Comprehensive Reviews in Food Science and Food Safety

Chapter VII

The Use of Indicators and Surrogate Microorganisms for the Evaluation of Pathogens in Fresh and Fresh-Cut Produce

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Scope

Indicators and surrogate microorganisms are used for a variety of purposes in food systems including evaluating quality or safety of raw or processed food products and validating effectiveness of microbial control measures. Although frequently used on an informal basis within a specific company, the use of indicators is highly dependent upon the microbiological criteria that are in place for the food product. All the considerations that must be addressed in establishing microbiological criteria must also be in place if indicators or surrogates are to be utilized in process verification. This chapter is limited to a discussion of the challenges of selecting an indicator or surrogate to assure safety for specific individual produce items and associated conditions (that is, growing, harvesting, processing, handling, storage and packaging) or for determining microbial reduction treatment effectiveness (for example, water treatment or manure composting effectiveness or decontamination procedures).

1. Introduction

When using indicators and surrogate microorganisms, a number of issues and questions need to be addressed, many of which may not have simple answers. Before attempting to select the best available systems, one needs to identify the role of the specific indicators or surrogate microorganisms in the control of microbial hazards in fresh and fresh-cut produce. There are many variables that are influential, such as the specific produce item, harvesting area, and other growth environmental conditions. Selecting the most significant microbial contaminants of concern and the analytical methods to detect or enumerate the indicator or surrogate are important aspects of choosing the most appropriate indicators or surrogates. For instance, as it will be described later, special methods are needed to retrieve the indicator or surrogate microorganisms or their metabolic or genetic components if injured survivors or systems (for example, due to processing or microbial reduction treatments) are to be enumerated accurately. An essential element of microbiological monitoring is a sampling plan that will assure statistical significance of the findings. Finally, there should be a scheduled review of the criteria for indicators or surrogates to determine if they continue to be appropriate for the conditions under evaluation. These are considerations that must be addressed if indicators or surrogates are to be used effectively and will be further discussed within the next sections.

1.1. Definitions

Indicators and surrogate microorganisms have been defined and described by a number of investigators. Mossel and others (1995) make the following statements:

The term 'index organisms' has been introduced for markers whose presence in numbers exceeding given numerical limits indicates the possible occurrence of ecologically similar *pathogens.*

On the other hand, Ingram suggested the use of the term indicator organisms for those markers whose presence in given numbers points to *inadequate processing for safety*.

A positive text for indicator organisms does not necessarily point to the presence of pathogenic organisms in the same commodity (Banks and Board 1983). The detection of an index organism in a food, however, provides evidence that a related pathogen may also occur—if not in the inspected consignment, then in a previous or later one. Clearly, a given marker can function both as an index and as an indicator organism, even in the same food.

Other names for markers have since been suggested. These include the terms model organisms (Havelaar and Pot-Hogeboom 1988; Sobsey and others 1988; Wolfe and others 1989), sentinel

Mossel and others (1995) include the following table to illustrate concepts and misconceptions:

Table 7.4—The use of marker organisms in the microbiological monitoring of foods, originating from operations complying with validated good manufacturing and distrubution practices (GMDP)

Concepts and misconceptions
• Ingram (1977) introduced the distinction between: —Indicator organisms = markers whose presence in given numbers points to failure to comply with applying GMPDs —Index organisms = markers whose presence in numbers exceeding given numerical values points to the possible occurrence of ecologically similar pathogens
• Indicators are consequently never to be considered as surrogate markers for the occurrence of pathogenic organisms in foods
• Index organisms may not be considered valid as surrogate markers for food pathogens, unless a correlation between their occurrence and that of well-defined pathogens—or at least a marker threshold level below which contamination with the pathogen under study is unlikely at a given *P*-level—has been firmly established

(Mossel and others 1995, p 289)

organisms (Drucker and others 1989) and surrogate organisms (Haas and others 1983; Lytle and others 1991; Payment and Franco 1993).

Buchanan (2000) made a distinction between direct analyses of single specific pathogens and indirect assessments for microbiological safety:

Indirect analyses are based on measuring a microbiological attribute that is related to an increased risk that a pathogenic microorganism may be present in a food. Two types of indirect analyses are differentiated, index microorganisms and indicator microorganisms. An index organism is a microorganism or group of microorganisms that is indicative of a specific pathogen.

An indicator organism is a microorganism or group of microorganisms that are indicative that a food has been exposed to conditions that pose an increased risk that the food may be contaminated with a pathogen or held under conditions conducive for pathogen growth. Thus, indicator tests are often employed to assess a process control attribute such as using the extent of mesophile growth as an indicator of inadequate refrigeration. Indicator analyses are widely used to measure improper sanitation.

