Microbiological Detection Methods — Assuring the Right Fit

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SUMMARY

The food safety industry is in the midst of rapid evolution. Leaders and scientists alike are approaching new regulatory requirements set forth by the Food Safety Modernization Act to ensure analytical methods, designed to detect hazards, are fit-for-purpose for their specific commodities. Simultaneously, the food industry is innovating at a tremendous rate. Unique ingredients and formulations are being developed, novel processing methods are being deployed, and new products are entering the market. The food safety community is scrutinizing analytical approaches to ensure that new and existing methods are appropriate for the bevy of products being tested. In addition, the industry is working to understand and agree upon the most prudent scientifically and economically sound approaches to method validation and verification. In this introductory article, the International Association for Food Protection Applied Laboratory Methods Professional Development Group discusses the needs and considerations for assessing fit-for-purpose approaches in the food analytical laboratory.

OVERVIEW

The first major change in U.S. food safety legislation since the Food Drug and Cosmetics Act of 1938 occurred in 2011, when the Food Safety Modernization Act (FSMA) was passed. This law emphasizes prevention of entry of foodborne contaminants into the market *(3)* and builds on approaches already implemented in industry, such as the Hazard Analysis Critical Control Point (HACCP) principles, to identify risks, apply control measures with defined critical limits, and verify effectiveness in mitigating those risks *(3)*. FSMA calls these control measures "Preventive Controls" and requires that "the owner, operator, or agent in charge of a facility" must verify that their food safety preventive controls "are effectively and significantly preventing the occurrence of identified hazards." This demand for verification is driving a large increase in laboratory testing, especially as food businesses expand environmental monitoring and increase the analysis of raw materials and finished products for pathogens, spoilage organisms, allergens and other adulterants. To facilitate this increase in testing, manufacturers are relying more and more on commercial or private laboratories to help them meet this demand by producing accurate results that are both efficient and cost effective.

In addition to testing that is driven by regulatory changes, globalization of the food supply, shorter product development timelines, and reformulation of existing products *(4)* to meet consumer trends create huge numbers of new food products that must be tested. In the U.S. alone, 21,435 new packaged food and beverage products for consumers were introduced in 2016, almost double the 11,853 introduced in 1998 *(11)*. These new products may be the result of incremental changes, such as the advent of Greek yogurt, which grew from nothing in 2005 to 44% of the yogurt market by 2014 *(10)*, or they may result from more radical innovations, such as the addition of probiotic cultures to various foods, including juices, chips, chocolate bars, pet food, and others. Products are also becoming more "exotic", as in the case of insect-based foods *(8)* such as energy bars made from cricket flour. All such foods may come in multiple flavors, varieties (e.g., nonfat, sugar free), and forms (e.g., freeze-dried bites), resulting in a complexity of forms and formulations that may interfere with pathogen detection methods.

The USDA Trends in Food Recalls *(12)* reported a doubling in recalls between 2004 and 2013 and suggested a number of possible reasons, including:

- increased regulatory oversight
- increased product and environmental sampling
- improvements in technology and detection
- better product and ingredient traceability
- increased audits and inspections, and
- new food types available in the market.

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Given the complexity resulting from such factors, it is more important than ever to ask, "How do we assure that test results are reliable and methods are fit for their intended purpose?"

RELIABILITY OF TESTING

Sampling

The focus of this article is on laboratory test methods; however we would be remiss if we failed to emphasize that a test can yield information about only the portion tested. Hence, it is crucial to ensure that sampling is representative of the material under study, and to understand the power of the sampling plan in making decisions based on test results. There are many excellent sources of information on sampling plans; thus they will not be addressed further here *(5)*.

Validation and verification

A key component of laboratory accreditation in line with ISO 17025 (updated in 2017) is that laboratories must show that the test methods used are both validated and verified *(7)*. Validation is the process of demonstrating that the method reliably detects the analyte, and verification demonstrates that the laboratory can effectively perform the method.

