

Blocking CCD-Specific IgE-Antibodies In A Multiplex Environment

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Introduction

Cross-reactive carbohydrate determinants (CCDs) are a common cause for elevated *in vitro* test results, especially when allergen sources of plant or insect venom origin are tested. This leads to results that can take a long time to interpret and explain to patients. A new multiplex diagnostic tool (Allergy Explorer®, ALEX) promises to block the binding of CCD specific IgE antibodies.

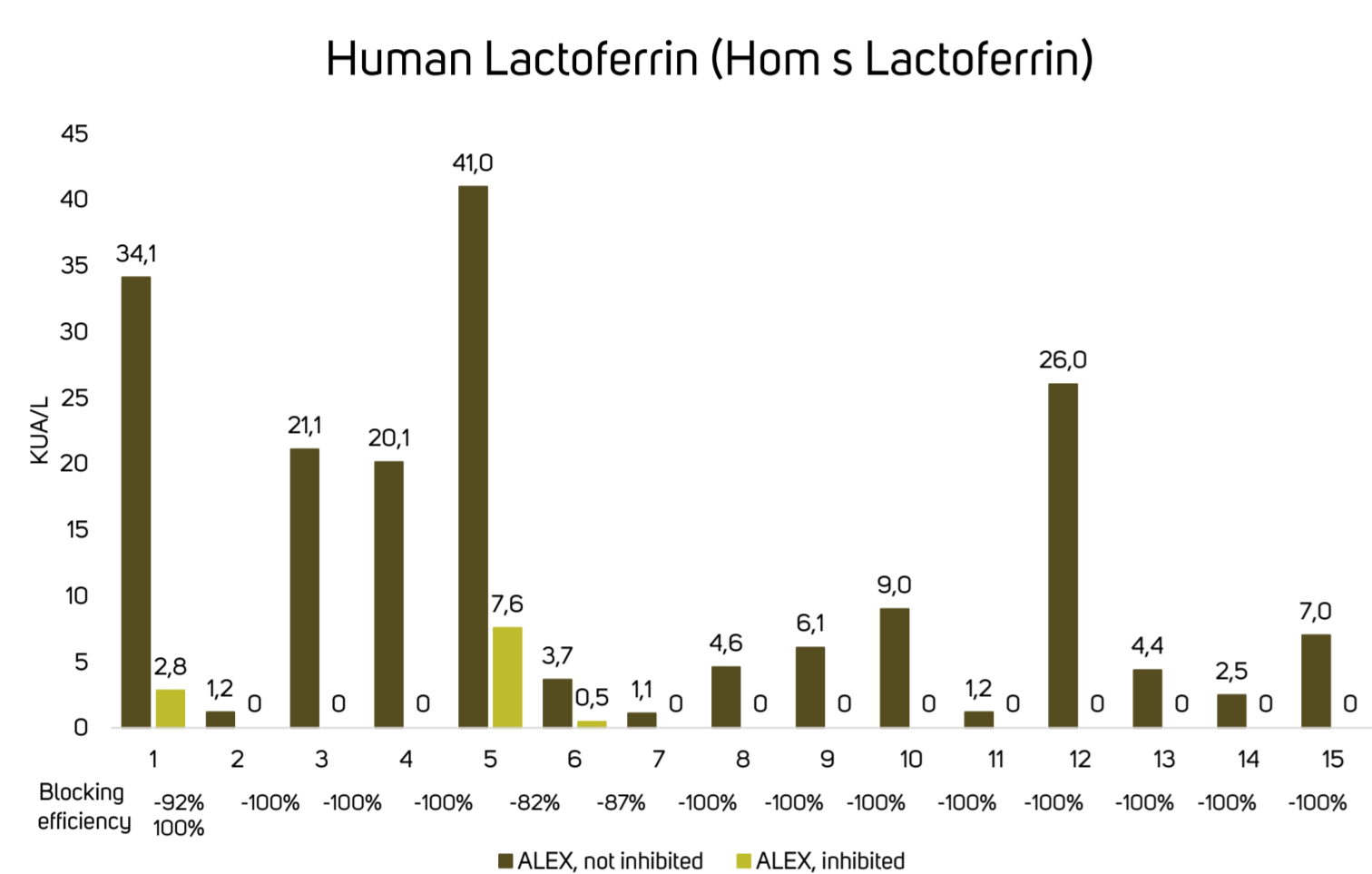
Goal: We investigated the CCD IgE antibody blocking efficiency of the ALEX test system and its impact on the effort of interpreting IgE test results. A further goal was to evaluate the performance of the two CCD markers spotted on this system (Ana c 2 & Hom s LF, in rice cells expressed human Lactoferrin).

