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 major 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 multiplex, in various sample types including iFAAM reference samples, milk powder, chocolate dessert, cookie and chocolate bar. The results were compared to ELISA.

RESULTS

- The food multiplex assay was able to measure multiple allergens in a small (≤50μl), single sample.
- Lower limit of detection (LLOD) out 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).

CONCLUSIONS

- ‘Proof-of-concept’ ELISA and multiplex immunoassays for quantification of major milk allergens have been developed.
- The ‘open-architecture’ multiplex platform allows for addition of further allergens to the list of analytes and creation of a wider ‘food-panel’.
- The array can provide a robust, rapid and cost effective alternative to existing methods for research, pharmaceutical, biotechnology and food industries.

ACKNOWLEDGEMENTS

We would like to thank Sabina Wünschmann for purifying Native Bos d 5. We would also like to thank Karine Adel-Patient and Hervé Bernard of INRA for purifying denatured Bos d 5 and Bos d 11 as well as the monoclonal antibodies.

The work was partly funded by iFAAM project (EU FP7, Integrated Approaches to Food Allergy and Allergy Risk Management).

REFERENCES