Validation involves a formal process of rigorous testing to ensure the method performs as expected and has acceptable inclusivity, exclusivity, sensitivity and robustness *(1)*. For third-party certified validation, there is also some element of inter-laboratory comparison. Third-party certification bodies include AOAC (*Performance Tested Method*℠ or *Official Methods of Analysis*℠), AFNOR, MicroVal, NordVal, and Health Canada. These organizations address the abundance of forms of matrix diversity already discussed by grouping foods and environmental samples into categories, and perhaps sub-categories, based on properties believed to influence their microbiological character. Validation certification can then be sought for a narrow or broad range of categories. For example, AOAC has over 100 subcategories of food *(2)*, and only 15 matrices are required for the broadest level of AOAC validation *(2)*. With the ever-expanding diversity of our food products, this leaves a great deal of room for incongruence between the food categories in the validated scope of a method and the range of food products in the market. To ensure food safety, we need to look beyond official validations.

Verification demonstrates that the validated method functions in the user's hands according to the method specifications determined during the validation activity *(6)*. Method verification studies have a more limited scope than validation studies and may include evaluation of only a small subset of performance characteristics (e.g., sensitivity verification for identification and qualitative methods; relative accuracy and precision verification for quantitative methods). A method can be verified for only those matrices or categories (including test portion size) that were included in the validation study. However, what should be done when a user wants to deviate from the intended use of a method by testing a different matrix or a different test portion size? Should a new, full-scale validation study be conducted on each new matrix addition? This process would be very time-consuming, costly, and, arguably, not necessary. Thus, a gap exists in guidance for the method validation and verification processes, which does not consider these types of situations and thus creates a need for alternative approaches.

Alternative approaches

One alternative approach mirrors the FDA internal guidelines on emergency testing, when samples must be analyzed immediately. These guidelines indicate that when a method will be used to test a food from the same category as in the validated method, laboratories can analyze the new matrix concurrently with a matrix spike. When the matrix has yielded at least seven positive and no negative results using matrix spikes, or a > 95% confidence level (19 of 20 positives) is achieved, the method will be considered verified for that food product *(13)*.

Another alternative approach draws on the suitability test approach of the United States Pharmacopeia (USP). This approach spikes every different formulation with the organism(s) of concern to demonstrate recovery and has some defined additional preparatory steps in the event that recovery is not achieved initially *(9)*.

It must be noted, however, that although abbreviated alternative methods may save time and money, they do come with added limitations as a result of the reduced scope of the data obtained.

WHAT DOES A RESPONSIBLE LABORATORY DO NEXT?

Responsible laboratories already recognize the complexity of the matrices analyzed and diligently strive to use appropriate methods. This objective is complicated by the relative dearth of guidance for independent and food industry laboratories. In working toward a sound scientific approach, food safety professionals in both laboratories and the food industry should take the following into consideration:

- Are alternative method verifications using an abbreviated approach acceptable? How is "acceptable" defined?
- Are food matrices appropriately grouped? What are the key criteria for grouping?
- What constitutes a significant difference in formulation if intrinsic parameters match?
- Should processes used in food manufacture be considered when grouping (e.g., fermentation byproducts, compounds produced during thermal processes)?
- How can historical data be used to substantiate fit-for-purpose?
- What is the applicability of shared matrix validation studies?

WHAT IS TO COME?

Validation, verification and fitness-for-purpose are important elements in assuring method performance, with clear guidance on how they apply in certain circumstances:

- Developers of diagnostic test kits can submit for validation through a formal scheme.
- Regulatory labs (e.g., US FDA, Health Canada, Public Health England) have internal validation guidelines to follow.

Unfortunately, the guidance for food companies and independent laboratories is less clear. In this article we have aimed to briefly review the complexity of this topic and raise a number of questions that we have found are generally recognized as difficult. We intend this to be the first in a series of articles in which we will address these questions and offer some sound approaches that can be readily adopted to simplify some of the difficult questions addressed in laboratories every day.

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