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Editorial Comment

A Christmas Present for the Food Allergen Community

Our dear colleagues and friends,

Another year is coming to an end and, again, it is time to reflect on all the accomplishments and also on all those things that could have been done better. This has been a good year for the Community, and we have had the pleasure of sharing information with you through the Newsletter and our face-to-face event during the AOAC annual meeting in Atlanta.

But the year is not over yet and we would like to put the cherry on the top of the cake. In this issue of the Newsletter, we are happy to share with you the publication of the **Special Issue of the Journal of AOAC International on food allergens**. Bert Popping and my-self have had the pleasure to serve as guest editors of this project and work with experts from all around the world. The issue contains **25 manuscripts** included in 6 different categories: regulations, surveys, food allergen management, organizations and projects concerned with food allergens, kit manufacturers' case studies and new methods. Bert and I would like to thank all the experts contributing to the special issue and also to the Editorial Board of the Journal for making all 25 manuscripts **open access**. This means that you can download all the articles free of charge. We cannot think of a better Christmas present for the Food Allergen Community.

In addition, we have worked to make available all past issues of the Newsletter. You will find the link to the repository site at the end of this publication.

The Editorial Board of the AOAC Food Allergen Community Newsletter wishes you a Merry Christmas and fruitful 2018. Enjoy the read!

Carmen Diaz-Amigo in **Editor in Chief**

Editorial Team

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Carmen Diaz-Amigo in

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James Roberts in, Bert Popping in, Samuel Godefroy in
Terry Koerner, Jupiter Yeung

European Regulations for Labeling Requirements for Food Allergens and Substances Causing Intolerances: History and Future

Bert Popping & Carmen Diaz-Amigo

Food allergens and intolerances have been diagnosed by doctors for decades, but have received heightened attention in the last two decades because diagnosis and awareness have increased. Consequently, regulators in many jurisdictions have addressed this topic by introducing labeling requirements for substances causing allergies and intolerance reactions in affected individuals. Mandatory labeling of food allergens allows persons suffering from these to make informed choices. However, regulations in some geographic areas have resulted in significant problems for manufacturers as well as consumers. This has been mainly due to frequent changes and amendments, and it has been difficult for all stakeholders to follow and understand the status quo of legislation. The present paper describes the development of European directives and regulations for the labeling of food allergens and intolerances to substances like gluten over the past decades and provides an outlook of what can reasonably be expected to change in the coming years. It also identifies existing gaps, like a lack of threshold levels for adventitious contamination and consequently a proliferation of precautionary allergen labeling, which neither benefits the consumer nor the food industry in its current form.

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Japanese food allergen labeling regulation – Update

Masahiro Shoji, Reiko Adachi, Hiroshi Akiyama

The Japanese food allergen labeling regulation was designed to match real Japanese food allergy circumstances and also to be enforced effectively; thus, (1) regulated food allergens were selected by prevalence and seriousness according to food allergy surveys in Japan; (2) the detection criterion for ELISA monitoring, 10 µg food allergen protein/g (or mL) food, was set up as the threshold value to regulate commercial prepackaged foods; and (3) official food allergen analytical methods, which can determine the threshold value accurately, were developed. These three points are distinctive from other countries. Furthermore, as an on-going project, the regulation has been amended according to food allergy circumstances and requirements of society. This paper presents recent changes regarding the Japanese food allergen labeling regulation. To date, the Japanese food allergen labeling regulation has been enforced for more than 15 years and seems to be working effectively. Now would be an opportune time to review the regulation for its next level of development.

