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Review

Indicator Organisms in Meat and Poultry Slaughter Operations: Their Potential Use in Process Control and the Role of Emerging Technologies

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ABSTRACT

Measuring commonly occurring, nonpathogenic organisms on poultry products may be used for designing statistical process control systems that could result in reductions of pathogen levels. The extent of pathogen level reduction that could be obtained from actions resulting from monitoring these measurements over time depends upon the degree of understanding cause-effect relationships between processing variables, selected output variables, and pathogens. For such measurements to be effective for controlling or improving processing to some capability level within the statistical process control context, sufficiently frequent measurements would be needed to help identify processing deficiencies. Ultimately the correct balance of sampling and resources is determined by those characteristics of deficient processing that are important to identify. We recommend strategies that emphasize flexibility, depending upon sampling objectives. Coupling the measurement of levels of indicator organisms with practical emerging technologies and suitable on-site platforms that decrease the time between sample collections and interpreting results would enhance monitoring process control.

In the United States, acquired foodborne illness has been estimated to cause 48 million illnesses, 128,000 hospitalizations, and 3,000 deaths each year *(34)*. FoodNet (Foodborne Diseases Active Surveillance Network) reports significant declines in the incidence of certain pathogens associated with illnesses; most of these reductions occurred before 2004. This program reported an incidence rate of salmonellosis of 16.2 cases per 100,000 in 2008, which is above the ''Healthy People 2010'' target of 6.8 per 100,000 *(12)*.

The plateau of salmonellosis corresponds to the plateau of *Salmonella* prevalence found in young chickens (broilers) and other meat and poultry products as measured by recent statistically designed Food Safety and Inspection Service (FSIS) surveys. From an FSIS survey conducted in 1994 *(39)*, the estimated prevalence of *Salmonella* on carcasses exiting chiller tanks was 20%. From a 2001 FSIS survey *(40)*, this value decreased to 8.7%; from the most recent FSIS survey (2007), the value was estimated to be 7.5% *(41)*. The *Salmonella*-salmonellosis relationship suggests that further reduction in *Salmonella* prevalence might be useful for reducing foodborne illnesses associated with this pathogen. As establishment-specific *Salmonella* prevalence diminishes, evaluating the progress of further reductions requires testing of more samples. Other methods of measuring process control need to be explored because of the expense associated with extensive testing for *Salmonella*.

The FSIS Pathogen Reduction/Hazard Analysis Critical Control Point (PR/HACCP) regulation specifies multiple approaches for monitoring process control. The regulation notably specifies using measured levels of generic *Escherichia coli* to assess process control *(38)*, through a three-class attribute sampling plan, where class demarcation values are based on the 80th and 98th percentiles of a nationwide distribution of *E. coli* levels determined from an FSIS survey, and a moving window of 13 samples. The PR/HACCP regulation stipulates a rate of sampling within a young chicken (broiler) slaughter establishment of about 1 per 22,000 carcasses *(38)*. Thus, the moving window of 13 samples represents about 286,000 carcasses. The time needed to process a flock varies with slaughter systems, which vary in line number and speed. Some of the commonly used systems are New Enhanced Line Speed, Streamlined Inspection System, and Maestro/NewTech *(4)*. For example, an establishment operating an evisceration line speed of 70 birds per min with two lines will process 286,000 birds in about 34 h of operation. In this scenario, the moving window of 13 samples generally represents different shifts and days of processing, introducing operationally related variables adversely affecting the development of a robust monitoring system.

