SPECIAL GUEST EDITOR SECTION: FOOD ALLERGENS NEW METHODS

Stakeholders' Guidance Document for Consumer Analytical Devices with a Focus on Gluten and Food Allergens

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Until recently, analytical tests for food were performed primarily in laboratories, but technical developments now enable consumers to use devices to test their food at home or when dining out. Current consumer devices for food can determine nutritional values, freshness, and, most recently, the presence of food allergens and substances that cause food intolerances. The demand for such products is driven by an increase in the incidence of food allergies, as well as consumer desire for more information about what is in their food. The number and complexity of food matrixes creates an important need for properly validated testing devices with comprehensive user instructions (definitions of technical terms can be found in ISO 5725-1:1994 and the International Vocabulary of Metrology). This is especially important with food allergen determinations that can have life-threatening consequences. Stakeholders—including food regulators, food producers, and food testing kit and equipment manufacturers, as well as representatives from consumer advocacy groups—have worked to outline voluntary guidelines for consumer food allergen- and gluten-testing devices. These guidelines cover areas such as kit validation, user sampling instructions, kit performance, and interpretation of results. The recommendations are based on (*1*) current known technologies, (*2*) analytical expertise, and (*3*) standardized AOAC INTERNATIONAL allergen community guidance and best practices on the analysis of food allergens and gluten. The present guidance document is the first in a series of papers intended to provide general guidelines applicable to consumer devices for all food analytes. Future publications will give specific guidance and validation protocols for devices designed to detect individual allergens and gluten, as statistical analysis and review of any validation data, preferably from an independent third party, are necessary to establish a device's fitness-for-purpose. Following the recommendations of these guidance documents will help ensure that consumers are equipped with sufficient information to make an informed decision based on an analytical result from a consumer device. However, the present guidance document

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The proficiency schemes mentioned herein are provided as examples and do not constitute an endorsement.

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emphasizes that consumer devices should not be used in isolation to make a determination as to whether a food is safe to eat. As advances are made in science and technology, these recommendations will be reevaluated and revised as appropriate.

Food consisting of a variety of matrixes, both in raw and processed forms, is a highly complex target for testing (even for trained scientists). Food matrixes can be grouped by relative fat content and carbohydrate and protein levels (1), as well as by other features, such as low or high pH. Each grouping presents a challenge for the analyst attempting to extract, purify, and detect the target analyte. Before extraction occurs, there is the sampling aspect to consider because most solid foods made of multiple food components are inherently nonhomogeneous, and any sample taken is unlikely to represent the entire food/product. This is even more relevant when food is tested from a plate containing multiple components, such as carbohydrate-rich foods (e.g., potatoes), vegetables, and meats. Each food may come in its own sauce or dressing, potentially containing different concentrations of the target analyte (2, 3). In addition, a kitchen presents a myriad of risks for cross-contact; e.g., breaded shrimp are deep fried, rendering the oil used in the fryer unsuitable for use to fry foods for consumers with shellfish allergies.

Therefore, until recently, food testing has almost always been exclusively performed by trained staff in a laboratory, with expert knowledge of sampling, applicable extraction methods, and fitness-for-purpose analytical methods that have been validated on common food matrixes. However, traditional food-testing methods are not suitable for restaurants, the home, or other nonlaboratory settings. Consumer demand for the detection of the presence of cross-contact or carelessness in food preparation exists to reduce the risk of adverse allergic or autoimmune reactions or to comply with strict dietary needs.

With advances in technology, consumer devices are now being made available for testing food constituents and contaminants. The analytes range from nutrients, pesticides, and mycotoxins to substances triggering food allergies and intolerances, with some of these analytes having potentially life-threatening implications.

There are currently no guidelines as to:

(a) how to provide information to consumers on the proper use of the devices and interpretation of results.

(b) how to validate and demonstrate fitness-for-purpose (e.g., via user trials).

(c) how to provide users with advice on the sampling and interpretation of results (and actions taken therefrom).

Therefore, stakeholders—including food regulators, food producers, food testing kit and equipment manufacturers, as well as representatives of consumer advocacy groups—produced the present guidance document to set voluntary guidelines.

