

Cellular Allergy Diagnosis

Bee & Wasp venoms
CAST® assays

There is more
to allergy than
just IgE

Clear results:

Significant reduction of false positive and false negative results

Optimized differentiation in case of double positivity

Successful monitoring of specific immunotherapy



Diagnosis of bee and wasp venom allergy

Relevance of hymenoptera allergy

Bee and wasp venoms are responsible for most hymenoptera venom allergies in the northern hemisphere. Each year 200 people die in Europe due to hymenoptera stings [1].

In case of suspected hymenoptera venom allergy, the European Hymenoptera Allergy Interest Group from EAACI suggests consulting an allergologist and starting a specific Immunotherapy (SIT) as a preventive treatment when allergy is confirmed by the diagnosis.

Diagnosis of hymenoptera venom allergy

Diagnosis of hymenoptera venom allergy is based on a history of anaphylactic sting reactions, positive skin test (ST) responses, and/or detection of specific IgE (sIgE) to bee or wasp venom [2].

In many cases however, the results remain ambiguous:

1. Up to **59% of patients with hymenoptera venom allergy show false double positivity for bee and wasp venom in skin and sIgE test whereas only around 10% suffer from allergy to both venoms.** [3, 4, 5]. Double positivity of bee and wasp is based on method-specific cross-reactivity issues. It is caused either by cross-reactive carbohydrate determinants (CCD) or by high sequence homology of proteins like hyaluronidases (Api m 2 and Ves v 2 [6]) and dipeptidylpeptidases (Api m 5 and Ves v 3 [7]).
2. sIgE tests available for **recombinant allergens (Api m 1 and Ves v 5)** have been fraught with risk for some patients because genuine sensitization to other major allergens might be missed. Also the sensitivity for Api m 1 is only 62-82% [8, 9].
3. **Depending on the method used, hymenoptera venom allergy patients often show false-negative results in ST and sIgE tests.** Golden et al. obtained negative results in ST in 99 (32%) of 307 patients with clinically confirmed insect venom allergy; in sIgE tests even 57% of these were negative [10].

Sensitivity and specificity of cellular tests compared to established diagnostic methods

	CAST® ELISA	Flow CAST®	sIgE	Skin Test
Sensitivity Bee (%)	94.0	89.5	92.5	93.7
Specificity Bee (%)	93.2	94.9	83.7	97.6
Relative Spec. Bee (%)	91.1	85.7	59.1	61.1
Sensitivity Wasp (%)	88.5	86.7	92.4	97.4
Specificity Wasp (%)	95.5	97.4	93.0	92.9
Relative Spec. Wasp (%)	98.4	92.1	50.0	63.2

Table 1: Sensitivity, specificity and relative specificity of CAST® ELISA, Flow CAST®, sIgE and skin test [11]; explanation refer to test.

4. A paper submitted for publication recently, shows that **only BÜHLMANN allergens contain all major components of honey bee and yellow jacket and thus offer a clear diagnosis.**

Thus ambiguous sIgE results can be clarified, if BÜHLMANN CAST® assays are included in the diagnostic algorithm as shown by Scherer et al. (Table 1). The study included 67 persons allergic to bee venom, 78 allergic to wasp venom, and 44 healthy subjects. Sensitivity and specificity of the CAST® assays for bee and wasp venom was between 87% and 94%, similar to those published for ST and sIgE. But considerable differences between these methods have been observed when patients were tested for double positivity. In ST and sIgE, approximately 37 – 50 % of patients only allergic to bee venom, result falsely positive for both insect venoms, while with CAST® assays this was reported in only 2-14% of the cases. The relative specificities favouring the BÜHLMANN CAST® assays are shown in Table 1.

Clarification of double positivity

We recommend checking whether the patient really reacts equally strong to both allergens. To identify the strength of the reaction, we suggest establishing dose response curves with both of our novel, highly concentrated venom check allergens (BAG2-I1CHK for bee and BAG2-I3CHK for wasp venom) in 4 different dilutions: undiluted, 1:5, 1:25 and 1:125. The concentration that triggers 50% (D50) of the maximum signal (ie. CD63 activation) has to be calculated and compared for both venoms. By calculation of the D50 ratio, the predominant reactive venom can be determined. Examples are shown in Fig.1 and Fig. 2. Recommendations: Laboratories should establish their own cut-off values for the D50 ratio in the following applications: Allergy to single venoms, double positivity and SIT monitoring.

Conclusion:

BÜHLMANN CAST® assays, used for first line diagnosis are powerful tools to clearly diagnose venom allergies with correct determination of culprit insect which is prerequisite for a successful SIT.

