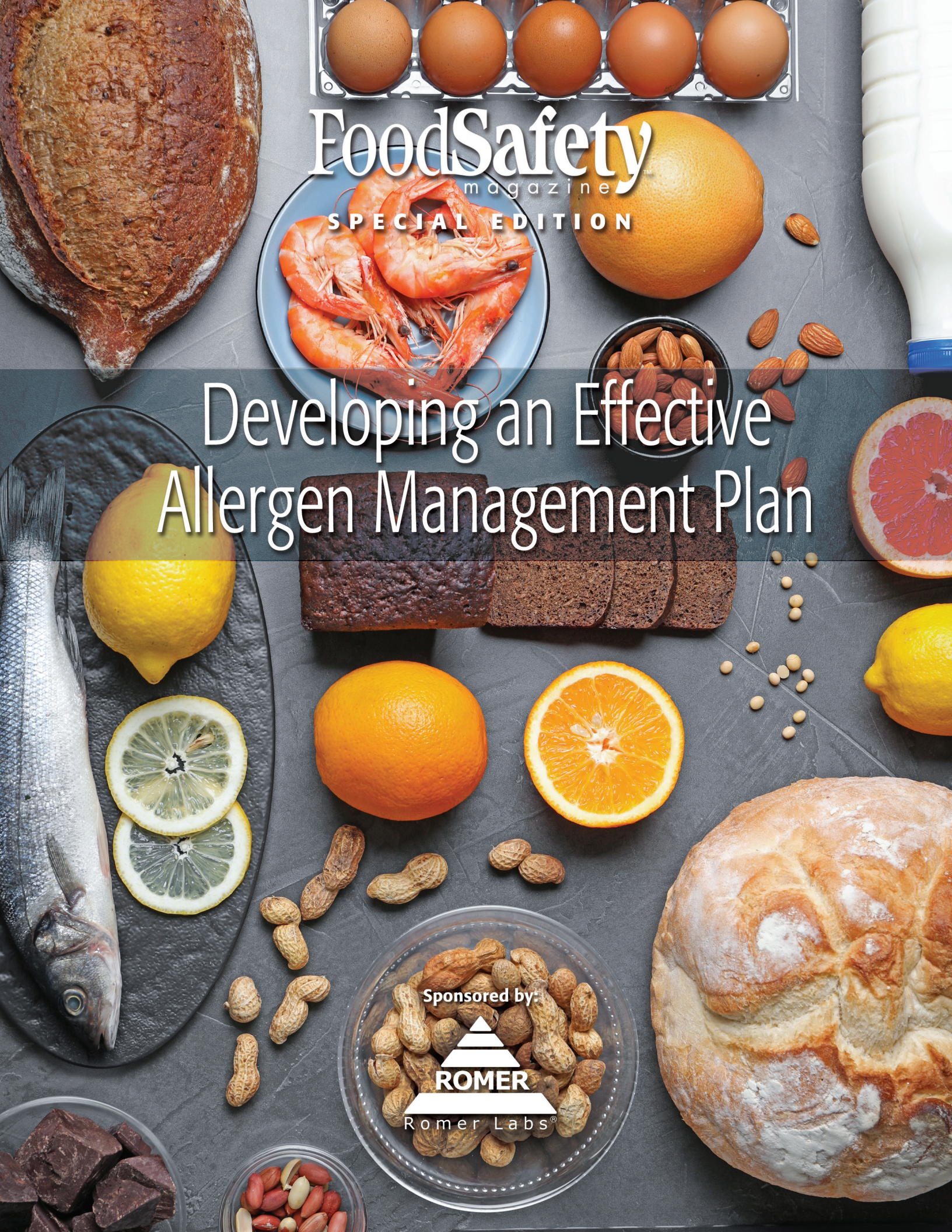

FoodSafety magazine

SPECIAL EDITION

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By Steve L. Taylor, Ph.D., and Maria Oria, Ph.D.

Report from the National Academies of Sciences, Engineering, and Medicine:

Recommendations to the Food Industry and Regulatory Agencies on the Management of Food Allergens

On November 30, 2016, the National Academies of Sciences, Engineering, and Medicine issued a report with wide-ranging recommendations aimed at providing a road map to improve the situation for individuals with food allergies. Entitled *Finding a Path to Safety in Food Allergy: Assessment of the Global Burden, Causes, Prevention, Management, and Public Policy*, the report includes recommendations on food allergy diagnostics, prevention, education of various stakeholders, emergency and daily management by caregivers and providers, allergen labeling, and development of policy guidelines for a variety of settings to improve safety. The National Academies study was supported by three federal agencies and eight nonfederal sponsors, including two consumer groups and several associations sponsored by growers and/or processors. The study was conducted over an 18-month period by a committee of 15 international experts representing a range of expertise and experience on the topic. This article will focus on the recommendations contained within the report that are aimed at the food industry and/or federal agencies that regulate the food industry. The scope of the report was global, but this article will focus on the impact of the recommendations on the North American (U.S. and Canada) food industry and the agencies that regulate that industry.

Recommendations on Mandatory Allergen Labeling

Several of the report's recommendations are directly aimed at the enactment of policies that will impact the food industry but, in the opinion of the committee, also improve the quality of life of consumers with food allergies. The labels on packaged foods are a primary conduit of critical information to consumers with food allergies and their caregivers. The primary advice given by healthcare providers to individuals with a food allergy is to avoid ingestion of their offending food(s) to prevent reactions. The ingredient declaration and the "Contains statement" (where used) provide critical information to food-allergic consumers.

Globally, various countries have established different lists of priority allergenic foods and associated labeling regulations that require the declaration of ingredients derived from these foods when they are intentionally used in product formulations (www.farrp.unl.edu/IRChart). Most countries rely on a list of priority allergenic foods established by the Codex Alimentarius Commission in 1999 as guidance to member countries of the World Health Organization and Food & Agriculture Organization (FAO) of the United Nations. That list of eight foods or food groups (milk, eggs, fish, crustacean shellfish, peanuts, tree nuts, soybeans, and cereal sources of gluten) was established based upon recommendations from an FAO expert consultation held in 1995. One of the key recommendations in the report focuses on this global guidance:

The Codex Alimentarius Commission and public health authorities in individual countries should decide on a periodic basis about which allergenic foods should be included in their priority lists based on scientific and clinical evidence of regional prevalence and severity of food allergies as well as allergen potency.

The committee recognized that the existing Codex list was reasonable and that regional differences in allergen prevalence exist but recommended that regional additions to the Codex list should be based upon sound scientific and clinical evidence. Clearly, as more scientific and clinical information becomes available over time, a reassessment of these lists should be considered.

The Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) in the U.S. and the Safe Foods for Canadians Act (SFCA) in Canada provide excellent labeling regulations to protect consumers with food allergies. FALCPA follows the Codex list with the exception that wheat is specifically recognized as a priority allergenic food because FALCPA focuses on food allergies rather than celiac disease associated with cereal sources of gluten. SFCA includes several additions to the Codex list, namely molluscan shellfish, sesame seeds, and mustard. Evidence of the prevalence and severity of allergic reactions to sesame seeds may warrant their

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inclusion on the priority allergen list in the United States.

The committee's task was not to make recommendations on specific foods that should be added to the priority allergen list; however, the committee concluded that although solid evidence on the prevalence of specific food allergies in the U.S. does not exist, the U.S. Food and Drug Administration (FDA) should consider the addition of sesame seeds to the U.S. priority allergen list.

> "The labels on packaged foods are a primary conduit of critical information to consumers with food allergies and their caregivers."

After the passage of FALCPA, FDA posted a list of 19 tree nuts on its website associated with FALCPA compliance. In the committee's opinion, clinical and scientific evidence is lacking to support the inclusion of beechnuts, butternuts, chestnuts, chinquapins, coconuts, ginkgo nuts, hickory nuts, litchi nuts, pili nuts, and shea nuts as priority allergenic tree nuts. In fact, litchi is a fruit and coconut is a palm drupe.

Globally, most countries follow the Codex guidance and require the labeling of the priority allergenic foods and any ingredients derived from them. However, the committee recognized that the allergenicity of ingredients from any allergenic source is based upon the protein (allergen) content of that ingredient. The European Union and Australia/New Zealand have provided exemptions from source labeling for certain ingredients where evidence exists that these ingredients are not hazardous to consumers with allergies to the allergenic source of the ingredients. In the U.S., Congress recognized that highly refined oils represent no risk and exempted such oils from the labeling provisions of FALCPA. In addition, FALCPA provided a mechanism for source-labeling exemptions, but FDA has granted only two exemptions (for certain confined uses of Solae soy lecithin and for ice-structuring protein derived from a fish gene). FDA should make its decisions about labeling exemptions for ingredients derived from priority allergenic sources based on a quantitative risk assessment (QRA) framework.

The report contains a comprehensive explanation of the QRA approach that, in the case of ingredients, involves an evaluation of the protein (allergen) content of the ingredient against the known range of individual threshold doses for individuals with allergies to the source of the ingredient.

Related to strengthening policies, the committee made a recommendation to FDA to continue working with other relevant federal, state, and local agencies to develop and implement labeling policies specific to allergenic ingredients in packaged and prepared foods that are distributed through airlines and other public venues, including schools and early care and educational facilities. Attention is needed in such venues where packaged food does not enter into interstate commerce and might not be subject to federal labeling laws.

