https://doi.org/10.4315/FPT-24-060

Role of Hazard Assessments in Driving Risk-Based Testing

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SUMMARY

The testing of food ingredients and products for microbiological contamination should be aligned with a food safety hazard assessment to verify control of the identified hazards. This verification testing is detailed in specifications used in the purchase of food products and test results are reported on Certificates of Analysis (COAs). Unfortunately, it sometimes appears that the specification is based on perceived or unsubstantiated hazards. Testing that does not verify the effectiveness of a food safety control does little to support the food safety plan. This misalignment may have consequences for public health, the food supply chain, and the efficient assignment of food safety resources. Positive results require an appropriate and rigorous response. However, troubleshooting and root cause analysis becomes difficult when the results are not underpinned by a relevant hazard analysis. This article summarizes the case for risk-based testing, gives examples of testing that is not risk-based, illustrating potential concerns, and illustrates an approach to linking the hazard analysis with suitable microbiological specifications and testing programs to more effectively demonstrate that hazards are under control.

INTRODUCTION

Microbiological testing in the food industry is mostly used to verify that preceding elements of the food safety program are working as intended and delivering products or intermediates with the safety and quality expected. For this assurance, test results should be compared against appropriate microbiological criteria. Some microbiological criteria are imposed by government agencies and regulations that may specify testing requirements. Others are recommended by organizations such as the United States Pharmacopeia (USP) *(37)* or International Organization for Standardization (ISO) *(16)*. However, in the United States (U.S.), most microbiological criteria are set by businesses as internal measurements or external contractual terms, as part of their supplier approval and verification programs. The microbiological criteria incorporated into contracts for the purchase of ingredients and for lot acceptance should verify the supplier's control of hazards identified through a well-developed hazard assessment, leaving the reduction of microbial contamination, and appropriate verification activities in the hands of the supplier.

Sometimes, it may appear that testing requirements have been based on perceived and, at times, unsubstantiated hazards for the purpose of providing a certificate of analysis (COA). This type of "COA-driven testing" could lead to a disconnect between the manufacturer's food safety plan and the hazards controlled if test results used for food safety decisions do not verify a relevant control. For instance, specifications for *Cronobacter* may be appropriate for dairy products used in the production of infant formula, which must be free from *Cronobacter* in finished product *(6, 34, 35)*. *Cronobacter* specifications are probably not relevant for dairy powders used in the production of cold pressed bars, which are not suitable for infant feeding. A testing requirement for *Clostridium botulinum* or its toxin in products that do not provide an anaerobic environment is another example of testing that is not aligned with an actual hazard *(32)*.

This paper discusses the importance of matching testing requirements to the identified microbial hazard or risk-based verification activity. Note that reliable results depend on using appropriately validated methods and throughout this paper, it is assumed that methods discussed have been appropriately validated. Discrepancies in results which may arise between methods are not a concern here and have been discussed at length previously *(22)*.

THE CASE FOR TESTING BASED ON A HAZARD-ASSESSMENT

Preventive controls for food safety must be verified to ensure they are effective and operating as intended *(36)*. For