During a brainstorming session, stakeholders discussed which aspects needed to be addressed, and three crucial areas were identified:

- providing information to users of such devices or methods;
- conducting food sampling, including aspects for consideration based on whether the foods have undergone processing; and
- validating such devices to prove fitness-for-purpose.

Figure 1 shows how these areas are interlinked.

Consumer Information/Kit Information

Every consumer device should come with a set of instructions that clearly describes its operation, performance characteristics, and limitations, as well as what constitutes proper food sampling technique. The instruction manual should emphasize that the test alone cannot determine the safety of any food, and that the device can only provide information to be considered in the context of other knowledge consumers have about the food supplier and their own sensitivity to the allergen/gluten.

Instructions included with the kit, as well as any online information, should consider diversity in user age, background, and education to help ensure the information is comprehensible to all consumers.

The kit manufacturer should determine what information should be provided in print and what additional information can be found online. Printed information should include any risks involved in the use of the kit, as well as basic kit performance information, including accuracy, confidence interval, sensitivity, result interpretation, types of samples that cannot be tested, and basic test-portion sampling instructions.

The kit manufacturer should also assess whether separate versions of the instructions are needed if the device is intended for use in different settings, such as testing in restaurants versus testing packaged goods in the home.

Customer Support or Help Line

The printed instructions should contain contact information for a customer support or help line, which can include e-mail, phone, or chat support provided by appropriately trained staff. The instructions should also encourage end users to use this help line to report any results that appear to be aberrant, as this may help uncover problems with matrixes that may not have been part of the initial kit validation. Kit manufacturers should exercise discretion in determining the credentials and training needs of the support staff responding to support requests. The kit manufacturer should also gauge the need for additional support options (e.g., in-depth training and/or supplemental consumer information), as education is likely to positively impact the accuracy, usability, and interpretation of test results.

Kit Performance and Limitations

The printed instructions should contain a section on kit performance and limitations, which should include the following:

(a) *A clear definition of what the kit measures.*—This should clarify the target analyte and the reporting units of the kit. For example, are the results or LOD expressed as milligrams of protein or milligrams of commodity per kilogram of food? (It is standard to express results in SI units, such as milligrams per kilogram. However, milligrams per kilogram is also often referred to as parts per million or ppm.]

(b) *The level of sensitivity with the stated accuracy (expressed as a percentage), including the confidence interval.*—This value should come from experiments across a specified number of food matrixes, and a description of how the value was obtained (e.g.,

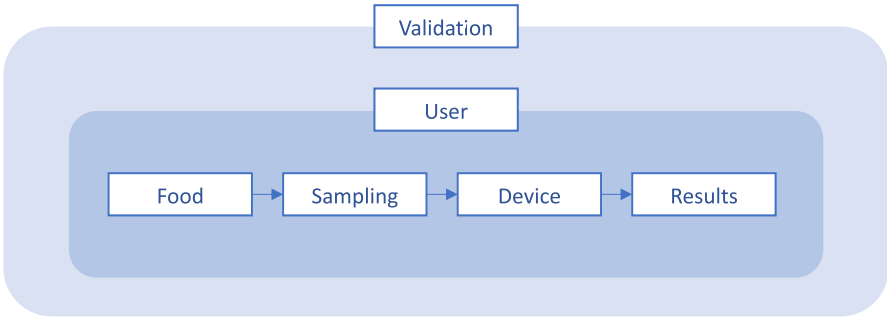

Figure 1. Interconnectivity of relevant factors related to validation of consumer analytical devices.

spiked samples, incurred samples, and/or specific matrixes) should be made available.

(c) *A list of the types of samples that have been proven to work with the kit.*—This can be done with a short list of representative foods that were used to validate the kit/device, and validation data should be available upon request (e.g., link to the complete validation data online).

(d) *A list of any products or product types that cannot be tested with the kit.*—This could include food categories, such as fermented or hydrolyzed foods, high-fat foods, hygroscopic foods, strongly colored foods, low- or high-pH foods, or specific foods that may produce false-positive or false-negative results. It could also include product categories, such as medications, dietary supplements, or personal care products. For categories, specific examples should be provided within the printed instructions as space allows (e.g., "fermented foods, such as soy sauce") or, at a minimum, be provided online. Materials known to cause false-positive or false-negative results should be clearly identified.