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Food Allergen Labeling: A Latin American Approach

María Cristina López

Food allergy is a public health concern almost all over the world. Although most of the countries that regulate the declaration of allergens in prepackaged foods include the list recommended by the Codex Alimentarius, some countries have added other allergens to this list due to prevalence data and regional incidence, whereas others have incorporated exceptions for some products derived from allergenic foods. Within this context, the situation in Latin America regarding these regulations is diverse. Data about prevalence of food hypersensitivity are very limited in the region. The countries that have established regulations are Brazil, Colombia, Costa Rica, Guatemala, Honduras, El Salvador, Nicaragua, Chile, Mexico, and Venezuela. Argentina has approved a regulation for the labeling of food allergens in November 2016. It only needs to be published in the Official Bulletin to go into effect. All countries follow the Codex list that includes latex and excludes sulfites, except Brazil. On the other hand, Argentina is the only country that includes exceptions. As for the methodologies for the detection of allergens in foods, this issue is a serious problem for both the food industry and control laboratories. Available methodologies are based mainly on commercial ELISA kits; currently, there are no Latin American companies that produce them, so ELISA kits are expensive and their acquisition is complicated. There is an initiative in Argentina to address all these gaps in the region through the Platform of Food Allergens (PFA), a nonprofit organization that integrates health professionals, patients, representatives of the food industry, government, and scientists. The different actions carried out by the PFA have made it possible to contact different scientific groups from other Latin American countries in order to expand this initiative and thereby promote and strengthen both public and private capacities in the region.

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Action Levels for Food Allergens: An Approach for Official Food Control in Germany

Hans-Ulrich Waiblinger and Gesine Schulze

Official food control laboratories in Germany have established internal action values for the assessment of analytical results of food allergens especially obtained from samples without declaration of the specified allergen. A pragmatic approach was chosen considering the current situation for European food information legislation. Accordingly, when a positive result is obtained for an unlabeled allergen, it is not necessarily an irregularity if it can be demonstrated that the result was caused by cross-contamination. Action values take into account current analytical experiences as well as published allergologic reference doses. They are considered

as internal de minimis thresholds by food control authorities that are used to support laboratories in the decision-making process and when a written expert opinion is requested by an enforcement authority. If only minor traces are detected at concentrations below the action values, further investigation of the issue and inspections at the location of manufacture can be abandoned. The present report includes a collection of results from official food control laboratories in Germany that have been evaluated in line with the aforementioned system of action levels.

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Undeclared Food Allergens and Gluten in Commercial Food Products Analyzed by ELISA

Andrew B. Do, Sefat E Khuda, Girdhari M. Sharma

Undeclared allergen(s) in commercial food products are responsible for many food recalls, as reported by regulatory agencies in various countries, including the United States. Correct allergen labeling practices are essential for the safety of food-allergic consumers. However, this practice may be hindered by the introduction of allergens all along the food supply chain, including unintentionally through cross-contact. To understand the pervasiveness of undeclared allergen(s) in commercial food products, the objective of this review is to summarize the prevalence of undeclared milk, egg, hazelnut, peanut, soy, and gluten as detected by ELISA from previously published surveys. The prevalence of undeclared allergen(s) in products with or without an advisory statement was also summarized and compared. As compiled by this review, there are some food categories that may be at higher risk for containing undeclared allergen(s). However, the data on prevalence and amount of allergen present may vary widely within any particular allergen or food category. Factors, such as food survey product selection, geography, awareness of allergen/gluten issues, and/or the choice of ELISA method, may be responsible for such differences.

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The Use of Visual Examination for Determining the Presence of Gluten-Containing Grains in Gluten Free Oats and Other Grains, Seeds, Beans, Pulses, and Legumes

Laura K. Allred, Cynthia Kupper and Channon Quinn

Obtaining representative test samples for antibody-based testing is challenging when analyzing whole grains for gluten. When whole grains are ground into flour for testing, confocal microscopy studies have shown that gluten tends to exist as aggregates within the starch background, making single-sample testing inaccurate and complicating the ability

to arrive at an accurate average from multiple samples. In addition, whole-grain products present a unique risk to gluten free consumers, in that any contamination is localized to specific servings rather than being distributed across the product lot. This makes parts-per-million values less relevant for whole-grain products. Intact grains, seeds, beans, pulses, and legumes offer an alternative opportunity for gluten detection, in that contaminating gluten-containing grains (GCGs) are visible and identifiable to the trained eye or properly calibrated optical sorting equipment. The purpose of the current study was to determine a Gluten Free Certification Organization threshold level for the maximum number of GCGs within a kilogram of nongluten grains sold as specially processed gluten free product and to determine the feasibility of this threshold by evaluating visual examination data from two major oat processors.

