Alternative Approaches for Qualitative Microbiological Method Matrix Additions

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SUMMARY

Most commonly used pathogen detection methods have undergone a rigorous validation through third-party certification bodies such as AOAC INTERNATIONAL, Association Française de Normalisation, MicroVal, and others. These validations focus on sensitivity, robustness, and inclusivity and exclusivity of the assay target(s) for the matrices submitted to the certification body. This creates a list of officially validated matrices that falls far short of what is seen routinely during end-user testing. Thorough validation of all matrices at all test portion sizes is neither cost efficient, practical, nor arguably necessary. Here, we provide guidance on alternate evaluation approaches using a food-similarity grouping and a risk-based questionnaire to help end-users determine an appropriate level of evaluation of their method of choice. In reducing the burden of evaluation for many matrices, these alternative approaches may allow more matrices to be evaluated, thus strengthening confidence in method application and ultimately leading to a safer food supply.

OVERVIEW

The Food Safety Modernization Act, passed in 2011, emphasizes prevention of entry of foodborne contaminants into the market *(33)*. This act focuses on the establishment of verified "preventive controls" to reduce or eliminate identified hazards in the food production environment. This has led not only to a dramatic increase in laboratory testing of raw ingredients, finished food products, and environmental samples but also to questions on what "verified" means. Most foodborne pathogen test methods are validated for specific applications by a third-party certification body such as AOAC INTERNATIONAL (AOAC), Association Française de Normalisation, MicroVal, NordVal International, or Health Canada. However, third-party validation studies often include only a small number of matrices or a different test portion size than is commonly tested in the field (e.g., 25 versus 375 g, respectively). Because test methods cannot be validated for every possible matrix at every test portion size, there is a substantial gap in data between third-party certified matrices and end-user fit-for-purpose analytical testing needs. In this article, we aim to provide suggestions for practical, risk-based approaches to address that gap in qualitative microbiological methods by focusing on matrix grouping and levels of test method evaluation. In support of this aim, we have created a Matrix Evaluation Level Assessment Tool (available at https://www.foodprotection.org/upl/downloads/library/matrix-evaluation-level-assessment-tool.xlsx) that guides the user through a set of questions to help determine the degree of test method evaluation needed for a new matrix.

Need for alternative method evaluation approaches

Rapid methods for the qualitative microbiological testing of foods are used extensively throughout the food industry for detection of low concentrations of pathogens. Typically, method validation studies are conducted through recognized third-party certification bodies by the rapid method developer or test kit manufacturer with a limited group of food matrices and associated method parameters such as test portion size, nutrient media, and enrichment conditions. Because the scope of the validation is limited to the matrices included in the method validation study, the responsibility for ensuring that methods are fit-for-purpose is left to end-users such as food manufacturers and third-party laboratories. This responsibility often means conducting matrix addition studies to extend the method scope to a new matrix or a new test portion size. Here, we use the term "evaluation" to encompass the process by which test methods are assessed for use with a matrix of interest. This is an attempt to distinguish this process from definitions of verification or validation used by regulatory and accreditation bodies.

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Guidelines for conducting matrix additions for third-party assessment are available from standard-setting organizations such as International Organization for Standardization (ISO) and AOAC *(4, 26)*. Laboratories operated by the U.S. Food and Drug Administration (FDA), U.S. Food Safety and Inspection Service (FSIS), Canadian Food Inspection Agency, and other global regulatory bodies are required to follow validation standards set forth by their own organizations. FDA validation guidelines *(36)* indicate that the FDA will not object to the use of commercial methods validated per AOAC *Official Methods of Analysis*℠ (OMA) Appendix J or ISO 16140-2:2016 so long as the methods are validated against U.S. reference methods, such as those found in the FDA's *Bacteriological Analytical Manual* (BAM). Methods validated against non-U.S. reference methods are not considered equivalent to U.S. reference methods, because there have not been extensive side-by-side comparisons of U.S. and non-U.S. reference methods (e.g., between the BAM and ISO reference methods).

When conducting matrix additions, are food manufacturers and contract laboratories required to follow these standardized schemes? Presently, novel food products are introduced at nearly double the rate from 20 years earlier. In 2016, 21,435 new consumer packaged food and beverage products were introduced into the marketplace *(31)*. Validating every new rapid method and matrix combination is inconceivable due to the challenges of cost, time, and often overwhelming workload. More importantly, the question can be asked as to whether public health is advanced or enhanced by conducting matrix validations as currently understood. Would a more risk-based approach allow for more matrix evaluation studies to be performed, thereby reducing instances of gross failure to detect? Can a method's past performance on a wide variety of matrices provide evidence to support its reliability for analysis of new matrices? How close is "close enough" when grouping matrices?

