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Detecting and Measuring Allergens in Food

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CHAPTER OUTLINE

INTRODUCTION

Food allergies affect an estimated 2 to 4% of the population around the world [1]. Allergic reactions to foods also account for a high proportion of emergency room visits, some of which result in hospital admissions, thus making food allergies a serious concern for public health around the world [2]. This increased awareness of the public health importance of food allergies has brought about increased regulatory oversight of food allergens. Various countries have highlighted the importance of food allergies by passing labeling legislation that requires a declaration of priority food allergens on the packaged food label [3]. While declaration of ingredients derived from allergenic sources when used as direct ingredients or processing aids has helped to provide allergic consumers with more transparent allergen information, these labeling laws do not address the potential risk involved with undeclared

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or 'hidden' allergens that may be in the food products due to cross-contact of the product produced on shared equipment, or due to commingling of ingredients at the supply chain level [4]. Cross-contact can occasionally occur despite the food manufacturer's best efforts to remove the allergenic residue. The food industry strives to mitigate this risk through use of allergen control plans and validated cleaning and sanitation procedures. Visual inspection of food contact surfaces is one of the key steps utilized to ensure the effectiveness of the cleaning procedure [5]. Analytical validation to ensure removal of allergenic residue from equipment surfaces or to ensure that the finished product does not contain the allergenic residue of concern is also utilized by the food industry.

Information on minimum eliciting doses of allergic individuals has emerged for various food allergens in recent years [6]. While there is interest by several stakeholder groups (i.e., food industry, regulatory agencies, allergic consumers, and clinicians) to evaluate the efficacy of using clinical threshold information for potential development of regulatory thresholds or action levels, currently many countries have not implemented regulatory thresholds [6]. Japan currently requires source labeling of its defined priority food allergens when the concentration of protein from the allergenic source is > 10 ppm (μg protein/g food) [7]. With the lack of regulatory thresholds, food industry is tasked with complying with essentially a zero threshold level of allergenic residue. This is operationally impossible given the complexity of manufacturing facilities and the numerous routes of allergen contamination and cross-contact that can occur throughout the supply chain.

As mentioned previously, visual inspection and analytical validation can be effectively used monitor the removal of allergenic residue and minimize the risk of hidden allergens in the next product after changeover. There are numerous quantitative and qualitative methods that are available for monitoring residues from allergenic sources [8–9]. It is quite important for food manufacturers to understand the advantages and limitations of the available analytical tools when selecting an appropriate method to ensure that the analytical results provide meaningful data that can be used for risk management purposes. Recently, food industry-led initiatives such as the Australian Allergen Bureau's VITAL (Voluntary Incidental Trace Allergen Labeling) program have been developed in an attempt to curtail widespread use of advisory labeling (www.allergenbureau.net/vital/vital). This voluntary risk management program relies on the accurate assessment of the level of potential allergenic residue that may be present in a packaged food product, along with information about the consumption of the product (i.e., serving size or other estimates of consumption). This is used to assess the need for using advisory statements when the exposure dose is above or below a defined reference dose. These reference doses have been developed based upon the available clinical threshold information for several priority food allergens. Quantitative (probabilistic) methods have also been developed for food allergen risk assessment

[10–13]. These risk assessment models rely on accurate determination of the concentration of allergenic residue. This chapter will discuss the analytical methods that are currently available for the food industry to detect residues from allergenic foods.

IMMUNOCHEMICAL METHODS FOR THE DETECTION OF FOOD ALLERGENS

Immunochemical methods are a broad classification of analytical methods that have been used for either clinical diagnosis of food allergy or for the detection of allergenic food residues. These methods rely on binding of allergen-specific antibodies to the allergenic food protein to be detected. Prior to the mid to late 1990s, rapid analytical methods that could be used by food industry for detection of food allergen residues were not readily available. Methods such as RAST (radio-allergosorbent) or EAST (enzyme-allergosorbent) assays were available primarily for clinical diagnosis of food allergy and for identification of allergenic proteins [9]. RAST and EAST rely upon the serum immunoglobulin-E (IgE) from food allergic individuals for qualitative detection of allergenic proteins. Protein from the allergenic source of interest is coupled to a solid phase. Allergen-specific IgE from allergic individuals is incubated with the allergen bound membrane, followed by detection of any bound IgE with a radioisotope labeled (in the case of RAST, e.g., ^{125}I) or enzyme labeled (in the case of EAST, e.g., horseradish peroxidase or alkaline phosphatase) anti-IgE antibody. Detection of bound IgE is achieved by measuring emitted radiation or color change.

RAST and EAST inhibition allow for quantitative detection of allergenic food proteins based on competitive binding of human IgE [14]. In these assays, protein from the allergenic source of interest is again coupled to a solid phase; however, the sample containing the potential allergenic protein to be quantified is incubated with allergen-specific IgE prior to adding this solution to the solid phase. The specific allergenic protein of interest will bind to the IgE, resulting in a decrease in the IgE that is available to bind to the solid phase. The concentration-dependent inhibition can be compared to a standard curve to allow for quantification of the concentration of food allergen in the sample of interest.