Methods

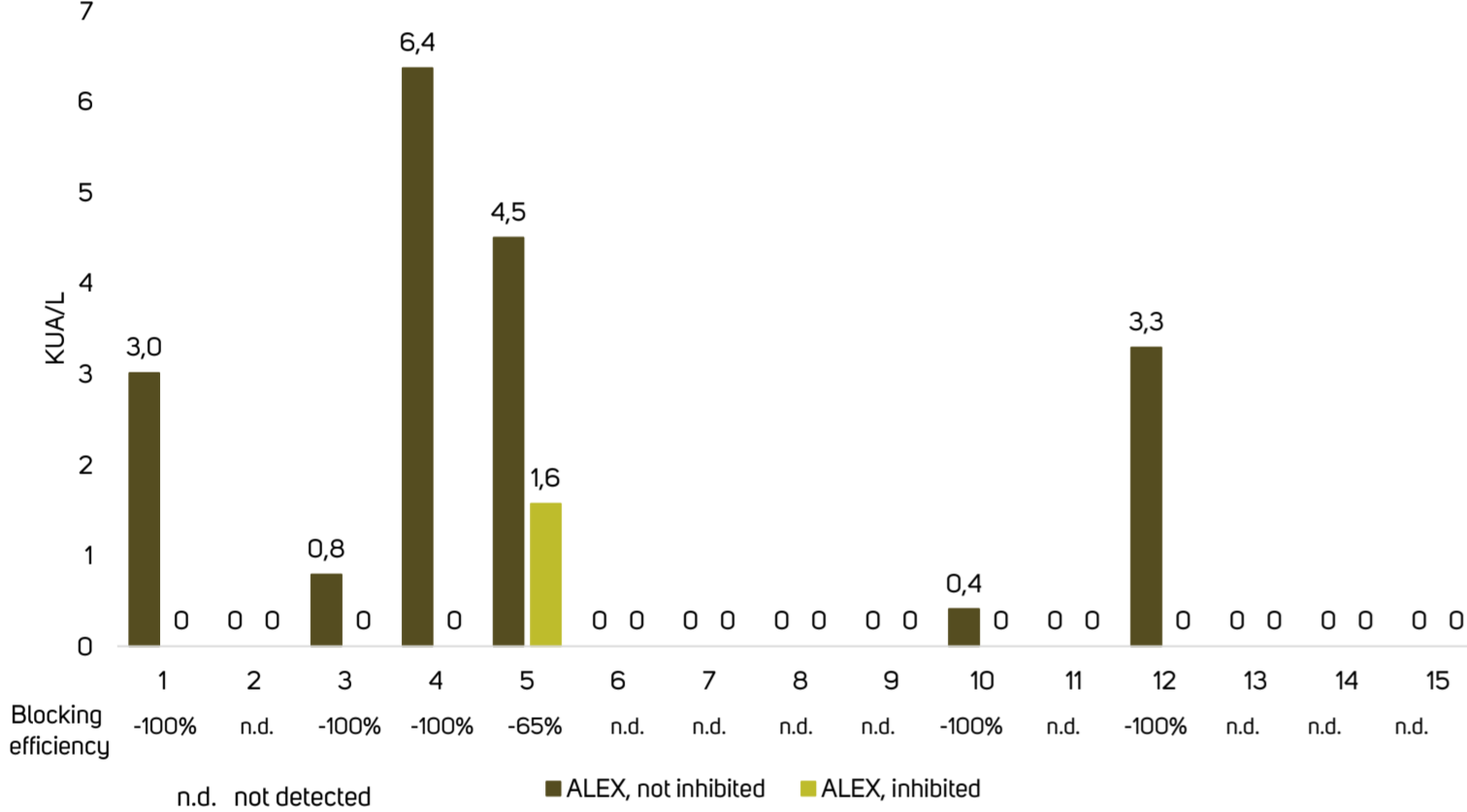
15 CCD positive samples (determined by ALEX and ImmunoCAP® o214, MUXF3) were tested by ALEX before and after CCD blocking (performed with ALEX CCD blocking reagent MUXF3-HSA).

