Selection of Pathogen Strains for Evaluating Rapid Pathogen Test Methods Applied to New Matrices

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SUMMARY

Before first use of a validated method, laboratories verify their ability to apply the method as designed. In routine laboratory operations, new matrices will appear occasionally, with insufficient data ensuring method performance for the matrix. Approaches have been documented to the "fitness for purpose" testing then required, but the question of how to select the pathogen strain or strains for this activity has received scant attention. This article reviews factors that may influence strain selection for method evaluation, including processing environment, geographical origin or proximity, seasonality, environmental factors, intrinsic characteristics of matrices, public health data, and the logistics, cost, and complexities involved in managing large challenge-strain collections. We conclude that food safety is served best when laboratories conduct method application studies for new matrices with one or more appropriately stressed members of a small, conveniently managed panel of challenge strains. However, if stakeholders have clear knowledge of a strong link between the matrix and a particular strain of concern, that would be a reason to favor acquisition and use of that strain. The worst approach is to not conduct application studies because of perceived limitations in accessing one or more highly specific strains.

OVERVIEW

Analytical methods for detecting microbial pathogens must be validated. Method validation is defined in International Standards Organisation (ISO) 16140-2 (43) as "the establishment of the performance characteristics of a method and provision of objective evidence that the performance requirements for a specific intended use are fulfilled." Validation is a rigorous experimental process that examines inclusivity, exclusivity, sensitivity, and robustness. Inclusivity testing determines a method's ability to detect strains or isolates of the target pathogen and should cover the genetic, serological, and biochemical diversity of the target. Certification bodies such as the Association of Official Analytical Collaboration (AOAC) International, Association Française de Normalisation, and others typically require 50 strains of the target pathogen for inclusivity testing. However, in the case of *Salmonella*, there are more than 2,500 recognized serotypes; therefore, the inclusivity requirement increases to at least 100 serotypes (3). At the time of writing, AOAC International is asking for these representative serotypes to include three strains from each of the *Salmonella enterica* subspecies and *Salmonella bongori* (36). Selection of suitable strains for method validation is critical to understanding method limitations (8, 10).

Once the method is formally validated and accredited, its performance in an individual laboratory should be verified before use. Method verification is defined in ISO 16140-3 (44) as "the demonstration that a validated method performs, in the user's hands, according to the method specification determined in the validation study and is fit for its intended purpose." Verification within a single laboratory may include only a single strain (44, 83).

The use of stressed microorganisms during validation of microbiological methods is intended to mimic the sublethal stress that may occur as a result of product manufacturing or environmental management procedures and thus the ability of the method to recover and detect low numbers of these viable organisms. ISO 16140-2 (43) prescribes stresses related to processing conditions, including heat (50°C for 15 min), cold or freezing, pH, and low water activity (a_w) , along with resource competition from a high intrinsic background microflora. Guidelines for AOAC International certification (3) have similar requirements. Parameters for imposing stress on the challenge strains may

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vary substantially. In dry products, for example, commercially available lyophilized cultures may be used or wet cultures may be dried on a carrier before inoculation of the matrix. In either case, general criteria then include a period of equilibrium. For dried foods, application of a dried inoculum is followed by storage at ambient temperature for a minimum of 2 weeks. For both raw and heat-processed foods, the inoculum is allowed to equilibrate in the matrix for 48–72 h at 4°C or for a minimum of 2 weeks at -20° C in the case of frozen foods (3, 43). In the case of environmental surface matrices, AOAC International recommends the use of microorganisms that have reached the stationary phase (3) before inoculation of the surface and surface drying at ambient temperature for 16-24 h before sample collection and testing. Where feasible, the laboratory should attempt to demonstrate the degree of stress or injury imposed by plating the inoculum pre- and postinjury with suitable recovery media. Another consideration is the microbial load on the matrix. Competitive microflora may suppress outgrowth of the target pathogen during enrichment steps. For validation studies, nontarget microorganisms that are known to share phenotypic characteristics with the target pathogen are inoculated at a concentration 10 times greater than that of the target pathogen. For validated and accredited methods, first use in a laboratory is preceded by verification. This is generally conducted using a small set of inoculated and uninoculated samples and demonstrating correct performance of the method with, usually, only a single strain of the target pathogen. First-use verification is not within the scope of this article but does have elements in common with matrix application studies.