This article addresses an important question to food safety: is a sampling plan using indicator organisms

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effective or useful for improving processes and food safety? Previous research *(5, 9–11, 20, 21)* pointed to the lack of correlation between the presence of indicator organisms and pathogens. National and international organizations (Food and Agriculture Organization/World Health Organization, the U.S. National Research Council, and the International Commission on Microbiological Specifications for Foods) have issued findings and reports suggesting that there is a lack of available scientific data to support a correlation of indicators with pathogens in food products *(13, 19, 29)*. One could easily surmise that there would be little benefit in using measurements of indicator organisms to help ensure food safety. However, correlations are complex statistical measures that depend upon the population being studied and the distribution of the variables within that population, as well as measurement and sampling error. Furthermore, the presence of indicators often just indicates conditions that are indirectly related to the actual presence of pathogen but, when existing, could affect the likelihood of their presence or numbers. For example, studies performed in establishments that have processes in control might result in only small correlations compared with what might have been observed if the process was not in control. Thus, the lack of a measurable or substantive correlation between indicator organisms and target pathogens (levels and incidence) in previously published articles does not conclusively negate the use of nonpathogenic organisms as indicators of process control with respect to pathogens.

An effort to define the role of indicator organisms to assess a process for controlling pathogen levels would require determining the cause-and-effect relationship of processing steps and their impact on the distribution of microbial levels. Such an investigation could be expensive; however, an investigation could lead to an effective use of indicator organism measurements. The following section provides a brief discussion of basic concepts followed by discussions of other issues related to the use of indicator organisms.

BASIC CONCEPTS

The concept of assessing food safety by testing for a nonpathogenic organism was first introduced in 1892 *(35)*. At that time, the measurement of coliform levels was used to assess possible contamination of water by a fecal source and thus an indicator of *Salmonella* Typhi. In 1927 and1932, pasteurized milk, initially subjected to bactericidal treatments, contained measurable units of coli-aerogenes (coliform) bacteria, which was indicative of recontamination following treatment or lack of sufficient lethality during the treatment *(24, 27, 37)*. This example highlights the value of using an indicator organism to determine the possible presence of target pathogens and for determining an appropriate process control to limit contamination.

The definitions of ''index organism'' and ''indicator organism'' differentiate how organisms are used. Mossel and colleagues *(25–27)* defined an index organism for some pathogen as an organism for which there is an ''association'' with the pathogen in the food matrix. Mossel continued by defining indicator organisms as ''used for the purpose of assessing the risk of inadequate bacteriological quality of a more general nature'' *(26)*. Similarly, Buchanan *(7)* defined indicator organisms as a ''microorganism or group of microorganisms that are indicative that a food has been exposed to conditions that pose an increased risk and that the food may be contaminated with a pathogen or held under conditions conducive for pathogen growth.'' Other authors have defined a similar concept by using hygiene marker terminology *(6)*. However, in Buchanan's definition, an ''indicator'' is connected to process control by a concept of ''cause''—conditions that underlie the relationship between the organisms, even though there (still) is a hint of correlative dependency between pathogen and indicator.

It is commonly assumed that the primary difference between the terms ''index'' and ''indicator'' as applied to organisms is that, while both could have some sort of association with the pathogen(s), for the former the association with associated pathogen(s) is less ambiguous and thus is more readily observed to be correlated with the pathogen(s) while for the latter it is not as explicit and thus not readily observed to be correlated with pathogen(s). Thus, the term ''index organisms'' has been used for organisms, e.g., *E. coli,* whose presence in numbers above certain limits indicates the possible presence of ecologically similar pathogens in potable water *(26, 27)*. ''Indicators'' are those organisms whose presence in numbers above certain limits indicates inadequate processing for ensuring that pathogens would not be present *(24, 27, 36)* or would be present in small numbers, assuming the organism was present, at possibly high levels, before the processing step of concern. Therefore, classifying an indicator organism for a pathogen or set of pathogens requires (i) an understanding of how the indicator organism's presence at high levels reflects probable or possible process deficiencies; (ii) acceptance of the assumption that, in response to monitoring levels of indicator organisms, actions that improve the process or introduce new process controls to help provide consistent processing could eliminate or reduce the number of indicator organisms; and (iii) that such actions could also affect the levels or presence of pathogens. For example, assume a processing deficiency has the effect of creating conditions favorable to bacterial growth resulting in higher-than-expected levels of the indicator organism; then, a corrective action causing the elimination of these favorable conditions and resulting in reduced levels of indicator organisms could also result in reducing levels of pathogens.