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Managing Food Allergens in the U.K. Retail Supply Chain

Michael J. Walker, M. Hazel Gowland and John Points

The U.K. food and grocery market is highly significant financially and dominated by 10 retailers within a regulated and extremely economically competitive environment. We summarize the approach of U.K. retailers to allergen risk assessment (RA) and risk management (RM) within the U.K. legal framework and explore public visibility of retailers' allergen policies. RA and RM of allergens appear effective in curtailing retail-triggered severe food allergy reactions. However, allergen recalls remain high, precautionary allergen labeling (PAL) remains an area of confusion, and there is no consistent Web-based provision of information for consumers who have allergies. Resolution of PAL awaits an agreed-on threshold framework, but a key challenge is to engage with patients and gain their trust rather than thrust education at them. It would be helpful for retailers to publish their allergen RA and RM policies. A target should be agreed on between government and retailers for a reduction in the proliferation of PAL wording variants by a given date within the next 3 years. A further hurdle is potentially flawed allergen analysis— development of reference methods and reference materials are acknowledged needs. Laboratories should report allergen results in an informative manner, communicating uncertainty and caveats. Ideally a laboratory representative would be included on any incident control team. Efforts must continue to standardize preparedness for protecting and defending food and drink from deliberate attack.

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Allergens: An Enhanced Focus

Kristen Spotz

Allergy Awareness Week serves as a reminder of the over 15 million Americans who suffer from food allergies. The importance of allergies and allergen labeling can be seen when looking at U.S. Food and Drug Administration recall data: of the 764 recalls in 2016, 305 (representing more than 40%) were due to undeclared allergens. However, recalls for undeclared allergens are a complex issue with numerous factors. The implementation of prevention-based systems with the necessary management components and further error-proofing the systems, along with allergen awareness embedded throughout a company's food safety culture, can likely help reduce the number of recalls for undeclared allergens. As a resource to manufacturers, the Grocery Manufacturers Association and the Food Allergy Research and Resource Program have developed several resources to assist with developing robust allergen management programs. By reducing the number of recalls for undeclared allergens, the food industry will likely increase and maintain consumer confidence and trust of the food-allergic community. This enhanced consumer confidence and trust could eventually open the door for further collaboration with the food-allergic community and, potentially, advance allergen-related policies.

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Food Allergen Management in Australia

Martina Koeberl, Dean Clarke, Katrina J. Allen, Fiona Fleming, Lisa Katzer, N. Alice Lee, Andreas L. Lopata, Maria Said, Pieter Scheelings, Neil Shepherd, Robin Sherlock and James Roberts

Food allergies are increasing globally, including numbers of allergens, the sensitization rate, and the prevalence rate. To protect food-allergic individuals in the community, food allergies need to be appropriately managed. This paper describes current Australian food allergen management practices. In Australia, the prevalence of food allergies, the anaphylaxis rate, and the fatal anaphylaxis rate are among the highest in the world. Interagency and stakeholder collaboration is facilitated and enhanced as Australia moves through past, current, and ongoing food allergen challenges. As a result, Australia has been a global leader in regulating the labeling of common allergens in packaged foods and their disclosure in foods not required to bear a label. Moreover, the food industry in Australia and New Zealand has developed a unique food allergen risk management tool, the Voluntary Incidental Trace Allergen Labelling program, which is managed by the Allergen Bureau. This paper summarizes insights and information provided by the major stakeholders involved to protect food-allergic consumers from any allergic reaction. Stakeholders include government; consumer protection, regulation, and enforcement agencies; the food industry;

and food allergen testing and food allergen/allergy research bodies in Australia. The ongoing goal of all stakeholders in food allergen management in Australia is to promote best practice food allergen management procedures and provide a wide choice of foods, while enabling allergic consumers to manage their food allergies and reduce the risk of an allergic reaction.