In this general-interest paper, we address only methods that have been properly validated and verified for use in a particular laboratory. A laboratory will inevitably face the occasional challenge of a novel matrix and the need to determine whether available methods are fit for purpose. This may involve additional method verification or validation studies to ensure appropriate application of the method for use with the new matrix (5). However, little attention to date has addressed how to determine which strain or strains of a target pathogen should be selected for such a study. This may be challenging when considering the more than 2,500 serovars for a pathogen such as Salmonella. Considerations may include known or documented associations with the matrix of interest, outbreaks, geographical and seasonal considerations, growth or survival characteristics, and even access to strains for the purpose of study.

In this paper, we discuss the relevance of these and other considerations to the selection of strains for matrix validation and verification studies.

WHAT AFFECTS THE STRAINS PRESENT IN A PARTICULAR MATRIX?

Process environment

For processed food products, the facility's environment is likely the most relevant factor for the selection of a

strain. Certain well-adapted subtypes can persist in the processing environment and be a source of recurrent product contamination (39). For example, *Listeria monocytogenes* found in ready-to-eat (RTE) foods is often connected to postprocess contamination from the production environment (87). Similarly, *Salmonella* has been shown on equipment within poultry houses (62), chicken processing plants (57), and rendering plants (33), as well as to transfer from slicing blades to tomato surfaces (88). Cross-contamination by *Salmonella* within multiple production processes and environments has been extensively reviewed by Carrasco et al. (11) and can account for a significant fraction of the incidence on a product, with genetically identical isolates found on equipment at different points in the process.

Agricultural source of raw materials

Peanuts, leafy greens, and other raw agricultural commodities may have multiple species, serotypes, and/ or subtype associations (9, 59, 75). Consideration to strain selection could include known affinity with a particular matrix or growing region, or it could be isolated to a particular local farm environment. Outbreak investigations have identified strain associations as localized, resulting from either point sources, e.g., sewage effluent spills or runoff from on-farm manure stockpiles, or nonpoint sources, including wildlife and/or livestock fecal droppings (80, 81, 82). These mechanisms can introduce a particular strain to the environment, where persistence increases the potential for food contamination (80). However, persistence factors are generally associated with a pathogen species rather than a particular strain. For example, L. monocytogenes thrives naturally in the environment and has great propensity to contaminate raw agricultural commodities. Salmonella and Escherichia coli O157:H7 endurance may be limited by temperature, sunlight (UV exposure), moisture level, pH, available nutrients, and related factors. However, if favorable conditions exist, either pathogen, regardless of strain identity, may persist and contaminate raw agricultural commodities (80). As such, it is appropriate to consider inclusion of localized and specific strains, when available, for any commodity or matrix if the stakeholder determines that the strain could potentially contaminate a product.

Geographical considerations

Raw agricultural commodities tend to dominate growing regions where they are adapted to the climate. In peanuts, for example, serotype association seems to be regional (9) (*Table 1*). However, the raw peanuts sampled (9) were shelled, and the apparent presence of regional strains may be more the result of contamination at the sheller than of the growing region. Similarly, beef, pork, poultry, or seafood products may be produced in particular geographical regions, each of which may have unique prevalent serotypes or strains. For example, *Salmonella* Enteritidis is most prevalent in