Results

CCD marker Hom s Lactoferrin versus Ana c 2 (Bromelain)



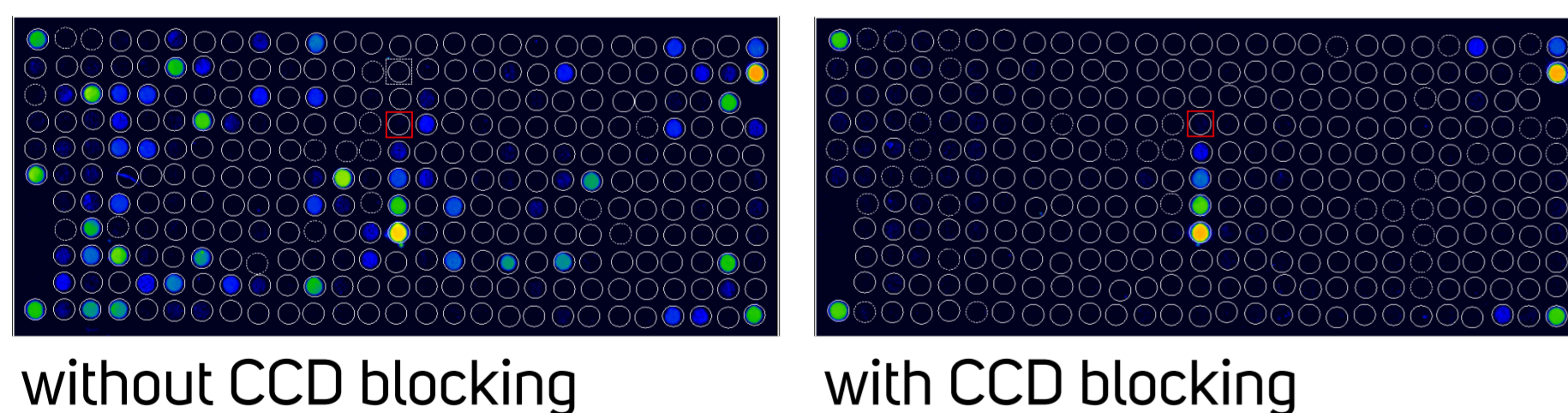
Ananas (Ananas comosus 2)



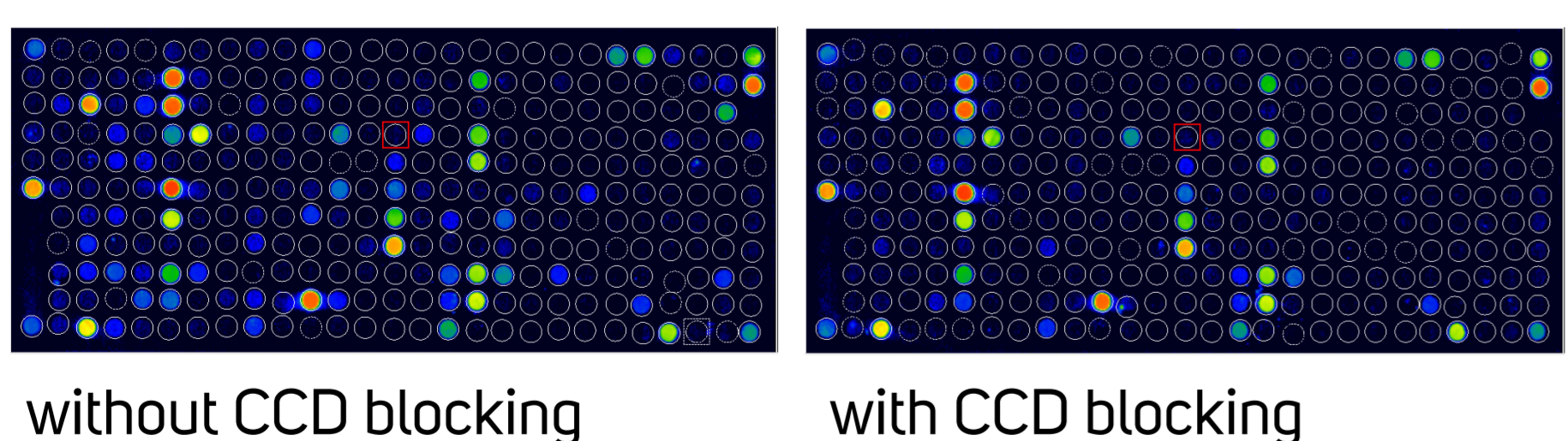
All samples showed positive results for Hom s LF and 6 out of 15 samples were found positive for Ana c 2 prior to CCD blocking. After blocking of CCD specific IgE antibodies, the results for Hom s LF were decreased by 100% in 12 out of 15 samples. The other three samples showed a blocking rate of 82-92%. The Ana c 2 positive results were reduced to one positive result, which was reduced by 65%.