Often, the decision of whether a method should be validated or verified for a particular matrix is based on an educated guess and a logical rationale for the direction chosen. The decision is based on whether the matrix in question falls within a category of matrices for which the method has been validated *(36)*. According to Thomas Hammack, Senior Policy Analyst for Microbiology at the FDA, if the matrix has been validated within a category, a minimal approach with positive spike controls of less than 30 CFU per test portion of a target organism is acceptable. If the matrix does not fall within a validated category, a full method/matrix validation is required *(20)*.

Alternative matrix evaluation approaches

When a validated matrix is used on a rapid testing method, a simple "first use" verification will suffice *(36)*. The FDA guidelines suggest testing six inoculated and six uninoculated samples with the new method and the reference method, with no false negative or false positive outcomes. Because this does not need to occur with every matrix in the official validation, most labs will have performed this during training steps or initial implementation of the method. More frequently, the exact matrix is not under the official validated scope, and a matrix addition is needed. Creating open access libraries of evaluated matrices for rapid methods could reduce the cost burden associated with evaluating every type of food product in every laboratory; however, this seems unlikely to occur as products frequently have proprietary ingredients or testing laboratories consider matrix evaluation data proprietary. Therefore, matrix grouping strategies and fit-for-purpose studies can balance the scientific validity, risk, and practicality (cost) in performing matrix additions.

Food matrix grouping approach: Intrinsic characteristics

Method validation schemes use food matrix categorization to simplify the work needed to demonstrate that methods are effective and fit-for-purpose across similar foods. Categorization or grouping of food types based on intrinsic factors that influence microbial growth such as pH, fat content, water activity, and salt content is a common way to address the number of studies and/or complexity of the studies used for matrix addition *(Table 1)*. This approach of food matrix categorization is supported by FDA, FSIS, AOAC, and ISO and is incorporated into accreditation body validation requirements *(3, 26, 32, 36)*. The Interagency Food Safety Analytics Collaboration *(23)* has also created a food categorization scheme that is broken down by type of food and further divided based on differences in food processing (e.g., pasteurized fluid dairy products, unpasteurized fluid dairy products, pasteurized solid, and semisolid dairy products, among others). However, these schemes only group select products, leaving many uncategorized (e.g., cheese powder concentrates, proprietary spice blends) for industry to assess.

In the United States, industry must decide how similar uncategorized products are to validated matrices and then determine whether a full matrix validation is required, if first use verification will suffice, or whether some other type of evaluation is appropriate *(36)*. This can be difficult because products that seem quite similar can have intrinsic properties or production processes that might affect testing results. For example, not all cheese types can be considered the same. Intrinsic properties, including fat, protein, salt, inclusions (e.g., onion powder), and starter cultures (including fermentation-produced compounds), can affect microbial detection *(19, 28, 37, 39)*. There are also examples where detection can fail between different matrices within the same category. For example, red lettuce often causes inhibition in immunoassays, whereas green lettuce does not *(17)*.

Overall, categorization schemes align quite well with public health risks associated with different foods as illustrated by the draft ISO 16140-3 matrix table *(27)*. This table provides a sensible summary of the "organisms of concern" within the constructed categories. As such, it is a reasonable "risk management" table.

TABLE 1. Chemical and physical food attributes (intrinsic factors) considered in grouping matrices

Intrinsic factor	
pH	Surface structure
Water activity	Salt
Natural occurring inhibitors – cocoa polyphenols, enzymes	Sugar
% Fat	Added humectants – polysaccharides, dietary fiber, hydrocolloid, pectin
% Protein	Emulsifiers
% Fiber	Fermentation products and by-products
% Carbohydrate	Microbial inhibitors and preservatives used in formulation
Added organic acids	Type of processing – roasted, high-pressure processing, irradiated
Microbial load – active cultures, raw agricultural product, meat	Physical form – dried, intermediate-moisture food, high moisture

Food matrix grouping approach: Commonality in enrichment procedures

When a matrix has not previously been evaluated, we are concerned with two primary risks to method performance: (i) the alternative method selected may not allow propagation of the target organism to detectable levels, and (ii) matrix effects may interfere with the assay's chemistry or technology.

