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Universal Allergen Standard for MARIA™ and ELISA

Background

Allergen measurements are widely used in the allergy and indoor air quality fields for environmental exposure assessments; monitoring the effects of remediation procedures; assessing the efficacy of control devices and procedures; and measuring the dose of specific allergens in diagnostics and vaccines.

The standards currently used in Indoor Biotechnologies ELISA kits are extracts of source materials containing an empirically determined level of a given allergen. In some cases, e.g. Der p 1 and Can f 1, the ELISA standards were sub-standardized against World Health Organization/International Union of Immunological Societies International Reference Preparations. Most measurements of Der p 1 and Can f 1 are based on WHO/IUIS International Reference Preparations which were prepared in the 1980's[1;2]. The *D. pteronyssinus* reference (NIBSC 82.518) was reported to contain 12.5µg Der p 1 per ampoule and used as such. In the 1990's two studies indicated that this level was an over-estimate and that the actual level was close to 5µg/ampoule. The dog allergen reference (NIBSC 84/685) contains 100,000 units of Can f 1 per ampoule which was approximately equivalent to 10µg Can f 1 per ampoule.

Advances in the structure and molecular biology of allergens over the past 10 years, have shifted the focus of allergen standardization towards developing purified natural or recombinant allergens that can be used as standards. In the European Union, this approach has been spearheaded by the CREATE project. The aim of CREATE was to develop purified allergen standards which satisfy criteria of protein purity and for which the absolute protein content is established by amino acid analysis, extinction coefficient or other protein assays[3;4]. The project successfully evaluated the structures of eight natural and recombinant allergens: Bet v 1, Phl p 1, Phl p 5, Ole e 1, Der p 1, Der f 1, Der p 2, and Der f 2. Two of these allergens (Bet v 1 and Phl p 5) are currently being formulated as international standards by the European Directorate for the Quality of Medicines (EQDM) and it is anticipated that other allergens will follow. The CREATE standards are based on using amino acid analysis to determine the protein content of purified allergens.

Universal Allergen Standard

Allergenic extracts contain many proteins and other macromolecules and are not suitable as standards in multiplex arrays. Scientists at Indoor Biotechnologies have formulated a Universal Allergen Standard (UAS) modeled on the approaches used in the EU CREATE project. We have developed a single Universal Allergen Standard that contains eight purified natural allergens and can be used for both MARIA™ and ELISA allergen detection systems[5].

Natural allergens used in the UAS were purified in our laboratories. Protein content was determined by amino acid analysis in an independent third party laboratory. Amino acid analysis was compared with two other methods for measuring protein: extinction coefficient at 280nm and a colorimetric assay (Advanced Protein Assay, Cytoskeleton, Denver, CO). The three methods showed reasonable agreement between the protein measurements of the eight allergens (Table 1) and the allergens showed a high degree of purity (Figure 1).

Relationship between UAS and ELISA standards

Overall, the dose response curves obtained with the UAS are shifted to the left compared with current ELISA standards and give lower values for most allergens (Figure 2). This would be expected since the UAS standard is based on absolute determination of protein composition by amino acid analysis. Comparison of the UAS with current ELISA standards (by ELISA) showed close agreement (<2-fold difference) between standards for Der p 1, Der p 2, Mus m 1, Rat n 1 and Bla g 2 (Figure 2). For Fel d 1 and Can f 1 the UAS curves were three fold and 4-5 fold lower than the ELISA curves, respectively. The UAS curves for Der f 1 were 8-10 fold lower than the ELISA standard. Similar dose response curves for the UAS and ELISA standards were obtained using MARIA™ testing (not shown).

Comments

Allergen standardization is moving into a new era based on purified allergen standards. Similar approaches are already in use for measuring other biologics, such as cytokines and growth factors. Purified proteins are essential for use as standards in multiplex arrays and Indoor Biotechnologies has developed the UAS in accordance with industry standards. This represents a bold move forward in allergen standardization. We appreciate that researchers and companies will have to make adjustments as we move from ELISA standards to the new UAS and will work with our customers to manage this process.

As a company which now markets, both ELISA and MARIA™ kits and services, we are obliged to use a common standard for both types of product. Thus, our policy moving forward is to use the Universal Allergen Standard for both product lines. As of January 2008 the UAS will be provided in MARIA™ kits and in ELISA kits for Der p 1, Der f 1, Mite group 2, Fel d 1, Can f 1, Bla g 2, Rat n 1 and Mus m 1.

We hope that our customers will embrace this change to a new standard. We also recognize that for some of our colleagues making this change may cause difficulties e.g. for those involved in long term studies or for companies using allergen measurements in their products. Indoor Biotechnologies will use its best efforts to help manage these changes. Indoor Biotechnologies is committed to allergen standardization and to efforts to develop certified international standards that can be used throughout the allergy and indoor air quality industry.

Please contact us if you have any questions about the Universal Allergen Standard or if you would like to replace any current ELISA standards with the UAS.