ALEX test arrays with and without CCD blocking

Sample #3



Sample #8

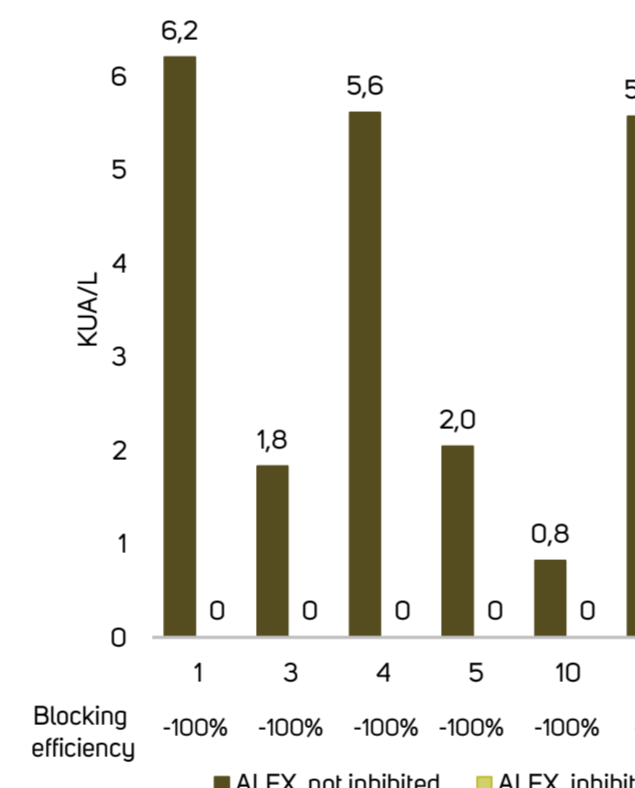


CCD antibody blocking efficiency is observed in various allergen extracts

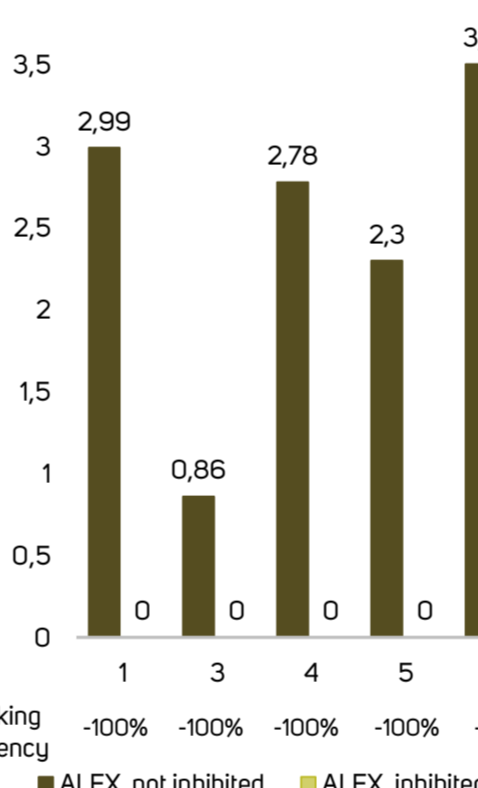
Allergen Group	100% CCD antibody blocking efficiency observed
Tree Pollen	Acacia, Ash, Cottonwood, Elm, Lilac, Mulberry, Oak, Privet
Fruit	Blueberry, Lemon, Litchi, Mango, Melon, Pear, Plum
Cereals	Barley, Millet, Quinoa, Rice, Rye, Wheat
Spices	Anise, Caraway, Mustard, Paprika, Parsley
Nuts	Hazel, Macadamia, Pecan, Walnut
Legumes	Green Bean, Pea, Peanut
Seeds	Pumpkin, Sesame, Sunflower
Weed Pollen	Annual Mercury, Nettle, Wall Pellitory
Vegetables	Lettuce, Onion
Other	Common Mussel, Latex, Wasp Venom, <i>Ficus benjamina</i>

In a plethora of samples a 100% CCD antibody blocking rate was observed for grass pollen, certain tree pollen, weed pollen, cereals, fruits, legumes, mussels, nuts, seeds, spices, vegetables, hymenoptera venoms, latex and *Ficus benjamina* (see Table).

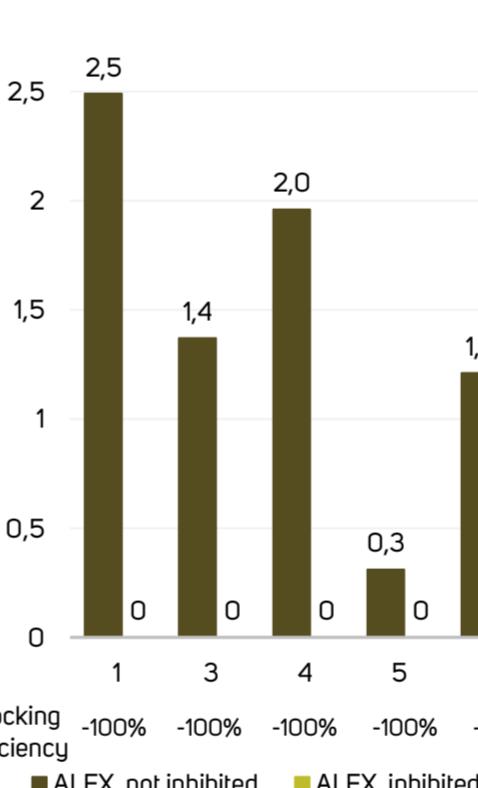
Peanut (Arachis hypogea)