Enrichment procedures are the foundation for detection of pathogens, requiring growth of the pathogen to sufficient numbers for detection by either traditional or rapid methods. Within a particular test method, there is often a significant common core in validated enrichment procedures between matrices *(Tables 2 and 3)*, demonstrating substantial robustness of the method, even when exceptions are noted. This common core of enrichment conditions increases our confidence that a method can recover the pathogen of concern, even from an unevaluated matrix, and suggests that a limited demonstration of efficacy may often be sufficient. This point is discussed further and explored using the Matrix Evaluation Level Assessment Tool available online.

Rapid test methods increasingly use proprietary enrichments, usually for some claimed reduction in the required incubation period or simplification from a two-stage to a single-stage enrichment. Newer methods may have more limited validations, and if faced with evaluating a new matrix it is best to consult the manufacturer for enrichment recommendations.

When conducting a matrix addition with an established and validated method, choosing the enrichment used by that method for a matrix from a similar validated category is a good starting point. If further method development is needed following evaluation, using validated enrichments for fdifferent matrix categories within the same method or a similar matrix with a different method may increase the chances of success. Validated enrichment schemes mitigate a portion of the risk for detection failure due to failure to grow the organism to a detectable level and move the risk element to matrix interference with the detection system. However, method modifications such as additional dilutions, modified enrichment media, or other significant method changes may require a "full" validation. Interference by matrix effects is addressed further in the fit-for-purpose approach described below.

Public health risk versus detection risk approach

Less rigorous evaluation approaches might be acceptable for use with food matrices that are rarely associated with foodborne illness, recalls, or pathogens due to intrinsic properties and evidence (as assessed through surveillance programs and reported isolations).

Figure 1 diagrams the interaction of public health issues and detection failure along a continuum in relation to the need for more rigorous matrix evaluation studies. As consumer risk increases, so does the level of matrix evaluation that may be required. In making this assessment, it is important to be current on the hazards associated with different matrices. For example, recently discovered associations include those between *Salmonella* and soy lecithin *(1)* or hydrolyzed vegetable protein *(18, 21)*. The inherent risk to the consumer presented by these matrices was previously considered to be low. Similarly, the association between Shiga toxin-producing *Escherichia coli* and flour came to prominence in the United States with some high-profile recalls in 2016 and again in 2019 *(2, 16)*.

TABLE 2. Core method conditions and associated validated matrix categories for five rapid screening methods and three reference methods for *Salmonella*

	Enrichment core conditions						Categories								
Reference	Test portion size	Enrichment stage	Ratio[a]	Broth identity	Time (h)	Temp (°C)	Dairy products	Meat and poultry	Egg products	Seafood	Fruits and vegetables	Miscellaneous foods	Animal feed	Spices	Environmental samples[b]
AOAC OMA 2011.03 *(8)*	Follow BAM or MLG	Primary	Follow FDA BAM or USDA MLG				X	X	X	X	X	X	X	X	X
		Secondary		SX2	22–26	42 ± 1									
AOAC OMA 2013.01 *(9)*	25 g, 375 g, 30 mL, sponge, swab	Single	0.1, 0.25	BPW + proprietary supplement	22–24	42 ± 1	X	X	X	X	X	X	X	X	s, p, t
AOAC OMA 2014.01 *(11)*	25 g, 100 g, 325 g, 375 g, sponge	Primary	0.1	BPW	18–24	41.5 ± 1		X	X	X	X		X		s
		Secondary		RV[c]	24[c]	41.5 ± 1[c]									
AOAC OMA 2016.01 *(12)*	100–375 g, 325 g, 30 mL, sponge, swab	Single	0.1	ISO BPW	24	37 ± 1		X	X			X	X		X
			0.25	ISO BPW (prewarmed)	18–24	41.5 ± 1[c]		X							s, c, t
AOAC OMA 2017.06 *(14)*	25 g, 375 g, 30 mL, sponge, swab	Single	0.1, 0.25, 0.5	BPW (prewarmed)	21 ± 1	37 ± 1	X	X	X		X		X		s, t, p, c
					10 ± 2[d]	36 ± 1[d]									
FDA BAM[e] Chap. 5 *(34)*	25 g, sponge, frog legs, pig ears	Primary	0.1	Lactose	24 ± 2	35	X	X	X	X	X	X	X		X
		Secondary		RV and TT	24 ± 2	42 ± 0.2 35 ± 2									
USDA MLG[f] Chap. 4.10 *(29)*	25 g, 100 g, 325 g, 30 mL, sponge	Primary	0.1, 0.2, 0.5	BPW	22–24	35 ± 2		X	X	X		X			X
		Secondary		RV and TT	22–24	42 ± 0.5									
ISO 6579-1:2017 *(24)*	25 g	Primary	0.1	BPW (prewarmed)	18 ± 2	34–38	X	X	X	X	X	X	X		X
		Secondary		RVS and MKTTn	24	41.5 37									

Core enrichment ratio varies for sponges and swabs and may vary for different test portion sizes.