Dose response curves with venom check allergens

Similar response to yellow jacket and bee venoms

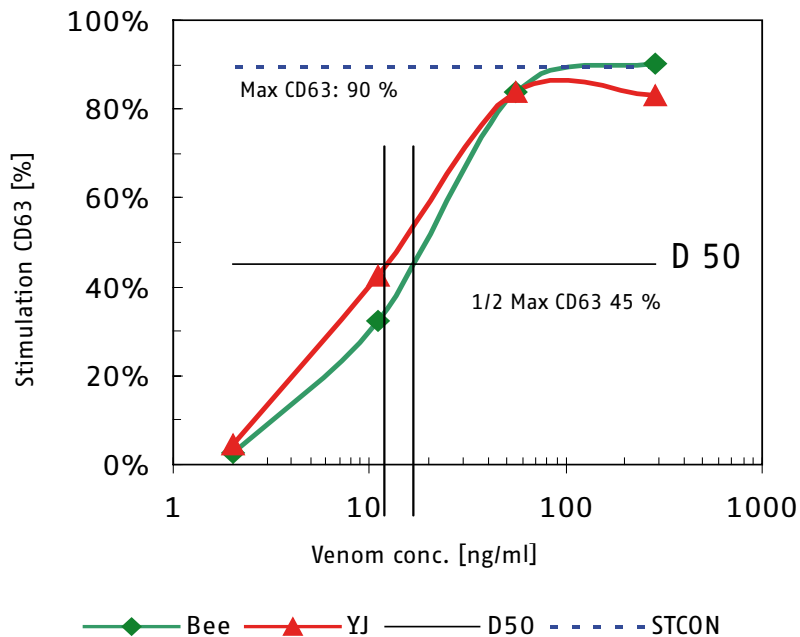


Figure 1: Dose response curve of patient A showing similar reaction to bee and yellow jacket venom.

D50 Honey bee venom: 18 ng/ml
D50 Jellow jacket venom: 12 ng/ml
Ratio D50 = 18 ng/ml / 12 ng/ml = 1.5

Stronger response to yellow jacket than to bee venom

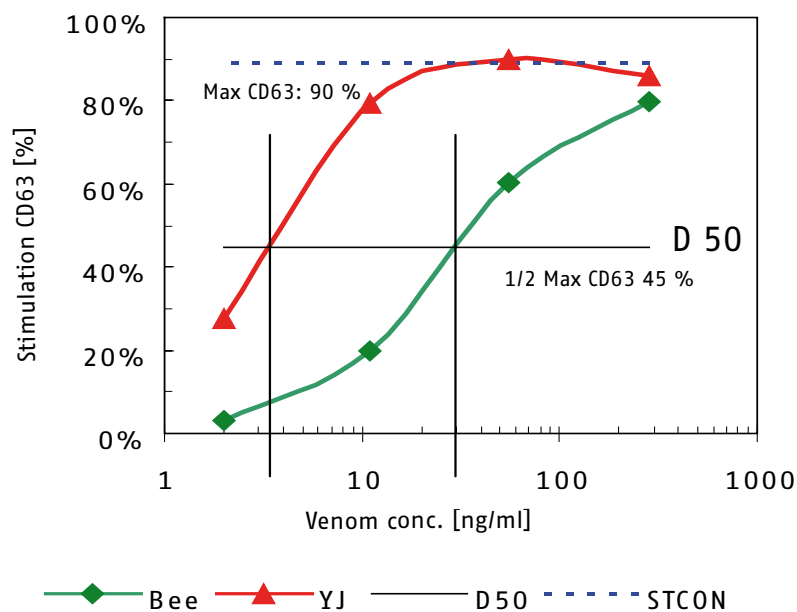


Figure 2: Dose response curve of patient B showing a predominant, 15-fold stronger reaction to yellow jacket than to honey bee venom.

D50 Honey bee venom: 30 ng/ml
D50 Yellow jacket venom: 2 ng/ml
Ratio D50 = 30 ng/ml / 2 ng/ml = 15

Therapy Follow-up and literature

SIT and success monitoring

In life-threatening hymenoptera venom allergy, SIT is the only specific and effective treatment. However, in 5 – 15% of SIT-treated patients complete immunologic protection cannot be obtained. Since reliable *in vitro* markers for monitoring the success of SIT have not been available, therapy failure can only be clearly diagnosed with sting provocation tests or after a field sting [13].