Surrogates have a specific niche among indicator systems. Surrogates are specifically utilized to evaluate the effects and responses to selected processing treatments. In the case of fresh and fresh-cut produce where no traditional processing inactivation steps are used (for example, heat pasteurization) surrogates would be used to assess and validate decontamination procedures. Surrogates may be selected cultures prepared in a laboratory and inoculated onto or into the produce (see Chapter VI), or they may be a naturally occurring inoculum that conforms to the requirements of a surrogate and has been confirmed to exist at adequate concentrations in the specific produce.

2. Indicators and their targets

Indicator compounds or organisms are used for a variety of reasons but for consideration here, they will be limited to evaluating safety of raw or processed food products (that is, fresh or fresh-cut fruit or vegetables) and to assessing, validating or verifying effectiveness of microbial control measures. The use of indicators is highly dependent upon microbiological criteria that are in place for the food product. These can be standards enforced by government agencies or guidelines (limits) recommended by government agencies or specifications stipulated in commercial contracts. All the considerations that must be addressed in establishing microbiological criteria obviously apply to indicators. The following discussion will be limited to naturally occurring indicators that could be used to determine apparent product safety or treatment effectiveness. There are challenges in the selection of such indicators due to the unique conditions of the specific produce category, which include situations related to growing, harvesting, processing, handling, storage, and packaging.

The principles of indicators and their use, with examples, have been addressed by many authors including Ray (1989), Mossel and others (1995), Jay (1996, 2000), Smoot and Pierson (1997), and Buchanan (2000). General preferred qualities of ideal indicators include the following:

- History of presence in foods at any time that target pathogen or toxin might be present;
- Concentrations (numbers, metabolic end-product, and so on) initially and after any growth opportunity that are directly related to that of the target pathogen or toxin;
- Absent from food when target is not present, or absent after a process that would eliminate the target;
- Growth or increase of indicator equivalent to, or slightly greater but not less than, target under all processing and storage conditions of food as well as in analytical situations;
- Easily and quantitatively detected as distinguishable entity even at low concentrations among other microorganism and food components;
- Measurable in a short period of time, preferably in less than routine holding time of product at any point of testing;
- Resistant to cellular injury or decrease in concentration from stress of handling conditions, processing or storage, unless the equivalent effect would occur with the target;
- Nonpathogenic or nonhazardous to testing personnel if handled properly.

The indicator could be a specific microorganism (for example, viable colony count, enrichment culture, indirect cell count), a metabolite (for example, lactic acid titration), a fragment of DNA (for example, PCR method), or some other indirect measure (for example, ATP in organic matter on surface measured by bioluminescence).

2.1. Role of indicators

Ideally, the absence or a low concentration of a specific indicator means that food has not been exposed to conditions that would permit contamination by a specific target pathogen or present the opportunity for its growth. This can be applied to raw produce directly from the field or after some decontamination process. The selection of the appropriate target(s) is key to the control of the microbial hazards based on some set of criteria. Chapter III presents a set of extensive tables listing the possible sources of pathogenic microorganisms (Table III-4), their incidence on or in various kinds of produce (Table Series I), foodborne illness outbreaks associated with consumption of produce (Table Series O), and the survival and growth of these many pathogens in various types of produce (Table Series G/S). Chapter II also delineates extensively the role of production practices (for example, water quality, manure contamination) as well as transportation on possible introduction or growth of pathogenic microorganisms onto or into fresh produce. The challenge is not only the selection of appropriate targets, but also the selection of an indicator that would relate to the target microorganism. In addition to production practices, possible contamination through a specific action (for example, rinsing of produce) could be monitored with an indicator.

2.2. Specific produce item and environmental considerations

It is possible to prioritize selection of a fresh or fresh-cut produce category for evaluation on the basis of a number of factors: total volume sold, the number of foodborne outbreaks attributed to the product/category, the potential for mishandling, the frequency that the source that has been associated with a problem or some other criterion. None will be acceptable to all parties nor will any be totally defendable. At present, there is not enough adequate data from well-designed scientific studies to make ideal decisions; nevertheless, these decisions are being made as best as can be done with limited information.

2.3. Challenges of selecting target contaminants

Again, Chapter III demonstrates that the microbial hazard of concern is not readily predictable. Nevertheless, some trends can be observed, possibly due to better surveillance over the past few years. For instance, Salmonellae and *E. coli* O157 appear to be the main concerns in seed sprouts, whereas *E. coli* O157:H7 appears to have been the cause of the majority of outbreaks in unpasteurized juice. With major intervention activity being implemented for these two products, will these concerns continue or will the problems be solved? Or will some other pathogenic microorganisms resistant to the intervention step, although heretofore unidentified, emerge as a problem? If the choice is salmonel-

lae, can we separate the to-date uncontrollable contamination by wild birds from contamination by production water or should we bother? Once we make the selection, will we test the indicator against laboratory culture strains or wild strains, in a well-controlled environment or in an open natural environment, under controlled pilot-plant conditions or under commercial conditions? With emerging molecular technology that may be able to discriminate between virulent and nonvirulent strains, will we need to collect much new survey data to accurately pinpoint the hazardous situations?