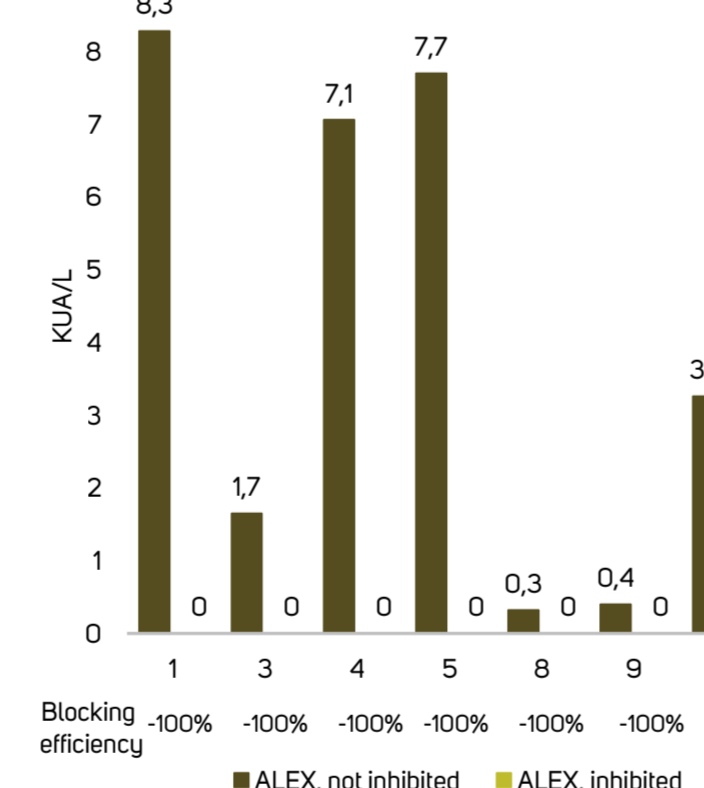
Latex (Hevea brasiliensis)



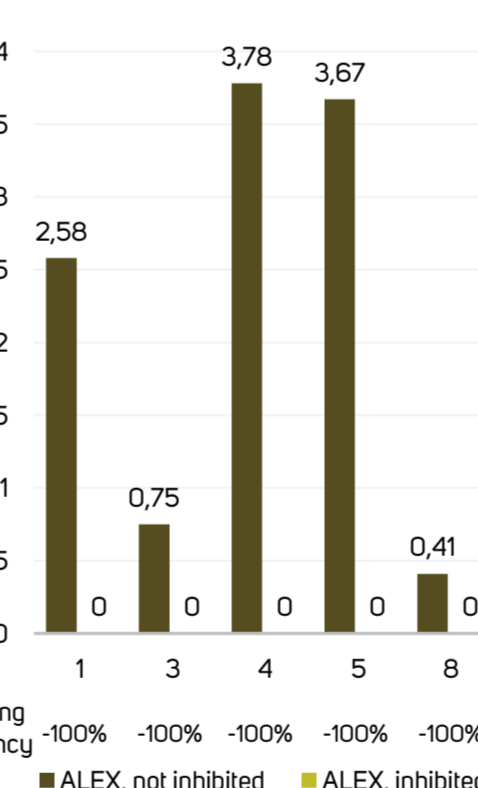
Common mussel (Mytilus edulis)



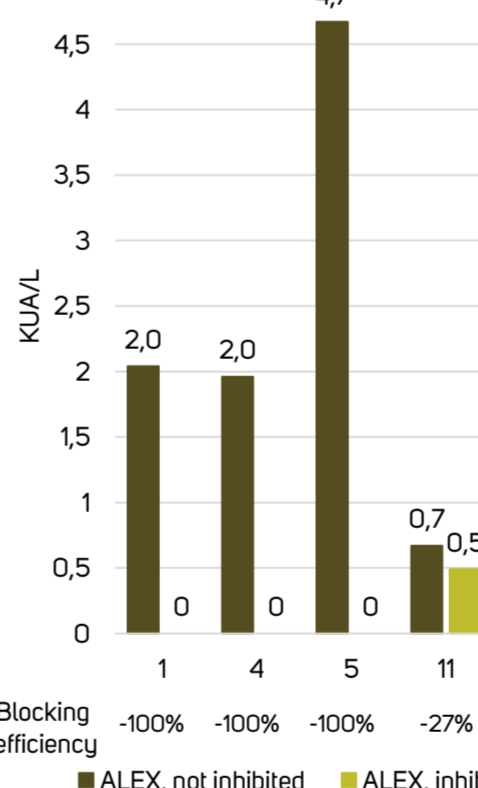
Millet (Panicum miliaceum)