Recommendation on Precautionary Allergen Labeling (PAL)

Unintentional allergens that might occur at low levels, but could still cause a reaction in some individuals, can be identified on the labels of packaged foods with PAL. Currently, PAL usage is voluntary but allowed in most countries. PAL takes dozens of specific formats, including "may contain x," "manufactured on shared equipment with x," and "packaged in a shared facility with x." PAL is not risk-based, and evidence from analytical surveys indicates that many products bearing PAL statements contain no detectable allergen residues. Consumer surveys indicate that consumers with food allergies attempt to assign variable levels of risk to products with PAL based upon the wording of the PAL statement. These surveys also reveal that some consumers with food allergies ignore PAL. Consensus expert opinions, including the opinion of the committee, indicate that PAL is confusing to consumers and has lost much of its intended effectiveness. Accordingly, the committee reached the following recommendation:

The food manufacturing industry, FDA, and the U.S. Department of Agriculture (USDA) should work cooperatively to replace the PAL system for low-level allergen contaminants with a new risk-based labeling approach, such as the VITAL (Voluntary Incidental Trace Allergen Labelling) program used in Australia and New Zealand.

To implement this risk-based approach, the committee recommended three further actions:

1. FDA and USDA should establish reference doses (thresholds) for allergenic foods, where possible. The committee concludes that at this time, sufficient data exist on milk, eggs, peanuts, certain tree nuts (cashew, walnut, hazelnut), wheat, soybeans, fish, and crustacean shellfish (shrimp) to establish reference doses. FDA and USDA should review the reference doses periodically, with particular attention to the remaining tree nuts for which data to establish reference doses are not currently available (i.e., almond, Brazil nut, macadamia nut, and pine nut).

2. Once reference doses are established, a food product would carry an advisory label (e.g., "peanut may be present") only in situations when ingesting the product would expose the individual to a level above the reference doses for that allergen. FDA should restrict the number of allowable advisory labels to one phrase. Because this labeling is voluntary, the product should clearly inform the consumer, through labeling as appropriate, as to whether a risk-based approach (such as VITAL) has been followed for each specific product. FDA and USDA should educate healthcare providers and consumers about the meaning of such a food allergy advisory statement.

3. FDA and USDA, together with the food industry and the analytical testing industry, should develop and validate detection methods and sampling plans for the various food allergens for which reference doses are established. A common unit of reporting also should be established, such as parts per million of protein from the allergenic source, so that comparisons can be made between methods and between levels in the food and clinical threshold values.

This particular recommendation is complex but, in the view of the commit-

tee, critically important to improving the quality of life for food-allergic consumers. Currently, many food products bear PAL statements. If food-allergic consumers avoid all foods with PAL statements, their food choices are seriously restricted. In the committee's view, PAL guidance from FDA and USDA, working with industry, would lead to more meaningful implementation of PAL where use of a PAL statement would convey the existence of a known level of risk. The committee recognized that considerable clinical data now exist on individual threshold doses for food-allergic consumers that could be used to estimate population thresholds or reference doses. The development of sufficiently robust analytical methods to support the risk-based approach to PAL will require more harmonization and guidance.

State, Local, and Tribal Policies for Food Establishments

An individual with a food allergy encounters many settings that offer food. Consumers with food allergies must depend upon personnel in restaurants, retail outlets, and retail foodservice establishments (e.g., ice cream parlors, bakeries, grocery stores, food carts) to obtain allergen-safe foods. The FDA Food Code is neither federal law nor federal regulation, but it is a key food policy that provides advice from FDA for uniform systems and practices that address the safety of food sold in operations such as restaurants, retail food stores, food vendors, and foodservice operations in institutions, such as schools, hospitals, assisted living, nursing homes, and child care centers. The code, which is updated and released every 4 years, is being developed by the Conference of Food Protection, a nonprofit organization created to provide a formal process to develop food safety guidance. Members of industry, academia, regulatory, and consumer and professional organizations contribute to the development of the Food Code. The 2013 FDA Food Code includes provisions on preventing food-allergic reactions. As of October 2015, all 50 states and the District of Columbia had adopted codes patterned after previous versions of the FDA Food Code, but not all have adopted the 2013 Food Code.

All state, local, and tribal governmental agencies should adopt the 2013 FDA Food Code, which includes provisions for food establishments on preventing food-allergic reactions. Working in collaboration with other stakeholders, the agencies should also propose that the next Food Code release require that the person in charge in food establishments pass an accredited food safety certification program that includes basic food allergy management to decrease or prevent the risk of food allergen exposure. In addition, agencies should develop guidance on effective approaches to inform consumers with food allergies in foodservice establishments.

The committee considered that guidance on effective approaches to inform consumers with food allergens could include menu designations of allergens, posters, and other forms of displaying information about food allergens in food establishments.

Recommendation on Training

The committee recognized that food allergens are an important public health issue that impacts the food continuum from farm to fork. Thus, awareness of and training on food allergens is essential. The committee made the following recommendation:

Food industry leaders should provide the necessary resources for integrating food allergy training (e.g., food allergen identification and preventive controls, effective risk communication with customers) into existing general food safety and customer service training for employees at all levels and stages in the food industry, as appropriate, encompassing processing, retail food and grocery stores, restaurants, and other foodservice venues.

As noted, this training recommendation impacts all sectors of the food industry from processing to various retail food outlets. The committee recognized that training does exist currently but that the use and the effectiveness of training across all sectors of the food continuum are variable and could be improved.

Other Recommendations

The report contains numerous other recommendations. While these other recommendations do not primarily impact the packaged food or foodservice industries, the implementation of these recommendations will have some effect on food industry stakeholders. One of the key recommendations in the report calls for assessment of the true prevalence of food allergies in the U.S. Currently, prevalence estimates are based upon clinical surveys of allergic individuals that rely upon self-reporting. A rigorous, clinically sound assessment of the prevalence of food allergies would help underscore the importance of this public health issue.

Another key area of emphasis within the report relates to preventing food allergies from developing. Clearly, the development of food allergies is a complex process, with many likely contributing variables. Disturbingly, the prevalence of food allergies appears to be increasing, and the factors behind the increase are not yet fully understood. However, as research points toward effective measures, the food industry will probably find opportunities to contribute to the implementation of these measures. As an example, the early introduction of peanuts into the diets of weaning infants has been shown to reduce the prevalence of peanut allergy development. The research leading to the discovery of this approach was initiated by the observation of a very low prevalence of peanut allergy in Israel, where a peanut-containing snack food (Bamba) was popularly used as a weaning food.

Thus, we encourage interested parties to read the entire report and consider all the recommendations. In summary, this landmark report provides numerous avenues for the food industry and regulatory/public health agencies to bring the recommendations to bear to improve health and safety for food-allergic consumers. ■

Disclaimer: The authors' views do not necessarily represent the views of the National Academies of Sciences, Engineering, and Medicine, their committees or convening bodies.

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By Tracie Sheehan, Ph.D., Joseph Baumert, Ph.D., and Steve Taylor, Ph.D.

Allergen Validation: Analytical Methods and Scientific Support for a Visually Clean Standard

Most food processing plants are designed to leverage the maximum number of different products on the fewest pieces of expensive equipment. One challenge for the food industry is changeovers from a product containing allergens to a similar product that does not contain allergens (or the same allergens) produced on the same equipment. Some companies employ precautionary allergen labeling such as "may contain" to all product on the same line or in the same facility; however, this may unnecessarily limit the choices of food-allergic consumers.

Furthermore, the U.S. Food and Drug Administration (FDA) has stated that precautionary labeling cannot be used as a substitute for Good Manufacturing Practices (GMPs), which implies that companies should try to clean between formulations. Other companies follow an allergen validation protocol to demonstrate an effective sanitation changeover and limit the use of precautionary statements to provide the allergic consumer with more food choices.

Historically, companies used a "visually clean standard" for inspections, with allergen checklists to assess the effectiveness of the cleanup.[1] Prior to the development of allergen test methods, companies had no data to verify whether this visual inspection system was effective or adequate to protect the health of food-allergic consumers. A consortium of major food companies sponsored research at the University of Nebraska to develop analytical methods (enzyme-linked immunosorbent assays or ELISAs) to measure allergen residues in food and on equipment. ELISA methods have now been developed for many of the common allergenic foods, and commercial kits are available on the market for most such foods. The University of Nebraska also sponsored research to measure the amount of allergenic food that would be needed to cause even a mild reaction in food-allergic consumers. Using food challenges conducted in allergy clinics has allowed the University of Nebraska to evaluate how much allergenic food can be tolerated without even a mild reaction, and the dosages have been above the limit of detection of the allergen ELISA kits. Now using these same allergen test kits, food manufacturing sanitation procedures can be validated and modified as necessary to produce a changeover product that does not need precautionary labeling. An allergen sanitation validation gives an additional assurance of safety and often supports the adequacy of using the visually clean standard to both plant personnel and allergic consumers.