2.4. Analytical methods to detect or enumerate the indicator

As stated above, the ideal indicator should be present and detectable at any time that the target may be present and at concentrations directly related to those of the target. Spatial, temporal and seasonal distribution must be considered. Growth of the indicator should be greater than the target in analytical situations. The indicator should be easily, accurately and quantitatively detected and differentiated even at low concentrations among other microorganism and matrix components. The indicator should be measurable in a short period of time at relatively low cost. Compared to the target, the indicator should be as, or slightly more, resistant to cellular injury from stress of conditions of handling, processing or storage. The analytical methodology should not introduce any conditions that would affect the accuracy of the determination. Furthermore, the choice of inappropriate methods or misinterpretation of test results can effectively destroy the value of any indicator system. The procedures as well as the indicator should be nonhazardous to testing personnel when handled properly.

An extensive list of considerations for examining raw produce for pathogens has been offered in III, Table III-1 and has been adapted here for use with indicator microorganisms (see Table VII-1). Furthermore, the many specific considerations listed in Chapter VI Table VI-1 for developing a standard method for evaluating the efficacy of sanitizers used on produce are also appropriate for analysis of indicators and surrogates (see Table VII-2).

Table VII-1—Considerations when examining raw fruits and vegetables for the presence or concentration of indicators (Adapted from Chapter III Table III-1)

Identification and origin of product and sample
Indicated target of marker system
Procedure for sampling
Location of source (field, packing shed, processing plant, retail location, food service, home)
Number and size of samples
Distribution of samples in test lot
Protection of samples for transport to laboratory
Handling samples between collection and analysis
Protection against cross-contamination
Temperature between selection and analysis of sample
Time between selection and analysis of samples
Processing samples
Weight or number of pieces to represent samples
Area or portion to be tested (whole piece, skin only, diced, cut)
Selection of wash fluid or diluent
Ratio of produce to wash fluid or diluent
Temperature of produce and wash fluid or diluent
Soaked or not soaked before processing
Type of processing (washing, rubbing, stomaching, homogenizing, macerating, blending)
Time of processing
Microbiological methods and culturing techniques
Enrichment and/or direct plating
Composition and volume enrichment broth
Composition of direct plating medium
Pour-plate or surface plate
Incubation temperature and time
Confirmation procedures

2.5. Methods for injured cells

Are special methods needed to retrieve the indicator or their metabolic or genetic components so that survivors damaged by any handling or processing treatment are not missed? All procedures should address this question and confirm that appropriate consideration has been given to resuscitation of stressed cells or masked metabolites or cellular components. Responses to stress by the indicator should be equivalent or slightly more resistant than the target microorganism. It is reasonable to believe that the bacteria of concern in the fresh produce industry could, at some point of the process, be sublethally stressed or injured by a food-related physical intrusion. Microbiological criteria are extremely dependent upon accurate and precise analyses of the microorganisms present in the sample of product under test. It is critical that the analytical method detects injured cells because pathogens existing in the food in a similar injured state could remain virulent, or could be resuscitated later to cause a foodborne infection upon ingestion. Some contemporary analytical techniques may not suffer from the same problems if they do not depend on an amplification step that requires growth in a selective or restricted growth system. With many methods that utilize selective agents, a nonselective growth step is needed to allow the opportunity for resuscitation of injured cells.

Irrespective of whether the analysis pertains to the target pathogen, the indicator microorganism or the surrogate, loss of specific identifiable characteristics as a result of sublethal injury can cause faulty observations. Injury may occur in any vegetative cells and endospores of pathogenic as well as nonpathogenic microorganisms. If the cell appears to be "dead" (that is, unable to multiply and demonstrate its viability) the food, the processing system or the environment may appear free of the pathogen only to experience subsequent resuscitation of the pathogen or the indicator under some special circumstances. Inaccurate underestimates of surviving microorganisms could significantly alter the determination of the kinetics of inactivation by a specific system and result in hazardous processes or inadequate process deviation responses (Doyle and Mazzotta 2000). This phenomenon is reflected in the intricate responses of microorganisms when exposed to sequential and different stresses. The following are a few of the published examples of the influence that stress may have on responses and outcomes of some pathogens in food systems: pressure-damaged *E. coli* were more acid sensitive than native cells (Pagan and others 2001); addition of 5% ethanol enhanced inactivation by organic acids and osmotic stress (Barker and Park 2001); growth at low aw increased the heat resistance of *Salmonella* spp. (Mattick and others 2000); induction of acid resistance in enterohemorrhagic *E. coli* increased radiation resistance (Buchanan and others 1999); environmental stresses in the form of temperature shifts influenced *Listeria monocytogenes* attachment to food contact surfaces (Smoot and Pierson 1998); stress as a result of exposure to sublethal concentrations of oxidative sanitizer produced an adaptive resistance response in *E. coli* O157:H7 (Zook and others 2001). These are appropriate considerations as we address indicators for decontamination treatments of produce, such as UVB treatment of surface waters (Obiri-Danso and others 2001), and sanitizer treatment of salmonellae attached to apples (Liao and Sapers 2000).