(e) *A description of any limitations on kit stability/performance.*—The printed instructions should include any environmental or other factors that could affect kit performance, such as "do not store device in a hot car" or "active components must be stored in a controlled environment."

Sampling

The printed instructions should include a section on sampling. Validation data should be available and referenced for all sampling criteria. This section should define:

(a) whether the kit is designed to test one food at a time or small, combined amounts of multiple foods;

(b) whether additional tools or steps are required to produce accurate results, such as a scale to weigh the sample or preprocessing steps that are required before taking the test portion;

(c) what steps to take to avoid cross-contamination;

(d) what concerns can arise from inhomogeneous samples, such as the dilution effect that can occur when testing multiple foods at once or nonuniform cross-contact on the surface of a food; and

(e) the impact of testing too small or too large a sample.

Interpreting Results

The printed instructions should contain a section on interpreting results, which should cover the following:

(a) *The meaning of an invalid result.*

(b) *The meaning of a positive result.*—The interpretation of a positive result should be related to the level of detection (shown in the validation data) in the product being tested, but should not identify a threshold for consumers. It should make clear that a positive result is one data point that only represents the sample tested and does not necessarily mean that the product is unsafe. This section may link to information regarding how to address positive results with the restaurant or manufacturer in question.

(c) *The meaning of a negative result.*—The interpretation of a negative result should also be related to the level of detection in the product being tested. It should make clear that a negative result does not necessarily mean that the food is safe.

Reagent Safety and Disposal

The printed instructions should include a section on reagent safety and disposal. Advice should be given in relation to recommended storage conditions and the reagents used (that are not reusable) and disposal thereof after use. Any safety concerns should be printed on the reagents themselves. This information should also be available online.

Independent Validations/Certifications

The printed instructions should identify any independent validations/certifications that have been performed, including the matrixes tested.

Sampling

The most important factor in the sampling of foods for estimating target analyte content from single or multiple measurements is the relative heterogeneity of the sample tested. It is a tenet of analytical measurement that the more uniformly distributed the target analyte within the sample matrix, the less tested material is needed to accurately measure the analyte to an adequate confidence level. When considering how to sample from a plate of food, there are two levels of heterogeneity to consider.

The first could be termed "macroheterogeneity," which is what portion of the entire plate is to be tested when the plate consists of multiple foods. For instance, a meal consisting of a hamburger on a bun with an array of condiments, accompanied by a side portion of French fries, requires a prudential decision by the user whether to test the meat, the bread, any of the condiments, or the side portion (or combinations thereof) or

to perform multiple tests. The decision requires some knowledge by the user of the relative likelihood that any of these elements of the meal might contain gluten, as well as adequate user instructions and guidance provided based on the device manufacturer's validation. In this example, it might be a straightforward choice to only test the bun because the other foods may be presumed to be gluten-free, whereas the grain-based bun might naturally be thought to carry a greater inherent risk.

The other level of heterogeneity could be termed "microheterogeneity," which is the granular distribution of target analyte throughout the tested food portion. Most commercial consumer-based devices available today can only test a few hundred milligrams of food at a time. The problem with detecting "hot spots" in any matrix in which gluten contamination occurs in small specks, or, perhaps, even one small speck, is thus present in the consumer-based scenario. Once again, adequate knowledge about the risk of hot spots in a particular food is needed for the user to make a proper inference about the safety of the food.

The underlying concepts of proper sampling in the complex environment of a plate of prepared food may be difficult to convey to diverse users, but proper sampling will increase the benefit and value of the test result. The risk of incorrect inferences about allergen/gluten content can be mitigated, but never eliminated, by rigorous testing and validation across a wide array of possible scenarios, as well as through proper user instructions.

Validation Procedures and Steps

According to the technical report, "Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis," (4) method validation tests the assumptions on which an analytical method is based and establishes and documents the method's performance characteristics, thereby demonstrating whether the method is fit for a particular analytical purpose. Typical performance characteristics of analytical methods are applicability, selectivity, calibration, trueness, precision, recovery, operating range, LOQ, LOD, sensitivity, and ruggedness. Measurement uncertainty and fitness-for-purpose could be additional performance characteristics.