A Regulatory Perspective

Currently, there is no regulatory standard for adequate food allergen sanitation globally. In 1996, FDA issued an Allergy Warning Letter[2] stating that "precautionary labeling should not be used in lieu of adherence to GMPs" and that manufacturers "take all steps necessary to eliminate cross-contamination." However, no regulatory definitions of these steps or adequate levels have been promulgated to date. The food industry can, however, see similar approaches in the drug industry's *Guide to Inspections of Validations of Cleaning Processes for Pharmaceuticals*[3] that FDA published in 1993. The Guide outlines the basics of preventative sanitation programs that a pharmaceutical facility may employ. Similar recommendations were outlined in a document produced by the University of Nebraska's Food Allergy Research & Resource Program for use by food manufacturers: *Components of an Effective Allergen Control Plan: A Framework for Food Processors*. This guide also states that "FDA does not intend to set acceptance specifications or methods for determining whether a cleaning process is validated. It is impractical for FDA to do so due to the wide variation in equipment and products used throughout the bulk and finished dosage form industries…. Some limits that have been mentioned by industry representatives in the literature or in presentations include analytical detection levels such as 10 ppm…and organoleptic levels such as no visible residue." FDA food division researchers have studied the adequacy of food allergen sanitation and stated that visual inspection and ELISA allergen kits were the most sensitive methods for detecting the presence of allergen residues compared with ATP swabs.[4,5]

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Choosing Methods

In most cases, companies should use a quantitative ELISA method to validate sanitation that is specific for the allergen to be measured. For example, if the facility is validating the absence of peanuts, it should use a quantitative peanut ELISA kit versus a total protein or an ATP swab due to the increased specificity and sensitivity of the quantitative peanut ELISA kit. Facilities should validate that the peanut can be detected in the peanut-containing food matrix or on the swab prior to use with the non-peanut product (i.e., the positive control that confirms that the ELISA kit is fit for the purpose). Some research has been done on the use of other methods such as polymerase chain reaction and liquid chromatography coupled to mass spectrometry, but these methods are certainly not suitable for routine analysis in the food industry. Thus, the preferred method for food companies remains quantitative, allergen-specific ELISAs because they are relatively simple and sufficiently sensitive to ensure that products with no detectable allergen residue by ELISA are safe for food-allergic consumers. Once the initial allergen validation is completed, the use of qualitative ELISA formats such as lateral flow strips and allergen-specific swabs may be employed in a facility as a more cost-effective method of ongoing monitoring. However, it should be noted that historical "visually clean standards" have generally been supported with these allergen test kit validations and therefore may be adequate for ongoing monitoring.

Only a few of the commercial ELISA kits have undergone the extensive AOAC validation procedures. However, in practice, these methods have been successfully applied for a variety of allergen residues in an even wider variety of food matrices. The choice of the most appropriate ELISA kit for a specific use must be carefully made and then evaluated using positive controls to ensure that the method will yield reliable results. For example, milk may be added to foods in a variety of forms, including nonfat dry milk (NFDM), casein, whey, etc. Some of the milk allergen ELISA kits measure total milk, some measure casein, and some measure ß-lactoglobulin. Thus, depending on the nature of the milk ingredient, one of these kits may be more appropriate than another. Furthermore, ELISA kits for milk can be calibrated in terms of ppm NFDM, ppm casein or ppm ß-lactoglobulin. It is possible to use a conversion factor to adjust the results obtained as ppm casein into ppm NFDM, but the conversion factors can be debatable. Another confounding factor is that milk and some other allergens may undergo significant conformational and degradation changes during cooking or fermentation to render the allergen residue less detectable by ELISA kits while still potentially causing allergic reactions. To validate this processing effect in a facility, samples should be taken before and after processing to determine the detectability in the specific food matrix.

Sampling Procedures

Never do testing until you have a plan about what to do if you encounter a positive result. Planning for allergen testing requires clear communication and coordination with senior management to hold or destroy product, pending results of the testing. Some companies employ a testing plan termed "safe mode" wherein they run the same allergen product before and after sanitation so that if the swabs indicate inadequate cleaning, they can proceed to ship and have not put the consumer at risk. They can then modify the sanitation procedures prior to the next allergen validation testing. Management should plan to run the formula with the highest percentage of allergen to effectively assess the sanitation. Consideration should also be given to the form of the allergen, as peanut butter may be cleaned differently than peanut granules. Particulate materials can present a sampling challenge in which numerous samples may need to be tested to offer assurance that some sample would contain a particle if any were present. Management should also consider the method of sample shipping, laboratory scheduling, and availability that may impact turnaround time of the results. Prior to testing, the swabs (certified allergen-free) from the kit manufacturer must be ordered and available for use (note: other swabs or sponges may

Location	Visible residue before cleaning	Tested result before cleaning	Visible residue after cleaning	Tested result after cleaning
Peanut hopper	P	7.6	-	ND
Pot	P	>25.0	-	ND
Pump	P	>25.0	P	3.2
Hoses	P	>25.0	-	ND
Cooking belt	-	ND	-	ND
Reject conveyor	P	9.0	-	ND
Vacuum belt/drum	-	ND	-	ND
2–3 sections of conveyors	P	8.4	-	ND
5–6 sections of conveyors before freezer	-	ND	-	ND
Freezer conveyor	-	ND	-	ND
3–4 sections of conveyors after freezer	-	ND	-	ND
Turrets	-	ND	-	ND
Stacker table	-	ND	-	ND
3–4 sections of conveyors to packing	-	3.1	-	ND
Packing equipment	-	ND	-	ND
Checkweigher belt	-	ND	-	ND

Table 1. Swab Results; P: visually present; ND: none detected (detection limit is 2.5 ppm); maximum is 25.0 ppm

actually contain the allergen due to recyclable materials or microbiological media in sponges). Other items to order include disposable gloves, phosphate buffer (certified allergen-free), labels for samples, and a shipping container.

Prior to the testing, identify all equipment and/or solutions contacting the allergen product. Prior to the sanitation and before discarding food contact solutions, swab each piece of equipment with a separate swab at multiple sites including crevices, as the size of the surface area is not important for qualitative testing. Recording whether there is visual allergen present on the equipment will allow comparison to test results later to support a visually clean standard. Ensure that all locations that have visual product residue are swabbed prior to sanitation, which can verify that the method can detect the allergen and this can identify equipment that may harbor more allergen. After sanitation, use separate gloves to swab each piece of equipment again and mark as "after sanitation" with the equipment name placed in a sealed plastic bag. These allergen kits are very sensitive and able to detect transfer of peanut from one sample to another, so the use of gloves and separate bags is critical. Other samples to consider include final rinse water from clean-in-place systems, utensils for ingredients or product sampling, gloves or hands from assembly operations, and internal surfaces of disassembled equipment. Ensure that all crevices and potential niches are swabbed prior to reassembly when possible. Lab analysis should begin within 48 hours of sampling. Report results as in Table 1.

> "One challenge for the food industry is changeovers from a product containing allergens to a similar product that does not contain allergens..."

There are basically three options for the next steps: 1) continue operation after sanitation with the exact same allergen product previously sampled so that positive swab results do not impact that product and plan for the next validation; 2) wait for all allergen swab sample results prior to start-up; samples taken after sanitation should be "None Detected" or "BLQ, below the limit of quantitation," prior to start-up of a nonallergen product. If the after-sanitation results are positive, immediately communicate so that the plant-intensive sanitation corrective action can take place, modify Sanitation Standard Operating Procedures (SSOPs) and retest until acceptable results are obtained or allergen-label as appropriate; or 3) if swab results after sanitation are positive at a low level, continue with product testing, focusing on the first product after start-up to assess the risk. Positive swab tests do not necessarily mean that the product will be positive, since swabs are so sensitive, the results are not quantitative, and the surface areas and contact times are variable.

After successfully completing the swab validation testing, proceed to product sampling. Manufacture the product through the same equipment that was previously swabbed and validated for food contact surfaces. Mark or indicate in some manner the first product produced through the line and sample adequately. Ship product to the lab in the temperature state as labeled (e.g., Keep Refrigerated or Frozen). Product from the entire sample lot must be held awaiting the lab results, destroyed, or labeled as containing the allergen. Successful allergen validation results should be summarized in a report including the version of SSOPs that was validated, which products and lines were validated, and the lab results. Some companies may employ a "push-through" method where the first 5 minutes (or more) of product after changeover is always discarded. However, this method should be validated sufficiently to ensure that the time or volume discarded is adequate. Quantitative ELISAs can also be used for this purpose. Use push through for 5 minutes, then 10 minutes, then 15 minutes, and sample at each time followed by testing. Once you get to the "None Detected" or "BLQ" result, then the validation of the push-through procedure is completed upon adequate documentation of this approach. Typically, dust in the air from dry products with adjacent lines does not accumulate to a significant level as to cause the nonallergen product to test positive. This may be facility dependent, however, and should be verified. Dust can be an issue when the adjacent line is idle, but swab testing will reveal if sufficient allergen residues have been deposited on the idle line to require sanitation prior to start-up.

This comprehensive sanitation validation testing can be used initially upon start-up of a new line to assess the need for labeling and the risk for the facility. Ongoing monitoring may include only visual inspection if the validation results support that approach, revalidation periodically or if the SSOP changes significantly, or routine monitoring in the case of packaging claims such as peanut-free or when consistent sanitation is especially difficult. Periodic revalidation can establish a history to confirm that SSOPs are consistently applied over time.

