

Measuring Food Safety: The Urgent Need for Food Allergen Metrology

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Introduction

Food allergies, affecting up to 10 % of the global population, represent a public health issue that demands immediate attention. A food allergy is an adverse immune-mediated health effect arising repeatedly from exposure to a given food commodity (e.g., milk, eggs, tree nuts, etc.). The health effects can range from a mild rash to life-threatening anaphylaxis. The chemical food constituents that are most frequently associated with an immune-mediated allergenic response are proteins. All allergenic food commodities contain several different allergenic proteins, although an individual's sensitivity to the various allergenic proteins can vary.

Since there are currently no cures for food allergies, the only way to address the health risks of food allergies is to avoid eating the allergenic food commodity. This is achieved through accurate food labeling, which is legally required by many countries worldwide, although the labeling requirements may vary. The United States Food and Drug Administration requires food manufacturers to label foods that may contain milk, eggs, wheat, soy, fish, crustacean shellfish, peanuts, tree nuts, and sesame.

Meeting food labeling requirements for allergens may involve testing when it is believed that a food inadvertently contains an allergen commodity. Food manufacturers also test to evaluate production equipment cleanliness to ensure minimal cross-contact between ingredients of different productions. Food allergen testing is also necessary to investigate food fraud when a cheaper allergenic commodity is substituted for a more expensive food ingredient. Routine testing for food allergens is performed with immunoassays and occasionally DNA-based methods. [Comparison studies](#) of commercial food allergen immunoassays show very little agreement between them.

To achieve better agreement between allergen testing methods, measurement traceability is needed. Without a metrological framework in place, achieving accuracy and comparability in food allergen measurements becomes impossible, compromising public safety. The necessary component of measurement traceability for allergen testing includes certified reference materials for allergen food commodities, reference measurement procedures, and consensus conversion factors.

Reference Materials

A key component of the measurement traceability scheme for food allergens is the availability of certified reference materials (CRMs) for all the food commodities needed to support labeling requirements. NIST currently has available the food allergen commodity reference materials (RMs) listed in Table 1 below.

Table 1. NIST CRMs/RMs for food allergen commodities.

NIST CRM/RM	Allergen Food Commodity
SRM 1549a "Whole Milk Powder"	Milk
SRM 1567b "Wheat Flour"	Wheat (Gluten-containing Cereal)
SRM 1845a "Whole Egg Powder"	Egg
SRM 3234 "Soy Flour"	Soy
SRM 3235 "Soy Milk"	Soy
SRM 2387 "Peanut Butter"	Peanut
RM 8404 "Almond Flour for Allergen Detection"	Tree nuts
RM 8405 "Hazelnut Flour for Allergen Detection"	Tree nuts
RM 8256 "Wild-caught Coho Salmon"	Fish
SRM 1947 "Lake Michigan Fish Tissue"	Fish
SRM 1974c "Organics in Mussel Tissue"	Crustacean Shellfish
RM 8258 "Wild-caught Shrimp"	Crustacean Shellfish

While the CRMs and RMs listed in Table 1 can be used to support food allergen measurements, none completely support measurement traceability for food allergen measurements because they lack certified measurement values for total protein. A certified total protein concentration measurement is necessary for traceability to the SI.

An important characteristic of a reference material, its commutability, also has not been evaluated for the materials listed in Table 1. Specifically, how well do materials listed in Table 1 represent similar materials in real consumer foods? Commutability of food commodities is very difficult to assess because food is such a diverse sample category. At NIST, we have performed proteomic comparisons between RM 8404/RM 8405 and commercial sources of almond and hazelnut flours using LC-MS/MS and commercial immunoassay analyses. Figure 1 below compares the number of peptides identified from each of the twenty hazelnut proteins identified in four commercial hazelnut flours and NIST RM 8405 "Hazelnut flour for allergen detection". Both RMs appear comparable at the protein level to the commercial tree nut flours tested.