A simple cause-and-effect diagram depicts this situation (Fig. 1). Condition C is said to cause event E if there is some way of manipulating C that would have an impact on E *(45)*. Figure 1 presents a cause-and-effect diagram depicting a relationship between indicator and target organisms. In Figure 1, ''C'' represents a set of causes that act on both indicator organisms (X) and pathogen target organisms (Y). The diagram implies that there could be factors that affect Y without affecting X (Uy) and other factors that could affect X without affecting Y (Ux). In this diagram, X and Y need not be correlated in the general sense, for example, when comparing results collected

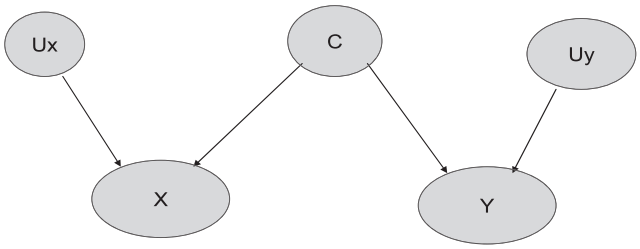

FIGURE 1. *Cause-and-effect diagram, depicting the relationship between indicator organism (X) and pathogen (Y), where C is a common cause for both and Ux and Uy are causes for each, independently.*

randomly over time within establishments, or from different establishments; rather, what this diagram implies is that some changes in values of ''C'' under some conditions could result in changes in levels of both X and Y. Thus, positive correlations between X and Y could exist under conditions that might not be present when data intended to measure a correlation are collected; Ux and Uy can mask the association (as determined from estimated correlations) between X and Y due to C. In practice, if X is to be an indicator for Y, then the changes made to C that impact X also would affect Y (and in the same direction). In the design of any processing system, the known components of Uy should be monitored independently of the monitoring for X. The degree to which the Uy factors exist and affect the levels of Y determines the usefulness of using X as an indicator organism for Y.

STATISTICAL PROCESS CONTROL (SPC)

Basically, when a process is in control, measurements of the levels (or some other appropriate measure) of indicator organisms would have a certain distribution, *F,* characterized by being unimodal and by certain parameters, such as the mean, median, various percentiles, standard deviation, and/or range of the results. Statistical tests can be performed to determine whether the underlying distribution of measured indicator organism levels, at any time, was significantly different from *F*—the null distribution. An out-of-control signal, as defined by some statistical criterion, is assumed to result from some cause, termed ''assignable cause'' by Wheeler and Chambers *(43).* Once the cause(s) is identified, consequent actions taken to address or eliminate the cause would presumably decrease the levels of indicator organisms. A new null distribution, reflecting this decrease, could exist because of changes in processing. Through time, the process would be improved by finding additional assignable causes and taking effective actions to eliminate them. These actions could consist of putting in place process controls and quality control (QC) measures to ensure that they are operating properly. Eventually, a steady state might be reached, wherein it would appear that significant improvement in processing could not be made; essentially the process would be stable and operating at its assumed capability. Further improvements could be achieved by investing in new systems or implementing new interventions, thus creating a new capability for the process.

By conducting a series of studies for optimization of the system and identifying the period of time when it is assumed that the process is in control, the distribution (*F*) can be determined. This identifies the first of three primary difficulties in applying SPC. The second one involves identifying ''causes,'' when there is an out-of-control signal, that impact levels of indicator and target pathogen organisms; and the third one is determining the frequency of sampling sufficient for detecting when the process is out-of-control. The first difficulty is addressed by establishing a distribution of results over a period in which the process is presumed to be in control. In this period, *F* should have certain steady-state characteristics with which there are low autocorrelations of results *(1).* Often, the first determination of *F* is not adequate—that is, the distribution displays skewness or autocorrelations that suggest that some factors have a certain degree of influence on the results that could be controlled. As these factors are identified through time, improvements would be expected, and a new null distribution would be established.