The study by Hausmann et al. [13] investigated the benefit of CAST® assays in identifying patients who became bee venom tolerant after successful SIT. This study analysed 49 subjects – 29 post-SIT and 16 pre-SIT subjects – using CAST® assays. The bee venom concentration ranged between 2 and 250 ng/ml. SIT-treated patients have shown significantly lower basophilic activation than „pre- SIT patients“; see Fig. 3.

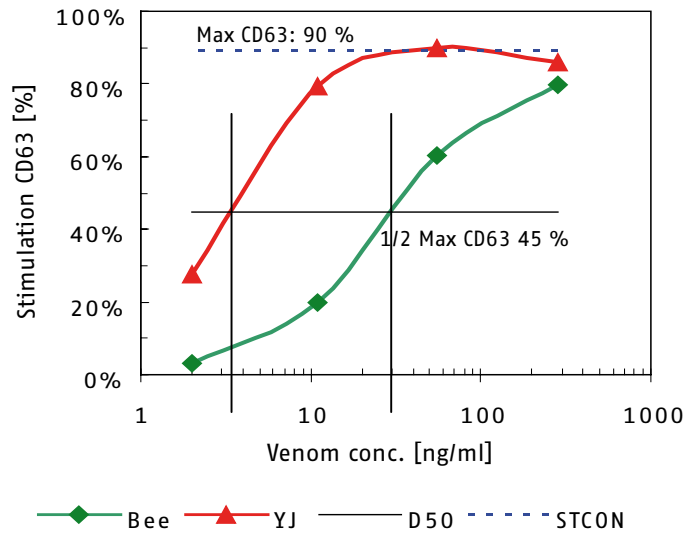


Figure 3: Follow-up of specific Immunotherapy of bee allergics before and after SIT [13]; D50: Allergen concentration at 50% of maximum stimulation.

Significantly different bee venom concentrations are needed to provoke the allergy before and after SIT.

Therefore this study demonstrates CAST® assays being valuable tools to monitor the success of hymenoptera venom SIT.

- 1 O.Hausmann et al.: Insektengiftallergie. Schweiz Med Forum 2010; 10: 698-704.
- 2 B.M.Bilo et al.: Diagnosis of hymenoptera venom allergy. Allergy 2005; 60: 1339-49.
- 3 R.G.Hamilton et al.: Diagnostic methods of insect sting allergy. Curr Opin Allergy Clin Immunol 2004; 5: 209-12.
- 4 M.Mertens et al.: Cross-reactive carbohydrate determinants strongly affect the results of the basophil activation test in hymenoptera-venom allergy. Clin and Exp Allergy 2010; 40: 1333-45.
- 5 U.R.Müller et al.: Hymenoptera venom allergy. Allergy 2009; 64: 543-8.

- 6 T.P.King et al.: Yellow jacket venom allergens, hyaluronidase and phospholipase. J Allergy Clin Immunol 1996; 98: 588-600.
- 7 S.Blank et al.: Identification, recombinant expression and characterization of the 100kDa high molecular weight Hymenoptera venom allergens Api m 5 and Ves v 3. J Immunol 2010; 184: 5403-13.
- 8 S.C.Hofmann et al.: Added value of IgE detection to rApi m 1 and rVes v 5 in patients with hymenoptera venom allergy. J Allergy Clin Immunol 2011; 127: 265-7.
- 9 G.J.Sturm et al.: Detection of IgE to rApi m 1 and rVes v 5 is valuable but not sufficient to distinguish bee from wasp venom allergy. J Allergy Clin Immunol 2011; 127: 265-7. Epub ahead of print.

- 10 D.Golden et al.: Insect sting allergy with negative venom skin test responses. J Allergy Clin Immunol 2001; 107: 897-901.
- 11 K.Scherer et al.: Cellular in vitro assays in the diagnosis of hymenoptera venom allergy. Int Arch Allergy Immunol 2008; 146: 122-32.
- 12 O.Hausmann et al.: Double positivity in insect venom allergy - diagnostic approach with basophil activation test. Poster, SGAI 2009.
- 13 O.Hausmann et al.: Usefulness of basophil activation tests in monitoring the immun response to bee venom immunotherapy controlled by sting challenge - pilot phase results. Poster, EAACI 2009.



BÜHLMANN Laboratories AG
Baselstrasse 55
CH-4124 Schönenbuch/Basel
Switzerland
Phone +41 61 487 12 12
Fax orders +41 61 487 12 99
info@buhlmannlabs.ch
www.buhlmannlabs.ch



Ordering Code:

Flow CAST® FK-CCR 100 tests
CAST® ELISA EK-CAST 192 wells
EK-CAST5 480 wells

CAST® is a registered trademark of BÜHLMANN Laboratories AG