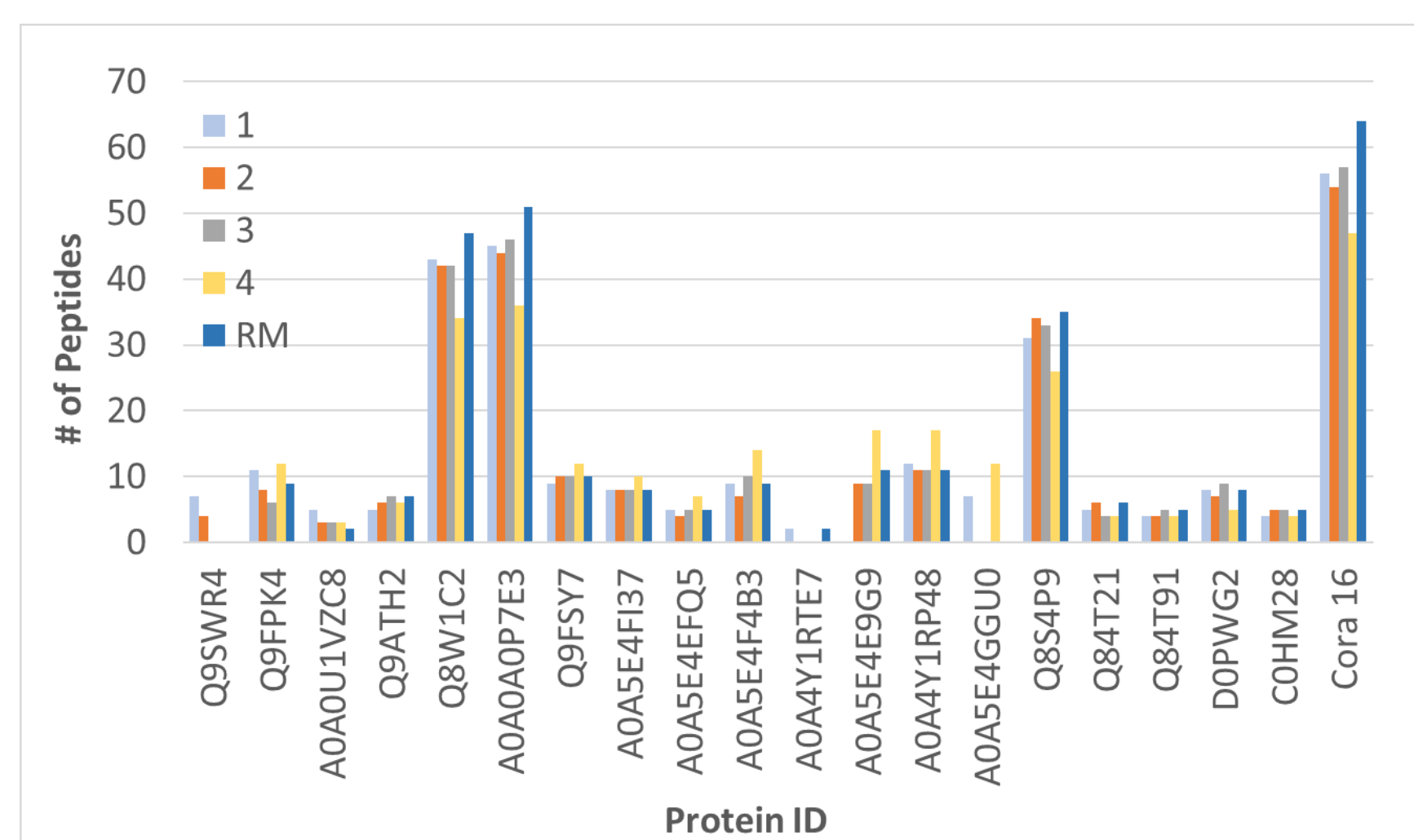


Figure 1. The number of peptides identified from each hazelnut protein is shown using the NCBI or SwissProt KB number. Peptides for different sources of hazelnut flour are shown as follows: American Hazelnut Co. (1), Bob's Red Mill (2), Corylus Farms (3), Mandelin (4), and RM 8405 (RM).