Yours sincerely,

Martin D. Chapman, PhD
President

Table 1 Comparison of Protein Measurements on Allergens in the UAS

Allergen	Lot Number	APA concentration mg/ml	Concentration determined by AAA mg/ml	OD 280 mg/ml
nDer p 1	6002004	1.4	1.07	1.15
nDer f 1	30074	1.1	0.69	0.91
nDer p 2	30037	1.1	0.85	0.98
nFel d 1	30035	1.1	1.38	1.08
nCan f 1	30081	0.56	0.65	1.01
nMus m 1	2843	2	1.2	1.41
nRat n 1	30063	1.3	0.8	1.15
nBla g 2	30095	3.1	3.6	5.97

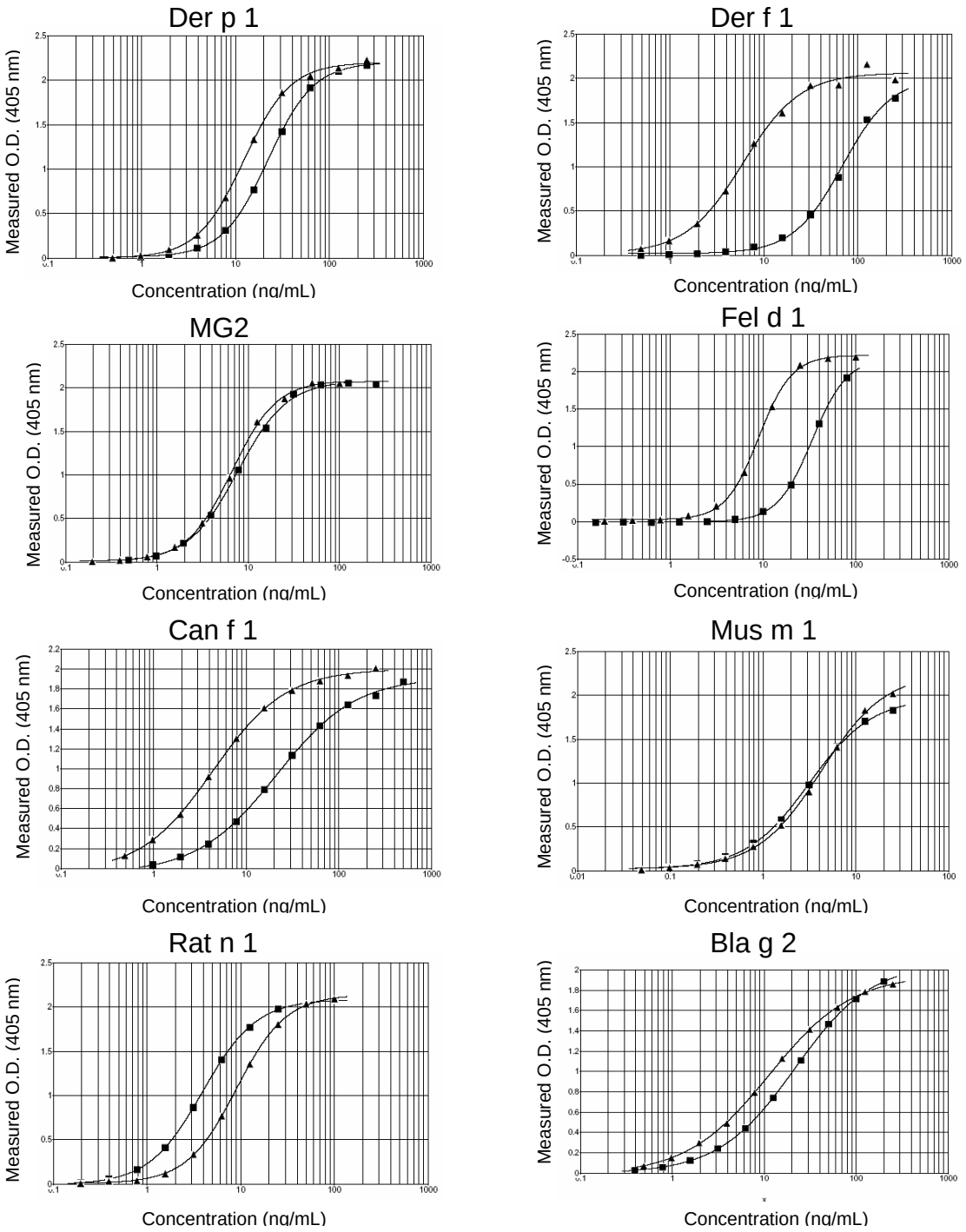
APA = Advanced Protein Assay; AAA = Amino Acid Analysis

Figure 1: SDS-PAGE Analysis of Purified Natural Allergens used in the UAS

Lane	Natural Allergen
1	n Der p 2
2	n Der p 1
3	n Der f 1
4	n Can f 1
5	n Fel d 1
6	n Mus m 1
7	n Rat n 1
8	n Bla g 2

Trace amounts of dimers are present in Der p 2, der p 1, der f 1, Can f 1 and Fel d 1. Mus m 1 and Rat n 1 show two isofoms at 18kd and 20kd. Natural Bla g 2 show the typical bands of the affinity purified allergen.

Figure 2: Comparison of dose response curves using current ELISA standards (■) and the UAS (▲), measured by ELISA.



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