Development and Application of Quantitative Immunoassays for Major Milk Allergens Bos d 5 (β-lactoglobulin) and Bos d 11 (β-casein)

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INTRODUCTION

In order to help allergic patients manage often severe symptoms, food manufacturers are required to list allergens on their products and researchers are working to develop effective immunotherapies. Due to limitations of the existing tools, precise quantification and standardisation of milk allergens in food, therapeutic and diagnostic products can be difficult.

Aim: We sought to develop accurate, sensitive and reliable assays that would enable quantification of multiple milk allergens.

MATERIALS AND METHODS

- Allergen specific mAbs were developed against Native Bos d 5, Denatured Bos d 5 and Bos d 11 (Table 1).
- These mAbs were used to develop allergen specific ELISA and Multiplex (Figure 1) immunoassays.
- Purified natural allergens (Figure 2) were used to generate a standard curve for each protein.
- Detection of the target allergens was accomplished using biotinylated specific mAbs antibodies (Table 1). and streptavidin conjugated fluorochrome.
- Allergen content was measured using the immunoassays in various sample types including iFAAM reference samples, milk powder, chocolate dessert, cookie, chocolate bar and infant milk formula products.

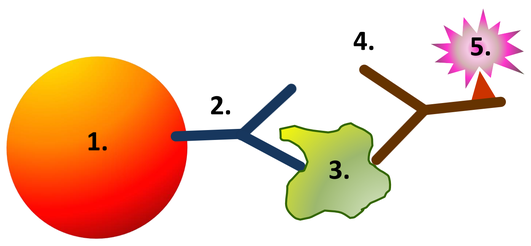

Figure 1. 1. Fluorescent microsphere; 2. Allergen specific antibody coupled to the bead; 3. Target protein; 4. Allergen specific biotinylated detection antibody; 5. Streptavidin-PE.

Figure 2. SDS-PAGE diagrams of purified native Bos d 5, denatured Bos d 5, and Bos d 11 (β-casein) used as standards in milk immunoassays.

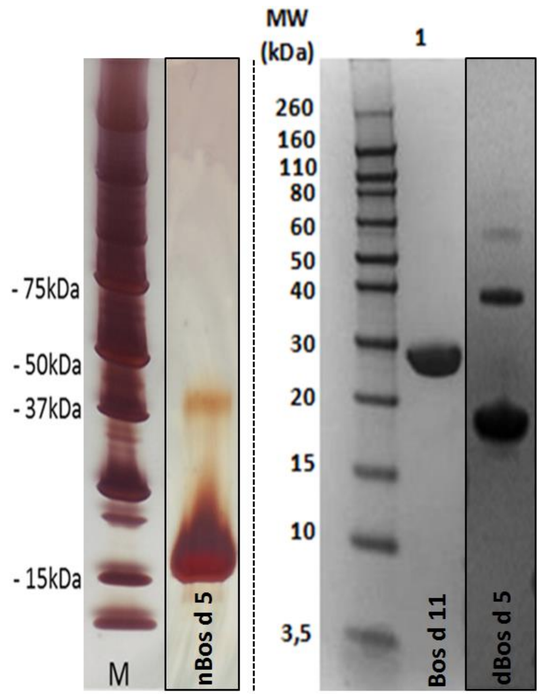

Table 1. Milk proteins and antibody pairs used for development of the milk immunoassays. *nBos d 5 was denatured via alkylation to create dBos d 5

Target food	Target protein	Standard	Capture Antibody	Detection Antibody
Milk	Native Bos d 5	N-Bos d 5	97N	117N
	Denatured Bos d 5*	D-Bos d 5	74R	92R
	Bos d 11	Bos d 11	CC11	VB1C

RESULTS

- The food multiplex assay was able to measure multiple allergens in a small (<50µl), single sample.
- Lower limit of detection (LLOD) of the assays was as low as 0.2 ng/ml for Native Bos d 5 (Table 2).
- Sensitivity of the multiplex assay was increased by up to 39-fold compared to ELISA.
- The multiplex food array produced reproducible results showing intra-assay CVs<8% and inter-assay CVs<15%.
- Multiplex standard curves range between 200-0.1 ng/ml for Native Bos d 5, 1000-0.49 ng/ml for Denatured Bos d 5 and 5000-2.44 ng/ml for Bos d 11 (Figure 3).
- There was a significant correlation between multiplex assays and ELISA for nBos d 5, dBos d 5 and Bos d 11 (Figure 4).
- The concentrations and ratios of specific milk allergens in infant milk formula varies across products. Some hypoallergenic milk formula tested appear to contain readily detectible levels of allergens (Figure 5).