Wall pellitory (Parietaria judaica)



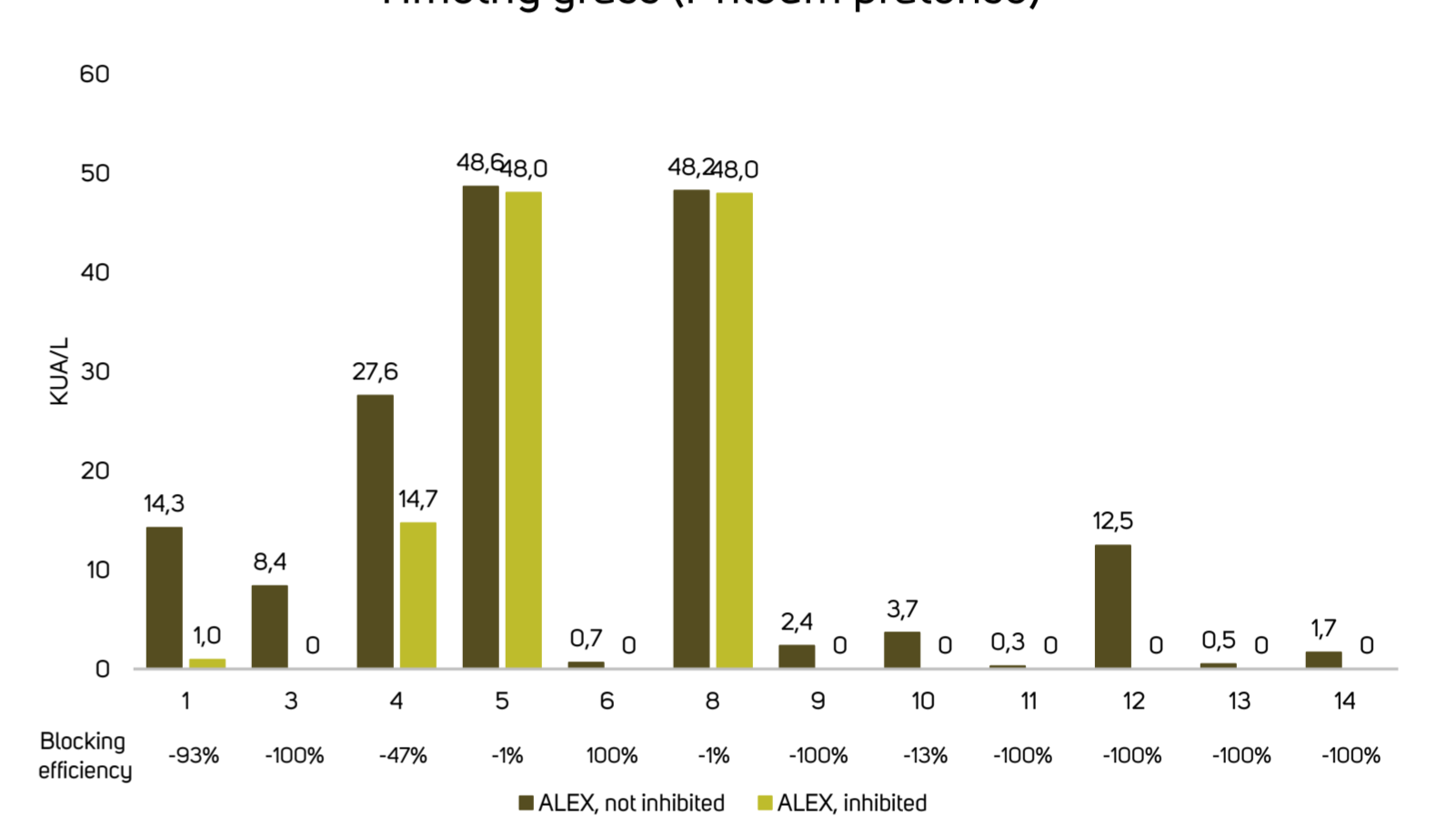
Wasp venom (Vespula vulgaris)



