.[2,3] Studies later indicated that a common epitope was involved that contained sugar molecules linked to the protein backbone. Asparagine-linked oligosaccharides (N-glycans) containing α1,3-linked fucose residues were recognized as foreign because human N-glycans contain a different configuration of these sugar-linked amino acids.

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Of greater interest, however, was that provocation tests in patients as well as empirical evidence indicated that this type of anti-CCD reactivity never caused any obvious allergic symptoms.[4] Therefore, it appeared that the incidence of false positive results related to anti-CCD reactivity was primarily an inconvenience that required additional testing rather than a clinically relevant phenomenon.

Unfortunately, this phenomenon was more common than most laboratorians recognized. The prevalence of CCD reactivity has been described in as much as 25% of the general population.[4,5] It is particularly prevalent for common food allergies like peanut, wheat and nuts; clinicians have been urged to utilize Component Resolved Diagnostics (CRD) to separate true positivity in patient results from the more trivial anti-CCD reactivity. Although CRD testing can identify false positive results related to the extract itself, many allergens do not have "CCD-free" components and this restricts their use as an appropriate tool for many affected samples. In addition, there are more recent publications demonstrating false positive results unrelated to the allergic sensitization. In these cases, the use of CRD only increases the cost of testing with no obvious benefit to the patient.

FALSE POSITIVE RESULTS IN CELLULOSE-BASED TESTING

Unbeknownst to most researchers, laboratories and key opinion leaders, recent data demonstrate that the ImmunoCAP™ cellulose matrix that is used as the solid-phase allergen carrier is recognized by anti-CCD antibodies in many patients at concentrations high enough to cause significant non-specific binding.[4] This non-specific binding triggers false-positive results as suggested in Figure 2.

Products should be designed to ensure that results accurately reflect the disease state of the patient. CCD itself is **NOT** an allergen, but CCD on the cellulose matrix is a source of non-specific, false positive reactivity. As shown by Hemmer and colleagues,[4] even the use of "CCD-Free" allergens does not address false positive anti-CCD reactivity seen on cellulose-based assays. The data demonstrated that for many patients, suspected allergies for peanut, tree nuts, wheat and venoms may simply be an artifact of the testing method rather than a true allergy:

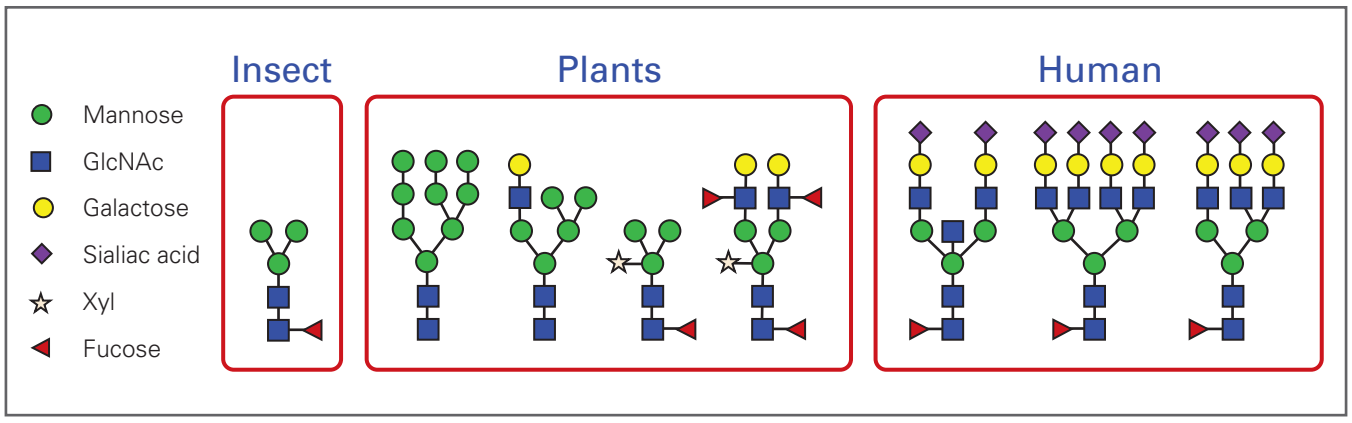


Figure 1: The configuration of N-glycans in insects, plants and humans. The presence of xylose and α1,3-linked fucose residues on insect and plant proteins are recognized as foreign because they differ from endogenous N-glycans in humans.