For devices destined for consumers, single-laboratory validation (SLV) is insufficient to demonstrate fitness-for-purpose, as are the other validation parameters. Therefore, a step-wise or parallel approach is recommended, which includes the following steps (Figure 2).

As a first step, the SLV of consumer analytical devices and other analytical assays destined for consumer use should be performed using common guidelines for SLV [e.g., Eurachem (5) and/or International Union of Pure and Applied Chemistry (4)]. Validation performance criteria assessed should include parameters, such as

- applicability
- sensitivity
- selectivity
- ruggedness
- calibration and linearity
- trueness
- precision
- recovery
- range
- LOD

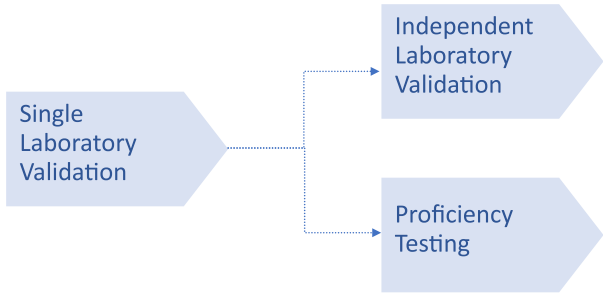

Figure 2. Proposed sequence to demonstrate the fitness-for-purpose of consumer analytical devices.

- fitness-for-purpose
- matrix variation
- measurement uncertainty

It should be noted that validated matrixes should be comparable with key matrixes expected to be tested by the target user or consumer. Matrix exclusions should also be clearly stated in the validation report and instructions for users.

Once validation has been successfully performed and meets the minimum performance criteria [e.g., as set by the International Organization for Standardization (6) or the European Committee for Standardization (7)], or, if such do not exist, performance criteria that are equal to or better than the routinely used assays for the analysis of the target analyte(s), additional assessments should be made. Other existing and specific guidance documents should also be considered [e.g., for food allergen validation; *see* Abbott et al. (8)]. External assessments that include an independent laboratory validation are strongly recommended. Unlike standard multilaboratory validations for laboratory analytical methods, which are conducted by trained staff only, such schemes should include consumers or untrained personnel as testers. The number of participants in the consumer group in a study should be such that it allows the statistical evaluation of results for both laboratory- and consumer-generated data.

Any performance statements made by the manufacturer for the assay or device may be reflected in the design of the validation and independent laboratory studies in accordance with industry standards and supported by the data generated. This includes, but is not limited to, the number of foods (or matrixes) that can be tested, the LOD, and the LOQ.

Validation data should be analyzed following commonly accepted guidelines for collaborative study procedures to validate characteristics of a method of analysis (9). Acceptable parameters for quantitative methods have been described, e.g., as in the publication by Abbott et al. (8). Acceptable parameters for binary methods have been described in publications by Koerner et al. (10) and Wehling et al. (11). To validate device performance (or chemistry), sample size should be equivalent for both the device under test and the reference device (predicate device) or assay. In an ideal situation, test portions of the same homogeneous sample are used.

In the event that no reference method or standard exists for this kind of analysis, alternative options can be considered. Analytical results, even for homogeneous samples, as shown in several proficiency testing (PT) rounds, can differ significantly between assays used by routine analytical laboratories. To evaluate consumer devices in these cases, kit manufacturers should consider PT schemes [e.g., the Food Analysis

Performance Assessment Scheme (12) and Dienstleistung Lebensmittel Analytik (13)], along with commonly used laboratory assays. Results obtained by consumer devices or assays should be satisfactory and fall within the same value range as laboratory assays used in the same PT.

Summary

Following this guidance document will help ensure that sufficient information is provided to consumers so they can make informed decisions based on analytical results and help device manufacturers validate their devices so they can ensure the device or method is fit-for-purpose. This guideline will significantly contribute to the consumer's decision-making process, and consequently, contribute to the consumer's safety and quality of life.

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