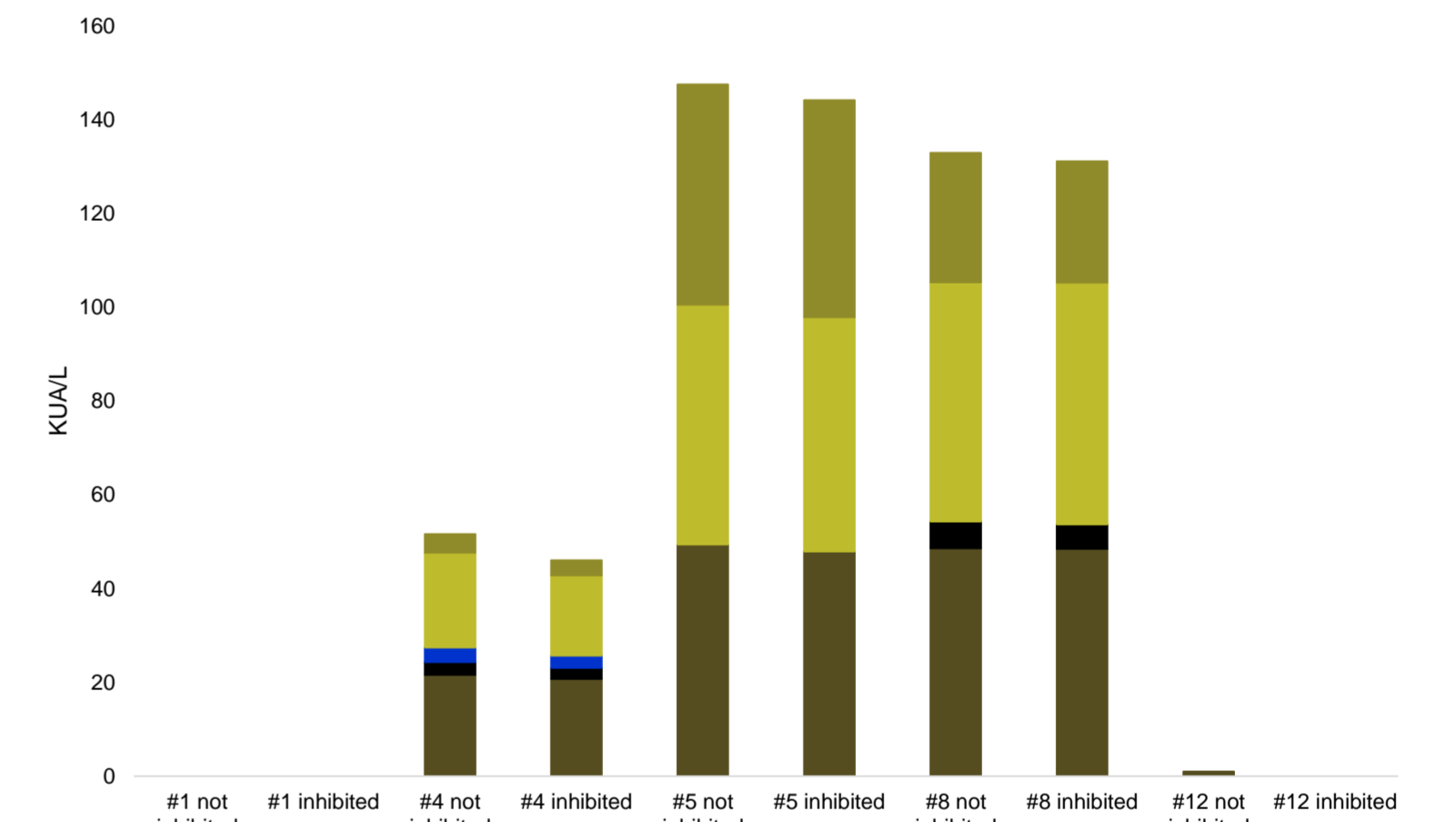
Prior to blocking 758 positive results were obtained with ALEX. After blocking 252 positive results remained. Sera with a lower Hom s LF value (<10 kUA/L) showed a CCD blocking efficiency (BE, based on Hom s LF) of 87-100% (9 out of 10 were blocked completely). Mid-range level sensitizations (10-30 kUA/L) against Hom s LF were blocked completely. Highly positive results showed a BE of 82 and 92%.