[b]s, stainless steel; p, plastic; t, ceramic tile; c, sealed concrete.

[c]For materials with high background counts.

[d]For simultaneous detection of *E. coli* O157:H7.

[e];FDA BAM has many specific variations; the most common core method is shown here.

[f]USDA-FSIS *Microbiology Laboratory Guidebook* (MLG) data for core conditions of the most common enrichments. BPW, buffered peptone water; ISO BPW, buffered peptone water (ISO formula); lactose, lactose broth; MKTTn, Muller-Kauffman tetrathionate-novobiocin broth; RV, Rappaport-Vassiliadis broth; RVS, Rappaport-Vassiliadis broth with soy; SX2, a proprietary selective *Salmonella* enrichment broth similar to RV; TT, tetrathionate broth.

Fit-for-purpose approach: Matrix Evaluation Level Assessment Tool

Although matrix grouping and similarity of enrichment approaches are helpful in reducing the burden of test method validation, these factors are only half of the story. Both options rely on knowing the composition of the matrix to be tested and understanding how that composition may affect method performance. With current matrix grouping schemes (e.g., ISO, AOAC, FDA), defining the matrix is essential to determining the level of evaluation required for a matrix addition. However, intrinsic properties of the matrix may be unknown to the food producer or testing laboratory, making assessment of the matrix difficult when quick turnaround times are essential. Rather than starting with the question "To which food category does my matrix belong?", a risk-based assessment approach could be used.

With the variety of products that are produced or come through the door for testing, how do you decide how much, if any, evaluation is needed? To help address these questions, we have developed a Matrix Evaluation Level Assessment

TABLE 3. Core method conditions and associated validated matrix categories for five rapid screening methods and three reference methods for *Listeria* spp.

		Enrichment core condition					Categories								
Reference	Test portion size	Ratio[a]	Stage	Broth identity	Time (h)	Temp (°C)	Dairy products	Meat and poultry	Egg products	Seafood	Fruits and vegetables	Miscellaneous foods	Animal feed	Spices	Environmental samples[b]
AOAC OMA 996.14 *(5)*	25 g, sponge, swab	0.1	Primary	Modified Fraser with LiCl	26–30	30 ± 1	X	X		X	X				s, c, r
			Secondary	BLEB	22–26	30 ± 1									
AOAC OMA 999.06 *(6)*	25 g	0.1	Single	BLEB	48–50[c]	30 ± 1	X	X			X				
AOAC OMA 2004.06 *(7)*	25 g	0.1	Primary	Demi-Fraser	24–26	30 ± 1	X	X		X	X				
			Secondary	Fraser without FAC	24–26	30 ± 1									
AOAC OMA 2013.10 *(10)*	25–125 g, sponge, swab	0.1	Primary	LPT	26–30	30 ± 1	X	X		X	X	X		X	s, c, p, t
			Secondary	LPT	22–26	30 ± 1									
AOAC OMA 2016.07 *(13)*	25–125 g, sponge, swab	0.1, 0.2, in 10, 100, or 225 mL	Single	Demi-Fraser	28–30	37 ± 1	X	X		X	X				s, c, p
FDA BAM Chap. 10 *(35)*	25 g, sponge, swab	0.1	Single	BLEB + pyruvate	24–48	30	X	X	X	X	X	X	X		X
USDA MLG Chap. 8.11 *(30)*	25 g, 125 g, sponge(s), filter	0.1	Primary	UVM	20–24	30 ± 2		X	X	X					X
			Secondary	MOPS-BLEB	18–24	35 ± 2									
ISO 11290-1: 2017 *(25)*	25 g	0.1	Primary	Demi-Fraser	24–26	30	X	X		X	X				X
			Secondary	Fraser	22–26	37									

[a]Core enrichment ratio of test portion size to total enrichment volume; varies for sponges and swabs and may vary for different test portion sizes.
[b]s, stainless steel; c, sealed concrete; r, rubber; p, plastic; t, ceramic tile.
[c]Usually no supplements for first 4 h.
BLEB, buffered *Listeria* enrichment broth; FAC, ferric ammonium citrate; LPT, proprietary broth for this assay; UVM, University of Vermont broth; MOPS-BLEB, 3-(*N*-morpholino) propanesulfonic acid-buffered *Listeria* enrichment broth.