	snelled peanuts [*]				
Crop year	Peanut type or grade	Region of origin	Salmonella serotype	MPN/g <0.003	
2013	Virginia extra large	Virginia–North Carolina	Bardo/Newport		
2013	Runner jumbo	Southeast	Bardo	< 0.003	
2010	Extra large	Virginia–North Carolina	Agona	< 0.03	
2010	Extra large	Extra large Virginia–North Carolina Agona		< 0.03	
2010	Extra large	Virginia–North Carolina	Agona	< 0.03	
2010	Extra large	Virginia–North Carolina	Agona	< 0.03	
2010	Extra large	Virginia–North Carolina	Agona	< 0.03	
2010	Extra large	Virginia–North Carolina	Agona	2.4	
2010	Medium	Southeast	Anatum	< 0.03	
2010	Runner split	Southwest	Braenderup	< 0.03	
2010	Runner split	Southwest	Muenchen	< 0.03	
2010	Runner split	Southwest	Anatum	< 0.03	
2010	Runner split	Southwest	Roodepoort	< 0.03	
2010	Runner split	Southwest	sp. C(1):m,t	< 0.03	
2010	Runner split	Southwest	sp. C(1):m,t	< 0.03	
2010	Runner split	Southeast	Dessau	< 0.03	
2009	Runner split	Southeast	Dessau	< 0.03	
2009	Runner split	Southeast	Dessau	< 0.03	
2009	Runner split	Southeast	Hartford	0.036	
2009	Runner split	Southeast	Tornow	< 0.03	
2008	Runner split	Southwest	sp. G(1):b;—	0.036	
2008	Runner no. 1	Southeast	Tennessee	< 0.03	
Unknown	Runner split	Southeast	Dessau	< 0.03	
Unknown	Runner split	Southeast	Meleagridis	< 0.03	

TABLE 1. Serology and most probable number (MPN) of Salmonella isolates from raw, shelled peanuts^a

^{*a*}Adapted from Calhoun et al. (9).

poultry in Asia, Latin America, Europe, and Africa, whereas *Salmonella* Kentucky, Typhimurium, and Sofia are most prevalent in poultry in North America (27) (*Fig.* 1). Within regions in North America, *Salmonella* Tennessee has been found in poultry in the Southeast United States (86), whereas *Salmonella* Litchfield has been found around the Seattle region in the Northwest (62).

Seasonality

Human salmonellosis has often been shown to exhibit seasonal variability (14, 54, 63, 67, 85, 96). For example, in Florida, the average annual peak is in October and the annual low is in February, and there is a clear annual cycle in case numbers (54). It is less clear that there is a seasonal association with the incidence of *Salmonella* in foods such

as chicken (85, 93), whereas there is a strong association between foodborne illness and human seasonal activities (85). Therefore, seasonality has a greater effect on the consequences of pathogen presence than on the specific strain present and is likely not an important consideration for strain selection.

Matrix characteristics

There is some association between pathogens and matrices. For example, *Listeria* is often associated with chilled foods such as soft cheeses; *E. coli* O157:H7 is often associated with ground beef and, increasingly, with leafy greens; and *Salmonella* is often associated with raw poultry. However, at the level of strain or serovar, it is less clear that there is any specific association with the matrix. For example, multiple serotypes

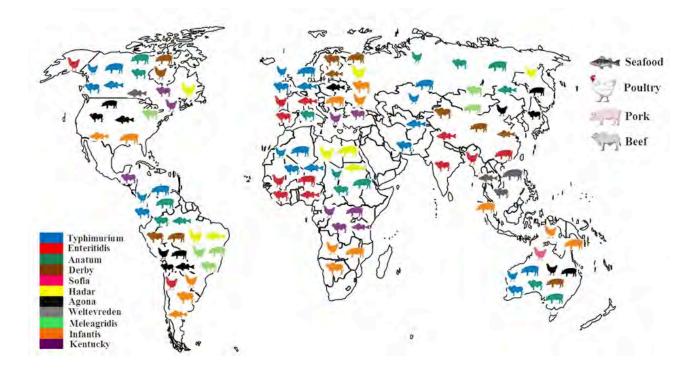


Figure 1. Worldwide distribution of *Salmonella* by Matrix. Republished with permission of American Society for Microbiology, from Ferraria et al. (27); permission conveyed through Copyright Clearance Center, Inc.