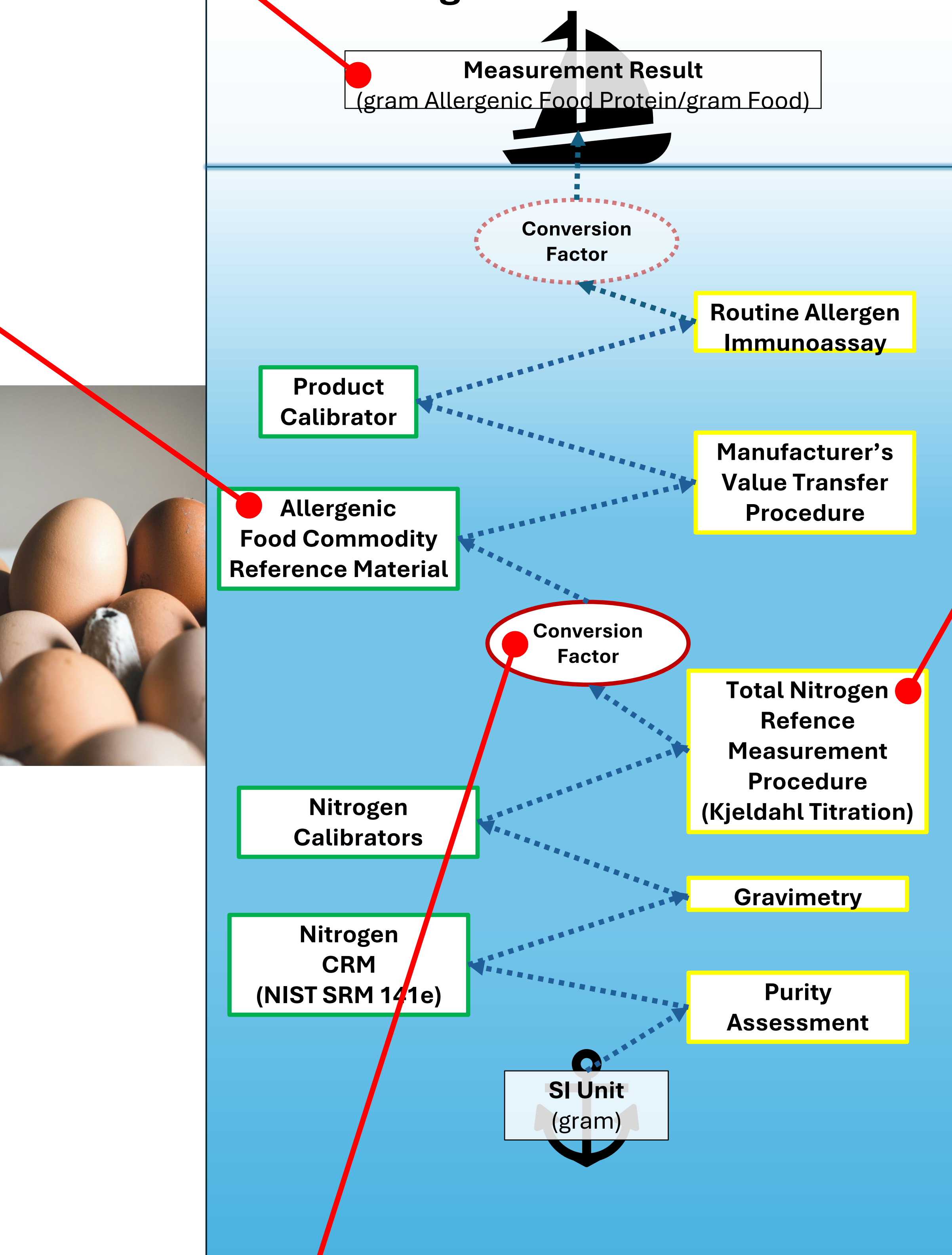
While food-matrix reference materials for allergens do not factor in the basic measurement traceability scheme, they would nonetheless be valuable quality control assets for evaluating routine immunoassays for matrix effects and extraction recoveries.

Measurement Reporting Units

In January 2023, AOAC International updated its "Guidance on Food Allergen Immunoassay Validation" and included recommended reporting units for commercial food allergen assays. The AOAC recommended that assays report measured food allergen concentrations in "units of mass of total protein from allergenic food per mass of food or environmental sample". For example, for the measurement of milk in a food item, the recommended reporting units might be "grams of total milk protein per kilogram of food", or in simpler terms "ppm total milk protein".

A survey of product information sheets from commercial assays to measure milk in food shows some concordance with the AOAC reporting units recommendation but not yet complete standardization. While many commercial milk allergen assays do report in units of "ppm total milk protein", others list reporting units of "ppm NFDM", "ppm total milk", and some just "ppm". It is also rare for commercial milk allergen assays to report the animal species of the milk their assay measures. Does it measure goat's milk with the same performance characteristics as it measures cow's milk?