Tool available through the International Association for Food Protection Applied Laboratory Methods Professional Development Group homepage at https://www.foodprotection.org/upl/downloads/library/matrix-evaluation-level-assessment-tool.xlsx. This tool is intended to guide an end-user through appropriate questions to consider before testing the product.

The first item to consider is whether the method planned for use is validated for the specific matrix at the intended test portion size. If so, the method is fit-for-purpose for that product, and no further evaluation needs to be completed, assuming a first use verification as described above has already been performed. If not, ask "Is the method validated for the food category of interest?" If the method is not validated for the food category, a full matrix validation based on AOAC Appendix J or ISO 16140-2:2016 may need to be performed or an alternative method or platform chosen. If the method is validated for the food category and more generally validated for a broad range of foods, consider whether a similar matrix has been validated at a similar test portion size and enrichment ratio. Depending on the answers to these questions, different levels of evaluation are recommended. For example, if the method is validated for the category of interest and the matrix tested as part of the validation is similar enough (with respect to the intrinsic properties listed in *Table 1*), a fit-for-purpose test can be performed. However, if the method is not broadly validated, a risk assessment may need to be conducted to determine the level of evaluation needed.

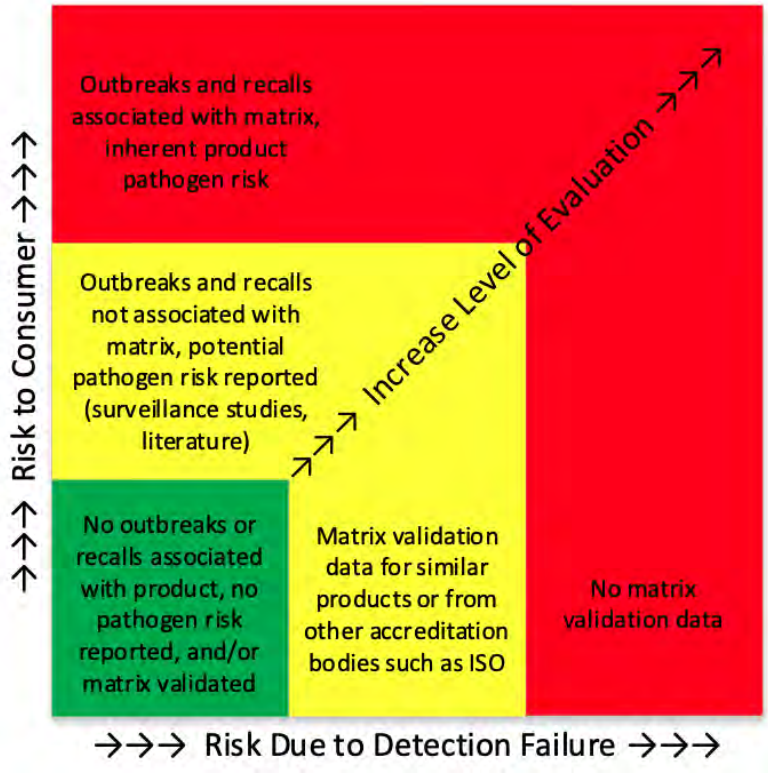

FIGURE 1. Evaluation level determined by public health risk versus detection risk.

Fit-for-purpose test

A fit-for-purpose test is a cost-sensitive approach that provides essential information as to whether the proposed method should be used at all for the new matrix by screening out obvious detection issues (e.g., false negatives). The test determines whether the enrichment protocol required by the method allows propagation of the target organism to detectable levels and whether obvious matrix effects are known that interfere with the assay's chemistry or technology. There are many known inhibitors for different technologies, such as collagen, humic acid, calcium ions, and polyphenolic compounds, among others, that inhibit polymerase chain reaction *(15, 22)* or, alternatively, create physical barriers to the process. Furthermore, certain herbs and spices may have antimicrobial or bacteriostatic properties, making growth of the target organism in the enrichment problematic. Therefore, additional dilution of the matrix during enrichment is common, if there are known spices or herbs in the sample. Knowing whether potential inhibitors are present in your matrix beforehand can be useful in choosing a method; however, a fit-for-purpose test can quickly provide evidence that the method is suitable for detection of the organism. This could provide an appropriate level of evaluation, clarify whether to proceed to a full AOAC OMA Appendix J-style matrix validation, or highlight the need to choose another method. Alternative matrix evaluation approaches are outlined using the decision tree in *Figure 2*.