of *Salmonella* have been associated with outbreaks linked to chocolate products, but none stand out as uniquely associated with the product (*Table 2*). Upon investigation, cross-contamination from the facility's production environment was reported as the root cause for most of these outbreaks (4). More examples were covered in "Process Environment."

Illness caused by *L. monocytogenes* serotypes 1/2a, 1/2b, and 4b is most often linked to cold-stored RTE foods (49), and *L. monocytogenes* contamination events involving RTE product outbreaks can often be traced to cross-contamination from the production environment. Root cause analysis often reveals multiple *Listeria* species and strains present that can even evolve, although minimally, in different harborage sites of processing facilities (73, 95).

Ryan et al. (68) showed that some strains can be particularly difficult to detect when evaluating rapid methods with challenging matrices, but that seems to be a feature of the strains, not the matrices. One hopes that such strains are present in validation inclusivity studies within the certification-body guidelines. Probably the most important effects of the matrix on pathogen detection include inhibition of outgrowth during enrichment culture or interference with the rapid detection technology. When a method is already validated, these effects have more to do with the new matrix and method of sample preparation than with any strain used to verify it.

STRAIN GROWTH AND SURVIVAL CHARACTERISTICS

Food matrices vary considerably with regard to physical and chemical composition. Several intrinsic factors of a sample, such as a_{μ} , pH, and antimicrobials, can influence whether an organism can survive or grow (79). Food manufacturers modify these intrinsic factors to preserve food, and such modification may trigger stress responses in bacteria that result in generally higher stress tolerance (35, 52). In some circumstances, cells may enter a viable, but not culturable, state, which makes detection by culture methods difficult or impossible, although culture-independent methods may still be useful (28).

The adaptation of particular strains to stresses applied during processing is critically important to process validation, in which surrogates for the pathogen of concern are often used for validation studies (12, 13), which are outside our scope. However, one could imagine that individual strains or serovars that are more tolerant of the intrinsic properties of a particular matrix would be more able to grow to detectable levels in an enrichment containing that matrix.

Heat resistance

The heat resistance of *Salmonella* is highly influenced by the strain tested, the type of experiment (log reduction versus end point), culture conditions before the experiment, and

Salmonella serotype	Alternate strain source	Alternate strain origin	Vehicle	Outbreak date	Reference
Durham	NAª	NA	Cocoa powder	1972	(30)
Eastbourne	NCTC 5771	Kauffmann F State Serum Institute, Copenhagen	Chocolate products	1974	(16,72)
Napoli	NCTC 6853	Italian food handler	Chocolate bars	1982	(31, 34)
Nima	NA	NA	Chocolate coins	1985–1986	(38)
Typhimurium	ATCC 14028	Heart and liver from 4-week-old chickens	Chocolate products	1987	(48)
Oranienburg	rg ATCC 9239 Outbreak of food poisoning at an Illinois state hospital Ch		Chocolates	2001-2002	(90)
Montevideo	ATCC BAA-710	Human clinical specimen: salmonellosis from tomatoes, 1993	Chocolate tablets	2006	(23)

TABLE 2. Salmonella serotypes associated with chocolate outbreaks

^aNA, not applicable.

heating and recovery conditions (21). The heat resistance of Salmonella and other bacterial species can increase if cells are preincubated (64) or heated in low a_w media obtained by adding solutes such as sodium chloride (15, 58, 65), sucrose, glycerol, and fructose (32, 71). However, this increase may be transient or depend on both the subsequent heating temperature and the solute used for a_w reduction (61). Although relevant to the protocol or protocols used for creating heat-stressed cells for inoculation studies, heat resistance probably has little to do with the ability of enrichment cultures to recover the survivors.