The second and third issues involve identifying the causes of an out-of-control signal. Information needs to be collected to aid in identifying possible causes. Thus, procedures should be established for collecting possible relevant processing information that could be used in investigating possible causes for poor processing, such as keeping records regarding important process operating parameter values, characteristics of source materials of processing intervention aids such as antimicrobial solutions used, product as it moves through the system, and more generally results of QC monitoring of process controls that have been put in place. From a statistical perspective, it is possible that the out-of-control signal is due to random chance or that the cause that is identified is not one that would be related to the target pathogen (e.g., factors in Ux as shown in Fig. 1). As the frequency of sampling increases, the likelihood of these occurrences increases. The first of these possibilities can be addressed by setting statistical criteria for deciding whether the system is out-of-control in order to achieve a ''balance'' between the chances of making a type I error (saying the system is out-of-control when it is not) and a type II error (not detecting a true out-of-control situation). Criteria that have a higher associated type I error rate make it more likely to initiate a search to find assignable causes but, perhaps, less likely to find one and thus could be inefficient. On the other hand, establishing criteria with high type I error rates can help ensure that causes be sought and identified when they occur; it is possible that even small deviations from the norm (the null distribution) could indicate the existence of causes. One situation in which a higher type I error rate might be desirable is when SPC procedures are being used for the first time in a system when, or whenever else, there is less confidence that the system's process controls can be maintained consistently over time.

Before discussing indicator organisms for meat and poultry processing, it is important to note two related aspects of SPC. First, SPC does not just provide evidence of poor processing but can also identify when a process is

doing better than originally anticipated by identifying sequences of results with a ''better'' underlying distribution than the null distribution, *F*. Such findings could be used to identify the cause(s) of the ''good sequences'' of results. Second, finding causes associated with Ux, even if they have no impact on the pathogen levels, is helpful because identifying and acting to eliminate the adverse effect of Ux on X should make identifying C easier. These features of SPC can be realized through an active search for causes enabled by the system and accompanying data collection designs; thus, auxiliary data that could be used for identifying factors associated or correlated with the microbial results could be used for exploratory data analyses, such as looking for differences of measured levels of indicator organisms among commodity or livestock suppliers.

Thus, SPC is distinguished by the development of measurable objectives that are statistically determined in an attempt to characterize the ''process capability'' (i.e., when the process is thought to be in control). Data are collected and results compared with these objectives through time (sequentially), wherein a decision is made to determine if the process is out-of-control after each sampling. The objective, *F,* might not be properly or correctly defined (e.g., during the period when *F* was determined and the system was not performing up to its capability), and thus, constant review of sample results is necessary, retrospectively, to help identify out-of-control periods. Once identified, effective action to address the causes could consist of putting in place multiple process controls and QC measures that could help ensure that these processes are working properly. In addition, new procedures or processes could be introduced. Thus, there is a dynamic component associated with SPC, where *F* can be changed over time, based on continuous data analysis, reflecting changes (improvement) in processing capabilities.

ISSUES RELATED TO THE USE OF INDICATOR ORGANISMS

To be useful, variation in the levels of indicator organisms should effectively and efficiently demonstrate variation in the degrees of control of a process. The effectiveness depends upon the relational properties of the concerned and candidate organisms, and efficiency of course depends upon the cost of implementing measurement procedures. Thus, when selecting a process control indicator, four broad issues *(8, 19, 31)* are important to consider: (i) the nature of the ecological association and correlation with pathogen growth and inactivation kinetics; (ii) the prevalence of the proposed indicator; (iii) the ease of measurement; and (iv) the purpose of use. The first issue addresses underlying conditions that pathogens and indicator organism share that could establish the rationale for the belief in the causal pathway(s) connecting the process. Fecal material has long been considered the main source of pathogens in contaminated raw meat and poultry products. During the slaughter process, it is difficult to avoid the contamination of carcasses with fecal enteric organisms that reside on hair, hooves, hide, feathers, or ruptured digestive tract contents. Thus, it could be noted that, for example, certain targeted pathogens are enteric and gram negative, and it would be expected that good indicator organisms for these pathogens should also share these characteristics including similar taxonomic groups and be enteric such as *E. coli,* coliforms, enterococci, and *Enterobacteriaceae (13, 27).* Different indicators might be appropriate for pathogens that are found primarily in the environment and are gram positive (such as *Listeria*). Knowing the locations of organisms within the carcass might also provide a rationale for assuming one organism to be a good indicator for another one. However, we point out that a natural association between indicator and pathogen organisms is not necessary; for example, introducing surrogates (markers) illustrates how an indicator and a pathogen need not be ecologically linked but might provide a valuable mechanism to monitor certain steps of a process *(5).* There might be, however, practical difficulties of such use since its introduction might affect the quality of the product in other ways, and of course the expense involved with setting up a delivery system that could effectively control the levels of the surrogate is an important consideration.