Consumer Protection with Thresholds

The food industry currently uses allergen ELISA kits to validate that the sanitation after allergen products is effective to eliminate the allergen from nonallergen-labeled products. In support of this approach, one can compare the detection limit for the peanut ELISA kits at 2.5 ppm (mg per kg of food) to the published threshold studies wherein the first dose of peanut to elicit a mild reaction was 0.4 mg whole peanut. Since threshold doses are expressed in amounts (i.e., mg) and ELISA kits measure concentrations (e.g., 2.5 ppm), the amount of food eaten that contains a particular concentration becomes critical to the evaluation of the risk. So if 0.4 mg whole peanut were contained in a 50-g serving of a food, it would equate to 8 ppm. Using a probabilistic risk assessment model with 450 peanut-allergic patients, the lowest elicitation dose of 0.4 mg was encountered with four of the 450 subjects.[6] Thus, a food containing 8 ppm peanut would put a rather low percentage of peanut-allergic consumers at risk of a mild reaction if they ate 50 g of the product. Of course, the risk would increase if one of these highly sensitive peanut-allergic consumers ingested an even larger amount

of that food. But if a food is tested to have less than 2.5 ppm peanut, then the food would be predicted to be safe for the vast majority of peanut-allergic individuals even with rather high consumption levels.

In summary, allergen validation studies provide a valuable tool to protect allergic consumers from reactions and to minimize unnecessary precautionary labeling statements to provide allergic consumers with more food choices. These allergen validation studies also provide the food industry and regulatory bodies with scientific support for the visually clean standard. ■

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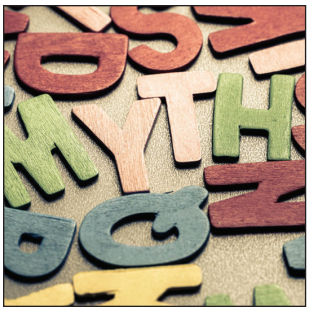
References

1. Deibel, K, et al. 1997. "A Comprehensive Approach to Reducing the Risk of Allergens in Foods." *J Food Prot* 60(4):436–441.
2. FDA. 1996. Allergy Warning Letter. NOTICE TO MANUFACTURERS Label Declaration of Allergenic Substances in Foods.
3. FDA. 1993. *Guide to Inspections of Validation of Cleaning Processes for Pharmaceuticals*. Division of Investigations, Office of Regional Operations, Office of Regulatory Affairs.
4. www.charm.com/resource/file/186.
5. Jackson, LS, et al. 2008. "Cleaning and Other Control and Validation Strategies to Prevent Allergen Cross-Contact in Food-Processing Operations." *J Food Prot* 71(2):445–458.
6. Taylor, SL, et al. 2010. "Threshold Dose for Peanut: Risk Characterization Based upon Diagnostic Oral Challenges of a Series of 286 Peanut-Allergic Individuals." *Food Chem Toxicol* 48:814–819.

By Jasmin Kraus

Six Common Myths about Food Allergen Testing



Ask anyone with a food allergy and they will tell you the same thing: There's not much that's simple about a quick trip to the grocery store. They have to check every label on every product that goes into the basket to make sure that their food is free from allergens. Because there is no treatment for food allergies, there's only one thing that works: completely avoiding the allergen or allergens in question.

This makes it all the more crucial for food producers to conduct routine tests for potential allergen contamination in their products.

Yet this isn't as simple as it sounds.

Food products can range widely from straight raw materials, such as cereals, to highly processed, ready-to-eat products. Their composition, moreover, varies according to the amount of protein, fat, salt, and other compounds present. Test methods are expected to analyze all food sample types for allergens with equally reliable results. This, however, is often far from achievable in reality.

With all the complexity surrounding food allergen testing, perhaps it's not surprising that there are a lot of half-truths and myths out there. Here, the allergen experts at Romer Labs dispel six of the most common misconceptions about food allergen testing.

Myth #1: A test kit off the shelf works with any food matrix.

The facts: Take a test kit from the shelf and start testing. Sounds tempting, doesn't it? The results would be quick but are they reliable? In reality, food products are highly diverse: Certain test methods may work better for certain food samples. The extent of processing adds further complexity to this equation.

With new or unfamiliar matrices, we always undertake a spike recovery validation at three different levels to make sure the matrix works with our kits and covers the detection range of the assay. Some matrices, such as chocolate, are full of tannins and other polyphenols that bind to allergenic proteins, creating insoluble complexes from which it is difficult to extract without adding extra protein to the extraction buffer.

While implementing an allergen control plan, it is highly recommended that the selected allergen test method be fully validated for the food producer's specific food matrices.

Learn more about test kits and food matrices here.

Myth #2: "May contain…" statements can solve all our problems.

The facts: Food allergen labeling–though intended to make the lives of people with allergies easier and safer–often causes confusion, as most laws fail to state the levels above which an allergen must be labeled. Advisory "may contain…" statements are voluntary and often serve primarily to prevent the producer from having to make potential allergen-related product recalls.

Studies have shown that up to 9 percent of products with advisory labels in fact contain detectable levels of allergens. This means that there is a real risk of allergen contamination in products that only make a precautionary statement. As there are varying reasons why manufacturers include such statements, consumers find it increasingly difficult to interpret them.

Consumers with allergies should avoid products with precautionary labels, as the risk is not assessable. In return, food producers should avoid using a "may contain…" statement without reasonable suspicion.

Learn more about "may contain…" labels here.

Myth #3: PCR is more reliable than immunological tests.

The facts: It depends. Polymerase chain reaction (PCR) assays are extremely sensitive and make sense when specificity is called for. For example, no antibodies have been developed that can reliably detect celery without also giving a signal for related species, such as fennel, carrot, or parsley. Hence, celery detection with an immunological test is not currently possible.
How can specific species be detected with PCR? It relies on DNA extraction and amplification, which is made possible by the

nature of DNA: It is a stable molecule that remains unaffected by most common food processing methods. Yet PCR has significant drawbacks: It requires specially trained personnel to perform the complex sample preparation and result interpretation. Furthermore, the DNA molecule itself is not responsible for the allergic reaction, meaning that the presence of DNA is, at best, an indicator of the allergenic potential of the sample.

Rapid immunological tests are still the gold standard and should be preferred in most cases, as they directly detect food allergens. However, when specificity is called for, PCR may be a great alternative.

Learn more about PCR in allergen testing here.

Myth #4: Mass spectrometry will soon replace rapid allergen tests.

The facts: Mass spectrometry (MS) is a high-end technology that is already used in several fields for routine analysis and shows some potential in allergen analysis: It can measure several allergens in parallel. However, it is still in its infancy and is currently restricted to research applications. As a result, it's not clear how MS will perform in routine analysis.

Additionally, MS is not yet able to deliver the highest level of accuracy. Its basic principle is one of fragmentation: A molecule—in this case the allergenic protein—is broken down into small pieces (peptides), and their masses are subsequently determined. However, food processing can affect the fragmentation process of proteins, resulting in varying peptide patterns.

Without a doubt, MS technology will continue to develop and improve in the future. Yet, since it relies on highly trained personnel and expensive equipment, there will still be demand for fast and inexpensive in-house testing, making it rather unlikely that rapid tests such as enzyme-linked immunosorbent assays will be replaced.

Learn more about MS in allergen testing here.

Myth #5: All test kits on the market are the same.

The facts: Commercially available test kits do not perform in the same manner. For each food allergen, there is a variety of different allergenic proteins, but there is no recognized standard defining which of them must be detected. Therefore, we cannot assume that all test kits detect the same allergens and consequently give comparable results.

> "While implementing an allergen control plan, it is highly recommended that the selected allergen test method be fully validated for the food producer's specific food matrices."

Kits do have one thing in common: the overall target (e.g., peanut or casein). But the similarities end there. Different kits use different buffers and procedures, which can have an impact on the extraction process and generate diverging patterns. Furthermore, kits differ in the antibodies used, which, in an added layer of complexity, need to take the various methods of food processing into account.

1So what should you do? A close discussion with the kit manufacturer is highly recommended, as they can provide information about the test kit's performance specifications. Also, analysts should carefully review and summarize all the processing steps that are applied to a food product to assess which kit is most suitable for their individual application.

Learn more about how test kits differ here.

Myth #6: Currently available "allergen reference materials" improve testing reliability.

The facts: It may be a controversial assertion, but it's the truth: There are no allergen reference materials, despite claims by some producers. In other fields of food safety, producing reference materials requires high-end technologies, but the procedures for doing so are well-established. If we take mycotoxins, for example, we have one defined molecule, allowing accurate calculations of final concentrations.

In contrast, with food allergens, there is not just one specific molecule; an allergenic commodity consists of a mixture of different proteins. To date, several allergenic proteins have been identified, but many have not yet been well characterized. Furthermore, the protein pattern varies between different cultivars of the same species. And to make matters worse, proteins can change their conformations as a result of processing, which may lead to a change in their allergenic potential.

So what are these so-called "allergen reference materials"? Typically, they are mixtures of allergenic food commodities in certain matrices. Such mixtures do have their uses in checking regular test performance, provided that they are used with care and in consideration of all known limitations. Materials that are produced in house using the matrix in question result in even more significant evaluations of test performance and represent the best possible alternative we currently have until standardization bodies define specifications for actual reference materials.