A fit-for-purpose test could take many forms, depending on the risk level of the sample. Performing a matrix extension following FDA guidelines (Section 5.1.1), where unknown samples are tested in parallel with spiked samples (20 to 30 CFU per test portion) until 7 of 7 or 19 of 20 spikes show recovery of the organism may be sufficient *(36)*. However, if spiked samples do not show recovery, sample results must be invalidated. When spiking samples, it is considered best practice to use appropriately stressed cultures, if practical. This would more closely replicate the state of pathogenic cells in "real-world" samples. There are options to purchase lyophilized cultures at different concentrations that are a convenient way to meet this guideline to mimic low moisture stress where applicable (e.g., dry powders). Another type of inoculum stress includes heat stress to mimic thermal processing of some food products (e.g., ready-to-eat deli meat). For higher risk, or quantity-limited samples, it may be best to perform a fit-for-purpose test before testing any samples. In this case, three test portions of the matrix would be spiked at 20 to 30 CFU per test portion and tested by the candidate method and reference method, and three test portions would be unspiked (0 CFU per test portion) controls. If all three spiked portions are positive and all

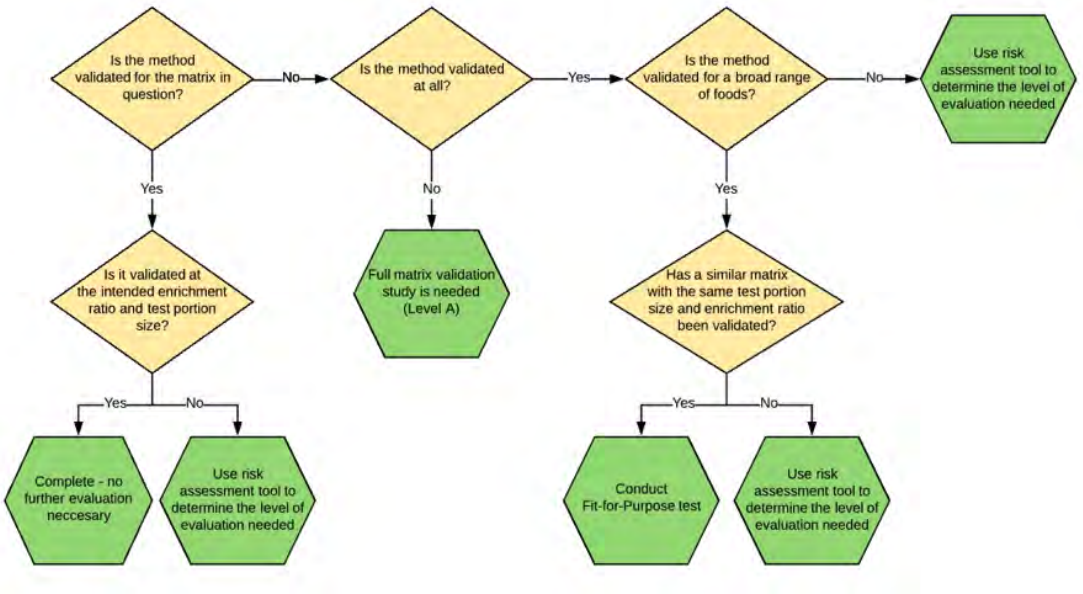

FIGURE 2. Decision tree for directing alternative matrix evaluation approaches.

three unspiked portions are negative by the candidate method, the method can be considered suitable for that matrix. Further evaluation may not be needed but can be conducted if desired based on the risk level of the product. However, if these results are not obtained, additional method development may need to be performed or an alternative method chosen. More details on specific outcomes of this fit-for-purpose test are discussed in the Matrix Evaluation Level Assessment Tool. Depending on the extent of method development needed, a fit-for-purpose test could be conducted again, or if method changes are substantial, an AOAC-style OMA Appendix J validation may need to be conducted for that matrix. Finally, if a very low risk for that matrix is anticipated, a simple United States Pharmacopeia (USP)-style suitability test can be performed. This option is inspired by the USP suitability tests *(37, 38)*. USP suitability tests spike a single sample at a higher level (<100 CFU per sample), and if the spike is recovered by the assay then the method is fit-for-purpose.