2.6. Sampling plans

Sampling plans are as critical in the assessment of indicators as sampling plans are in the evaluation of the presence of pathogens. These plans include sampling procedures as well as decision criteria. The International Commission on Microbiological

Criteria for Food (ICMSF 1986, 2001 forthcoming) has described in extensive detail the statistical concepts behind sampling, population probabilities, decision criteria, and the general validity of specific sampling plans. If the approach to assessing control of pathogens revolves around the use of indicators, it is imperative that the stringency of the sampling plan and the measure of the presence or relative population of the indicator stipulate the statistical probability of the prediction of the target microorganism of that indicator. Buchanan (2000) offered some examples of data and their interpretation.

2.7. Evaluation of appropriateness of indicator

After an indicator program has been adopted, it should undergo regular evaluation. There should be a scheduled review of the criteria for indicators to determine if the indicators were appropriate selections initially and if they continue to be appropriate for the conditions under evaluation. An appropriate indicator will continue to be present in foods at any time that target pathogen or toxin might be present. Concentrations initially and after handling will directly relate to those of the target. The indicator will be absent from food when target is not present, or absent after a process that would eliminate the target. Growth or increase of indicator will be equivalent to, or slightly greater but not less than, target under processing and storage conditions of food as well as in analytical situations. It will be easily and quantitatively detected as a distinguishable entity even at low concentrations among other microorganism and food components, and it will be measurable in a short period of time, preferably in less than routine holding time of product at any point of testing. Resistance to cellular injury or no decrease in concentration from stress of conditions of handling, processing or storage, would be expected unless the equivalent effect would occur with the target. Ideally the indicator will continue to be nonpathogenic or nonhazardous to testing personnel if handled properly.

With the plethora of data and information generated on a regular basis, it is imperative that criteria set down for an indicator be regularly evaluated. Methodologies improve; pathogens find new situations in which to flourish; production practices, transportation, handling, and processing evolve; and scientific research uncovers new findings that potentially modify the indicator criteria or the indicator conformance to the criteria. Furthermore, if the indicator system was selected for ease and speed of detection compared to a pathogen and the methodology to detect and identify the target dramatically improves, the value of the indicator approach may decrease. Overall, as the capability of the molecular methodology increases and permits very precise differentiation of strains, relationships and sources of indicators or pathogens could be determined and used to identify and solve the problem.

It should be emphasized that indicators do not test for the presence of a pathogen but rather indicate an exposure that increases the risk that the food may be contaminated or permitted unwanted growth. Consequently, indicators are frequently used to validate and verify the adequacy of GMP, GAP, SSOP, and other programs related to the identification of non-hygienic practices that could influence food safety.

2.8. Examples of the use of indicators

As described in Chapter II, fecal coliforms have been proposed or used as indicators of acceptably conditioned manure. A low level of this class of microorganisms apparently suggests that the pathogenic microorganism population has diminished to an acceptable level. Similarly, water for irrigation has utilized fecal coliforms as an indicator of acceptable quality. Beyond the fecal coliforms, some organizations have used the entire family *Enterobacteriaceae* as an indicator or index of potential pathogen contamination while others have used the general category of coliforms or generic *E. coli*. Coliphage has also been proposed as an indicator of the possible presence of *E. coli*. "Fecal streptococci" or enterococci also have been used or proposed. Each of these groups of microorganisms has shortcomings as indicators of enteric pathogens.

The use of coliforms or "generic" *E. coli* as indicators of possible enteric contamination in other systems such as potable water sometimes stimulates consideration of them for similar use in fresh produce. A number of recent publications continue to address this issue. In a white paper on the significance of these microorganisms in fresh and minimally processed produce, NFPA (Anonymous 2000) stated that the absence of correlation between these microorganisms and pathogens on fresh produce makes testing for coliforms or generic *E. coli* an unreliable indicator for the presence of pathogens. In some cases with products stored at refrigerated temperatures, elevated numbers of coliforms or generic *E. coli* may be an indication of temperature abuse or long-term refrigerated storage; however, the quality of many refrigerated produce items will not tolerate temperature abuse and generic *E. coli* rarely reaches high numbers at refrigeration temperatures. The white paper indicates that these elevated populations would be quality issues; however, temperature abuse with certain kinds of fresh or fresh-cut produce may also indicate a possible safety concern (see Chapter II). This white paper also cites a personal communication from Kvenberg (1999, cited in Anonymous 2000) stating that NACMCF considers the use of "fecal coliforms" (or "thermotolerant coliforms") as an indicator of fecal contamination not appropriate for fresh produce. This is consistent with a statement by Nguyen-the and Carlin (2000) indicating that fecal coliforms had poor value as fecal indicators in fresh vegetables. This is in part based on the capability of commonly present *Klebsiella* spp. and *Enterobacter* spp. to grow under thermotolerant test conditions. NACMCF (1999) discussed extensively the limits of "fecal coliforms" as an indicator of fecal contamination and recommended *E. coli* or similar organisms as more appropriate. Brackett and Splittstoesser (2001) address briefly the interpretation limitations of coliform indicators in produce; however, neither ICMSF (1998) nor Lund and Snowdon (2000) addressed the concept of fecal indicators in fresh fruits. Recently, Kornacki and Johnson (2001) indicated that "numerous studies have determined that *E. coli*, coliforms, fecal coliforms and *Enterobacteriaceae* are unreliable when used as an index of pathogen contamination of foods."