Learn more about "allergen reference materials" here. ■

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By Monique Bremer, Ph.D.

Selecting a Suitable Food Allergen Detection Method

Undeclared traces of allergenic substances in food may cause problems for allergic individuals as they are inadvertently exposed to the offending substance. To improve consumer safety, labeling of the eight major allergens became mandatory within the U.S. in 2006. These so-called "Big Eight" include tree nuts, peanuts, soy, eggs, milk, fish, wheat, and shellfish.

To comply with allergen labeling laws and to protect their own reputation and business, food producers need analytical methods to monitor the presence of allergens during production and to avoid cross-contamination in production lines. How can food producers effectively select and implement a detection method from the range of methods available? In this article, the most widely used methods available at present and upcoming ones are described, and the pros and cons of the various methods are analyzed to facilitate the selection procedure.

Immunological Screening Methods

Immunological methods are most widely used to detect allergenic products at trace levels (i.e., a low mg/kg range) in foods. These methods are based on the binding of an allergenic protein by specific antibodies. Immunological methods are available in different formats, with the more conventional formats being strip tests and enzyme-linked immunosorbent assays (ELISAs). Over the past few decades, many ELISAs and strip tests for the detection of different allergens have been developed and have become commercially available. More recently, research has focused on multiple-allergen detection (i.e., the development of methods in which several allergens or allergenic compounds can be detected simultaneously). These assays are usually developed on biosensors and microsphere-based flow cytometric systems. It is anticipated that within the next few years, these assays will also become commercially available. With the introduction of these types of assays, routine screening of products for the presence of the "Big Eight" will become possible.

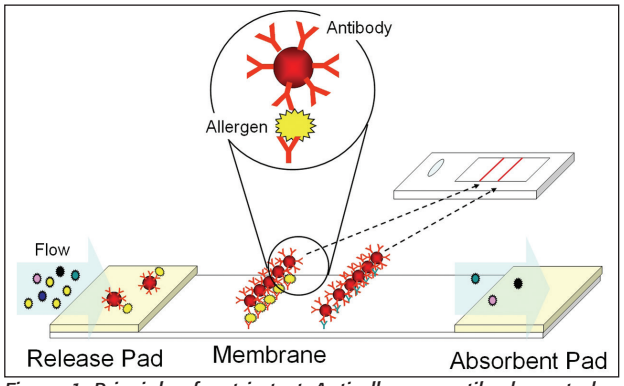

Figure 1: Principle of a strip test: Anti-allergen antibody-coated colored beads form a complex with allergens in the sample and anti-allergen antibodies on the strip. This leads to a colored test line indicating a positive (i.e., allergen-containing) sample. A colored control band indicates correct performance of the test.

Strip tests

Strip tests (Figure 1) are based on the formation of complexes between anti-allergen antibody-coated colored beads with allergenic proteins in the sample and anti-allergen antibodies on the test strip. These complexes give rise to a colored test line on the strip, indicating a positive (i.e., allergen-containing) sample. In a similar way, a colored control band is formed, indicating that the test has been carried out correctly.

Strip tests are very easy to use, inexpensive, rapid (analysis time of a few minutes), do not require instrumentation, and can therefore be used in the field. Today, most available strip tests are only qualitative; however, it is anticipated that in the near future, more and more suppliers will deliver simple handheld readers with which semi-quantitative results can be obtained. With strip tests, only single samples can be analyzed for the detection of a single allergen at one time.

ELISAs

ELISAs are carried out in multiple-well strips or 96-well microtiter plates. The proteins of the allergenic compound are detected by a specific enzyme-labeled antibody and visualized by an enzymatic reaction that leads to the formation of a colored product (Figure 2). The color is read in a microplate-compatible spectrophotometer. The concentration of the

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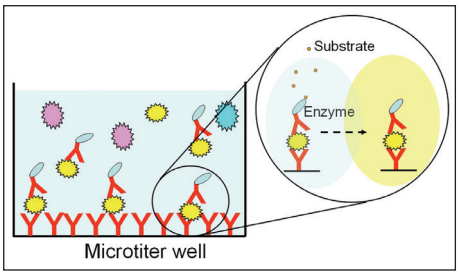

Figure 2: Principle of an ELISA: Allergens are detected by a specific enzyme-labeled antibody and a specific capture antibody on the wall of a microtiter well. After conversion of a substrate by the enzyme, a colored product is formed. The color is read in a microplate-compatible spectrophotometer.

allergenic compound in the sample can be determined from a calibration curve constructed by analyzing standards. To carry out an ELISA, trained laboratory personnel, standard laboratory equipment, and a microtiter plate spectrophotometer are necessary. Using an ELISA, more samples (i.e., 48 or 96 including standards) can be analyzed simultaneously for a single allergen. The analysis time ranges from 30 minutes for fast ELISAs to 3 hours for standard ELISAs. At present, ELISAs are the most widely applied methods for detecting allergens by food processors and food control authorities.

Biosensors

In food analysis, biosensors and, in particular, surface plasmon resonance (SPR)-based biosensors have become increasingly accepted tools. SPR detection is based on changes in the refractive index at the surface of a sensor chip, caused by the binding of an analyte to an immobilized ligand. For detection of high molecular-weight compounds such as allergens, specific antibodies are usually immobilized on the chip surface (Figure 3). The binding of allergens in the sample is followed in real time, and from the change in the signal, the concentration in the sample can be calculated from a calibration curve.

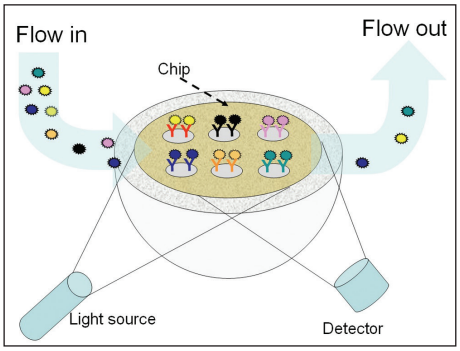

Figure 3: Principle of a surface plasmon resonance biosensor immunoassay: Binding of allergens to different anti-allergen antibodies on the sensor chip leads to changes in the refractive index at the antibody spots on the surface.

The majority of new biosensors are aimed at high-throughput and multi-analyte measurements. The major advantages of these systems are their short assay time (minutes), their high degree of automation that reduces labor time, the option to simultaneously detect several analytes, and label-free detection. A major disadvantage of the majority of these systems is the relatively high price of both the machines and chips. Furthermore, only a single sample can be tested at one time, and trained laboratory personnel are needed. A few biosensor immunoassays for the detection of allergens have been developed by research groups and are described in the literature. It is expected that within the next few years, allergen test kits will become commercially available and will come to be applied in food control agencies.

Microsphere-based flow cytometric systems

These assays are based on the flow cytometry detection of sets of differently colored micron-size beads (Figure 4). To each color-coded set of beads, antibodies against different allergenic compounds can be coupled. Specific, fluorescently labeled, second antibodies are used to visualize the binding of allergens to the beads. For analysis, different bead sets (to detect different allergens) are added simultaneously to a sample in a microtiter well. The beads are drawn up into a fluidic tube that causes the microspheres to line up in single file before they pass through the detection chamber. In this chamber, one laser identifies each bead and categorizes it into the appropriate bead set (based on which allergen is detected), while another laser scans the beads for the quantity of fluorescently labeled antibodies per bead (and determines the concentration of the detected allergen). In this way, multiple allergens can be detected simultaneously in a sample. The advantages

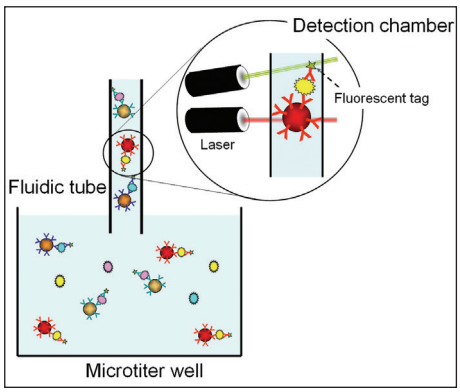

Figure 4: Principle of a microsphere-based flow cytometric assay: Differently colored beads are coated with antibodies against different allergens. Fluorescently labeled second antibodies are used to visualize binding of allergens to the beads. Multiple allergens can be detected simultaneously.