How much evaluation is needed: Risk assessment

What if the method is validated, but not for a wide range of foods or for the particular matrix of interest? The Matrix Evaluation Level Assessment Tool provides a scoring system to determine how complex the new matrix is and suggestions for experimental designs to ensure the method is fit-for-purpose for the new matrix. These suggestions are based on a numeric score obtained by answering a set of risk-based questions about the matrix. It is important to note that this tool only considers potential scientific risk factors for why a method may not be ideal for the new matrix. It does not consider any intent-of-use (e.g., ready-to-eat foods) or market risk factors (e.g., quantity of product for distribution) that may be present with the new matrix.

This tool guides consideration of how closely related the new matrix is to a matrix in the validated scope of the method. In general, the more closely related the new matrix is to a validated matrix in the method, the less extensive the matrix evaluation needs to be. Similar to the method assessment, if a similar food matrix has been validated at the intended test portion size and enrichment ratio, there is less risk of the method being unsuitable. Most official validations test performance at 25-g test portion size; however, larger composite test portions are common for end-user testing. Composite test portions require a lower limit of detection of the method (i.e., 1 CFU per 25 g versus 1 CFU per 375 g) and rigorous evaluation is highly recommended. If the matrix has a high-risk association with the target analyte, such as previous known outbreaks, the risk, and the score for that matrix, will increase. In addition, additives or variations of food products may impact method performance. Therefore, the tool also guides consideration of inclusions that might present pathogen risk or impact growth conditions. Inclusions that are present (or sufficiently similar) in the method validation and use the same growth conditions (e.g., incubation time, temperature, and media) will require less evaluation than those that do not fit this criterion. Finally, if there are any known inhibitory properties (see above discussion) of the matrix or inclusions, a fit-for-purpose test is recommended to screen out obvious detection issues.

Evaluation levels

Results of the Matrix Evaluation Level Assessment Tool will provide a score, with higher numbers indicating higher risks associated with the matrix. This numerical score

TABLE 4. Evaluation levels

Evaluation level	Number of spiked test portions	Inoculation level, CFU/test portion	Inoculating cells	Analysis
Full matrix validation	5	2-10	Fresh culture or heat stressed	Presumptive results compared with confirmation results and reference method to demonstrate no statistical difference between the methods
	20	0.2-2		
	5	0		
Moderate matrix evaluation	2	2-10	Fresh culture or heat stressed	Presumptive results compared with confirmation results to demonstrate no deviation in candidate method result compared to culture confirmation
	10	0.2-2		
	2	0		
Minimal matrix evaluation	1-7	20-30	Fresh culture or heat stressed	Candidate detection results for inoculated anduninoculated samples should match input
	0-1	0		

translates into a suggested evaluation level for that matrix *(Table 4)*. For matrices with highest risk, a full matrix validation based on AOAC OMA Appendix J or ISO 16140-2:2016 is recommended *(4, 26)*. This includes test portions analyzed at a "high" inoculation level (2 to 10 CFU per test portion), a "fractional positive" inoculation level (0.2 to 2 CFU per test portion), and uninoculated controls. For each level of each matrix, the candidate method is compared with the reference method and a probability of detection (POD) for each is calculated. These PODs are compared to conclude whether the two methods are statistically different. The next lower level of evaluation follows a very similar experimental design but approximately halves the total number of samples analyzed. This can speed up evaluation and reduce costs, while providing a substantial amount of data, although not enough for statistical analysis.

The final suggested level of evaluation provides a range of options for low-risk samples. It includes the FDA's method verification guidelines, where seven of seven spiked test portions resulting in presumptive positive results are required for the method to be considered fit-for-purpose *(36)*. In addition to the spiked test portions, unspiked test portions can be analyzed to account for any interference in the matrix that may result in false positive results. However, for some low-risk, rarely tested samples, this much evaluation may not be feasible or practical. This level also provides an option inspired by USP suitability tests, where a single sample is spiked at a level of <100 CFU per sample; if the spike is recovered by the assay, the method is fit-for-purpose.

