GLOSSARY:

**Carbohydrate determinant:** A protein-linked carbohydrate (sugar) chain that can bind IgE

**Covalent:** A bond between two atoms involving the sharing of an electron pair

**Cross-reactive carbohydrate determinant (CCD):** A protein-linked carbohydrate (sugar) chain that can bind IgE and be responsible for cross-reactivity of sera from allergic animals towards certain allergens (such as plants and insects)

**Epitope:** The part of a antigen molecule that a antibody attaches itself to

**Glycosylation:** An example of protein modification involving the reaction of a carbohydrate with an amino acid side chain

**Motif:** A small region/pattern (in this case of sugar residues which form the carbohydrate side chain)

**Multi-reactive sera:** Sera that contains IgE that binds to multiple allergens across a group e.g grasses

WHAT IS A CARBOHYDRATE DETERMINANT?

The aim of allergen-specific IgE serological testing, is to detect the amount of IgE antibodies an individual animal has, in it’s blood, against each particular allergen. The allergen extracts used for testing are predominantly proteins.

In nature, after they have been synthesised, the vast majority of proteins are further modified, this includes proteins which are common allergens such as plant pollens and insects.

Glycosylation is an example of this protein modification; it involves the linkage of sugar residues to the protein via an amino acid side chain (see Figure 1). The unique patterns the sugar residues form are called a ‘motif’. When these sugar residues join together in a chain it is referred to as a ‘carbohydrate determinant’.

The two most common forms of glycosylation are:

1. **N-glycans** - where the carbohydrate determinant is linked to the protein via the nitrogen of the asparagine amino acid side chain and;
2. **O-glycans** - where the carbohydrate determinant is covalently linked through the oxygen of the serine or threonine amino acid side chain.
Mammalian proteins do not have the same carbohydrate determinants as plants and insects. This means that the mammalian host immune system is more likely to develop IgE antibodies against plant and insect carbohydrate determinants on exposure, than autoantibodies to itself (against mammalian carbohydrate determinants).

**HOW DO CARBOHYDRATE DETERMINANTS CAUSE ALLERGEN CROSS REACTIVITY?**

Carbohydrate determinants are widely distributed between various proteins; it is therefore likely that different plant and insect proteins will be glycosylated with similar sugar motifs. As a consequence, any IgE antibodies against a particular motif on one plant or insect will also bind when they find it on another, and so immunologically cross-reactivity will be observed. Hence the term ‘cross-reactive’ carbohydrate determinants (CCDs). This could account for the frequent occurrence of multi-allergen responses (runs of positive results) historically seen in serological assays (Figure 2).

**Figure 2.** An example of allergen cross-reactivity

Two proteins (1 & 3) are glycosylated with the same sugar motif and so both are recognised by the IgE. The IgE binds to the sugar motifs regardless of the protein they are associated with. Protein 2 is glycosylated with a different sugar motif and is therefore not recognised by the IgE in this case.
HOW DO CCDS AFFECT SEROLOGY RESULTS?

The majority of serological results are unaffected by CCDs, but in some cases the presence of CCD-binding IgE could lead to a false positive result. Table 1 outlines possible serological samples received, and the affect that CCDs can have on the results.

Table 1. Possible serological results

<table>
<thead>
<tr>
<th>What’s in the animal’s serum?</th>
<th>What results would you expect?</th>
</tr>
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<tbody>
<tr>
<td><strong>GROUP 1</strong>&lt;br&gt;Animals without any IgE to the allergen under investigation</td>
<td>There are no IgE antibodies to bind to either the protein of the allergen under investigation or the CCD.&lt;br&gt;The result is a negative which is correct.</td>
</tr>
<tr>
<td><strong>GROUP 2</strong>&lt;br&gt;Animals with IgE antibodies to the protein of the allergen but not to the CCDs.</td>
<td>The IgE antibodies bind to the protein of the allergen under investigation but there are none to bind to the CCD.&lt;br&gt;The result is positive which is correct.</td>
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<tr>
<td><strong>GROUP 3</strong>&lt;br&gt;Animals with IgE antibodies to both the protein of the allergen and to CCDs.</td>
<td>The IgE antibodies bind to both the protein of the allergen under investigation and the CCD.&lt;br&gt;The result is positive. This is correct <strong>BUT</strong> the result could be much higher due to CCD binding in addition</td>
</tr>
<tr>
<td><strong>GROUP 4</strong>&lt;br&gt;Animals with IgE antibodies to the CCDs of the allergen under investigation but not to the protein.</td>
<td>The IgE antibodies bind to the CCD of the allergen under investigation but there are no IgE antibodies against the protein portion.&lt;br&gt;The result is positive however, in the majority of cases IgE binding to a CCD is not currently thought to be clinically relevant so this is a false positive.</td>
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From the current available evidence, anti-CCD IgE antibodies in animals are not thought to be associated with disease in the vast majority of cases. Any resultant CCD-specific IgE response is, therefore, of questionable clinical significance and could lead to serological false positive reactions. This is particularly true for multi-reactive sera (serum where many allergens are positive often from the same group e.g. grasses). In dogs with atopic dermatitis, the prevalence of sensitisation to CCDs has been reported to be 24%.

**WHAT IS A CCD BLOCKER AND WHY DO WE USE THEM?**

A CCD blocker prevents binding of anti-CCD IgE antibodies to CCDs. Incorporation of CCD blockers into the IgE serology assays is the most widely-used approach (Figure 3) to reduce the impact of CCDs on *in vitro* tests.

CCD blockers that comprise a mix of natural glycoproteins have the potential to cause unwanted inhibition (preventing genuine hypersensitivity reactions where binding of IgE to the protein component occurs from being detected). The use of a CCD blocker where all visible protein epitopes have been destroyed is therefore more desirable.

**Figure 3.** The use of CCD blocker in an IgE serological assay

<table>
<thead>
<tr>
<th>WITHOUT CCD BLOCKER</th>
<th>WITH CCD BLOCKER</th>
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</thead>
<tbody>
<tr>
<td>IgE binds to relevant allergens AND non-relevant CCD</td>
<td>Non-relevant binding is blocked by the CCD blocker. This can then be washed away prior to detection</td>
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</table>
Without CCD blocking, IgE binding to both proteins and sugar motifs is detected. With the addition of the CCD blocker, antibodies that would have previously bound to the sugar motifs are blocked and removed in the assay wash step. The detection reagent (anti-IgE antibody-alkaline phosphatase) then binds the non-N-Glycan (non-CCD) associated IgE. The alkaline phosphatase subsequently reacts with a substrate to produce a soluble yellow compound which is proportional to the amount of IgE present.

THE AVACTA ANIMAL HEALTH CCD BLOCKER
Following extensive research, Avacta Animal Health has developed its own unique CCD blocker. This is incorporated into all of our canine and feline IgE environmental assays, to inhibit binding of N-glycan specific IgE so that only serum IgE antibodies specific for protein portion of allergens (which are those thought to be of clinical significance) are detected.

PUBLICATIONS


Gedon NKY, Boehm TMSA, Klinger CJ, Udraite L and Mueller RS. “Agreement of serum allergen test results with unblocked and blocked IgE against cross-reactive carbohydrate determinants (CCD) and intradermal test results in atopic dogs.” (2019) Vet Dermatol 30: 195-e61