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Challenges and Path Forward on Mandatory Allergen Labeling and Voluntary Precautionary Allergen Labeling for a Global Company

Jupiter Yeung and Marie-Claude Robert

For food manufacturers, the label on a food package is a tool meant to alert consumers to the presence of specific allergens, allowing consumers to make informed decisions and not unnecessarily limit their food choices. Mandatory allergen labeling is used when the allergen is an intentionally added ingredient, whereas voluntary allergen labeling is used when the presence of the allergen is unintentional and may be in the finished product as a result of cross-contact. In a globalized economy, ensuring food safety is a growing challenge for manufacturers. When ingredients and technologies are sourced worldwide from multiple business partners, complexity rises, which can increase the chance for errors, leading to potential harm. Threshold science, Voluntary Incidental Trace Allergen Labelling (VITAL) reference doses, fit-for-purpose analytical technology, and common sense enable us to optimize allergen management for the benefit of allergic consumers. This is a good strategy because all stakeholders share the common goal of making foods safe and wholesome for all. Herein, we recommend that (1) senior management make science-based thresholds a priority for both regulatory authorities and the food industry; (2) VITAL 2.0 be adopted as a risk assessment and risk management tool for precautionary allergen labeling (PAL); (3) a standardized message for PAL, i.e., "may contain x," be used to make it easily understandable to allergic consumers so they can make informed food choices; and (4) validated fit-for-purpose allergen methods be used to meet analytical needs. This is an opportunity for us to speak with one voice and demonstrate that food safety is not a competitive issue, but a shared responsibility. This approach could significantly improve allergic consumers' lives.

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The Allergen Bureau VITAL® Program

Simon Brooke Taylor, Georgina Christensen, Kirsten Grinter, Robin Sherlock and Lisa Warren

This paper sets out the role of the Allergen Bureau and the Voluntary Incidental Trace Allergen Labelling (VITAL) Program from its origin in 2007 to its current iteration, VITAL 2. Herewith are outlined the scientific principles that support the program; the program's application in the food chain; and the benefits of the program's use to the food industry, clinicians, and the allergic consumer. VITAL was developed by the Australian and New Zealand food industry in consultation with multiple stakeholders, including consumer organizations, industry bodies, regulators, and retailers, to provide a standardized, science-based risk assessment process for the investigation of the potential presence of food allergens due to cross-contact and to determine whether, for cases in which the allergen is unable to be removed or controlled consistently, precautionary statements are required. The aim of the program is to provide a consistent process, a standardized approach, and a relevant cross-contact statement to allow the allergic consumer to make an informed decision regarding consumption of food.

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Integrating Allergen Analysis Within a Risk Assessment Framework: Approaches to Development of Targeted Mass Spectrometry Methods for Allergen Detection and Quantification in the iFAAM Project

Chiara Nitride, Victoria Lee, Ivona Baricevic-Jones, Karine Adel-Patient, Sabine Baumgartner and E. N. Clare Mills

Allergen analysis is central to implementing and monitoring food allergen risk assessment and management processes by the food industry, but current methods for the determination of allergens in foods give highly variable results. The European Union-funded "Integrated Approaches to Food Allergen and Allergy Risk Management" (iFAAM) project has been working to address gaps in knowledge regarding food allergen management and analysis, including the development of novel MS and immuno-based allergen determination methods. Common allergenic food ingredients (peanut, hazelnut, walnut, cow's milk [Bos domesticus], and hen's egg [Gallus domesticus]) and common food matrixes (chocolate dessert and cookie) have been used for both clinical studies and analytical method development to ensure that the new methods are clinically relevant. Allergen molecules have been used as analytical targets and allergenic ingredients incurred into matrixes at levels close to reference doses that may trigger the use of precautionary allergen labeling. An interlaboratory method comparison has been undertaken for the determination of peanut in chocolate dessert using

MS and immuno-based methods. The iFAAM approach has highlighted the need for methods to report test results in allergenic protein. This will allow food business operators to use them in risk assessments that are founded on clinical study data in which protein has been used as a measure of allergenic potency.