Acid tolerance and pH

Foods with a pH below 4.6 generally do not support pathogen growth (41, 79). However, the acidity of the matrix can influence the pathogen or pathogens of concern. In *Salmonella*, acid tolerance is induced below pH 4.5 (51). The sustained acid tolerance response works through the alternate sigma factor (σ^{s}) produced on expression of the gene *RpoS* and can trigger protection against other stresses, including salts, H₂O₂, and more (1, 20, 26, 89). Acid tolerance can play a role in pathogen recovery. For example, recovery of Shigatoxigenic *E. coli* can be improved when using a short-duration acid treatment to reduce background flora before plating. However, this property is not considered to differentiate among strains (78). Acid stress is not considered in AOAC International method validation guidelines (3), although pH stress is included in ISO 16140-2 (43).

a and salt tolerance

a is a measure of the availability of water in a matrix. It declines as the concentration of salt, sugars, or other solutes increases. Most bacteria have an optimum a value between 0.97 and 0.99 for growth, and foods within this a_{μ} range are recognized as highly perishable, including fresh meats, vegetables, and fruits. As a decreases, microbial growth can decline to a state of dormancy (41, 42), but as noted earlier, reduced a may modify heat resistance. Habituation of Salmonella at an a_{y} of 0.95 increased heat resistance, but this effect was stronger with some solutes (glucose-fructose) than with others (sodium chloride or glycerol). The increase was greatest after around 12 h of habituation and faded after 72 h (60). Enrichment protocols for dry foods have long recognized that more care may be needed to appropriately resuscitate cells stressed by long exposure to a low a environment; however, it is unclear whether this should affect strain selection for matrix validation or verification; rather, this is more likely a consideration for inducing stress in the protocol.

Antimicrobials

Some foods and samples may contain natural or synthetic compounds that exert antimicrobial activity against target pathogens. These may be naturally occurring in the matrix, deliberately added (e.g., preservatives), or a result of carryover from processing or environmental exposures (i.e., sanitizers). If present at sufficiently high concentrations, these compounds may inhibit growth during the enrichment culture step. This should be considered when designing a matrix validation or verification study based upon a stakeholder's insight into the respective formula, source, environment, etc., but it is unclear whether it would affect strain selection.

Strain genomics

The abundance of a strain in a host or ecosystem might correlate with how well that organism can infect and spread. *Salmonella* strains that predominated in a beef slaughterhouse carried plasmidborne type IV secretion systems, which have been linked to persistent infections in numerous pathogens (17). This study showed that identifiable genetic elements could be linked to persistence of particular strains in a defined environment. However, this study did not explore the effect of these genetic elements on outgrowth of the strains in culture media. Hence, we can draw no conclusions about their significance for matrix validation and verification.

CLINICAL CONSIDERATIONS AND OUTBREAK DATA

An analysis of FoodNet data from 1996 to 2006 differentiating Salmonella serotypes by clinical severity found that the serotypes Salmonella Enteritidis and Salmonella Heidelberg had significantly higher case fatality, hospitalization, and incidence rates, with a higher proportion of invasive disease than Salmonella Typhimurium. Conversely, Salmonella Newport had a significantly lower proportion of invasive disease than Salmonella Typhimurium, as well as a lower case fatality rate (47). In a study of spatial and temporal patterns of Salmonella infections in Florida, the most prevalent Salmonella serotypes were Enteritidis, Newport, Javiana, Sandiego, and Braenderup (54). Risks would seem to rise when the prevalent serovars are those associated with more severe clinical outcomes. We would clearly want to be able to detect strains with the greatest potential to cause illness. This is addressed by inclusivity testing during method validation, for which purpose the foodborne relatedness measure of Luvsansharav et al. (56) could help with serotype selection.