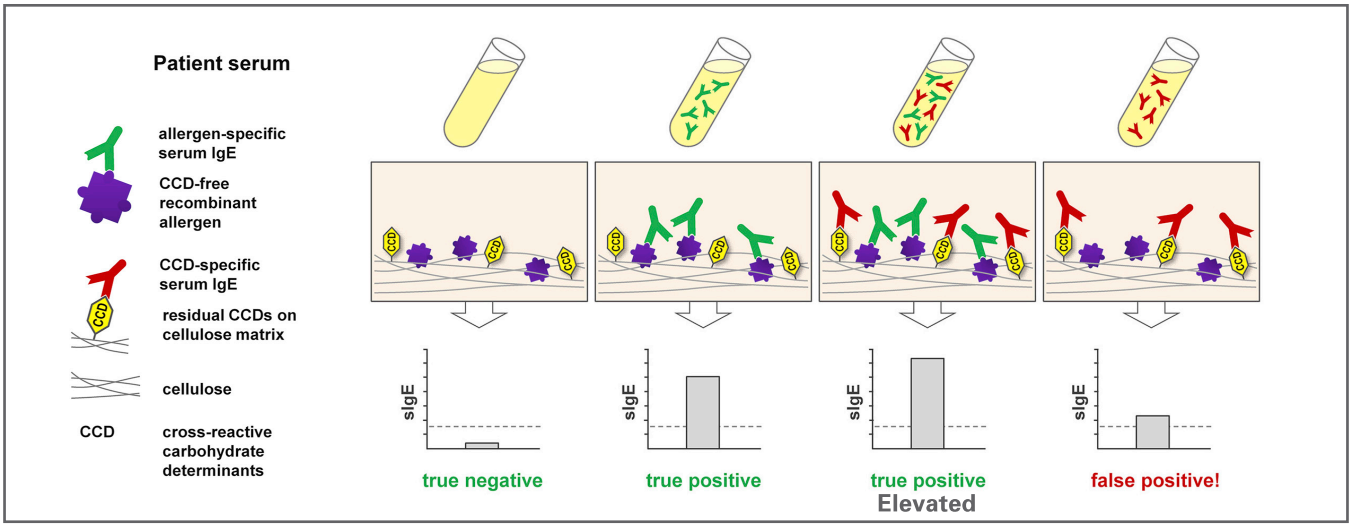


Figure 2: Residual CCDs on cellulose matrix creates false positive results. Since the cellulose matrix contains residual CCDs in addition to the bound allergens, a patient with CCD reactivity will exhibit abnormally high values for allergies that they have (column 4) and false positive values for allergies that they do not have (column 3).

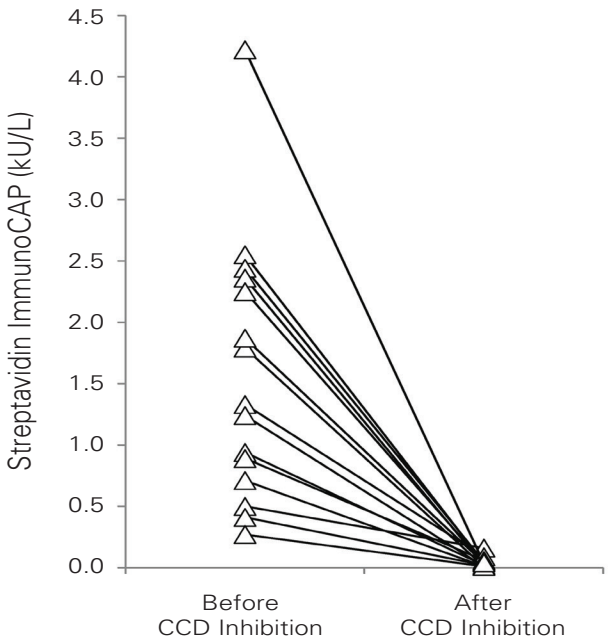


Figure 3: CCD reactivity with the cellulose matrix despite the use of CCD-Free allergens. Patients were analyzed for CCD reactivity either before CCD inhibition or after addition of a CCD inhibitor used to block binding to CCDs present on the cellulose matrix. The data demonstrates why laboratories should be cautious when using cellulose-based assays since substantial cross-reactivity created by the system can lead to the erroneous results.

Anti-CCD reactivity is more common in the general population than originally believed.[4,5] What is more difficult to determine is the incidence of patients with no obvious allergic symptoms who are obtaining false positive results on the cellulose-based systems due to this CCD reactivity. In addition, it is not clear whether other patient results on these platforms are mildly or grossly "elevated" due to the additive impact of anti-CCD reactivity to the baseline of true allergen sensitivity.