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ILSI Europe's Food Allergy Task Force: From Defining the Hazard to Assessing the Risk from Food Allergens

Crevel R W R, Ronsmans S, Marsaux C F M3, Bánáti D

The International Life Sciences Institute (ILSI) Europe Food Allergy Task Force was founded in response to early public concerns about the growing impact of food allergies almost coincidentally with the publication of the 1995 Food and Agriculture Organization-World Health Organization Technical Consultation on Food Allergies. In line with ILSI principles aimed to foster collaboration between stakeholders to promote consensus on science-based approaches to food safety and nutrition, the task force has played a central role since then in the development of risk assessment for food allergens. This ranged from consideration of the criteria to be applied to identifying allergens of public health concern through methodologies to determine the relationship between dose and the proportion of allergic individuals reacting, as well as the nature of the observed responses. The task force also promoted the application of novel, probabilistic risk assessment methods to better delineate the impact of benchmarks, such as reference doses, and actively participated in major European food allergy projects, such as EUROPREVALL, the European Union (EU)-funded project "The prevalence, cost and basis of food allergy across Europe;" and iFAAM, "Integrated approaches to food allergen and allergy risk management," also an EU-funded project. Over the years, the task force's work has evolved as answers to initial questions raised further issues: Its current work program includes a review of analytical methods and how different ones can best be deployed given their strengths and limitations. Another activity, which has just commenced, aims to develop a framework for stakeholders to achieve consensus on acceptable risk.

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Assessing Almond and Peanut Allergens Using Commercially Available Immunoanalytical Kits and LC-MS/MS: A Case Study

Daly M, Ansari P, Häubl G, Rogers A, Brunner K

With an ever-increasing allergic population and an emerging market for allergen-free foods, accurate detection of allergens in foods has never been more important. Although ELISA-based methods are the most widely used for detection of allergens in food, there is a need for the development of orthogonal approaches. A commercial ELISA detected a relatively high concentration of peanut and almond in an allergen-free product. However, another commercial ELISA declared a low peanut concentration and was negative for almond. Further testing using a commercial almond lateral-flow device confirmed the results from the second ELISA kit and demonstrated that the positive detection of almond was due to cross-reactivity. An MS method was used for final confirmation that the reported results were negative for both almond and peanut.

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Commercial ELISA Measurement of Allergens and Gluten: What We Can Learn from Case Studies

Markus Lacorn, Stella Lindeke, Susanne Siebeneicher, Thomas Weiss

During the last decade, results from ELISA test kits have often been criticized as being flawed. Therefore, it may appear counterintuitive that ELISAs are used for most food allergen and gluten analytical needs. One reason, in addition to the nonavailability of comparable alternative methods, is the fact that the methods used underwent a long validation and learning period in the market. This publication presents several case studies on interference, cross-reactivity issues, calibrators, fragmented allergens and gluten, matrix influences, and misunderstood intended-use statements. Afterward, the relevant validation parameter LOD, LOQ, selectivity, and precision are discussed. Finally, a comprehensive list of practical recommendations for ELISA test kit users is presented.

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A Targeted LC-MS/MS Method for the Simultaneous Detection and Quantitation of Egg, Milk, and Peanut Allergens in Sugar Cookies

Chelsea C. Boo and Christine H. Parker

Food allergy is a growing public health concern, with many individuals reporting allergies to multiple food sources. Compliance with food labeling regulations and prevention of inadvertent cross-contact in manufacturing requires the use of reliable methods for the detection and quantitation of allergens in processed foods. In this work, a novel liquid chromatography-tandem mass spectrometry multiple-reaction monitoring method for multiallergen detection and quantitation of egg, milk, and peanut was developed and evaluated in an allergen-incurred baked sugar cookie matrix. A systematic evaluation of method parameters, including sample extraction, concentration, and digestion, were optimized for candidate allergen peptide markers. The optimized method enabled the reliable detection and quantitation of egg, milk, and peanut allergens in sugar cookies, with allergen concentrations as low as 5 ppm allergen-incurred ingredient.