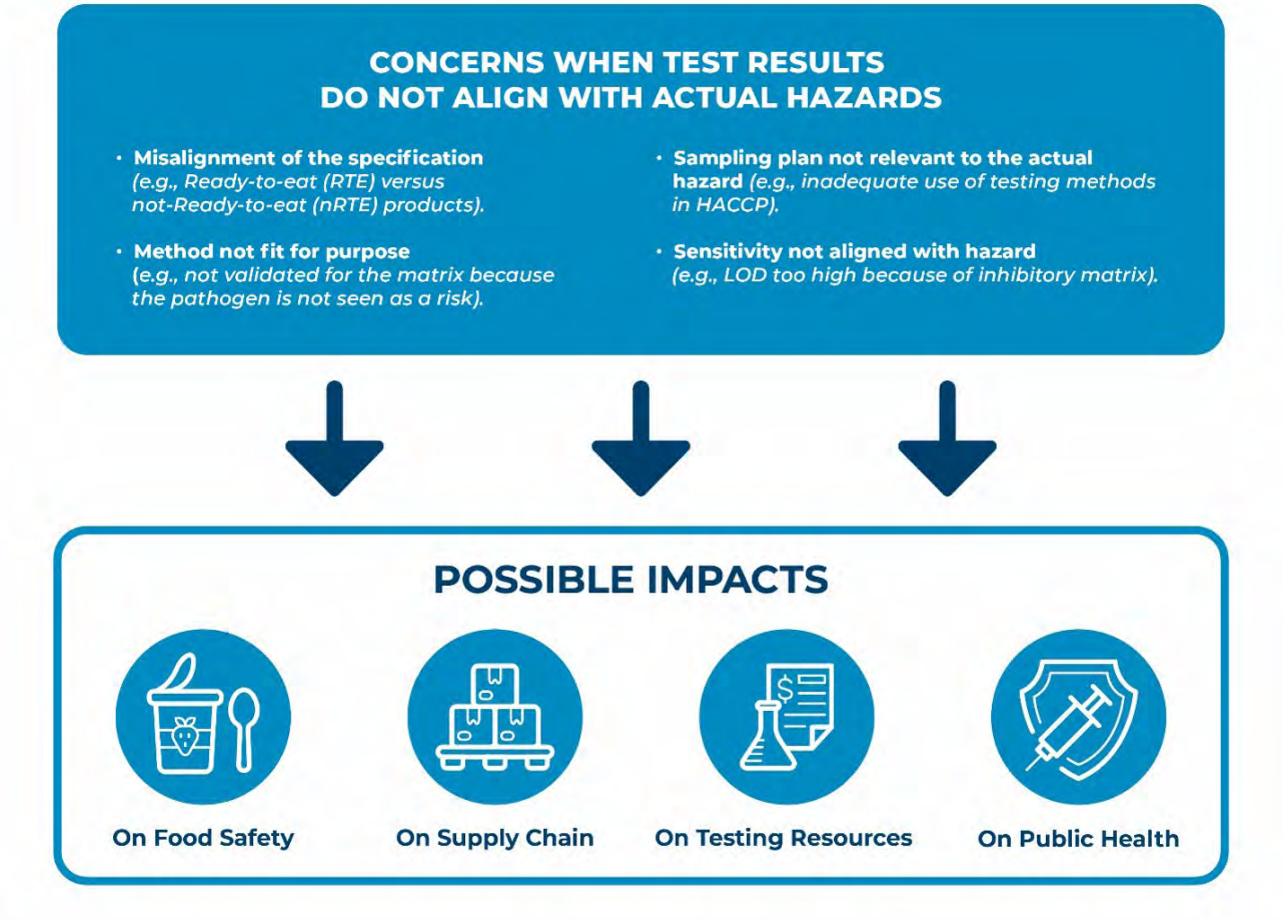

Figure 1. Schematic of possible impacts arising from misalignment of testing programs and actual hazards.

example, pathogen controls in the manufacture of chocolate would target *Salmonella*, a known risk according to a hazard analysis of the ingredients and process *(36)*. Testing of the manufactured chocolate product, ingredients and processing environment for *Salmonella* could be used in part to verify that process controls and sanitation controls are working effectively to mitigate the pathogen hazard.

Pathogen controls for acidified foods, outlined in 21 CFR part 114 *(31)*, require a thermal process to mitigate vegetative pathogens and a finished equilibrium pH of 4.6 or below to prevent the outgrowth of spore-forming pathogens *(36)*. Control of vegetative pathogens is verified by showing that required time and temperature parameters are met *(3, 4)* Spore-forming pathogens are likely to survive thermal processing but remain dormant in the finished product at low pH. Their control is verified by measuring the pH of the product. A specification requiring a pH of <4.3 would be appropriate for control of *Bacillus cereus* or *Clostridium botulinum (36)*.

In another example, *E. coli* O157:H7 has been associated with multiple outbreaks in apple cider *(2, 10, 18)*. A producer of apple cider must ensure that their established preventive controls properly mitigate this hazard by performing an inactivation study to demonstrate that the preventive control measure (such as a UV-light kill step, or use of preservatives) achieves the required 5-log reduction of *E. coli* O157:H7 *(33)*. Subsequent routine microbiological testing for the pathogen and related indicator organism should be used as an on-going verification of the process control.

Product testing for identified microbiological hazards serves to verify that the preventive measures are operating as intended and the hazards are controlled.

CONCERNS WHEN TESTING DOES NOT ALIGN WITH HAZARDS

Several different concerns can arise if testing is not aligned with identified hazards. These concerns are summarized in *Figure 1* and explored below.

