



The original ALLERCEPT test for the detection of specific IgE was launched in 1997. It revolutionized dog, cat, and horse IgE measurements, assuring for the first-time absolute specificity for IgE detection.

Since it was introduced, the ALLERCEPT test has been recognized as the reference in *in vitro* IgE detection.

ALLERCEPT continues leading the IgE detection area by establishing new accuracy standards in IgE detection. The Heska approaches are unique, and innovative:

- **Preventing interfering CCD-IgE reactions**
- **Absolute specificity for IgE detection**
- **Use of a purified IgE standard curve**
- **Result evaluation according to test detection limit**

**Preventing interfering CCD-IgE reactions (IgE against cross-reactive carbohydrate determinants)**

When testing patient serum to measure specific IgE, it is commonly observed an unexpected number of multi positive pollen results. These results, which cannot be confirmed by IDST (intra-dermal tests), are confusing and mislead the result interpretation. As a result, the allergen selection process becomes doubtful leading to incorrect allergen selections for immunotherapy.

In the allergic pathway, hypersensitive patients react with a type-I reaction (producing allergen specific IgE) against offending allergens. Each allergen is characterized for its specific proteins.

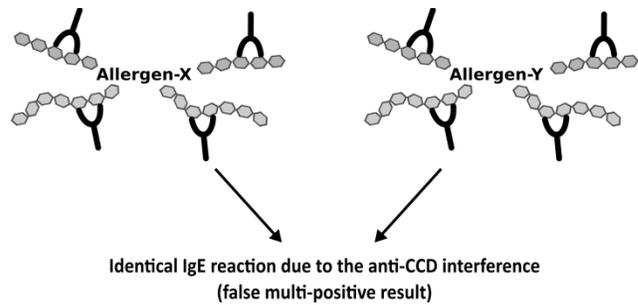
However, most proteins are glycosylated. The glycosylation pattern in plants leads to common glycosylated structures between the different allergens.

A substantial number of allergic patients (1/3 in dogs) react with a type-I response against the carbohydrates producing specific IgE (IgE anti-CCD).

As shown in the illustration, IgE anti-CCD antibodies react with the carbohydrate chains.

As a result, CCD reactions are revealed together with the allergen specific reactions giving rise to false multi positive reactions.

It is a major problem since IgE anti-CCD reactions are detected in 20-40% of allergic patients.



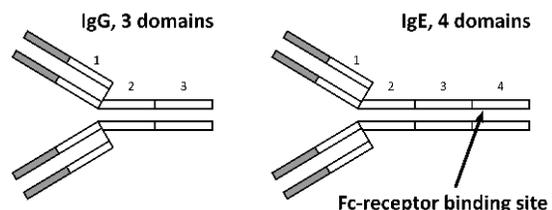
IgE anti-CCD reactions are not allergen specific and give rise to false multi positive results.

Heska was the first to point out to problem in back in 2016. ALLERCEPT was the first commercial test who implemented a pre-test to identify which patients produced these reactions and an IgE anti-CCD blocker to overcome this problem.

**Absolute specificity of IgE detection**

The use of the Fc-ε receptor alpha chain (FcεR1α) overcomes the potential problem of cross-reactivity with IgG which is the major and most current problem when using polyclonal and monoclonal antibodies to detect specific IgE.

The Fc-ε receptor binds to the 3<sup>rd</sup> and 4<sup>th</sup> constant domains of the IgE. The 4<sup>th</sup> domain is missing on the IgG molecule. The Fc-ε receptor cannot bind to IgG.





Hypersensitive patients react specifically against offending allergens with both IgE and IgG reactions. IgG is present in the serum in concentrations that are 10.000 to 100.000 higher than IgE. The specificity of the IgE detecting reagent is critical.

The capacity to distinguish the IgE molecules from IgG at these ratios, is very difficult. The binding strength and avidity of the Fc-ε receptor for IgE is one of the strongest found in nature. Its dissociation constant (Kd) is  $10 \times 10^{-10} \text{M}$ .

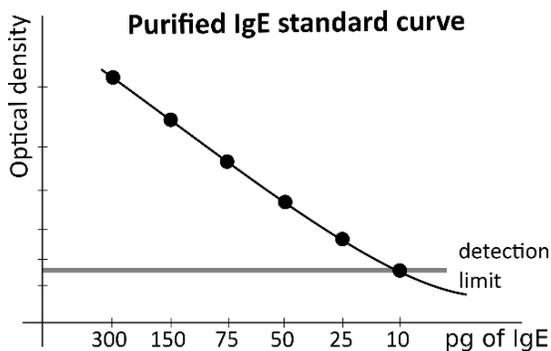
The specificity of the Fc-ε receptor for IgE is absolute (Garman, 2000).

### The use of a standard curve

To quantify a molecule in an ELISA test, it is necessary to run standards containing known concentrations of that molecule. For an IgE test, purified IgE is required.

ALLERCEPT is the only in vitro test that employs an IgE standard. The IgE standard curve, allows achieving reliable and reproducible IgE measurements.

IgE standard curves are not generally employed in veterinary tests due to the complexity and difficulty of obtaining purified IgE in sufficient quality and amounts.



### Result evaluation: detection limit

For most analytical parameters, clinical reference values have been established. This is not the case in specific IgE measurements.

The amount of specific IgE against an offending allergen is distinctive and individual to each patient.

The level of specific IgE does not necessarily correlate with the severity of the clinical disease.

The presence of allergen specific IgE in a patient diagnosed with atopic disease must be considered as potentially significant, even in cases where low IgE concentrations are present.

The use of a standard curve allows ALLERCEPT reaching a detection limit of 10 picograms of specific IgE in the sample.

Accurate IgE measurements, together with comprehensive clinical observations and detailed history of the patient must be used combined with a sound IgE test in the design of valuable specific immunotherapy treatments.

### References

- Structure of the Fc fragment of human IgE bound to its high-affinity receptor FcεR1α. Scott C. Garman et al. Nature 406 (2000) 259-266.
- Measurement of canine IgE using the alpha chain of the human high-affinity IgE receptor. K. Stedman et al. Veterinary Immunology and Immunopathology 78 (2001) 349-355.
- Intra- and interlaboratory variability of allergen-specific IgE levels in atopic dogs in three different laboratories using the Fc-ε receptor testing. N. Thom et al. Vet. Immunol. & Immunopathol. 133 (2010) 183-189.
- Specific IgE to CCD's strongly affect the in vitro diagnosis of allergic diseases. A. Mari et al. J. Allergy Clin. Immunol. 103 (1999) 1005-1011.
- Agreement of serum allergen test results with unblocked and blocked IgE against cross-reactive carbohydrate determinants (CCD) and intradermal test results in atopic dogs. N.K.Y. Gedon et al. Vet Dermatol (2019) 1-8.
- Carbohydrate Epitopes and their relevance for the diagnosis and treatment of allergic diseases. R, van Ree. Int. Arch. Allergy Immunol. 129 (2002) 189-197.
- Inhibition of IgE binding to CCD's enhances diagnostic selectivity. F. Holzweber et al. Allergy 10 (2013) 1269-1277.