The second and third issues relate to operational practicality; the less expensive and more accurate measurements are, the better their use in a QC setting. These issues are generally accepted for most conceivable applications and affect sampling frequency. The last issue, purpose, relates to defining desired processing capability and identifying the type of actions that would be taken as a result of a presumed out-of-control process. It is the last component that is often problematic for process control—the extrapolation of the assumed relationship to define appropriate actions might be difficult because of the highly complex microbial ecologies that exist for processes of these products *(2, 14).* These complexities might contribute to why correlations have not been observed, hampering the ability to find causes for out-of-control processes. Considerations of these issues affect the frequency of sampling for designing a sampling plan that is both effective and efficient.

TYPE II ERRORS

The ability to identify ''false-negative'' results associated with the selected indicator organism requires an in-depth examination of the process (e.g., high pathogen levels without the corresponding out-of-control signal). A variety of QC measures might be needed to mitigate the consequences of false-negative occurrences.

Some studies have identified factors or conditions that might interfere with identification of positive correlations and thus might lead to type II errors (e.g., nonfecal source) *(15–17, 21, 44).* These factors or conditions include different binding properties of indicator and pathogenic organisms *(23, 30),* pathogen-free herds and flocks, different environmental survival rates for indicator and target pathogens *(32),* competitive microflora *(17, 46),* stressed and injured cells, and sampling and measurement variability *(22).* Despite these possible factors or conditions,

we have not found many examples that would imply type II errors. Russell provides a most illustrative example of conditions leading to false-negative events *(33)*; this study reported levels of *E. coli* and *Campylobacter* on carcasses from flocks identified as air-sacculitis positive (AS) compared to flocks identified as AS negative. From the information reported, the mean log levels of *Campylobacter* measured on carcasses from AS-positive flocks at postchill were about 1.0 log greater than the mean log levels of *Campylobacter* measured on carcasses from AS-negative flocks (all carcasses processed came from birds that passed antemortem inspection). The mean of the log levels of *E. coli* on AS-positive flocks was greater by only about 0.16 log, which was assumed to be due to poor processing. At the same time, it was reported that there were substantially more tears and ruptures on carcasses from the AS-positive flocks during the evisceration process, which could increase the amount of enteric bacteria from the intestines on the carcass. The number of samples in the study was not large, and thus the conclusions are not definitive; however, this provides an example of conditions under which using measured *E. coli* levels at postchill might not detect poor processing, with a possible detrimental effect on the safety of the product due to an increase in *Campylobacter* levels. This is consistent with findings by Berrang et al. *(3),* who reported that *Campylobacter* levels in the entire crop, ceca, and colon samples are proportionally higher relative to other parts of the carcass than *E. coli* levels on commercial broiler carcasses. Thus, it would be expected that poor evisceration processing could lead to an increase of *Campylobacter* levels, if the organism is present, while *E. coli* levels might not show a corresponding increase. This suggests that *E. coli* might not be a good indicator for *Campylobacter*.