It should be emphasized that the negative comments directed at fecal coliforms related to the presence of pathogens in the product do not contradict the value of *E. coli* as an indicator of fecal contamination from manure or other sources. This does not indicate that pathogens are present but rather that the risk is increased that the food might be contaminated.

Indicators for other pathogens of concern have been approached differently. The absence of generic listeria has been used as an indicator for the absence of *L. monocytogenes.* Alternative indicators have not been adopted. Although usually considered an indicator of quality, some organizations use total aerobic colony counts as a measure of overall plant good manufacturing practices and consider this an indirect indicator of safety.

Beyond the pathogenic bacteria, there are needs for indicators of pathogenic viruses such as *Calicivirus* (Norwalk-like viruses) and parasites such as *Toxoplasma gondii, Cryptosporidium parvum, Cyclospora cayetanensis*, and *Giardia* spp. Obviously, the requirements for indicators with their qualifications are different for viruses and parasites than for bacterial pathogens; their inability to grow in the product presents different considerations, such as responses to storage temperature, inconsequential growth consideration, relative resistance to environmental stresses, and limited detection method.

3. Surrogate microorganisms

3.1. Introduction

Since the production of whole and cut produce does not use a processing kill step to eliminate pathogens but may have a decontamination treatment, surrogates may be inoculated onto produce or equipment surfaces as test organisms to determine the efficacy of cleaning/sanitation/disinfection regimes. In contrast to the evaluation of the effect of a process on naturally occurring indicators or pathogens that may be present at some relatively low concentration in or on the incoming produce, the assessment of the efficacy of a decontamination treatment on an exogenously inoculated surrogate can be experimentally controlled and evaluated. Prior to selection of a surrogate, the pertinent pathogen of interest must be determined. As with the previous discussion of indicators, this requires knowledge of previous outbreaks, if any, from the produce in question or possibly the isolation of specific human pathogens from the produce, survival characteristics of various pathogens exposed to environmental stresses, and the influence of production practices on pathogen survival. In some cases, more than one pathogen may be pertinent for the produce item in question.

The validity of the established produce decontamination process can be confirmed using an inoculated test pack study. An inoculated pack study would be tested under actual plant conditions, which includes processing and control equipment, product handling and packaging to reproduce the process in detail. Since pathogens should not be introduced into the production area, surrogate microorganisms should be used in the inoculated pack study, and their survival or growth can be measured to validate the process. Surrogates should not create a problem for or compromise routine microbiological assessment of factory conditions; consequently, they should be completely eliminated from the environment after the test or not be part of the routine testing program. Surrogates play an important role as alternative biological indicators that can mimic survival and growth properties of a pathogen and can help to detect peculiarities or deviations in decontamination, processing, and storage procedures. An important difference between surrogates and indicators is that the latter is naturally occurring and the former is introduced as an inoculum.

One of the challenges in using new washing or sanitizer treatment technologies for pathogen removal or inactivation is to determine if traditional methodologies can be used to establish and validate the new process. It is also appropriate to use surrogate microorganisms to assist in determining and validating the effectiveness of treatment or storage conditions in killing cells or controlling growth during subsequent storage. Nonpathogenic surrogates need to be identified and their significance evaluated for use with produce. To accomplish this, more research is needed in the area of inactivation kinetics of pathogens by new decontamination, packaging and storage technologies as well as in the identification of nonpathogenic candidates useful as surrogate organisms.

3.2. Importance of surrogates

Surrogate microorganisms are invaluable in validating the efficacy of produce decontamination processes. Their use, as opposed to using actual pathogens, derives from the need to prevent the introduction of harmful organisms into the production facility. It is recognized that when an appropriate and valid surrogate cannot be identified, experimental isolated pilot facilities have been used to test effects of processes on actual pathogens; however, the consequences of mishandling a pathogen in the presence of workers, product and equipment (from safety to legal liability issues) could be devastating. Therefore, the use of surrogates by processing companies is of great importance to ensure microbiological safety of the process. For instance, surrogates have been used for many years in the low-acid canning industry to establish and validate the destruction of *Clostridium botulinum* spores. The use of nonpathogenic spores of the putrefactive anaerobe *C. sporogenes,* or spores of the flat-sour thermophilic organism *Bacillus stearothermophilus* as surrogates for *C. botulinum,* have helped the industry develop thermal processes that ensure products are safe and commercially sterile.

