

Methods for the Determination of Allergenic Substances in Foods

K. TOMKOVÁ¹, F. ŠTUMR¹, P. DVORSKÁ¹, P. ŠAFÁŘOVÁ¹, J. RYSOVÁ²,
D. GABROVSKÁ^{2*}, P. HANÁK² and J. PLICKA³

¹SEDIUM RD, s.r.o., 530 02 Pardubice, Czech Republic; ²Food Research Institute Prague, 102 31 Prague, Czech Republic; ³Immunotech a Beckman Coulter Company, 102 27 Prague, Czech Republic, *E-mail: d.gabrovska@vupp.cz

Abstract: Within the framework of the research project ELISA methods for the quantitative determination of allergenic substances in foodstuff and raw materials were developed. ELISA kits for allergenic proteins of milk (casein, beta-lactoglobulin and BSA) egg white proteins and mustard proteins were validated and collaborative studies were performed to prove the validation of the ELISA methods developed. Various methods of extraction were tested. The parameters as a limit of detection, as a limit of quantification, robustness, repeatability and accuracy were determined. A broad range of zero matrices for allergens were tested as well. The ELISA kits are suitable for the determination of allergens according to EU legislation Directive 2005/26/EC and Directive 2006/142/EC in the laboratories focused on this topic.

Keywords: food allergy; ELISA methods; allergenic protein determination

INTRODUCTION

Food allergy, for the purposes of this brief review, is an IgE-mediated abnormal response to a normally tolerated food protein. The reasons for an individual to become intolerant towards a specific food protein are unclear. The amount of protein required, i.e., the threshold, to elicit an allergic response in a sensitised person varies considerably from patient to patient and protein to protein. Thresholds for many allergenic proteins remain unknown. Food allergy symptoms vary from mild localised symptoms to severe anaphylaxis that, at times, may be fatal. The spectrum of food allergy symptoms may include flushing, urticaria, angioedema, laryngoedema, diarrhoea, nausea/vomiting, bronchospasm, or hypotension (ANONYMOUS 2004).

Since there are no medical treatments currently available for curing food allergies, the best way to prevent unintended exposure to a food allergen is the complete avoidance of the offending food. For various reasons such avoidance may not always be possible, and in certain instances, impossible.

Accurate food labelling in conjunction with good manufacturing practices can enhance consumer safety and aid food processors, manufacturers, distributors, packers, transporters, and retailers to utilise foods/food ingredients in an efficient and safe manner. However, despite best intentions and practices, the presence of trace contaminants of offending agents cannot be ruled out at all times unless accurate methods are available to detect their presence. For these reasons, reliable, robust, sensitive, and specific detection methods capable of detecting trace quantities of the targeted food allergens must be developed (SATHE *et al.* 2005).

MATERIAL AND METHODS

Casein ELISA Kit (Cat. No. FA 00208). The format of the newly developed ELISA kit for casein determination in food and raw materials is a competitive assay based on commercial polyclonal antibody used for solid-phase coating and signal commercial conjugate with horse-radish peroxidase. Commercial casein from bovine milk was

Table 1. Limits of detection and quantification for ELISA kits

ELISA kit for	Limit of detection (mg/kg)	Limit of quantification (mg/kg)
BLG determination	0.07	0.22
Casein determination	0.24	1.30
BSA determination	0.20	3.70
Mustard proteins determination	0.06	0.15
Egg white proteins determination	0.08	0.28

used for standard solutions preparation. The range of calibration scale is from 1.5 ppm to 45 ppm (5 solutions of calibrators).

Bovine Serum Albumin ELISA kit (Cat. No. FA 00308). The format of the newly developed ELISA kit for bovine serum albumin determination in food and raw materials is a competitive assay based on commercial polyclonal antibody used for solid-phase coating and signal commercial conjugate with horse-radish peroxidase. Commercial bovine serum albumin was used for standard solutions preparation. The range of calibration scale is from 35 ppm to 300 ppm (5 calibration solutions).

Beta-lactoglobulin ELISA kit (Cat. No. FA 00107). The format of the this ELISA kit for beta-lactoglobulin determination in food and raw materials is a 2-step sandwich assay based on the

commercial polyclonal antibody used for solid-phase coating and signal commercial conjugate with horse-radish peroxidase. Commercial beta-lactoglobulin was used for standard solutions preparation. The range of calibration scale is from 0.5 ppm to 20 ppm (6 solutions of calibrators).

Mustard ELISA kit (Cat. No. FA 00508). The format of the newly developed ELISA kit for determination of mustard proteins (*Sinapsis alba*, *Brassica juncea*, *Brassica nigra*, etc.) is a 2-step sandwich assay based on the developed polyclonal rabbit antibody used for solid-phase coating and signal conjugate with horse radish peroxidase. Lyophilised protein extract from the seeds of *Sinapsis alba* L. was used for standard solution preparation. The range of calibration scale is from 0.5 ppm to 20 ppm (5 solutions of calibrators).



Figure 1. The Mustard ELISA Kit

Egg ELISA kit – native (Cat. No. FA 00408). The format of the newly developed ELISA kit for determination of egg white proteins (the temperature of the heat processing should not exceed the 70°C) is a 2-step sandwich assay based on the developed polyclonal sheep antibody used for solid-phase coating and signal conjugate with horse radish peroxidase. The mixture of lyophilised egg white proteins were used for standard solution preparation. The range of calibration scale is from 0.5 ppm to 15 ppm (6 solutions of calibrators).

RESULTS AND DISCUSSION

The newly developed sandwich and competitive ELISA kits for quantification of above mentioned allergens were properly tested and validated. Elaboration of extraction method (extraction buffer, time and temperature of extraction) was tested. Analytical sensitivity, functional sensitivity, recovery, accuracy and other parameters were determined in in-house validation. A broad range of exercise and stability tests were carried out as well. The collaborative studies in which always 8 laboratories participated were organised for all five ELISA kits. All kits are commercially available.

Acknowledgement: The work was supported by the Ministry of Agriculture of the Czech Republic, Project No. 0002702202 “*Food Quality and Safety in Modern Society*”

References

- ANONYMOUS (2004): Opinion of the Scientific Panel on Dietetic products, nutrition and allergies [NDA] on a request from the Commission relating to the evaluation of allergenic foods for labelling purposes . The EFSA Journal, **32**: 1–197.
- Directive 2005/26/EC of the European Parliament and of the Council of 21 March 2005.
- Directive 2006/142/EC of the European Parliament and of the Council of 22 December 2006.
- Decree No. 101/2007 Coll. (the Czech Republic), on the method of labelling food and tobacco products. Date of entry into force: 12 April 2005.
- SATHE S.K., TEUBER S.S., ROUX K.H. (2005): Effects of food processing on the stability of food allergens. *Biotechnology Advances*, **23**: 423–426.