However, based on present measuring procedures, *Campylobacter* does not seem to be as widespread (i.e., testing yields many nondetects) as *E. coli* at postchill and is considerably more difficult and expensive to measure. Consequently, it would be preferable to use *E. coli,* another bacterium, or a different measurement method (e.g., aerobic plate count [APC]) as an indicator. In this example of poor processing, *E. coli* and *Campylobacter* levels were both higher in the AS-positive flocks, but the *E. coli* levels were higher to a much lesser degree. This relationship suggests that a QC plan based on *E. coli* should be sensitive to small deviations from process evaluation criteria, and many measurements would need to be collected within a short time, depending upon how timely detection is desired, minimizing type I and type II errors. To achieve QC charting that is sensitive to small systematic level changes, sequential analysis statistical techniques for QC such as cumulative sum deviations from a target (CUSUMs; see equation below) or exponentially weighted moving averages (EWMA) of deviations should be used, because these methods are more sensitive to small deviations from a target than other statistical methods *(1, 42).* This approach along with the use of direct measurements of the incidence of fecal contamination on carcasses (e.g., visually or with instruments) might be sufficient for monitoring the evisceration process.

TABLE 1. *APC (swab) before and after processing of carcass surfaces*[a]

Pair no.	Day	Initial APC	Ending APC	Reduction (difference)
1	1	4.41	2.34	2.07
2	1	5.03	1.93	3.10
3	1	6.56	3.60	2.95
4	1	3.25	2.88	**0.37**
5	1	4.38	2.99	**1.39**
6	1	4.00	3.83	**0.17**
7	1	5.18	3.51	**1.68**
8	1	3.48	3.58	**−0.10**
9	1	3.99	3.58	**0.41**
10	1	4.08	4.98	**−0.90**
11	1	4.16	1.78	2.39
12	1	4.13	2.34	1.79
13	2	3.41	3.45	−0.03
14	2	3.41	1.60	1.81
15	2	4.89	1.40	3.49
16	2	3.75	2.20	1.54
17	2	3.81	1.30	2.51
18	2	4.58	1.00	3.58
19	2	4.89	2.08	2.81

[a] Values are in log CFU per milliliter; values in boldface indicate lower-than-average reductions. Pairs of samples were taken 10 min apart. Different carcasses were sampled within each pair.

USES OF INDICATOR VARIABLES

The type II error concerns illustrated above, along with a high variability of measured values associated with indicator organisms, suggest that measuring indicator organisms could be beneficial, provided that a sufficient number of measurements are made over a short time using sensitive QC charting procedures for detecting trends, such as a CUSUM charting procedure. An example from actual data is given here to highlight some of the issues with sample design.

Swab samples were collected from paired carcasses, one taken before processing and one after processing, at 10-min intervals for 2 h over the course of 1 day, resulting in 12 sample pairs. On day 2, paired samples were collected for 1 h, resulting in seven paired samples. In total, 19 paired samples were collected. FSIS microbiologists determined APC for each pair of samples, and the reductions of levels, in log units, were computed. The reasons for using logarithmic-transformed results are that the expected measurement variances of the transformed results are more constant than the original results over the range of results and the distribution of the transformed results is more symmetric than that of the original results. These two properties (uniformity of variances over the range and greater symmetry) greatly simplify the statistical analysis. In addition, the logarithmic-transformed results provide a better scale for presenting and graphing differences. Table 1 presents the log of the measured APC and the differences (reduction) for the 19 paired samples. Over a period of 70 min on the first day, the lower reductions occurred from the 4th to 10th pairs of samples. There are a few other occasional low results, and in general, it appears that the