Table 2. Performance characteristics of the milk multiplex assay (*Mean CV% of average allergen concentration for duplicate samples run on the same plate; ** Mean CV% of average allergen concentration for samples analysed on at least two separate days; *** Mean CV% of average allergen concentration for at least 3 serial dilutions).

	ELISA LLOD (ng/ml)	Multiplex LLOD (ng/ml)	LLOD fold change	Multiplex intra-assay CV%*	Multiplex inter -assay CV%**	Multiplex assay parallelism***
Native Bos d 5	7.8	0.2	39.0	5	13	16
Denatured Bos d 5	7.8	2.0	3.9	5	14	19
Bos d 11	31.3	9.8	3.2	7	15	11

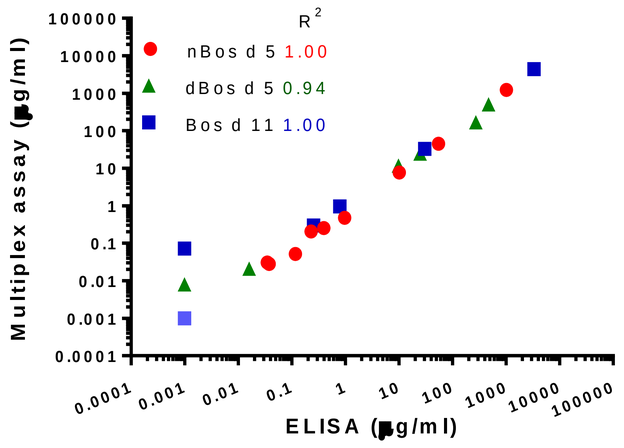

Figure 4. Correlation between results obtained using ELISA and multiplex milk assays. Analyzed samples were: allergen spikes, milk powder, research chocolate dessert with allergens and placebo; chocolate bar and placebo; and research cookie.

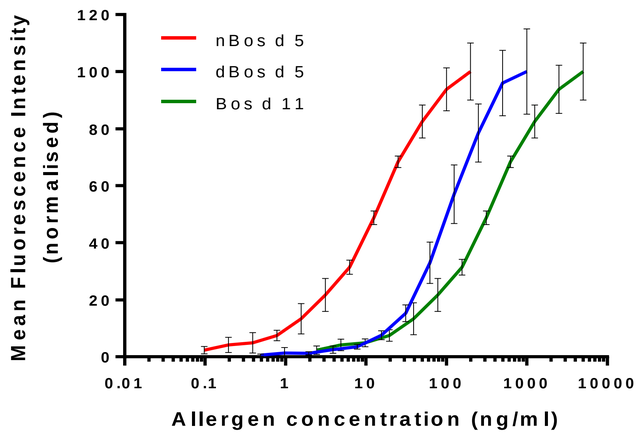

Figure 3. Standard curves for nBos d 5, dBos d 5 and Bos d 11 in the milk multiplex immunoassays.

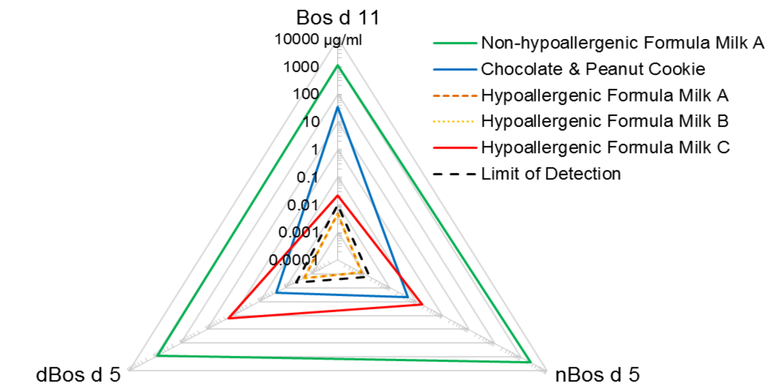

Figure 5. Concentration of the specific milk allergens native Bos d 5, denatured Bos d 5 and Bos d 11 in infant milk formula products. A research cookie known to contain milk is also shown for comparison.

CONCLUSIONS

- ELISA and multiplex immunoassays for quantification of major milk allergens have been developed.
- These immunoassays provide accurate, sensitive and reliable methods for quantification of specific milk allergens in research, pharmaceutical, biotechnology and food industries.
- Analysis of infant milk formula highlights important differences in the levels of allergen between various products.

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Disclosure: In relation to this poster I declare the following, real or perceived conflicts of interest: Ross Yarham, Anna Kuklinska-Pijanka, David Gillick, Elizabeth Young, Martin Chapman and James Hindley are employees of Indoor Biotechnologies. Karine Adel-Patient and Hervé Bernard are employees of INRA.