Although there are many potential paths to ensuring a fit-for-purpose method, this screening tool can provide an independent assessment with suggested actions to ensure the highest quality data and safest outcomes while minimizing time and cost. Notably, it should be in the food manufacturer's interests when developing new products to consider the analytical needs that will be required to release the products. Being proactive in this area during new product development could reduce long-term risk and cost.

CONCLUSION

Balancing practicality, risk, and science

Industry often groups similar products in food safety plans and uses one process validation for like products as part of the verification procedures. For example, verification of time, temperature, or flow rate of similar food products produced with the validated process is often considered adequate. Likewise, would science-based grouping for evaluation of testing methods be acceptable for products similar to those already validated? Clearly not all of the 20,000 new food products developed each year can undergo full matrix validation as defined by the various method validation bodies such as AOAC. Neither the resources nor the justifications are available in terms of time or associated costs. Instead, it may be better to reduce risk by evaluating more matrices with alternative approaches for matrix additions than comparatively evaluating fewer matrices due to prohibitive testing requirements. Increasing confidence in testing applications and reducing barriers for new method innovation and adoption will aid in moving industry food safety forward.

Matrix evaluation level assessment tools only provide part of the input intended for conducting a risk analysis for food safety. Additional important components include risk management and risk communication *(40)*. Risk management involves weighing other factors such as cost, time, and potential public health threat. Communicating decisions based on risk assessment findings and risk management completes the risk analysis and allows industry to determine the most appropriate approach to ensure food safety.

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GLOSSARY

1. **Evaluation** - Process by which test methods are assessed for use with a matrix of interest.
2. **Fit-for-purpose** - Degree to which data produced by a microbiological detection method enables a user to make technically and administratively correct decisions for a stated purpose (modified from ISO 16140).
3. **Fit-for-purpose test** - An initial screening to rule out gross method detection failure with a specific matrix.
4. **Full validation** - A full validation, e.g., AOAC Appendix J *(4)*, is recommended for a risk assessment score of 13+ by using the Matrix Evaluation Level Assessment Tool.
5. **Matrix addition** - Process of extending a test method for use with a new matrix that was not part of the validation study.
6. **Matrix Evaluation Level Assessment Tool** - Tool designed to help assess the risk level and subsequent recommended study design for using a desired test method on a matrix that is not part of the validation study.
7. **Matrix extension** - The means to demonstrate that the method can detect and identify an analyte (without any modification) in the user's laboratory on matrices for which it has not been validated and the food is not within the same category (AOAC, ISO) included in the original study. An accredited matrix validation study protocol is used *(4, 36)*.
8. **Matrix verification** - The means used to demonstrate that the method can detect and identify an analyte (without any modification) in the user's laboratory in a new food from the same category (AOAC, ISO) used in the original method validation. A matrix spike protocol is used *(36)*.
9. **Method implementation verification, first use verification** - An initial demonstration of acceptable laboratory capability to perform a method accredited by a third-party method validation body (e.g., Association Française de Normalisation, MicroVal, AOAC). The laboratory demonstrates that a matrix included in the scope of the method performs as expected in their laboratory. Six replicates of both an inoculated and uninoculated food matrix are tested with both the new alternative and the reference method. If no false negative or positive results are obtained, the method is verified to function in the user's laboratory on any matrix included in the scope or added through a matrix extension.
10. **Method validation** - Confirmation by examination and provision of objective evidence that the particular requirements for the specific use of a method are fulfilled. Serves to demonstrate that the method can detect and identify an analyte or analytes reliably for its intended purpose with a demonstrated sensitivity, specificity, accuracy, trueness, reproducibility, ruggedness, and precision to ensure meaningful results. Methods are typically validated by method developers using protocols developed by method validation bodies such as AOAC or standards bodies such as ISO *(36)*.
11. **Method verification** - Confirmation by examination and provision of objective evidence that the specified requirements for the performance of a method have been fulfilled by an individual laboratory. Also, the means used to demonstrate that the method can detect and identify an analyte (without any modification) in the user's laboratory on matrices not included in the original method validation *(36)*.
12. **Minimal evaluation** - Recommended for a risk assessment score of 2 to 5 using the Matrix Evaluation Level Assessment Tool.
13. **Moderate evaluation** - Recommended for a risk assessment score of 6 to 12 using the Matrix Evaluation Level Assessment Tool.
14. **Validation of an alternative method** - Demonstration that adequate confidence is provided when the results obtained by the alternative method are comparable to those obtained using the reference method using the criteria contained in an approved validation protocol *(36)*.

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