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Development and Validation of a Quantitative ELISA for the Detection of Almond Residues in Foods

Ewa Slotwinski, Dave Almy, Ryan Viator, Mohamed Abouzied, Frank Klein and Jennifer Rice

Neogen Corp. has developed Veratox for Almond Allergen for use in the quantitative analysis and screening of almond protein residues in food products, such as cookies, crackers, chocolate bars, cereals, beverages, and clean-in-place rinses. Quantitation with Veratox for Almond Allergen ranges from 2.5 to 25 ppm and, with dilution, it can be extended for highly positive samples. This paper describes the findings of internal testing and validation studies designed to establish product claims for the assay of Veratox for Almond Allergen.

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Novel Approaches for the MS-Based Detection of Food Allergens: High Resolution, MS3, and Beyond

Jens Brockmeyer

The prevalence of allergic reactions to food is believed to be increasing in industrialized countries worldwide. One of the major tasks in risk management is, therefore, the analytical surveillance of allergen contamination in food and targeted proteomics using MS, which is of hugely growing interest due to its specificity and sensitivity and the possibility to analyze multiple allergens in parallel. Though approximately 200 different foods have been described as having the potential to elicit allergic reactions, current regional labeling requirements are focused on the 5–14 priority allergens that elicit the vast majority of severe reactions or that pose a risk as hidden allergens in food production. MS-based detection

methods have been published for the majority of priority allergens, and this review provides an overview of the different methodological approaches, namely multiple-reaction monitoring-, high-resolution MS-, and triple-stage MS-based methods. In addition, requirements for the identification and validation of specific marker peptides and the influence of thermal processing and structural heterogeneity of allergens are discussed.

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Simultaneous Analysis of Multiple Allergens in Food Products by LC-MS/MS

Lee Sun New, Andre Schreiber, Jianru Stahl-Zeng and Hua-Fen Liu

There is currently no cure for food allergies, and sufferers can only rely on the correct labeling of foods to avoid allergens. Hence, it is important that analytical methods are sensitive and accurate enough to screen for the presence of multiple allergens in food products. In this study, we developed an LC-tandem MS method that is able to simultaneously screen or quantify the signature tryptic peptides of multiple allergen commodities. This method is capable of screening and identifying egg white, skim milk, peanut, soy, and tree nuts (almond, Brazil nut, cashew, hazelnut, pecan, pine nut, pistachio, and walnut) at a detection limit of 10 ppm in incurred bread and cookies. It was further tested for the quantitative analysis of whole-egg, whole-milk, peanut butter, and hazelnut commodities, which are incurred or spiked into selected food matrixes as defined in AOAC INTERNATIONAL Standard Method Performance Requirement (SMPR®) 2016.002. The method demonstrated excellent sensitivity with a Method quantitative limit of 3 ppm for whole egg and 10 ppm for the remaining three allergen commodities. It also demonstrated good recovery (60-119%) and repeatability (RSDr <20%), with an analytical range of 10–1000 ppm for each allergen commodity and was able to meet the minimum performance requirements of the SMPR.

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Target Selection Strategies for LC-MS/MS Food Allergen Methods

Melanie L Downs and Philip Johnson

The detection and quantitation of allergens as contaminants in foods using MS is challenging largely due to the requirement to detect proteins in complex, mixed, and often processed matrixes. Such methods necessarily rely on the use of proteotypic peptides as indicators of the presence and amount of allergenic foods. These peptides should represent the allergenic food in question in such a way that their use is both sensitive (no false-negatives) and specific (no false-

positives). Choosing such peptides to represent food allergens is beset with issues, including, but not limited to, separated ingredients (e.g., casein and whey), extraction difficulties (particularly from thermally processed foods), and incomplete sequence information, as well as the more common issues associated with protein quantitation in biological samples. Here, we review the workflows that have been used to select peptide targets for food allergen detection. We describe the use and limitations of both in silico-based analyses and experimental methods relying on high-resolution MS. The variation in the way in which target selection is performed highlights a lack of standardization, even around the principles describing what the detection method should achieve. A lack of focus on the food matrixes to which the method will be applied is also apparent during the peptide target selection process. It is hoped that highlighting some of these issues will assist in the generation of MS-based allergen detection methods that will encourage uptake and use by the analytical community at large.

