

# Accuracy of *in vivo* and *in vitro* tests

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Any new diagnostic test should be validated by standard procedures, and must be validated by the outcome of double-blind, placebo-controlled food challenges (DBPCFC), except in infants, with strict adherence to the EAACI guidelines. Available data should include calculations on sensitivity, specificity, predictive values, and concordance and must be calculated on the basis of patients in whom the diagnosis has been established by DBPCFC. Furthermore, data on possible cross-reacting foods and their clinical relevance should be available.

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The diagnosis of food hypersensitivity must rely entirely on the outcome of double-blind, placebo-controlled food challenges (DBPCFC) (1). A properly performed DBPCFC is the only confirmatory test in children and adults, whereas, in infants, open challenges, administered by trained personnel, may be sufficient. This does not mean, however, that *in vivo* or *in vitro* diagnostic tests are without value in the overall diagnostic process.

The applied diagnostic tests must be carefully validated, and it is necessary that any new diagnostic test should be validated by standard procedures, and the results must also hold up against the outcome of DBPCFC (1–3). The full validation program for a new test (*in vivo* or *in vitro*) should include data enabling the client to answer the following questions (2):

- 1) Can the test diagnose the disorder in question with sufficient specificity and precision?
- 2) Are abnormal values of the parameter associated with clinical disease, and has an age- and sex-related normal range been established?
- 3) Can other conditions (pathologic or physiologic) give rise to values outside the normal range?

Therefore, data comparing the test in question to the reference standard, DBPCFC, must be available, and should include calculations on sensitivity, specificity, and accuracy (3).

## Allergen extracts

In many cases, the allergen extracts used for *in vitro* or *in vivo* testing in inhalant allergy are standardized and have been shown in controlled studies to correlate well with clinical disease. In contrast, no

standardized extracts are yet available for use in the diagnosis of food hypersensitivity; therefore, variable and often poor correlations are seen. The commercial extracts used in skin prick tests (SPT) are generally of poor quality and should be used with caution. In many cases, it is better and more convenient to use fresh foods for SPT, by placing a drop of liquid food on the forearm and pricking through it, or, in the case of solid food, by placing a piece of the food on the forearm and pricking through it (the prick-prick method) (4, 5).

Except for milk, egg, shellfish, peanut, and fish, very few commercial extracts used in SPT or for determinations of specific IgE (RAST) have been evaluated in patients with food allergy proven by DBPCFC – most new *in vitro* tests are based on comparative studies with the classical Phadebas RAST or its successor, the CAP System, instead of patients and controls, whereas new extracts for use in SPT are mainly based on a few patients with a convincing case history, but rarely on patients proven clinically allergic by DBPCFC (3).

It is very important to realize that every food item is a unique system and must be validated by itself. Therefore, it is not possible to predict how a test will perform in, say, milk allergy from knowledge of how that test performed in another disorder such as cod allergy (Fig. 1) (2–4, 6).

Failure to comply with these criteria may have serious effects on the reliability of the test in normal daily routine (Table 1).

## Cross-reactions

Knowledge of immunologic cross-reactions and their potential clinical value are also important

Table 1. Some limitations in use of diagnostic *in vivo* or *in vitro* tests in diagnosis of food hypersensitivity

| Phenomenon, or characteristic, event                                    | Consequence                                                                                                                                                                         |
|-------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Lack of important epitopes in extract                                   | Sensitivity of test will be reduced                                                                                                                                                 |
| Cutoff level and normal range have never been established               | Sensitivity and specificity reduced. Cutoff levels different for different foods?                                                                                                   |
| Technical validation                                                    | Data on reproducibility not available                                                                                                                                               |
| New test is launched based on comparisons to market leader              | Every new test should be investigated in patients, as validated by DBPCFC, and sensitivity, specificity, accuracy, and predictive values should be calculated for every single food |
| All foods are considered to be similar and therefore treated equally    | Every food is unique. Data should be available for every single food on variables such as normal range, IgE classes, and differences between children and adults                    |
| Cross-reactivity between related or nonrelated foods or groups of foods | Data generally not available on possible clinical significance of given serologic or skin cross-reaction                                                                            |

when using RAST or SPT for diagnosis. For example, more than 60% of grass pollen-allergic patients show positive values of specific IgE against wheat in the CAP System (3), but this immunologic cross-reactivity is not reflected clinically. Similar results have been obtained with SPT to peanut and peanut-cross-reacting foods (7). Therefore, the performance (sensitivity and specificity) of a given test should always be calculated in comparison to the outcome of DBPCFC with the actual food item in question, and not with any related food.

## Challenge

Properly performed, DBPCFC (or open, controlled challenges in infants) constitute the ultimate tool for diagnosing adverse reactions to foods. There are several variables which must be considered in the actual situation, among which nature and source of food, timing, dose interval, and dose increment are important.

However, the most important variable is the reaction of patients to placebo challenges. As a hypothetic example, take a fictive trial investigating 100 patients claiming to experience symptoms of subjective intoxication when exposed to a food. To all patients, one active challenge and one placebo challenge were administered (DBPCFC), resulting in 25 patients claiming reaction to the active and the placebo, 25 to the active and not the placebo, and 25 to the placebo and not the active, whereas the remaining 25 patients reacted to neither the active nor the placebo challenge.

If all 100 patients were included in the statistical analysis, the conclusion would certainly be that no

## Accuracy of tests

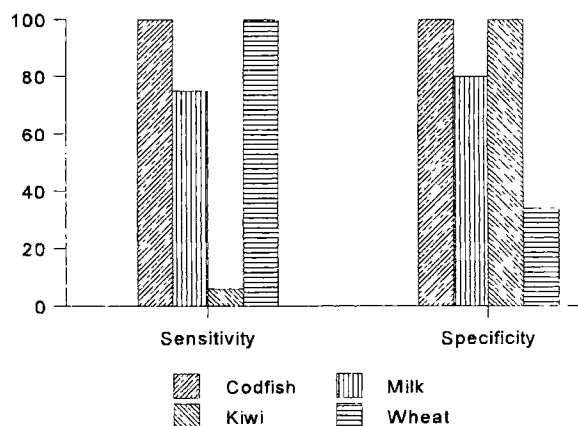


Fig. 1. Sensitivity and specificity of specific IgE for four different foods. Performance of codfish test (Magic Lite) is in fact diagnostic; i.e., in total correlation to outcome of DBPCFC. This is not case with test performance in milk, wheat, or kiwi allergy. Both CAP and Magic Lite are valuable tools in diagnosis of milk allergy, whereas CAP demonstrates clinically irrelevant grass cross-reactivity in wheat allergy, and Magic Lite demonstrates insufficient sensitivity in reactions to kiwi. Modified from data in Reference 3.

statistical connection between active challenge and symptoms could be established, whereas if patients with reactions to placebo challenges were excluded from the statistical analysis, 50% of the patients would be classified as reacting to the food.

Patients who react to a placebo challenge should not be excluded from statistical evaluation, but instead they should be compared with those reacting to an active challenge. However, this is not the case in most published papers on food hypersensitivity.

In conclusion, any new diagnostic test should be validated by standard procedures and against the outcome of DBPCFC, which in the future must be standardized, to enable direct comparisons between different protocols in different countries.

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