3.3. Criteria for surrogates

The ideal surrogate would be a nonvirulent strain of the test pathogen that retained all other characteristics except pathogenicity. Such an approach to surrogate selection is generally not followed due to possible reversion to pathogenicity or possible detection of false positives during routine testing. Generally, surrogates are selected from the population of well-known organisms that have well-defined characteristics and a long history of being nonpathogenic. In selecting surrogates, the following microbial characteristics are desirable:

- Nonpathogenic
- Inactivation characteristics and kinetics that can be used to predict those of the target organism
- Behavior similar to target microorganisms when exposed to raw fruit and vegetable processing parameters (for example, pH stability, temperature sensitivity, and oxygen tolerance)
- Stable and consistent growth characteristics
- Easily prepared to yield high-density populations
- Once prepared, population is constant until utilized
- Easily enumerated using rapid, sensitive, inexpensive detection systems
- Easily differentiated from other microflora
- Attachment characteristics, including influence of commodity surface and rate, that mimic those of target
- Genetically stable so results can be reproduced independently of laboratory or time of experiment
- Will not establish itself as a "spoilage" organism on equipment or in the production area
- Susceptibility to injury similar to that of target pathogen

Ideally, surrogates used in produce challenge studies or sanitizer efficacy studies should have many of these criteria, as is the case with the traditional surrogates used in low-acid canned foods processing validation.

3.4. Surrogates for produce

To obtain quantitative information to support the development and validation of produce decontamination processes, it is necessary to use microbial surrogates or indicator organisms that serve a surrogate role. Pathogens of public health significance in raw fruits and vegetables are vegetative cells of both gram-positive and gram-negative bacteria as well as viruses and protozoan cysts (see Chapter III). In cases where *L. monocytogenes* is the pathogen of interest, strains of *Listeria innocua* have served as nonpathogenic surrogates. In addition, nonpathogenic strains of *E. coli* have served as surrogates for *E. coli* O157:H7. In cases such as these, where surrogates are utilized, the suitability of the surrogates for use should be proven based upon the above criteria.

3.5. Other considerations

The use of surrogate organisms to determine and validate the efficacy of produce decontamination processes may be challenging. The following are some further points to consider while undertaking this endeavor:

- Keep the approach as easy, accurate, and simple as possible.
- Design the process so that the surrogate response is predictable.

Table VII-2—Considerations for a standard surrogate procedure for determining the efficacy of a process for control of pathogens on fruits and vegetables (Adapted from Chapter VI Table VI-1)

Selection of surrogate
Prioritize targets of concern Select appropriate and valid surrogate microorganisms Gram-negative or Gram-positive bacteria, parasite, yeast or mold Mixture of strains or a single strain Marker or no marker Conditions for preparing inoculum Number of cells in inoculum
Type of produce
Whole or cut Washed, brushed, waxed, or oiled Botanical part (fruit, leaf, stem, flower, root, tuber)
Procedure for inoculation
Composition of carrier Temperature of produce and inoculum Dip, spray, or spot inoculum Temperature and relative humidity between time of inoculation, testing, and analysis
Procedure for evaluating processing test condition
Define treatment, condition, or processing situation Methods for measurement of processing treatment delivered Temperature and condition of produce entering process Application of specific process (for example, dipping, spraying, fogging or atmosphere) Time of exposure of inoculated produce to processing treatment Produce load and rate of exposure to process
Retrieval of surrogates
Sample weight, size, or number of pieces Composition of diluent Blending, homogenizing, macerating, or washing Time of treatment Composition of neutralizer (for sanitizer studies) Detection and enumeration media Conditions for incubating plates and broth Confirmation procedures
Reporting results
Number of replicates and samples/replicate CFU/g, CFU/cm^2, CFU/piece, MPN, Fraction negative Appropriate statistical analysis and interpretation Scale-up considerations for routine processing situations

- Be attentive to the introduction of system modifications or variables that could alter the properties of the produce leading to inaccurate results (for example, pH and temperature of produce and treatment solution, time of exposure to treatment, ratio of produce to treatment solution).
- Validate the susceptibility or tolerance of a surrogate.
- Work with a mixture of potential surrogate strains.
- Follow the standardization recommendations presented in Table VII-2.

4. Summary

Indicators and surrogate microorganisms may be used for evaluating safety of fresh or fresh-cut fruit and vegetable products by assessing or validating the effectiveness of microbial control measures. Although frequently used on an informal basis within a specific company, use of indicators is highly dependent upon microbiological criteria that are in place for the specific produce item or category. All the considerations that must be addressed in establishing microbiological criteria must also be in place if indicators are to be utilized in process verification. Sampling design, stringency, and statistical significance are critical to the evaluation of indicators or surrogates in the assurance of food safety. General ideal qualities of indicators and surrogates are valuable starting points when developing a safety program. The importance of selecting the significant target pathogen for the specific product, its source, handling practices, and distribution practices cannot be overemphasized. The same is true for selection of the indicator or surrogate to represent those pathogens. The extensive lists of considerations and procedures should be helpful when using indicators and surrogates with fresh and fresh-cut produce. The use and limitations of indicators and surrogates to determine or validate treatment effectiveness have been delineated. Challenges are identified for selection of an indicator or surrogate for the specific situation and conditions of an individual produce item, including growing, harvesting, processing, handling, storage, and packaging.