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Assessment of Recovery of Milk Protein Allergens from Processed Food for Mass Spectrometry Quantification

Kate Groves, Adam Cryar, Michael Walker, Milena Quaglia

Assessing the recovery of food allergens from solid processed matrixes is one of the most difficult steps that needs to be overcome to enable the accurate quantification of protein allergens by immunoassay and MS. A feasibility study is described herein applying International System of Units (SI)-traceably quantified milk protein solutions to assess recovery by an improved extraction method. Untargeted MS analysis suggests that this novel extraction method can be further developed to provide high recoveries for a broad range of food allergens. A solution of α-casein was traceably quantified to the SI for the content of α-S1 casein. Cookie dough was prepared by spiking a known amount of the SI-traceable quantified solution into a mixture of flour, sugar, and soya spread, followed by baking. A novel method for the extraction of protein food allergens from solid matrixes based on proteolytic digestion was developed, and its performance was compared with the performance of methods reported in the literature.

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Almond or Mahaleb? Orthogonal Allergen Analysis During a Live Incident Investigation by ELISA, Molecular Biology, and Protein Mass Spectrometry

Michael J Walker, Malcolm Burns, Milena Quaglia, Gavin Nixon, Christopher J Hopley, Kirstin M Gray, Victoria Moore, Malvinder Singh, and Simon Cowen

It is now well known that an incident investigated in the United Kingdom in 2015 of cumin alleged to be contaminated with almond, a risk for people with almond allergy, was caused by the Prunus species, Prunus mahaleb. In the United Kingdom, the Government Chemist offers a route of technical appeal from official findings in the food control system. Findings of almond in two official samples, cumin and paprika, which had prompted action to exclude the consignments from the food chain, were so referred. Herein are described the approaches deployed to resolve the analytical issues during the investigation of the incidents. The cross-reactivity of ELISA to Prunus species was confirmed, and although this is useful in screening for the genus, orthogonal techniques are required to identify the species and confirm its presence. Two novel PCR assays were developed: one specific for P. mahaleb and the other a screening method capable of identifying common Prunus DNA. Peptides unique to almond and mahaleb were identified, permitting LC-tandem MS and criteria were developed for peptide identification to forensic standards. This work enables a staged approach to be taken to any future incident thought to involve Prunus species and provides a template for the investigation of similar incidents.

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Highly Sensitive Matrix-Independent Quantification of Major Food Allergens Peanut and Soy by Competitive Real-Time PCR Targeting Mitochondrial DNA

Eva-Maria Ladenburger, Markus Dehmer, Ruben Grünberg, Hans-Ulrich Waiblinger, Dieter Stoll, Jörg Bergemann

The development of two competitive real-time PCR assays for the quantitative detection of trace amounts of two major food allergens, peanut and soybean, is reported. In order to achieve very low detection levels for both allergens, we established PCR primers and probes targeting mitochondrial DNA sequences. We were able to demonstrate that this approach led to an increase in detection sensitivity in the range of at least 1 order of magnitude compared with published assays targeting nuclear DNA. Furthermore, we generated corresponding competitor molecules, which were used as internal standards to compete with matrix effects that are evident during DNA extraction and PCR amplification in heterogeneous analytical matrixes like food. According to

the recently described competitive quantitative PCR method published by Holzhauser et al. (2014), we performed threshold calibration against milk powder spiked with 10 ppm peanut and soy. Matrix-independent quantitative determination of peanut and soy could be demonstrated for three different calibrated food matrix standards in a range between 1 and 100 ppm. The data presented indicate that both assay concepts are powerful analytical tools for the quantitative detection of trace amounts of peanut and soy in commercial food products.