RAST and EAST inhibition do provide quantitative detection of allergenic food proteins; however, one of the main limitations of these immunochemical assays is that they require serum from allergic humans, which is not readily available and which varies from one allergic individual to another. Use of serum from allergic individuals also requires thorough characterization to ensure that IgE is not present that would recognize other allergenic proteins and thus lead to potential false-positive results. Finally, human serum poses a potential biological safety hazard that does not make these assays suitable for use within the food processing facility.

Enzyme-Linked Immunosorbent Assays (ELISAs)

Enzyme-linked immunosorbent assays (ELISAs) are the methods most widely used by the food industry for detecting specific allergenic protein [8,15−16]. ELISAs provide several advantages including:

1) They detect protein(s) from the allergenic source of interest, which make these assays ideal for validation of the removal of specific allergenic proteins,
2) They are sufficiently sensitive to ensure the safety of the allergic consumer (detection limits generally range in the low milligram per kilogram (ppm) range),
3) The reagents used in the assay are suited for use within the food processing facility, and
4) They provide a rapid assessment that can be run in the food processing facility or in a food industry laboratory [8,16].

ELISAs use immunoglobulin-G (IgG) antibody from animal sources such as rabbits, goats, or sheep that are directed against the allergenic protein(s) of interest rather than IgE from human serum. Use of an animal source for IgG antibodies allows the generation of suitable quantities of the antibody and also decreases the variability that is typically observed with human serum IgE.

Quantitative ELISAs can be developed in the sandwich or competitive formats. The sandwich ELISA is the most common format used for detection of food allergens [9]. In this format, an IgG antibody (referred to as a capture antibody) is immobilized onto the surface of a solid phase (typically a polystyrene microtiter plate or strip). The extracted sample is then added to the microwell and allowed to incubate. Any specific allergenic protein of interest will bind to the capture antibody. A second allergen protein specific antibody that is labeled with an enzyme (e.g., horseradish peroxidase or alkaline phosphatase) will bind to any captured allergenic protein. Two IgG binding epitopes must be present on the protein of interest in order to complete the binding to both the capture antibody and the secondary antibody. A substrate is added and allowed to interact with the antibody bound enzyme, which results in the development of a colored product, which can be measured by a spectrophotometer. The intensity of the color is proportional to the concentration of allergen present in the sample. Quantification can be accomplished by comparing the absorbance of each sample to the absorbance of the standard curve [17].

Competitive ELISAs (also referred to as competitive inhibition ELISAs) can also be used to quantitatively determine the presence of allergenic protein. With competitive ELISAs, the antigen (allergenic protein(s) from the source of interest) are coated onto the surface of a microwell plate. The sample extract is pre-incubated with the allergen-specific IgG antibody, which allows the antibody to bind to any specific allergens of interest present in the sample [17]. This solution is then added to the antigen-coated microwells. Any allergenic protein present in the sample will competitively inhibit binding of the IgG to the plate. After several washing steps to remove any unbound antigen, an enzyme-labeled secondary antibody is added, followed by the

appropriate substrate, which results in the development of a colored product. Unlike the sandwich ELISA format, the color intensity is inversely proportional to the concentration of the allergen present in the sample. In this format, the more color product produced, the lower the concentration of the allergen in the sample (i.e., less allergen present to compete with the coated antigen for binding to the allergen-specific IgG antibody). One advantage of competitive ELISA is that only one IgG binding epitope is needed on the allergenic protein of interest. This makes this format useful for the detection of fermented or hydrolyzed proteins where the allergenic proteins may be partially digested. Hydrolysis of the proteins can result in disruption of antibody binding epitopes, which can decrease the number of epitopes available for detection with a sandwich ELISA. Currently, commercially competitive ELISAs are available for the detection of gluten peptides that have gone through partial hydrolysis or fermentation.

Lateral Flow Assays

Lateral flow assays (LFAs) are a qualitative immunochromatographic form of an ELISA. These assays are also referred to as lateral flow strips (LFSs) or lateral flow dipsticks (LFDs). LFAs are comprised of five primary components:

1) The sample filter,
2) The conjugate pad,
3) The membrane,
4) The reservoir, and
5) The test and control lines [18].

An extracted food sample, swab, or final rinse water sample is first applied to the sample filter area consisting of a simple paper-like material where any solid food particles are excluded and soluble protein is wicked into the assay. The conjugated pad consists of a fiberglass-type material that is carefully coated within known quantities of allergen-specific IgG antibody coupled to latex or colloidal metals such as gold [8]. The coupled antibody is not bound to the surface of the LFA. When a sample is applied to the LFA, the allergenic proteins of interest will bind to the coupled antibody and continue to wick through the LFA by capillary action. The membrane of an LFA is generally constructed from polyvinylidene difluoride (PVDF), nitrocellulose, or nylon, where allergen-specific IgG is immobilized in the first zone of the LFA called the test zone. The coupled antibody-allergen (if present in the sample) will migrate to the test zone, and the coupled allergen will bind to the IgG present in this zone, forming a visible line that indicates the positive presence of the specific allergen of interest. The intensity of the line can be correlated to the concentration of the allergen present in the sample. Semi-quantitative results can be obtained if a strip reader is utilized. Some of the coupled antibody will not have bound antigen and will continue to migrate towards the second zone (control zone) where species-specific IgG is immobilized. This anti-species IgG antibody is developed to bind to the coupled IgG. For example,