5. Research needs

- Identify indicators to determine if produce has been exposed to conditions that would permit contamination by or survival/growth of a pathogen after a given decontamination process.
- Identify indicators for use in sensitive specified ready-to-eat fresh and fresh-cut produce that would signal the presence of bacterial pathogens, pathogenic viruses, or pathogenic parasites.
- Identify surrogate microorganisms for use in sensitive specified ready-to-eat fresh and fresh-cut produce that would measure the effectiveness of intervention treatments targeted at decontamination from bacterial pathogens, pathogenic viruses, or pathogenic parasites.
- Develop comprehensive standardized and validated protocols that address the criteria and special considerations for use in selection and application of surrogate microorganisms in testing efficacy of pathogen control procedures and processes.
- Consider and evaluate nonvirulent strains as possible indicators and surrogates.
- Propose, design, and test evaluation program(s) for indicators of safety by systematically assessing possible sources of contamination, total volume sold, number of foodborne outbreaks attributed to the product/category, potential for mishandling, incidence data, and other quantifiable measures.
- Determine if pathogenic microorganisms placed in a specific decontamination situation may increase resistance, as a stress response to the intervention step.
- Differentiate between the influence of point of origin and processing environment on the stress responses.
- Identify and validate approaches to test the elected indicator(s) against wild and laboratory culture strains in a well-controlled pilot plan environment and in commercial conditions in an open natural environment.
- Collect new survey data with emerging molecular technologies that discriminate between virulent and nonvirulent strains to accurately pinpoint the hazardous situations and true pathogen targets for which indicators and surrogates are needed.
- Consult the entire list of considerations for examination of produce, whenever a new product, general geographic origin, decontamination procedure, or fresh-cut process is introduced and evaluate changes if differing from established normal procedures.
- Identify or develop special methodology to accurately and quantitatively retrieve indicator microorganisms, their metabolite or genetic material, especially when a stress has been administered that may result in damaged cells or VNC organisms.
- Assess existing and new testing procedures and sampling plans to verify that they have appropriate stringency with stipulat-

ed statistical design and probability considerations to accurately predict presence of target organisms or probable contamination.

References

[Anonymous]. 2000. NFPA White Paper—Significance of coliforms and generic *Escherichia coli* on fresh and minimally processed produce. <http://www.nfpa-food.org/members/science/wp_ecoli.html>. Accessed 2001 Aug 29.

Banks JG, Board RG. 1983. The incidence and level of contamination of British fresh sausages and ingredients with salmonellas. J Hygiene 90:213-23. (cited in Mossel and others 1995).

Barker C, Park SF. 2001. Sensitization of *Listeria monocytogenes* to low pH, organic acids, and osmotic stress by ethanol. Appl Environ Microbiol 67:1594-600.

Brackett RE, Splittstoesser DF. 2001. Fruits and Vegetables. In: Downes FP, Ito K, editors. Compendium of methods for the microbiological examination of foods. Washington: American Public Health Association. p 515-20.

Buchanan RL, Edelson SG, Boyd G. 1999. Effects of pH and acid resistance on the radiation resistance of enterohemorrhagic *Escherichia coli.* J Food Prot 62:219-28.

Buchanan RL. 2000. Acquisition of microbiological data to enhance food safety. J Food Prot 63(6):832-8.

Doyle ME, Mazzotta AS. 2000. Review of studies on the thermal resistance of *Salmonellae*. J Food Prot 63:779-95.

Drucker E, Webber MP, McMaster P, and others. 1989. Increasing rate of pneumonia hospitalizations in the Bronx: a sentinel indicator for human immunodeficiency virus. Int J Epidemiol 18:926-33. (cited in Mossel and others 1995).

Haas CN, Meyer MA, Paller MD, and others. 1983. The utility of endotoxins as a surrogate indicator in potable water microbiology. Water Res 17:803-7. (cited in Mossel and others 1995).

Havelaar AH, Pot-Hogeboom WM. 1988. F-specific RNA-bacteriophages as model viruses in water hygiene: ecological aspects. Wat Sci Technol 20:399-407. (cited in Mossel and others 1995).

[ICMSF] International Commission on Microbiological Specifications for Foods. 1986. Sampling for microbiological analysis: principles and specific applications. 2nd ed. Toronto: Univ Toronto Pr. (Microorganisms in foods: 2).

[ICMSF] International Commission on Microbiological Specifications for Foods. 1998. Fruits and fruit products. London: Blackie Academic and Professional. 252-73 p. (Microorganisms in Foods: 6).