Although all strains of *Salmonella* are considered infectious, some are more virulent than others. For example, multi-drug-resistant *Salmonella* strains cause increased severity and more prolonged disease in infected patients (24) and are becoming more prevalent in outbreaks, including from chicken in 2014 (*Salmonella* Heidelberg), cucumbers in 2015 (*Salmonella* Poona), papayas in 2017 (*Salmonella* Urbana), poultry in 2018 (*Salmonella* Infantis and *Salmonella* Reading), and pig ear pet treats in 2019 (19, 75). However, these strains are no harder to detect with rapid methods. Hence, it may be appropriate to use less virulent nonoutbreak strains for matrix validations.

L. monocytogenes serotypes 1/2a, 1/2b, and 4b are responsible for 98% of documented human listeriosis cases (55). Serotype 4b strains are isolated mostly from epidemic outbreaks of listeriosis, whereas serotypes 1/2a and 1/2b

are linked to sporadic *L. monocytogenes* infection (46, 92). The serotypes most frequently involved in human listeriosis (1/2a, 1/2b, and 4b) should be part of the inoculum for *Listeria* challenge studies in foods (70) and among the inclusivity strains for validation of *Listeria* detection methods. In an outbreak investigation associated with a known strain and unvalidated matrix, it would be valuable to extend the matrix validation using the outbreak strain, if available. For most other purposes, outbreak strains probably have less relevance for matrix validation, which is convenient because they can be challenging to obtain and only 42% of outbreak strains can be traced back to a specific source (18).

Availability: isolate sourcing and considerations

Sourcing and maintenance of isolates can be both logistically challenging and costly, often involving shipping permits and/or material transfer agreements and possibly restrictions on, or additional fees for, commercial use. The risks associated with maintaining a culture collection must be managed, e.g., through access restriction and appropriate measures for disposal. In addition, isolates must be properly stored, maintained, and monitored to ensure viability and purity over time.

Many microbial strains and variants can be secured through organizations that specialize in culture collection maintenance and distribution, such as the American Type Culture Collection (ATCC) and Agricultural Research Service (ARS) Culture Collection at the Northern Regional Research Laboratory (NRRL). These bodies have diverse banks of foodborne pathogen isolates from agriculture, food, environmental, and human origins (2, 77). An example of the regional presence of different *Salmonella* serovars available from ATCC is shown in *Table 3*.

There are also many reference collections for specific pathogens, generally in government or academic institutions. These may be ideal sources of genus, species, serovar, and strain diversity. As an example, Dr. Martin Wiedmann and colleagues in the College of Agriculture and Life Sciences at Cornell University were selected by the technical committee of the former International Life Sciences Institute of North America, now the Institute for the Advancement of Food and Nutrition Sciences, to house their Salmonella, Listeria, and Cronobacter collections (29, 40, 45, 91). These are maintained with additional pathogen and spoilage organism collections, and many of these collections have been described in the literature (37, 74). Wiedmann and his colleagues have also worked to create tools such as the Food Microbe Tracker (https://www.foodmicrobetracker. net) to collaboratively capture information on biodiversity in foodborne pathogens (84).

Federal and state agencies, particularly those with a research arm, may serve as sources of isolates. Such centers include the U.S. Department of Agriculture (USDA) Meat Animal Research Center, the U.S. Food and Drug

Serovar	Isolates in the ATCC catalog	Geographical association					Other association			
		SE US (86)	Maryland (66)	Louisiana (53)	Seattle (62)	India (50)	Egypt (22)	General	Clinical	Common
Typhimurium	61	Х	Х		Х	Х	Х	Х	Х	Х
Enteritidis	10	Х	Х	Х	Х	Х	Х	Х	Х	Х
Thompson	4			Х						Х
Montevideo	2			Х		Х			Х	
Newport	2					Х			Х	
Pullorum	2					Х		Х		
Senftenberg	2				Х			Х	Х	Х
Braenderup	2			Х						
Cerro	2								Х	Х
Anatum	2									Х
Javiana	2									Х
Virchow	1							Х		
Dublin	1								Х	Х
Worthington	1									Х
London	1									Х
Muenchen	1									Х
Bredeney	1								Х	
Hadar	1									Х
Mississippi	1									Х