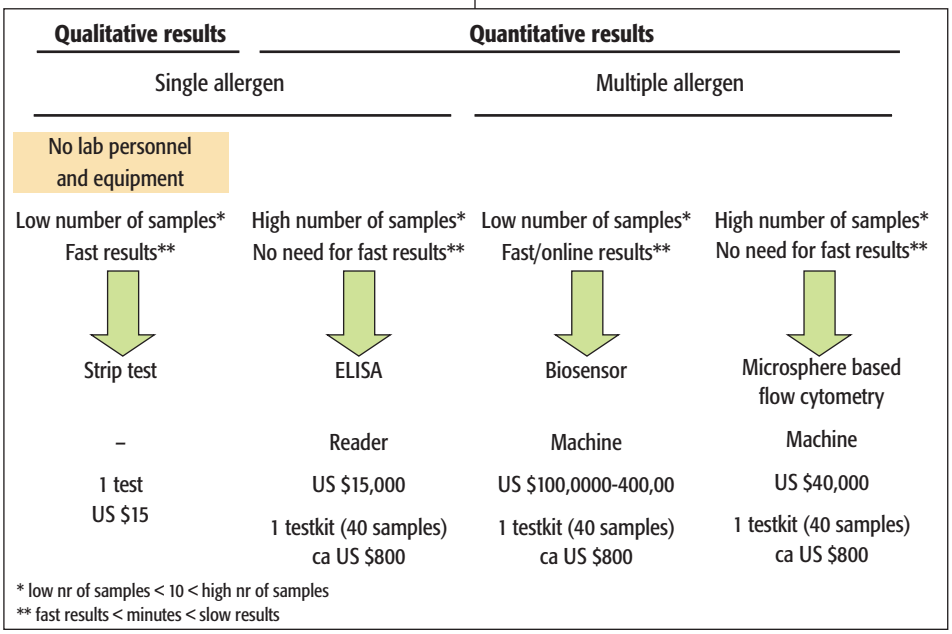

Figure 5. Scheme for Selecting a Suitable Immunoassay

of these types of assays are their very short analysis time (seconds), simultaneous detection of multiple allergens, small sample volume, and relatively low-priced machines (compared with biosensors). However, labor times are comparable to those of ELISAs.

Selecting a Detection Method

When selecting a suitable detection method, the following criteria should be considered: availability of laboratory personnel and standard laboratory equipment, number of samples, number of allergens to be detected, frequency of testing, need for short analysis time, need for quantitative results, ease of use, degree of automation, and the resulting costs. In Figure 5, a simplified selection scheme is presented.

> "The choice for a particular method should be based on the specific circumstances at the testing location..."

When a company doesn't have trained lab personnel or equipment, the only tests that employees can perform by themselves are strip tests. When a high number of samples are required to be analyzed for only a single allergen, ELISA is the best option as the necessary equipment is relatively inexpensive. When multiple allergens need to be quantitatively analyzed in a few samples and when the results need to be obtained within an hour (e.g., before accepting incoming materials at receiving docks), biosensors are the method of choice. When a qualitative result is sufficient (i.e., "present at concentrations above the detection limit"), strip tests are an inexpensive and rapid alternative (no expensive machines needed). When there is no need for an especially short analysis time, microsphere-based methods are a better choice as the price of the necessary equipment is generally much lower than that of biosensors.

Before an immunological assay can be implemented, it is necessary to check whether the method is suitable for the matrix (or matrices) in which it will be used. This is important as the applied antibodies may cross-react with food-matrix components, leading to false-positive test results. Furthermore, food processing may affect the detection of allergenic proteins by antibodies as the structure and integrity of the target proteins and the recognition/binding by the antibody may be altered due to processing. In general, the kit supplier can provide validation data and is generally willing to validate the method in the required matrices or after processing. Otherwise, in-house validation for specificity and recovery is necessary. Samples suspected to contain allergens can be confirmed by using a mass spectrometry (MS) method. Using MS, parts of the unique amino acid sequence of a protein can be determined, and the protein can unambiguously be identified. MS, however, is labor-intensive, requires expensive equipment and materials, and is not suitable for routine analysis.

In summary, there are several different immunological screening methods available for allergen testing, and each has its advantages and disadvantages. The choice for a particular method should be based on the specific circumstances at the testing location (e.g., number of samples, number of allergens to be tested, and required analysis time). The simple selection scheme presented can be used as a guide to select a suitable method. It is important that before implementation, the method is tested in the appropriate matrices after the applied production processes. ■

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FINDING THE RIGHT FOOD ALLERGEN TESTING LAB IN 9 QUESTIONS

HOW DO YOU KNOW WHETHER A 3RD-PARTY LAB IS THE RIGHT ONE FOR YOUR FOOD ALLERGEN TESTING NEEDS?
LIKE EVERY NEW RELATIONSHIP, YOU'LL HAVE A FEW QUESTIONS FOR THEM, AND THEY'LL HAVE A FEW QUESTIONS FOR YOU.
SO WHAT QUESTIONS SHOULD YOU EXPECT FROM A QUALIFIED LAB? WHAT SHOULD YOU ASK THEM?

QUESTIONS FROM THE LAB

1 WHAT IS THE PURPOSE OF THE ANALYSIS YOU NEED?

FOR MANY LABS, THIS IS ANOTHER WAY OF ASKING WHETHER YOU NEED QUALITATIVE OR QUANTITATIVE TESTING. ARE YOU VERIFYING THE ALLERGEN PROFILE OF A SAMPLE, MONITORING THE EFFECTS OF A CHANGE IN THE RECIPE OR CONFIRMING THE RESULT OF AN ON-SITE METHOD? ALL OF THEM REQUIRE DIFFERENT METHODS OR LEVELS OF SENSITIVITY.

2 WHAT TYPE OF SAMPLE WILL YOU BE SENDING US?

ARE YOU TESTING A SWAB OR RINSE WATER? RAW MATERIAL OR FINISHED PRODUCT? ONCE AGAIN, THE SUITABILITY OF A METHOD, ITS PERFORMANCE AND EVEN THE SENSITIVITY WILL DIFFER DEPENDING ON THE MATRIX. EXPERIENCED LABS CAN ANTICIPATE PROBLEMS AND HELP YOU COLLECT AND PREPARE SAMPLES PROPERLY.

3 WHAT SENSITIVITY DO YOU NEED?

MAKE SURE THAT THE LABORATORY CAN PROVIDE AN ASSAY WITH THE NECESSARY SENSITIVITY. THIS COULD BE TRICKY IF YOU USE THRESHOLD GUIDELINES: SOME GUIDELINES HAVE THRESHOLDS SO LOW THAT THEY MIGHT NOT BE AVAILABLE GIVEN THE PORTION SIZE OF YOUR PRODUCT.

4 WHAT MATRIX ARE YOU TESTING?

MATRICES CAN BE TRICKY: THEY CAN HAVE SUBSTANCES THAT INTERFERE WITH THE EXTRACTION OR DETECTION OF THE TARGET MOLECULE. THE PHYSICAL PROPERTIES OF MATRICES CAN ALSO MAKE THE EXTRACTION PROCESS INEFFICIENT. THIS IS ONE OF THOSE QUESTIONS YOU CAN'T ANSWER IN ENOUGH DETAIL, SO DON'T BE AFRAID TO TELL THE LAB TOO MUCH!

QUESTIONS TO THE LAB

1 WHAT ACCREDITATIONS DOES YOUR LAB HAVE?

ANYONE CAN OPEN A LAB, BUT ONLY A FEW HAVE THE COMPETENCE TO GET AN ISO CERTIFICATION OR RELEVANT NATIONAL CERTIFICATIONS. LOOK FOR A LAB WITH ISO 17025 ACCREDITATIONS AND MAKE SURE THE CERTIFYING BODIES ARE ON THE LEVEL. IN THE UK, MAKE SURE A LAB IS UKAS-ACCREDITED BEFORE DOING BUSINESS WITH THEM!

2 CAN YOU PROVIDE ME WITH THE UNITS I NEED?

20 PPM PEANUT IS NOT THE SAME AS 20 PPM PEANUT PROTEIN. MAKE SURE YOUR LAB CAN PROVIDE YOU WITH THE UNITS YOU NEED OR THE APPROPRIATE CONVERSION FACTOR. THIS CAN BE PARTICULARLY TRICKY FOR FISH, MOLLUSCS AND CRUSTACEANS. THE AMOUNT OF PROTEIN, PROPORTION OF TARGET PROTEINS AND HOMOLOGY BETWEEN THEM WILL DIFFER FROM SPECIES TO SPECIES!

3 IS YOUR LAB'S METHOD VALIDATED FOR MY MATRIX?

THE MATRIX MIGHT NOT ONLY COMPLICATE THE EXTRACTION PROCESS, BUT ALSO INTERFERE WITH THE DETECTION SO THAT THE LOD AND LOQ ARE DIFFERENT FROM THOSE STATED AS THE MINIMUM PERFORMANCE CRITERIA. MAKE SURE THAT THE LAB HAS VALIDATED YOUR TYPE OF MATRIX BEFORE SUBMITTING A SAMPLE.

4 WHAT ANALYTES DO YOUR METHODS ACTUALLY DETECT?

TAKE THE EXAMPLE OF MILK. EVEN IF THE RESULTS OF THE LAB'S METHOD ARE EXPRESSED IN PPM MILK, THE REAL ANALYTE OF THE METHOD IS LIKELY TO BE A SURROGATE, SUCH AS CASEIN. IF THE FOOD COMPONENT IN YOUR SAMPLE DOESN'T MATCH THE ACTUAL METHOD ANALYTE, YOU MIGHT WIND UP WITH FALSE NEGATIVES!

5 HOW DOES YOUR LAB GO ABOUT RESULT CONFIRMATION?

INSIST ON PRECISE INFORMATION ABOUT HOW THE LAB CONFIRMS ITS RESULTS. ASSAYS WITH POSITIVE AND NEGATIVE CONTROLS ARE THE MINIMUM. IT'S EVEN BETTER IF THE LAB USES ADDITIONAL, ALTERNATIVE METHODS OF ANALYSIS TO CONFIRM THEIR RESULTS.

