

Standardization of *in vivo* and *in vitro* diagnostic procedures in food allergy

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For most foods, true standardization is not yet feasible because there is insufficient information on the relative importance of individual allergens and their variants. Standardization without sufficient information may easily be counterproductive because improvements are less likely to be implemented. In the analysis of natural test material, the following principles apply:

- 1) RAST inhibition is usually inferior to other means of allergen quantitation.
- 2) Immunoblot is inefficient for some “important” allergens and over-efficient for some “unimportant” allergens and may therefore be deceptive.
- 3) Single-component assays are the only satisfactory way to describe complex mixtures.

Improving the actual food-testing procedure is important, but will not alone result in a reliable diagnostic procedure. Tests for measuring “effect modifiers” will have to be developed in order to predict *in vivo* reactions to foods.

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Diagnostic procedures in food allergy have a poor reputation. Lack of reproducibility and false-positive and false-negative tests are too common to be ignored. Some of the problems related to the reputation of these tests could be solved by standardization of the tests. However, standardization is not the answer to all problems. More importantly, standardization may actually prevent the problems from being solved.

Standardization implies settling for the suboptimal; why do it?

Standardization is often confused with optimization. It must be clear, however, that a standardized test is rarely an optimal test. This is particularly true in situations with as much (interpatient) variability as food allergy. An optimal diagnostic test for one patient is not necessarily optimal for another.

Moreover, standardization can easily slow down progress: any procedure that yields a result different from the standard is unacceptable until very substantial evidence proves the standard

inferior. Furthermore, if a test is fully standardized, any problem with it may no longer be noticed, as a result of the lack of competing tests.

So: why standardize? The two main reasons are as follows:

- 1) *Reproducibility*: a diagnostic test performed according to a fully standardized procedure gives the same result wherever and whenever performed (or, to be more precise: the inter-assay variability will not be markedly larger than the intra-assay variability).
- 2) *Quality assurance*: manufacturers and control authorities need criteria for acceptance of products; without standards, this is hard to do (but not necessarily impossible).

Food allergens are more problematic than other allergens

Variability of the source material

Food allergens are at least as variable as airborne allergens. One reason is that foods, particularly vegetable seeds, contain highly variable amounts of

storage proteins, which, furthermore, are less constrained with respect to the primary structure and thus often consist of a multitude of variants. The biochemically heterogeneous family of wheat gliadins is a well-known example.

False-positive reactions

Enhancing factors (that do not contribute to the test) can be involved, as in exercise-induced reactions. Of course, such “effect modifiers” are well known in the case of allergen exposure in the airways (e.g., bronchial hyperreactivity). In the case of food-induced reactions, the intraindividual variability seems to be more marked than with inhaled allergens. Furthermore, foods are far more likely to contain lectins and irritants (as protective factors against pests) that may cause nonimmune reactions mimicking allergic reactions.

False-negative reactions

Gastrointestinal digestion is clearly a major reason why food allergens are more problematic diagnostically than airborne allergens. Many subjects will tolerate the food despite the presence of IgE antibodies. Allergens that are very easily digested are unlikely to be important food allergens, at least not after being swallowed. Some IgE-binding components are less important as symptom-inducers than others.

Regrettably, the terms “major” and “minor” allergens have become associated with prevalence rates rather than with clinical relevance; it would perhaps be useful to introduce the terms “important” and “unimportant” allergens to indicate the contribution of allergens to clinical symptomatology. For the sake of discussion, it is furthermore convenient to distinguish three categories of clinical relevance of IgE-binding food components:

- 1) true food allergens
- 2) secondary food allergens
- 3) irrelevant food allergens.

True food allergens are food components that first act as immunologic sensitizers and upon subsequent contact induce symptoms. Peanut may act as a true food allergen. Secondary food allergens, i.e., nonsensitizing allergens cross-reactive with primary sensitizers (which are often pollen-derived), usually cause milder symptoms. Irrelevant allergens (or, to be more precise, irrelevant epitopes, because some relevant allergens may contain both relevant and irrelevant epitopes, whereas other allergens contain only irrelevant epitopes) usually cause little if any symptomatology. One group of

such “irrelevant” epitopes are the cross-reactive carbohydrate determinants (1).

A diagnostic test should ideally discriminate between these different types of allergens. Such a “discriminating” test would yield fewer positive test results and could thus easily be considered inferior in a comparison aimed at selecting a “standard” test.

Immunologic quantitation of allergens

Not all food proteins are allergenic. As long as we do not know much about the allergenic components of a food, it is an obvious choice to use human IgE antibodies to analyze food allergens immunochemically, by either direct RAST/EIA, RAST/EIA inhibition, or immunoblot. Because IgE antibodies are usually not monospecific, the next step is to develop monospecific reagents to the IgE-binding components. The most obvious choice is to produce monoclonal antibodies, but polyclonal antibodies often prove to be even more useful, particularly in an inhibition test in combination with purified (recombinant) allergens.

When we know which protein is of allergologic interest, there is no well-documented reason to use IgE antibodies for quantitation. The one reason invariably mentioned in this context is that IgE epitopes may be different from epitopes detected by hyperimmune animal antibodies, and the stability of the two types of epitopes may be different. While this argument is valid in principle, we do not know of any example where a relevant difference in reactivity has been documented (provided that the animal antibodies have been selected with proper care with respect to affinity and specificity). The use of monospecific animal antibodies has many advantages. In addition to the monospecificity (without which analytic quantitation is unreliable), the sensitivity and accuracy are usually substantially better, and the ample availability of the reagents makes exchange of these reagents for standardization purposes feasible. In contrast, human sera from patients with well-documented food allergies are usually valuable reagents.

What would be the ideal situation? do we know what we want?

- 1) *No double-blind, placebo-controlled food challenges* – but is it realistic to expect that we will ever have fully reliable skin tests and/or *in vitro* tests? We would certainly need to develop additional tests to measure the contribution of “effect modifiers”, analogous to the bronchial

hyperreactivity tests used to help to predict responses to exposure to airborne allergens.

- 2) *Well-defined, stable, irritant-free test material with all the relevant "important" allergens* – but we still have to identify many important allergens, and not everyone may agree whether all the "unimportant" allergens (which will hardly if ever cause serious clinical symptoms) have to be included. If we would have the important allergens available in pure form and could prepare mixtures for testing, do we want all allergens to be present in equimolar quantities or in their "natural" proportions?
- 3) *Undisputed estimates in mass units for allergens present in natural foods* – but this information

(not easily obtainable) will not reflect allergen exposure at the target site, because of the variable effects of digestion (and mucosal permeability).

Even if we still have a long way to go, we are making real progress. To try to take a shortcut (i.e., define standards before we know what to standardize) is likely to be counterproductive in the end.

Reference

1. Aalberse RC, van Ree R. Crossreactive carbohydrate determinants. Clin Rev Allergy Immunol 1997;15:375-87.