Impact on the supply chain

If a food commodity is withheld from commerce, it can affect any downstream part of the food supply chain that needs it as an input and result in shortages of key consumer

staples and/or logistical and cost issues for brand owners, processors, and retailers. The ripple effects of a shortage of even one relatively minor ingredient can be larger than anticipated given the complexities of the modern food supply chain *(5)*. In 2013, a global recall of infant formula was announced, after suspected *Clostridium botulinum* was detected during testing and created a false alarm for what was later confirmed as *C. sporogenes (1)*. Substantial trade disruption ensued based on the results of a test not indicated by any documented history of *C. botulinum* in this product type. In this case, consumers temporarily lost access to a safe product because risk-inappropriate testing was done.

Impact on public health

Not-ready-to-eat (nRTE) products traditionally relied on consumer cooking for food safety and testing for *Salmonella* seemed unnecessarily stringent. However, from 1998 to 2021, public health officials investigated 14 salmonellosis outbreaks attributed to consumption of nRTE breaded stuffed chicken products that may have appeared ready-to-eat (RTE) to consumers *(28)*. The USDA Food Safety and Inspection Service (USDA-FSIS) has since concluded that nRTE breaded stuffed chicken products that contain *Salmonella* at levels of 1 CFU/g or higher are adulterated *(30)*. That determination represents a modified hazard analysis which better aligns the required testing, by a quantitative *Salmonella* test with a sensitivity of 1 CFU/g or better, with the identified risk. Similarly, frozen corn and other frozen vegetables have been considered nRTE and labeled with cooking instructions and a statement that they are not ready to eat. However, consumers can readily find recipes for salads prepared with frozen corn using any popular search engine, despite both *Salmonella (25)* and *Listeria monocytogenes (27)* outbreaks having been linked to frozen corn. *L. monocytogenes* outbreaks have been linked with other frozen vegetables too and consumption of thawed individually quick frozen (IQF) vegetables (including nRTE products) without cooking is not unusual; for example, in salads or smoothies *(7)*. In these examples, consumers can be sickened when risk-appropriate testing is not done.

Impact on food safety resources as a whole

Non-detection of a specific pathogen in a product where it has not been determined to be a hazard could be interpreted as showing that the process is effective when, in fact, that pathogen has no value in process verification. At worst, this approach could lure the processor or customer into a false sense of security, leading to release of product that has not been appropriately assessed. At best, this example simply represents a waste of resources.

All businesses make decisions on allocation of resources from a finite pool. It follows that resources allocated for testing, whether through an in-house or a third-party laboratory, cannot be used for something else. If those resources come from elsewhere in the food safety and quality budget, other elements of the food safety system may be compromised by misallocation of resources to inappropriate testing.

EXAMPLES OF COA DRIVEN TESTING THAT DO NOT ALIGN WITH HAZARD ASSESSMENTS

Surprising as it may seem, many examples of misalignment between hazard and testing applications may be found, giving rise to the concerns outlined in the previous section.

Some ingredients and products possess antimicrobial properties which can inhibit the growth and survival of pathogens making detection of pathogens difficult or impossible. For instance, inhibitory compounds in ground mustard seed are lethal to *Salmonella* in standard 1:10 enrichment dilutions *(38)* but might not guarantee food safety. *Table 1* lists food ingredients or products which may exhibit intrinsic bacteriostatic or bactericidal properties, reducing viability of contaminating pathogenic organisms. To recover low levels of pathogens in these types of products one must counter the antimicrobial activity. Often, high dilution ratios and enrichment media modifications are used, however, this approach may reduce the concentration of the target pathogen below the detection limit. The combination of inhibited recovery and reduced method sensitivity puts great emphasis on appropriately assessing the hazard associated with the matrix and its intended use since a negative test result may lead to a false sense of security.

Another example would be requests to test citric acid powder for pathogenic bacteria. Citric acid is antimicrobial, depending on concentration, can be effective against both planktonic and biofilm encased bacteria *(19)*, and acts as a preservative in many processed foods. Given this known antimicrobial activity and the low water-activity of citric acid powder, the value of testing this matrix is questionable.

Requiring testing for Shiga-toxigenic *Escherichia coli* (STEC) in chocolate would not be appropriate because STEC is not considered a pathogen of concern for chocolate. While the process preventive control for *Salmonella* (thermal treatment of cocoa nibs) may be lethal to STEC, validating the control for effectiveness against STEC would not be substantiated by available evidence.

In other examples, customers may require suppliers to provide a COA with results for a standard list of tests. An extreme example would be requiring a panel of tests including *Salmonella*, *Listeria monocytogenes*, coliforms and *E. coli*, aerobic plate count and yeast and mold count on a thermally processed canned product. In this case, microbiological hazards are controlled by the thermal process and verification should be by examination of process records and, if desired, incubation of cans and examination to determine commercial sterility *(20)*.