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By Adrian Rogers

Challenges in Allergen Testing: Spiking and Recoveries

When I started developing immunoassays for the detection of allergens in food, the first thing that struck me was the wide range of different food types or matrices that the assays had to work with. Coming from a medical immunoassay background, there was a limited number of different matrices to work with. In my case, this was blood serum. With food, there is an almost infinite range of different sample types, each with their own specific properties.

How Do I Choose the Right Test Kit?

So how do we ensure that the test kit produced is suitable for use with such a diverse and challenging range of samples? This is where sample validation comes in. The process involves adding a known amount of an allergen of interest to our matrix (spike) and then trying to get that allergen back out again (recovery).

An important thing to remember is that, as the name implies, immunoassays use biological components (antibodies) to achieve the detection of the allergenic proteins of interest. As with all biological systems, the kits are sensitive to extremes.

In the case of foods, the kits may not work as they should in the presence of strong acid or alkali, high salt, high fat, etc. Many of these extremes can be countered during the extraction process: Kits may use a buffer system to cope with changes in pH and the addition of the buffer to the sample helps reduce and dilute some of the other problematic components such as salt and fat.

Is My Recovery Acceptable?

When it comes to the recovery of a known amount of allergen from a sample matrix, what is deemed acceptable? Before answering this, we need to define where we are starting from. Is it an incurred sample or a spiked one?

Incurred samples are defined as samples in which a known amount of the food allergen has been incorporated during processing, mimicking as closely as possible the actual conditions under which the sample matrix would normally be manufactured.

Here, I will concentrate on outlining the more accessible method of spiking a known amount of allergen into a matrix as received from the supplier or manufacturer, and measuring its recovery.

With regard to recovery, the Association of Analytical Communities (AOAC) guidance states that:

"Ideal percent recovery levels would range from 80 to 120 percent. Recovery levels are affected by both the efficiency of the extraction step and the enzyme linked immunosorbent assay (ELISA) procedure.

"With ELISA methods for food allergens, this level of recovery is not always possible, particularly when certain difficult matrixes are analyzed. In addition, the recovery from incurred samples can be substantially different from those obtained using spiked samples.

"For this reason, recoveries between 50 and 150 percent will be considered acceptable so long as they can be shown to be consistent."

The guidelines were published in 2010 by AOAC with particular reference to quantitative ELISA methods. Many of the key points are also applicable to qualitative or semi-quantitative lateral flow device (LFD) methods.

The Science behind Spiking

When we receive or encounter a new food type that has not been tested before, we will undertake spike recovery validation to ensure it works as it should with our test kits. We will spike in at three different levels of allergen–low, medium, and high–to cover the range of detection of the assay.

The low allergen spike will be close to the lower limit of quantitation of the ELISA (in this case, the lowest value calibrator above 0 ppm) or close to the limit of detection of an LFD. The medium spike will be in the middle of the ELISA calibration curve, and the high spike will be at or near the upper limit of quantitation (the highest ppm value calibrator). The sample is extracted and tested in accordance with the product insert supplied with the kit.

So, for example, if we spike 5 ppm of almond into chocolate, we would expect to see a recovery of 4 to 6 ppm. If the result

is outside of this range, then there are steps that can be taken to help improve the recovery. From experience, chocolate is one of the most challenging food matrices to test–it is full of tannins and other polyphenols that can bind to any allergenic protein that may be present and form insoluble complexes which are difficult to extract.

Such difficulties can be overcome by adding extra protein to the extraction buffer. The excess protein binds to the polyphenols and makes the allergens available for extraction. My protein of choice is fish gelatine, although other material such as milk powder can be used to improve the extraction efficiency from high polyphenol-containing foods. If using milk powder, be careful not to contaminate your laboratory space, especially if you are carrying out milk allergen testing.

LFDs, or strips or dipsticks as they are sometimes referred to, can be validated for spike recovery in a similar way to an allergen ELISA test kit. The thing to be aware of when choosing a high spike level is that although LFDs are capable of detecting very high ppm levels, you can actually overload the device by adding too much allergen. This can occur in amounts greater than 1 percent of the allergenic food.

Maintaining Quality and Test Precision

It may be necessary for a kit manufacturer to work closely with customers who routinely test challenging food matrixes. It is important to verify that the kit is working as it should and to the customer's satisfaction. This can be achieved, as detailed above, by undertaking allergen spike recovery experiments into the problematic matrix.

In some cases, it may be desirable to modify or change the standard kit method to meet the demands of the sample and/or the customer; this should always be undertaken with the guidance of the kit manufacture to ensure the quality and reproducibility of the test kit. ■

***Adrian Rogers** has been with Romer Labs for 9 years in his role as a Senior Research Scientist. He is responsible for research and development within the Romer Labs allergen competence centre based in the UK.*

Before joining Romer Labs, Adrian was an R&D Scientist involved in the development of ELISA and lateral flow immunoassays for the detection of food allergens.

Adrian is a microbiologist by training and has 15 years' experience in the development of immunoassays, 13 years of which have been spent developing test kits for the detection of food allergens.

Over the years Adrian has been involved in a number of food allergy projects including EuroPrevall, an EU funded multidisciplinary integrated project which investigated the prevalence of food allergy across Europe. He is currently a member of the University of Manchester's Food and Health Network allergy cluster and co-ordinates Romer Labs' contribution to the "Innovate UK Knowledge Transfer Project", with the University of Manchester looking at improving soya allergen analysis.

By Steven M. Gendel, Ph.D., Jianmei Zhu, Ph.D., Nichole Nolan, M.P.H., and Kathy Gombas

Learning from FDA Food Allergen Recalls and Reportable Foods

Seven years after the Food Allergen Labeling and Consumer Protection Act[1] (FALCPA) went into effect, unlabeled allergens continue to be the leading cause of recalls and a leading cause of reportable foods for U.S. Food and Drug Administration (FDA)-regulated foods. The presence of unlabeled allergens presents a significant health hazard for food-allergic consumers, and allergen recalls represent an economic burden for industry and a resource need for FDA.

Allergic consumers rely on food labels to be complete, clear, and accurate so that they can avoid exposure to foods or ingredients that can provoke potentially life-threatening reactions. This is particularly important because data from the U.S. Centers for Disease Control and Prevention (CDC) show that the number of food-allergic consumers is increasing as are the number of hospital visits related to food allergies and allergic reactions.[2] To help food-allergic consumers find and understand the information they need, FALCPA designated those food allergens of greatest public health concern in the U.S. as the major food allergens (milk, egg, peanut, soy, fish, crustacean shellfish, wheat, and tree nuts), described the two formats that can be used to declare the presence of major food allergens and required the use of the common or usual name of the food source for the major food allergens (e.g., declaring milk when casein is used as an ingredient). FALCPA also requires the declaration of major food allergens that are components of flavorings, colorings, and incidental additives.

Before FDA and the food industry can develop practical approaches to reducing the number of food allergen recalls, it is important to understand the nature of the problems that lead to these recalls, the foods that are most often affected, and the allergens that are most often involved. For FDA-regulated products, this information can be found in the FDA Reportable Food Registry (RFR) and the FDA Recall Enterprise System (RES) databases. A closer look at the allergen-related entries in these databases shows that there are clear patterns and trends, and suggests that the number of food allergen recalls can be significantly reduced through improved awareness and simple changes in the way that packages, labels, and ingredients are handled and tracked within production facilities.

Reportable Foods and Allergen-Related Recalls: The Numbers

The RFR collects mandatory reports from industry and voluntary reports from public health officials related to foods that represent serious health risks. These risks include the presence of undeclared allergens, microbial pathogens, foreign objects, and other hazards. Over the RFR's first 3 years (September 2009 to September 2012), about 90 percent of all reports involved three hazards: *Salmonella*, *Listeria monocytogenes*, and undeclared allergens, with undeclared allergens accounting for essentially the same number of reports as *Salmonella* (34 percent and 36 percent of all reports, respectively) (Figure 1). During these 3 years, the proportion of reports for undeclared allergens increased from 30 percent of all reports in the first year to 40 percent of all reports in the third year.