In vitro allergy testing is used as an aid in diagnosis of allergic disease. False positive or elevated results due to non-specific binding of CCD in the assay are misleading and may interfere with proper diagnosis, require additional diagnostic testing or unnecessary treatment, and cause undue stress to patients.

It is unknown whether the leading manufacturer of cellulose-based assays can or will address these issues since altering the basic nature of the cellulose support matrix would introduce significant performance, cost and regulatory complications. Moreover, customer confidence in the product would be shaken once laboratories understood the ambiguous nature of results in the presence of anti-CCD reactivity.

False Positives from CCD despite using CCD-Free Allergen Components

	Number	(%)
Total patients tested	236	100%
Number with CCD reactivity	110	47%
Number CCD false positive (cutoff 0.35 kU/L)	19	8%

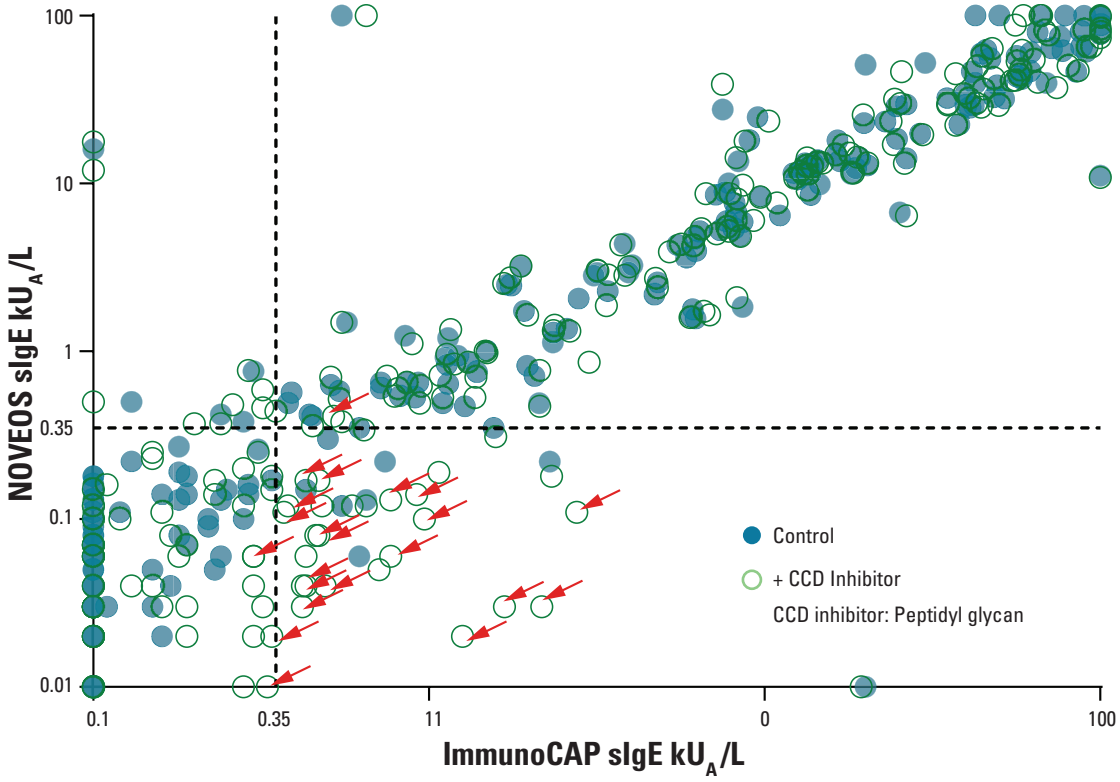
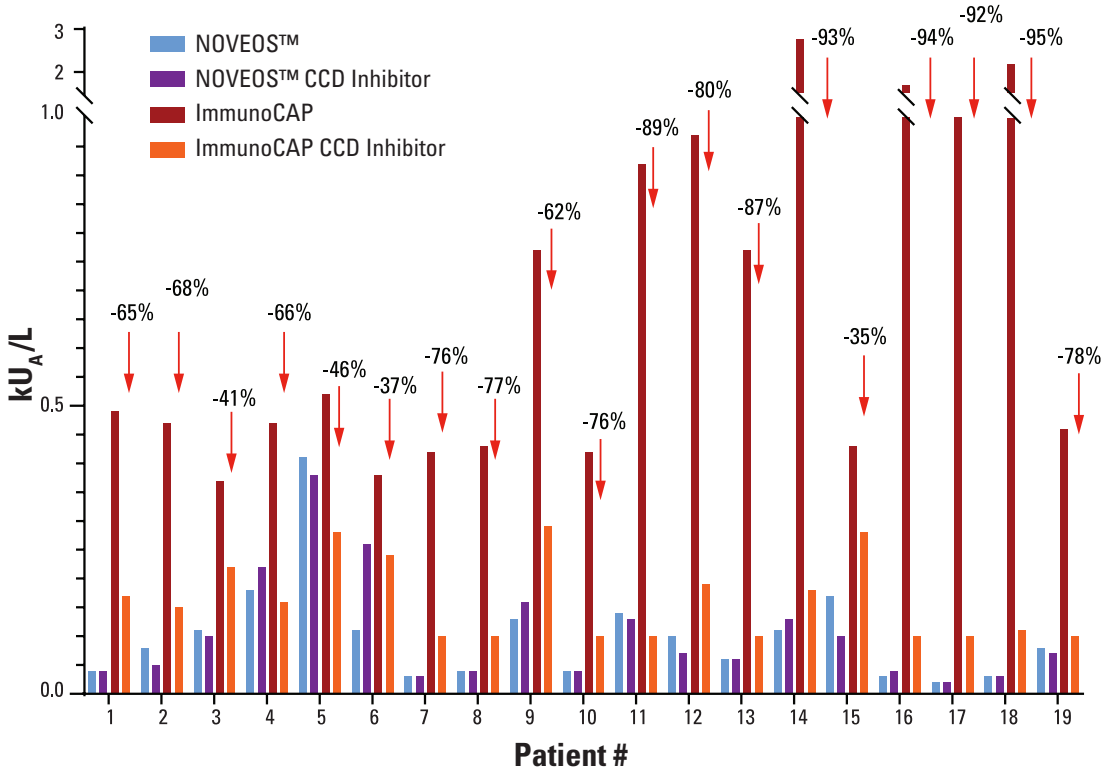


