

SPECIAL SECTION ON FOOD ALLERGEN TESTING

Food Allergens in the Focus

Since the introduction of allergen labeling in several countries between 2003 and 2006, the central question to be asked is: How can allergen labeling be reliably enforced, with sound, fit-for-purpose methods?

The problem arises because: (1) Legislative frameworks, with the exception of Japan and Switzerland, do not provide threshold or action levels, nor are specific analytical methods prescribed. (2) Methods have deficiencies and there is generally a lack of validated methods and reference materials. This results in high measurement uncertainties, with divergent results between even validated assays for specific matrixes and some cross-specificities

While enzyme-linked immunosorbent assay (ELISA)—an immunological technique relying on antibody recognition of the target structure—is the most common method being used for detection and quantification, very few methods have been thoroughly validated. AOAC INTERNATIONAL is one of the very few organizations with experience in validating ELISA methods for allergen detection, having validated some methods under the auspices of the AOAC Research Institute. The German Ministry of Health and Consumer Protection is the only other institution, having conducted several validation studies, in accordance with the AOAC/IUPAC harmonized protocol, for hazelnut and celery allergens, despite existing Europe-wide legislation and the need for enforcement.

However, in reviewing the information on ELISA kits publicly available, it appears that ELISA kits have specific requirements for validation in addition to the AOAC/IUPAC harmonized protocol. Specific requirements include statistical evaluation of results, testing for cross-reactivity (i.e., specificity), and reference materials to name just a few. An international expert group has worked on these requirements for several years now and the final result is presented in the paper by Abbott et al., giving guidance to kit manufacturers or other interested parties in order to harmonize validation of ELISA assays for allergen detection.

Now that this guidance is available and followed, the next question needs to be: To which threshold or action level should allergen labeling be enforced? Only Japan and Switzerland have clear thresholds of 10 and 1000 mg/kg, respectively, for labeling of products destined for the final consumer. But in other countries, discussions are ongoing in industry and government alike on the introduction of labeling of allergen thresholds and action levels. The problem is that, especially with highly processed products and particularly difficult matrixes, different available ELISA kits provide often significantly different results. But since no direct measurement or quantification of the actual target protein(s) is possible, the true value remains elusive. Legislation, to be enforceable, needs to account for the lack of accuracy of quantitation. The paper by Diaz-Amigo and Popping discusses these issues by describing the difficulties of establishing the correlation between clinical allergic reactions and analytical results, as well as the advantages, limitations, and the potential use of current and emerging analytical techniques as core tools available for compliance under HACCP programs and enforcement. The authors also suggest how these issues can be addressed.

Another unresolved issue is the cross-reactivity (specificity) of ELISA-based assays. As an example, tropomyosin, often the target protein to detect crustacean contamination, has very similar structures to the tropomyosin of the *Blattaria* family of insects to which cockroaches belong. Since cockroaches are ubiquitous, it cannot be distinguished if the positive signal of the ELISA assay truly comes from crustacean contamination or from a different source. As another example, celery is, as an allergenic produce, a major issue for food producers. Unfortunately, the proteins typically targeted in celery ELISAs (lipid transfer proteins) have very similar structures to proteins from carrots and potatoes. The paper by Fæste et al. describes the problem of identifying and quantifying celery in processed samples where potatoes and carrots may also be present.

Beyond ELISA, with all its advantages and disadvantages, a new technology application is on the horizon. While mass spectrometry (MS) has been used for the analysis of diverse food contaminants, actual advances in the technology are allowing the analysis of larger and more complex molecules like proteins, opening a promising analytical avenue for food allergens. The advantages of this technology are obvious: direct detection of the peptide reduces the risk of false positives as found with ELISA, and the capability of multitarget screening, i.e., in one run it is possible to screen for many allergens, not only one or two, as in the case for ELISA and polymerase chain reaction (PCR). The paper by Lee et al. compares all those technologies and shows benefits and problems for the detection of protein in egg, a major allergenic food ingredient. While the extraction of detectable egg protein presented problems in the paper, it should be noted that the Morinaga kit, previously evaluated by Garber (1), Diaz-Amigo (2), and Westphal, Pereira, and Williams (3), for detection of egg traces in processed products and found to work best, was not included in the study. This kit should be considered for a judgment on the ability of ELISAs to detect processed egg protein. An important aspect that Lee et al. present is certainly the fact that MS, while still having some extraction issues, has already proven to be a powerful tool in the hands of the skilled user.

In summary, this special section describes the most current issues and developments in allergen analytical trial methods, and their suitability for compliance and regulatory use, giving a comprehensive overview of the status quo in this field.

References

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- (2) Garber, E.A.E., & Brewer, V.A. (2005) *Evaluation of Commercial ELISA Assays for the Detection of Egg in Food*, Presented at 119th AOAC INTERNATIONAL Annual Meeting, Orlando, FL, April 26–28, P-1004
- (3) Westphal, C.D., Pereira, M., & Williams, K.M. (2008) *Commercial kits for egg allergens—What the end user cannot see*, Presented at Fifth Workshop on Food Allergen Methodologies, Halifax, NS, Canada, May 11–14, P-9

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