[ICMSF] International Commission on Microbiological Specifications for Foods. 2001. Microbiological testing and food safety management. [unknown]: Aspen. (Microorganisms in foods; 7). Forthcoming.

Ingram M. 1977. The significance of the index and indicator organisms in foods. In: Presented at the Tenth Intl Symposium of the IUMS Committee on Food Microbiology and Hygiene; 1977 Sept 5-10; Szczecin, Poland. Unpublished: for an account of this paper, see Mossel, D.A.A., Coliform test for cheese and other foods. Lancet ii, 1425. (cited in Mossel and others 1995).

Jay JM. 1996. Indicators of food microbial quality and safety. In: Modern Food Microbiology. 5th ed. New York: Chapman & Hall. p 387-407. (Food science texts series).

Jay JM. 2000. Indicators of food microbial quality and safety. In: Modern food microbiology. 6th ed. Gaithersburg (MD): Aspen. p 387-406.

Kornacki JL, Johnson JL. 2001. *Enterobacteriaceae*, Coliforms, and *Escherichia coli* as quality and safety indicators. In: Downes FP, Ito K, editors. Compendium of methods for the microbiological examination of foods. Washington: American Public Health Association. p 69-82.

Kvenberg J. 1999. Report of the NACMCF qualified through verification working group. Personal communication. (cited in Anonymous 2000).

Liao C-H, Sapers GM. 2000. Attachment and growth of *Salmonella* chester on apple fruit and in vivo response of attached bacteria to sanitizer treatments. J Food Prot 63(7):876-83.

Lund BM, Snowdon AL. 2000. Fresh and processed fruits, Chapter 27. In: Lund BM, Baird-Parker TC, Gould GW, editors. The microbiological safety and quality of food, Volume I. Gaithersburg (MD): Aspen. p 738-58.

Lytle CD, Truscott W, Budacz AP, and others. 1991. Important factors for testing barrier materials with surrogate viruses. Appl Environ Microbiol 57:2549-54. (cited in Mossel and others 1995).

Mattick KL, Jorgensen F, Legan JD, Lappin-Scott HM, Humphrey TJ. 2000. Habituation of *Salmonella* spp. at reduced water activity and its effect on heat tolerance. Appl Environ Microbiol 66:4921-5.

Mossel DAA, Corry JEL, Struijk CB, Baird RM. 1995. Essentials of the microbiology of foods: a textbook for advanced studies. Chichester (England): John Wiley & Sons. 287-9 p.

[NACMCF] National Advisory Committee on Microbiological Criteria for Foods. 1999. Microbiological safety evaluations and recommendations on fresh produce. Food Control 10:117-43.

Nguyen-the C, Carlin F. 2000. Fresh and processed vegetables. In: Lund BM, Baird-Parker TC, Gould GW, editors. The microbiological safety and quality of food. Gaithersburg (MD): Aspen. p 620-84.

Obiri-Danso K, Paul N, Jones K. 2001. The effects of UVB and temperature on the survival of natural populations and pure cultures of *Campylobacter jejuni, Camp. coli, Camp. lari* and urease-positive thermophilic campylobacters (UPTC) in surface waters. J Appl Microbiol 90:256-67.

Pagan R, Jordan S, Benito A, Mackey B. 2001. Enhanced acid sensitivity of pressure-damaged *Escherichia coli* 0157 cells. Appl Environ Microbiol 67:1983-5.

Payment P, Franco E. 1993. *Clostridium perfringens* and somatic coliphages as indicators of the efficiency of drinking water treatment for viruses and protozoan cysts. Appl Environ Microbiol 59:2418-24. (cited in Mossel and others 1995).

Ray B. 1989. Injured index and pathogenic bacteria: occurrence and detection in foods, water and feeds. Boca Raton (FL): CRC Pr.

Smoot LM, Pierson MD. 1997. Indicator microorganisms and microbiological criteria. In: Doyle MP, Beuchat LR, Montville TJ, editors. Food microbiology: fundamentals and frontiers. Washington: American Society for Microbiology. p 66-80.

Smoot LM, Pierson MD. 1998. Effect of environmental stress on the ability of *Listeria monocytogenes* Scott A to attach to food contact surfaces. J Food Prot 61:1293-8.

Sobsey MD, Fuji T, Shields PA. 1988. Inactivation of hepatitis A virus and model viruses in water by free chlorine and monochloramine. Wat Sci Technol 20:385-92. (cited in Mossel and others 1995).

Wolfe RL, Stewart MH, Liang S, McGuire MJ. 1989. Disinfection of model indicators organisms in drinking water pilot plant by using peroxone. Appl Environ Microbiol 55:2230-41. (cited in Mossel and others 1995).

Zook CD, Brady LJ, Busta FF. 2001. Peroxidative stress adaptation and thermal cross-resistance in *Escherichia coli* 0157:H7 933 subjected to sublethal doses of peroxyacetic acid sanitizer. J Food Prot 64:767-9.

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