Figure 4: Population of 236 samples tested on the Phadia and NOVEOS systems using CCD-free components and in the presence (filled circles) or absence (open circles) of CCD inhibitors. Red arrows designate samples false positive results on the Phadia system. The data suggests that samples near the cutoff of 0.35 are affected and generate false positive values that can be misleading.



Whole Allergen	Component
F013	Ara h 1
	Ara h 2
	Ara h 3
	Ara h 8
T003	Bet v 1
	Bet v 2
G006	Phl p 1

Figure 5: Closer examination of nineteen (19) false positive patients on the NOVEOS and Phadia instruments in the presence or absence of a CCD inhibitor. Assays were tested using CCD-free peanut components suggesting that the change in reactivity in the presence of a CCD inhibitor was due to reactivity to the assay solid phase rather than any interaction with the proteins themselves.

The NOVEOS™ Chemiluminescent Method

Cost-Effective, Accurate and Precise

- Highly-automated
- Superior walk-away time/ability
- Intuitive user interface for ease of training and operation
- Liquid, ready-to-use reagents

Uses only 4µL of Specimen per Test

- Improves lab workflow and operational costs
- Reduces Quantity Not Sufficient (QNS) errors
- Reduces patient resampling due to insufficient volume
- Reduces trauma for hard-to-draw patients

Reduction of variability

- Large reagent lot sizes
- Use of standardized extracts when available
- Every allergen receives extensive biochemical characterization to ensure performance
- Assay design is unaffected by biotin or cellulose-related cross-reactive carbohydrate determinants interferences

Trusted Analytical Performance

- Cutting-edge immunochemistry technology
- Paramagnetic microparticles
- High sensitivity and excellent low-end precision

About HYCOR® Biomedical

With over 40 years of experience, HYCOR Biomedical is a global manufacturer and marketer of in vitro diagnostic products.

Since its founding in 1981, HYCOR has supported clinical laboratories, hospitals and doctors' offices worldwide with allergy and autoimmune instrumentation and reagents. Among its products, HYCOR markets the HYTEC™ and AUTOSTAT® instruments and most recently the NOVEOS™ Immunoassay System. Each has received CE Mark for the European Union and FDA clearance in the United States.

The company is focused on delivering innovative technology products and comprehensive services that provide the highest value to clinicians and laboratories.

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