Measurement Traceability for Allergen Proteins in Food



Conversion Factors

There are two potential ways that conversion factors are used in the measurement of allergens in foods. The most common way is in the determination of the total protein concentration of allergenic food commodities either in the form of reference materials or the master calibrators used by assay manufacturers. Total protein concentration determinations are made as total nitrogen measurements using Kjeldahl analysis; the measured total nitrogen concentration is reported as total protein using a conversion factor. For food samples, the Jones factors are most frequently used. The Jones factors originated from work done in 1941 to establish food-specific nitrogen-to-protein conversion factors.

While the food-specific Jones factors are based on laboratory measurements, they have never been reported with any measurement uncertainty. As such, when they are used as part of a measurement traceability scheme, their lack of reported measurement uncertainty prevents the calculation of a complete uncertainty budget. Additionally, the accuracy of Jones factors has not been thoroughly evaluated.

A second way in which a conversion factor can be used in the reporting of food allergen measurement results occurs when protein-specific measurements are used to determine allergen commodity amounts. For example, a milk assay that measures β -lactoglobulin may require a conversion of measured β -lactoglobulin concentrations to reported total milk protein concentration units. There is currently no consensus on these types of conversion factors, and they are used without their measurement uncertainty.

Reference Measurement Procedures

Because the most basic measurement traceability scheme for food allergens doesn't require food matrix-based certified reference materials, reference measurement procedures to quantify food allergens are not necessary for measurement traceability. However, reference measurement procedures would be valuable to produce food-matrix reference materials for QC assessment of routine assays or to value-assign any food matrix-based calibrators for routine methods or their manufacturer's master calibrators.

Given that the consensus units for routine food allergen assays include the total protein concentration of the allergen commodities, the reference measurement procedures needed to produce certified reference materials measure total protein concentrations. Currently, no reference measurement procedures for total protein exist.

The AOAC official methods for foods are all based on measuring total Kjeldahl nitrogen and using food-specific conversion factors to convert measured total nitrogen concentrations into total protein concentrations. As discussed below, the accuracy of food-specific conversion factors has not been validated, and no measurement uncertainty is associated with them even though they are all empirically derived. As such, these conversion factors cannot be used in a complete assessment of measurement uncertainty and create a broken link in the unbroken chain of comparison needed for measurement traceability.

There has been discussion of using total amino acid analysis to determine total protein concentration. The measurement of total amino acid concentration is more specific to proteins than the measurement of total nitrogen due to the presence of non-protein nitrogen-containing molecules in foods. However, the quantitation of all twenty amino acids from protein samples is not without considerable challenges. The largest challenge is that during hydrolysis, to convert proteins to amino acids, many of the amino acids are not stable and become degraded. This degradation leads to bias in the determination of total protein through total amino acid measurements.

Food Allergen Extracts for Clinical Applications

Extracts of allergenic proteins from foods are used as reagents for clinical diagnostics and oral immunotherapy treatments for food allergies. For diagnostics, allergen extracts are used in "skin prick" tests to evaluate a patient's response to the food allergen in a controlled clinical environment. The quality of the allergen extract is important so as not to produce a false negative allergy diagnosis. It is important that the allergen extract is representative of allergenic food in its composition of protein allergens. There are currently no consensus methods to evaluate the commutability of allergen extracts.

Extracts of allergenic foods are also used in oral immunotherapy treatments. In oral immunotherapy, controlled amounts of allergens are introduced to the patient over time to desensitize the patient's immune system. The goal of oral immunotherapy is not to cure a food allergy but to increase the patient's tolerance for an allergen to reduce severe allergic reactions. Knowing the amount of the food allergen in extracts is critical to controlling exposure. However, quantifying allergens in extracts face the same challenges that are faced in quantifying allergens in foods for food safety goals. There are no metrology underpinnings in place to ensure the safety and efficacy of therapeutic allergen extracts.

Conclusions

To achieve more reliable food allergen measurements for food safety and clinical applications, stronger metrological support is needed.

For food safety applications:

- More commodity (i.e., milk, fish, sesame, etc.) reference materials are needed that have certified values for total protein content.
- The commutability of commodity CRMs must be assessed.
- Improved reference measurement procedures for total protein determination are needed.
- When using the Kjeldahl method for total protein measurements, more scrutiny must be given to the conversion factors used.

For clinical applications:

- Higher-order methods, both quantitative and qualitative, are needed to improve the assessment of food protein extracts.