Phl p positive results after CCD blocking are due to their sensitivities against individual components

Timothy grass (Phleum pratense)



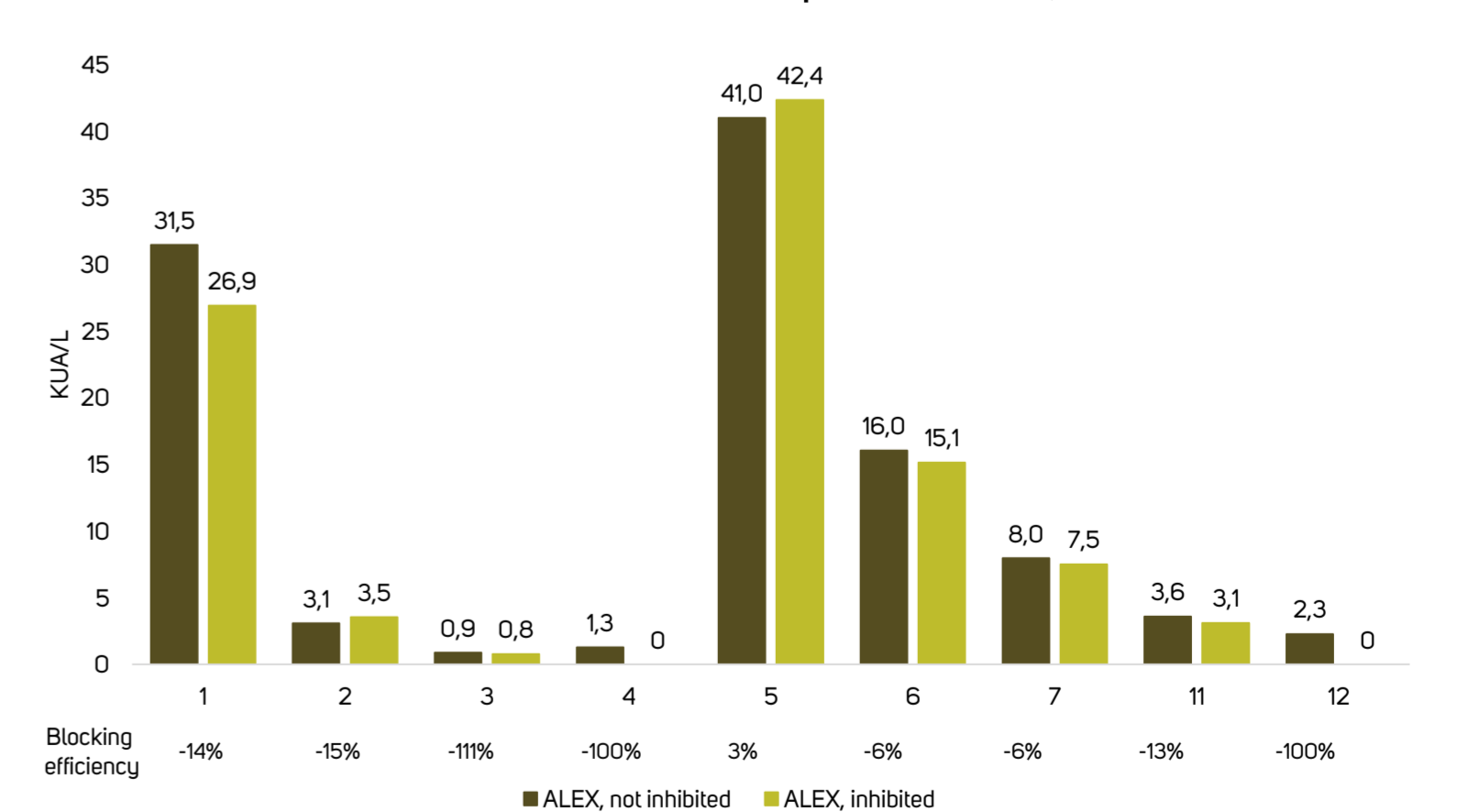
CCD inhibition - Timothy grass components (Phl p 1, Phl p 12, Phl p 2, Phl p 5.0101, Phl p 6)



For some Phleum pratense (Phl p) samples (as well as Apis mellifera samples) positive results remained also after CCD blocking. This could be explained by their genuine sensitization to the individual Phl p components (Phl p 1, 12, 2, 5.0101 and 6).

Api m positive results after CCD blocking are due to their sensitivities against individual components

Bee venom (Apis mellifera)



Conclusion

Based on our results Hom s LF is superior to Ana c 2 as a CCD marker, as 15 out of 15 samples were found positive compared to 6 out of 15 for Ana c 2. The dramatic decrease in positive s IgE results (67% fewer positive results), especially for pollen, foods, latex and hymenoptera venoms leads to a shortened time for interpretation and explaining results to patients. Patient's anxieties over positive test results can also be reduced, especially for high risk allergen sources.

Acknowledgment

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