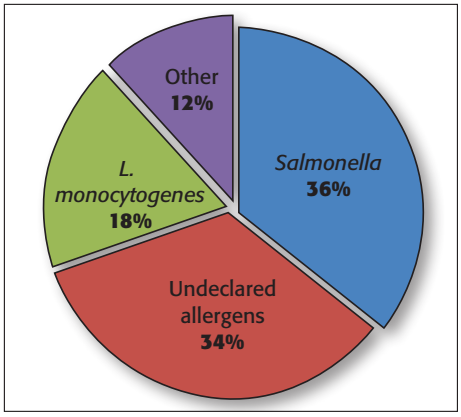

Figure 1: Distribution of Primary RFR Reports, 2009–2012

Reportable foods are foods that pose a risk of serious adverse health consequences or death to consumers. This standard is the same as that for FDA Class I recalls, so the number of primary RFR reports for unlabeled allergens is similar to the number of Class I allergen recalls. In addition to these recalls, there are a large number of Class II allergen-related recalls. Allergen recalls are considered to be Class II when the only allergen involved is wheat or when other mitigating circumstances reduce consumer risk. For example, a food label that declares the presence of one tree nut on a food that contains a different tree nut is typically considered to be a Class II hazard because most tree-nut-allergic consumers avoid all tree nut-containing products. In addition,

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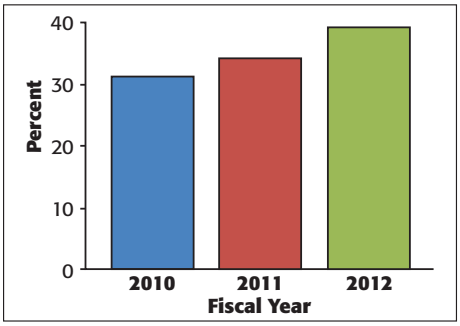

Figure 2: Percent of All Recalls Caused by Undeclared Allergens

labels that declare the presence of an allergen-containing ingredient (such as butter) without declaring the allergen (milk) by using the common name of the food are often classified as Class II recalls when the ingredient is commonly known to contain the allergen. Allergen recalls increased steadily over this 3-year period (Figure 2).

Food Allergen Recalls: Beyond the Numbers

To dig into the details behind the numbers and to better understand what problems were leading to these recalls, the information for each food allergen recall contained in the FDA RES database was reviewed. RES contains information on all recalls of FDA-regulated products entered by agency recall coordinators and field investigators. Each entry includes a description of the product(s) involved, the nature of the problem that led to the recall, and may contain additional information on the root cause. The RES entry for each primary food allergen recall that occurred during the same 3-year period was examined to identify the food involved, the unlabeled allergen(s), and the root cause (when this information was available). The foods involved were classified using the same system used for the RFR database. (A detailed description of these commodity definitions can be found on the FDA website.[3])

The five food types that were most often involved in food allergen recalls during this period were bakery products, snack foods, candy, dairy products, and dressings (Table 1). Bakery products accounted for almost as many food allergen recalls as all of the other top five foods combined. The proportion of Class I recalls ranged from 63 percent for candy to 58 percent for dairy.

Food Class	Number of Recalls	% Class I
Bakery	153	62
Snack	62	62
Candy	45	63
Dairy	39	58
Dressing	38	59

Table 1: Foods Most Often Involved in Allergen Recalls

The allergens most often involved in recalls were milk, wheat, and soy (Table 2). There were fewer recalls involving peanuts and tree nuts combined than for any of these top three allergens. Just over 20 percent of the recalls involved mislabeling for multiple allergens. Many of the recalls that involved more than one allergen combined milk, wheat, soy, and egg. This may reflect the many different ways that these foods are used and the variety of different ingredients that are derived from each of them.

Allergen	Number of Recalls*
Milk	174
Wheat	130
Soy	118

**Some of the recalls involved multiple allergens*

Table 2: Food Allergens Most Often Involved in Recalls

Only about 20 percent of the recalls for soy involved soy lecithin; however, it was not possible to tell whether these involved the use of lecithin as a release agent or as an emulsifier.

Because of the variety of foods involved in allergen recalls during this period, it was not possible to carry out a detailed analysis of the most common allergen/food combinations. However, information in the RFR database showed that within the bakery products category, cookies, and cakes were the most often reported food types. Within the candy products category, a large number of reports were caused by the presence of undeclared milk in products containing dark chocolate. Among the snack foods, there were several recalls for chocolate-coated snack bar products that carried "dairy-free" or "vegan" labels. In many cases, these snack bar products were made on shared equipment that was also used to manufacture products with milk chocolate. The levels of milk found in some of the vegan products represented a significant risk for milk-allergic consumers, particularly if the consumer assumes these products are completely dairy-free.

Looking at Root Causes

Understanding why food allergen recalls occur is critical for finding ways to reduce the number of these recalls and, therefore, the risk to allergic consumers. Root cause information was available in the RES database for about 67 percent of the allergen recalls. Overall, 13 distinct root causes were identified. Of these, use of the wrong package or the incorrect label for a product was the most common problem (Table 3). Although there were a variety of reasons why manufacturers used the wrong package or applied the incorrect label, one frequent problem was that packages for similar products made with different allergens, or products with and without allergens, looked very similar. In many cases, it was difficult for workers to distinguish packages or labels when they were handled in bulk. When packages look similar, it is easy for a worker to grab the wrong package or roll of film, especially in the middle of a production run. The use of similar-looking packages also makes it difficult to maintain accurate and detailed inventory records that might be used to recognize that mislabeling has occurred. Problems related to failure to remove unused packages or labels after a production run or during product changeover were also common.

Cause	Number of Recalls
Wrong package or label	82
Terminology	59
Failure to carry forward information from an ingredient to final label	41
Cross-contact	28
Ingredient mislabeled from supplier	21

Table 3: Causes of Food Allergen Recalls

The second most frequent cause of allergen recalls was the use of the wrong terminology in the ingredient list or the allergen "contains" statement. For example, a product might declare the presence of butter but not milk, the presence of tree nuts but not the specific type of nut, or the presence of flour but not wheat. It is not clear why there are so many terminology problems or why the number of these recalls is not decreasing over time. About one-half of the recalls caused by this problem were for bakery products.

The third most common cause of al-

lergen recalls was failure to carry forward allergen information from an ingredient to the final product label. In some cases, the failure to carry forward allergen information resulted from changes in an ingredient formulation by a supplier; in others, it was caused by changing ingredient suppliers to one with a different formulation. In other cases, allergen information was difficult to find on the ingredient labels, because it was not declared in a standard format or location on containers for bulk ingredients. Frequently, the certificate of analysis or product specification sheet for a bulk ingredient was sent to a customer separately from the actual lot of the ingredient, and the allergen information in these documents was not reconciled with the expected allergen content for the ingredient. These situations show that it is important for an ingredient user to develop procedures to recognize and respond to changes in ingredient formulations.

Emerging Issues Indicated Through RFR Reporting

Several trends seen in food allergen-related RFR reports may be signals of emerging issues that will become common in the next few years. One of these is an increase in labeling errors caused by the use of incorrect, outdated or damaged data files in computerized on-site label printing systems. The ability to print labels during manufacture or directly on packaging provides tremendous flexibility and cost savings for producers, but also creates new opportunities for errors and omissions unless controls are in place to ensure that the correct data or template files are used and that these files have not been corrupted. For example, several recalls have been caused by an employee clicking on an old (outdated and incorrect) version of a label file when loading data into a printer system. In several other cases, a label file generated at a central corporate location was used at a remote production site without checking to see if the ingredients actually being used matched those on the label.

Reports of problems related to allergen labeling of imported foods and ingredients are increasing. Within the RFR database, reports involving undeclared allergens increased from 13.2 percent of the reports for imported foods in year 1 to 19.6 percent of the reports for imported foods in year 3. Imported foods present some unique and difficult challenges, particularly when they involve a chain of suppliers. Foods or ingredients that contain major food allergens (as defined in the U.S.) might not need to be identified in the country (or countries) where they are produced or combined, leading to the presence of undeclared allergens in imported products. Problems like this are likely to become more common as the food production system grows more complex and more international over the coming years. A related problem stems from the increasing number of food products that are imported in consumer-ready packages. In some cases, these packages carry allergen declarations that are appropriate in the country of origin, or in other markets such as the European Union, but not in the U.S. One significant source of confusion is the different lists of allergens of public health concern in different countries. It is important to note that products for the U.S. market must meet U.S. requirements, including requirements about allergen declarations.

What Have We Learned from This?

The most important lesson learned from this analysis of food allergen recalls and reportable foods is that many of these recalls were caused by simple problems and could have been easily avoided. For example, the food industry could implement a regular review process to look for formulation changes in products and ingredients, which is not complicated or time-consuming but can provide insurance against unexpected serious problems. Similarly, double checks of packages and labels before they are used to ensure that they match the product being produced can be carried out in seconds and can avoid costly mistakes.

A second important lesson is that packing and label controls are as important for allergen control as are sanitation and Good Manufacturing Practices (GMPs). Allergic consumers rely on food labels to be accurate and complete. While GMPs and preventive controls are critical in averting the unintended presence of allergens through cross-contact, it is just as important to be sure that all the allergens that are used or that are components of ingredients are declared.

The third important lesson is that allergen-related problems occur more frequently in some types of foods than in others. In some cases, such as when using shared equipment to make different types of chocolate products, this reflects the difficult nature of the product. In other cases, such as the production of dry mixes, this reflects the nature of the production environment.

The final lesson is that ongoing monitoring of recalls and RFR reports provides important early warning signals that can be used to identify emerging issues and trends.[4] ■

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References

1. www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/Allergens/default.htm.
2. www.cdc.gov/nchs/data/databriefs/db10.htm and www.cdc.gov/nchs/data/databriefs/db121.htm.
3. www.fda.gov/downloads/Food/ComplianceEnforcement/UCM211534.pdf.
4. Gendel, SM and J Zhu. 2013. "Analysis of U.S. Food and Drug Administration Food Allergen Recalls after Implementation of the Food Allergen Labeling and Consumer Protection Act." *J Food Prot* 76(11):1933–1938.

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