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Stakeholders' Guidance Document for Consumer Analytical. Devices with a Focus on Gluten and Food Allergens

Bert Popping, Laura Allred, Francois Bourdichon, Kurt Brunner, Carmen Diaz-Amigo, Patricia Galan-Malo, Markus Lacorn, Jennifer North, Salvatore Parisi, Adrian Rogers, Jennifer Sealy-Voyksner, Tricia Thompson, Jupiter Yeung

Until recently, analytical tests for food were performed primarily in laboratories, but technical developments now enable consumers to use devices to test their food at home or when dining out. Current consumer devices for food can determine nutritional values, freshness, and, most recently, the presence of food allergens and substances that cause food intolerances. The demand for such products is driven by an increase in the incidence of food allergies, as well as consumer desire for more information about what is in their food. The number and complexity of food matrixes creates an important need for properly validated testing devices with comprehensive user instructions (definitions of technical terms can be found in ISO 5725-1:1994 and the International Vocabulary of Metrology). This is especially important with food allergen determinations that can have life-threatening consequences. Stakeholders— including food regulators, food producers, and food testing kit and equipment manufacturers, as well as representatives from consumer advocacy groups—have worked to outline voluntary guidelines for consumer food allergen- and gluten-testing devices. These guidelines cover areas such as kit validation, user sampling instructions, kit performance, and interpretation of results. The recommendations are based on (1) current known technologies, (2) analytical expertise, and (3) standardized AOAC INTERNATIONAL allergen community guidance and best practices on the analysis of food allergens and gluten. The present guidance document is the first in a series of papers intended to provide general guidelines applicable to consumer devices for all food analytes. Future publications will give specific guidance and validation protocols for devices designed to detect individual allergens and gluten, as statistical analysis and review of any validation data, preferably from an independent third party, are necessary to establish a device's fitness-for-purpose. Following the recommendations of these guidance documents will help ensure that consumers

are equipped with sufficient information to make an informed decision based on an analytical result from a consumer device. However, the present guidance document emphasizes that consumer devices should not be used in isolation to make a determination as to whether a food is safe to eat. As advances are made in science and technology, these recommendations will be reevaluated and revised as appropriate.

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A Global Reflection on Food Allergen Regulations, Management, and Analysis

Carmen Diaz-Amigo & Bert Popping

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Call for Scientific Sessions for the 132nd AOAC Annual Meeting in Toronto (Aug 26-29, 2018)

The AOAC Technical Programming Council (TPC) invites all AOAC Members, Community groups, and other interested individuals to submit proposals for the scientific sessions that will be offered at the 132nd Annual Meeting & Exposition in Toronto, Ontario, Canada at the Sheraton Centre Toronto Hotel (August 26–29, 2018). Proposals may be submitted no later than **December 13, 2017**.

All requirements for the submission of session proposals are listed in **this document** ⬇

FOOD ALLERGEN COMMUNITY NEWSLETTER ARCHIVE

available at:

www.focos-food.com/food-allergen-community

Upcoming Events

10th Workshop on Food Allergen Methodologies

DoubleTree by Hilton Toronto Downtown

Toronto, Canada

May 7 – 9, 2018

Keerati / freedigitalphotos.net

AOAC Food Allergen Community Newsletter

Contribute with articles, news items or suggestions.

Submission deadline for the 1st issue of 2018: March 23

Send your articles to AOAC.Allergens@gmail.com

Topics for publication

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Article requirements*

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✓ **Length: 400 words max.**

✓ **1 figure or table (optional)**

✓ **Author & Affiliation**

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* All articles are subject to review by the Editorial Board.

The AOAC Food Allergen Community is a forum serving the scientific community working on Food Allergens: The community aims to help AOAC INTERNATIONAL in its consensus-based scientific and advisory capacity on methods of analysis for allergens in foods and other commodities. It is also meant to serve the broader Stakeholder Community whose objectives it is to enhance the protection of food allergic consumers worldwide.

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