A clue that a request for testing a combination of pathogen and matrix is not substantiated by a hazard assessment could be if there is not a reference method available for that

TABLE 1. Examples of food components with intrinsic antimicrobial activity that can make detection of pathogens difficult or impossible. However, presence of these components should not be relied upon for food safety without verification. Gottardi *et al.* provide a more detailed list *(9)*

Ingredient or Product	Antimicrobial Compound(s)	Activity
Honey	Hydrogen Peroxide Methylglyoxal Bee defensin-1	Inhibit wide range of bacteria
Garlic	Allicin	Inhibit bacteria, fungi & viruses
Ginger	Gingerol, Shogaol	Inhibit bacteria & fungi
Turmeric	Curcumin	Inhibit pathogenic bacteria
Cranberries	Proanthocyanidins, Flavonoids	Inhibit bacteria
Oregano	Carvacrol, Thymol	Inhibit bacteria, fungi & parasites
Tea Tree Oil	Terpenes (terpenin-4-ol)	Inhibit bacteria & fungi
Zinc	Zinc Ions	Inhibit bacteria & viruses
Citrus Fruits	Citric Acid, Essential Oils	Inhibit bacteria & fungi
Cinnamon	Cinnamaldehyde, Eugenol, Cinnamic Acid	Inhibit bacteria, fungi & antibiotic resistant bacteria
Grapefruit Seed Extract	Polyphenols, Flavonoids	Inhibit bacteria
Raw Apple Cider Vinegar	Acetic Acid	Inhibit bacteria & fungi
Green Tea	Polyphenols	Inhibit bacteria, fungi & viruses
Mustard	Allyl Isothiocyanate	Inhibit bacteria
Cocoa	Flavonoids, Polyphenols, Alkaloids	Inhibit bacteria, fungi & viruses

pathogen and matrix, which suggests that a hazard has not been identified by regulatory agencies nor by a science-based approach. However, it takes time to respond to newly identified hazards, so absence of a reference method does not prove absence of a hazard.

SPECIFICATION AND TESTING PROGRAM DEVELOPMENT

The first step in developing appropriate specifications and testing requirements is to conduct a thorough hazard assessment of the product and process including as much as possible of the upstream supply chain. Without this extensive upstream analysis, it is easy to overlook hazards such as produce growing operations downslope from a cattle feedlot or dairy operation and the associated potential for cross-contamination. Or the potential exposure of cocoa and many spices to birds, rodents, and other animals during sun-drying processes. Risk profiles, assessments and surveys have been completed on many food commodities to identify the public health risk and evaluate mitigation or control options. For example, the Institute of Food Technologists report on the Evaluation and Definition on Potentially Hazardous Foods *(11)* and the "known or reasonably foreseeable hazards" in Appendix 1 of the FDA Draft Guidance to Industry *(36)*. Processors can use these sources to help develop their own hazard-assessments and risk-based testing plans but should take care not to overlook hazards that may be unique to their operations and not covered in these general guidance documents.

Testing plans must consider both the primary flow of ingredients as they are processed to the final product and potential sources of contamination in the surrounding environment. These two circumstances are quite different and testing plans will reflect the differences.

Specifications and testing for the ingredient and product stream.

For incoming raw materials, testing should be designed to verify that supplier controls are operating effectively and may be achieved by testing for one of several "indicator organisms", often closely related to pathogens of concern and having similar ecological niches and tolerance for inactivation