TABLE 3. Common Salmonella serovars available from ATCC on 2 February 2021, with geographical and other source indications

Administration (FDA) Center for Food Safety and Applied Nutrition, the USDA Animal and Plant Health Inspection Service's National Veterinary Services Laboratories, or as previously mentioned, the ARS Culture Collection at NRRL. Members of many institutions are also networked through organizations such as the U.S. Culture Collection Network sponsored by the National Science Foundation, the European Culture Collections' Organisation, or the World Federation for Culture Collections (*6, 25, 69, 76,* 77) The International Depository Authority via the World Intellectual Property Organization retains a list of culture collection institutes by country (*94*).

Contract laboratories may retain culture collections, particularly laboratories specializing in method and matrix validation or verification activities.

DO STRAINS FOR METHOD AND MATRIX VALIDATIONS AND VERIFICATIONS DIFFER, AND WHY?

Given the differences between the method validation and the matrix validation and verification outlined in the introduction, we can see that these activities have different purposes. Hence, the strains for method validation do not need to be the same as those for matrix validation and verification, and there are good reasons why they should be different.

For method validation, selection of the inclusivity strains should include examples reflecting the potential range of strain origins and the characteristics described earlier. Preparation of inoculated samples takes into account the stresses needed. These considerations are covered in the validation guidelines from AOAC International (3) and others.

Verifying or validating the method for use with a matrix not included in the original validation is intended to identify major deficiencies in performance and, if necessary, illustrate method performance with that matrix. The amount of testing needed can depend on the similarity of the new matrix to a validated matrix, as well as other factors affecting the riskiness of the matrix, and an approach to this decision was presented by Brown et al. (7). For this purpose, a single strain generally is selected, commonly one associated with the matrix, and preferably is isolated from the matrix production environment, ingredients, or final product. Use of strains that have created previous contamination issues in a plant environment or persistent contaminators that have eluded sanitization techniques can give increased confidence in the method. However, this approach is not always possible, it ties the matrix validation to a specific manufacturer's product, and it does not allow generic use of the matrix validation study.

CONCLUSION

Small and large inhouse laboratories and commercial laboratories often do not have the resources to gather, identify, and isolate strains or serotypes from naturally contaminated samples or the ability to maintain large collections. Although pathogen and serotype or serovar selection is an important consideration to method and matrix application studies, it appears that the best course of action is to rely on the inclusivity data produced during a method's validation and accreditation as the primary source documenting the method's ability to recover strain variability of the target pathogen. Detailed selection based on strain-level phenotypic or genetic determinants appears unjustified for evaluation with new matrices and could lead to inaction, but where stakeholders know of strong links among a particular process environment, matrix, and strain of pathogen, this would be a reason to favor that strain. More generally, the commercial availability of standardized strains can help laboratories facilitate method application work in a harmonized manner within the industry and could benefit from the expansion of collection diversity. It is arguably better to conduct matrix verification and validation activities with one or more appropriately stressed pathogen- or serovar-level representatives readily available to any laboratory than to take no action as a result of inaccessibility or complexity in selecting specific strains. The stresses applied to these cultures could be tailored for food matrix validations and verifications based on the probable injury sustained in processing. For instance, standardized, mass-produced, lyophilized strains are readily available and can provide a level playing field for slow-growing strains in complex processed food products.

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In Memory Bobby Meeker

We extend our deepest sympathy to the family of Bobby Meeker who recently passed away. Ms. Meeker joined the Association in 2012. IAFP will always have sincere gratitude for her contribution to the Association and the profession.