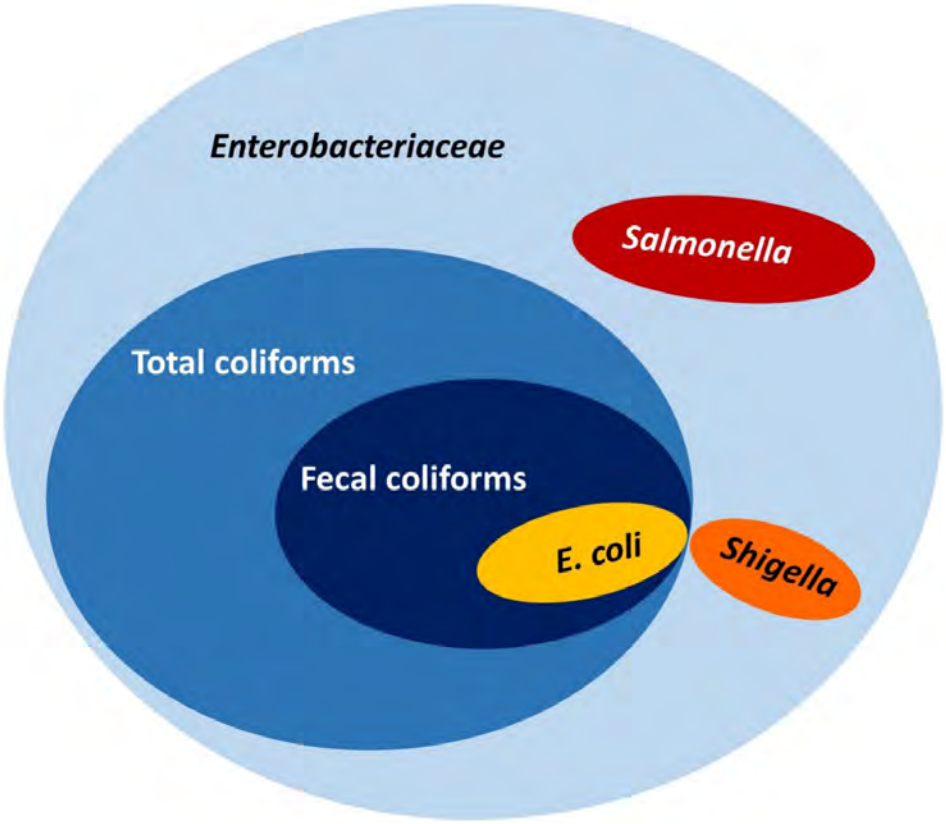

Figure 2. Relationships within the *Enterobacteriaceae* adapted from Roychowdhury et al., 2018 *(26)*.

processes. For example, *Enterobacteriaceae*, coliforms, fecal coliforms or *E. coli* are progressively narrower groups of microorganisms that may be much more easily detected or quantified than *Salmonella* but are closely related to it. These relationships are illustrated schematically in *Figure 2*. Alternative indicators might be such tests as aerobic plate count, where an upstream process should have reduced the overall microbial load to low levels *(24)*, or yeast and mold count where the primary control on the raw material is to maintain a low water activity. High yeast and mold counts might then indicate that a failure to protect the material from exposure to moisture had occurred, permitting yeasts and/or molds to grow.

To be useful, indicator organisms share certain characteristics *(17, 28)*:

- They have some connection with the indicated hazard: ideally a unique connection.
- They should survive at least as well as the indicated hazard in the environment or matrix.
- They should be readily detectable (or quantifiable).
- If the method is quantitative, results should be proportional to the level of the indicated hazard.
- Results can be used to initiate action to mitigate the indicated hazard with no delay or need for additional testing.

A processor can develop an internal specification for the maximum acceptable level of their chosen indicator based on their understanding of "normal" operation. It is quite common in food manufacturing to run a multi-shift review of product quality during initial startup of a new product. Collecting quantitative microbiological data during this period can establish a baseline on which to build a specification. For example, aerobic plate count, *Enterobacteriaceae* count, or mold and yeast count could all serve for this purpose. Determine the range of counts achieved during normal operation by taking the results from 20–60 shifts and calculating the mean and standard deviation of the log counts. This approach can be done easily in a spreadsheet program. If the processor sets the acceptable limit at two standard deviations above the mean, 97.5% of all production from future runs should be below this limit when the process or operation remains under control. An action to investigate results above this level is likely to discover some loss of control that must be corrected. Results greater than three standard deviations above the mean almost certainly mean that the process or operation is out of control since 99.85% of all results should be less than this level.

Where control of infectious pathogens such as *Salmonella* or *L. monocytogenes* must be verified directly, specifications

TABLE 2. Examples of microbiological specifications illustrating how they vary according to the nature and level of the risk identified in the hazard assessment. These are all intended to be illustrative and should not be taken as recommendations. *n* is the number of samples to test, *c* is the number permitted to exceed the quality limit *m*, and *M* is the safety limit which no samples may exceed. Adapted from Legan (2023) *(21)*

Matrix	Consumer	Concern	Target	Risk case	Sample size (g)	*n*	*c*	*m*	*M*	Source
Infant formula	Infants	Pathogen	*Salmonella*	14	25	60	0	0	0	*(34)*
Infant formula	Infants	Pathogen	*Cronobacter*	13	10	30	0	0	0	*(34)*
Frozen cooked rice	General	Pathogen	*B. cereus*	8	50	5	1	10^3	10^4	*(12,13)*
Dried milk	General	Indicator	Coliforms	5	50	5	1	10	100	*(12,13)*
Dried milk	General	Quality	Aerobic plate counts	2	50	5	2	10^4	10^5	*(12,13)*
Frozen fish	General, to cook	Quality	Aerobic plate counts	1	50	5	3	5×10^5	10^7	*(12,13)*

would normally set limits as "not detectable" in some defined mass or volume of the matrix. For toxigenic pathogens such as *Staphylococcus aureus* or *Bacillus cereus*, some low acceptable level may be set below population densities required for toxin production *(8)*. In all cases, the specification should include the sampling plan to be used (number of samples to be taken and acceptable marginal conditions, if any). Microbiological sampling plans most commonly follow an "attributes" scheme, in which nothing needs to be known about the distribution of contamination. Instead, limits are set for safety (M) and quality (m) and the maximum number (c) of marginal samples that exceed the quality limit but not the safety limit. The number of samples to be tested (n) is determined by the required plan stringency (more stringent plans test more samples) within a framework of 15 risk cases from 1 (least risky) to 15 (most risky). Sampling plans have been discussed extensively by the International Commission on Microbiological Specifications for Foods (ICMSF) and others *(12, 14, 15)*. *Table 2* shows illustrative examples of testing aligned with the nature and severity of risks identified through a hazard analysis.

Many sources provide guidance on acceptable limits for pathogens and indicator organisms in various food matrices e.g., *(8, 12, 14, 15, 23, 29, 37)*, though most have been presented as public health limits without specific knowledge of any particular company's product or process. Hence, if using this guidance, it is the processor's responsibility to choose wisely and adapt as appropriate to the situation. They may choose to use more stringent specifications to protect quality, provided that all parties to a transaction are agreeable. Equally, they may choose to use less stringent specifications if other controls are in place that will ensure equivalent product safety.

If testing is used for the purpose of "positive release" of a food product into commerce, specifications, sampling plans and testing requirements should again be driven by the hazard assessment, but the stakes become higher. The test result now becomes a primary determinant of product safety, rather than a verification that other controls were working as intended. As such, sampling plans should tend to become more stringent. This approach typically involves testing more samples per lot, to improve the chances of detecting a sporadic unacceptable condition, rather than changing the value of the test result that triggers a corrective action.

Specifications and testing for the environmental monitoring program.

A large portion of all microbiological testing in food safety management is done on sponges, swabs or other samples taken as part of an environmental monitoring program intended to demonstrate the effectiveness of plant sanitation. For these samples, acceptable limits are set to trigger appropriate actions when operations are out of control or moving out of control, but the lot concept does not apply. Instead, it is common to divide food production environments into zones:

- Zone 1 – food contact surfaces.
- Zone 2 – areas immediately adjacent to zone 1.
- Zone 3 – surfaces adjacent to zone 2 within the production space, e.g., floors, walls, drains, cleaning tools, fork-lift trucks, etc.
- Zone 4 – areas outside the production space, e.g., hallways, break rooms, locker rooms, etc.

Environmental sampling also does not usually follow a statistical sampling plan; instead, covering the facility such

that more attention is paid to areas with greater opportunities to act as sources of contamination *(15)*. Specifications for routine environmental samples should be based on what can normally be achieved when the operation is under control, with data for indicator populations collected and analyzed as suggested for internal specification above, for the ingredient and product stream. It is also useful to understand the baseline prevalence of pathogens in environmental samples but generally, detection of foodborne pathogens in the processing environment should trigger some remedial action or investigation.

CONCLUSIONS

Effective and appropriate microbiological testing of finished product, ingredients and the manufacturing environment is a critical component in a food safety plan to verify the correct operation of the multiple controls needed to assure a safe product. There can be good reasons to supplement this testing with tests to learn more about the operation but sometimes testing is done simply to place a number against a customer's "standard test list". It is, of course, a customer's right to request any testing they desire from their suppliers. However, testing not supported by a hazard analysis consumes resources that may provide a bigger improvement in food safety if deployed elsewhere. It can be argued that testing should be justified by a hazard analysis and not simply follow a standard list of tests applied to every situation to obtain a "clean" COA. If concerned, or in doubt, about what testing may be appropriate, processors may seek advice from their commercial laboratory, local university microbiology department or many independent microbiology consultants.

ACKNOWLEDGMENTS

The authors thank Gabriella Lopez-Velasco of Neogen for careful review and helpful comments on the manuscript.

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