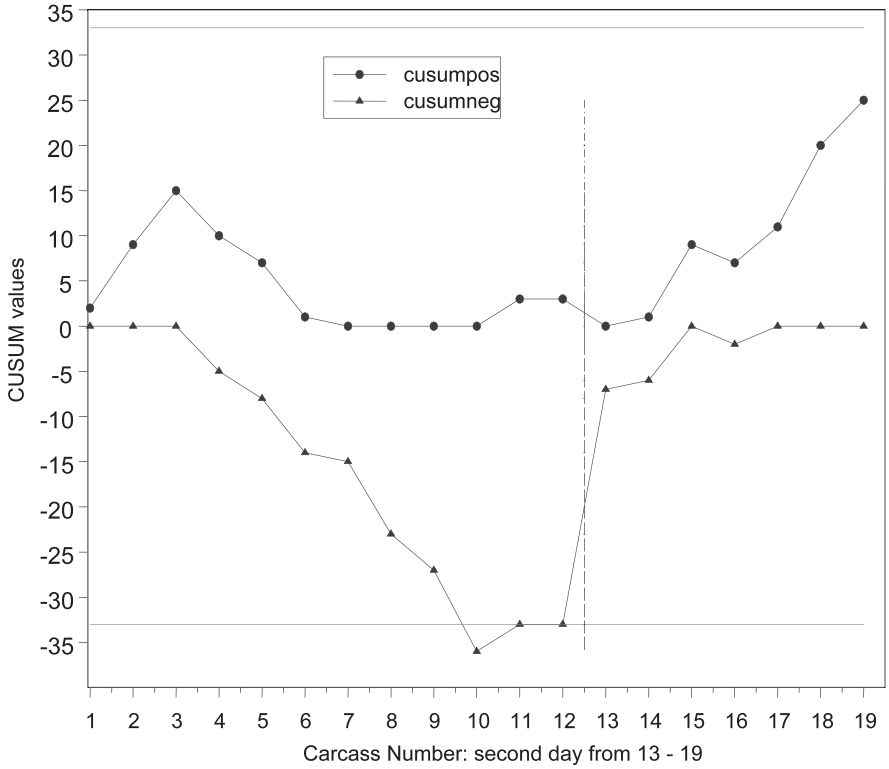

FIGURE 2. *Positive- and negative-trend CUSUMs of log APC reductions shown by plotting of Table 1 data. Horizontal lines at −33 and 33 indicate boundaries of nonsignificant trends: any CUSUM value greater than or equal to 33 or less than or equal to −33 indicates a statistically significant trend with a* P *value of <0.05. The vertical line distinguishes the two days of sampling.*

distribution of reductions is bimodal, where $P = 0.19$ based on the likelihood ratio statistic when comparing fits of bimodal normal distributions versus a single normal distribution. There are not enough data points to determine a parametric null distribution *(1),* so a retrospective statistical analysis testing for trends was performed using the ranks of the reductions over the 19 samples and positive- and negative-trend CUSUMS. The negative-trend CUSUM, CUSUM-N (k) for the kth sample, was computed as follows:

$$\text{CUSUM-N}\,(k) = \min\left[0,\ \text{CUSUM-N}\,(k-1) + (r_k - 10)\right]$$

where r_k is the rank of the kth observation, $k = 1, \ldots, 19$; CUSUM-N (0) was assigned a value of 0, and for the second day, the CUSUM was started anew from 0. The positive-trend CUSUM was computed similarly, except that the maximum instead of the minimum was used. Figure 2 is the plot of both CUSUMs, together with horizontal lines at 33 and −33, demarking the statistically significant results ($P < 0.05$) outside this region (−33, 33). A significant trend with a CUSUM value of less than or equal to −33 would indicate a ''process out-of-control'' signal, which should initiate an investigation regarding the possible cause. A significant trend with a CUSUM value of greater than or equal to 33 would indicate greater reductions than expected, initiating another investigation to find the reason for better-than-expected results. The demarcation interval was determined by performing 100,000 simulations.

We assume that the measurements are correct and are not due to sample mishandling; as such, the results represent the performance of the process. Given this assumption, the example demonstrates that a clear, statistically significant trend of an assumed out-of-control process occurred in a rather short time (~1 h). An hourly sample plan might not have caught this presumed egregious processing deficiency.

Whether this example would or should imply sampling every 10 min depends on context. If there were a desire to ensure the process is functioning properly at all times, then it would be important to identify and correct the process failure as soon as possible. During a development and validation phase, for example, extensive sampling to identify areas of poor processing might be desirable and extensive sampling would thus be reasonable. On the other hand, the perspective could be that short-term infrequent events of poor processing, while not desirable, are not necessarily critical to identify. The concern instead might be whether such egregious events occurred with some regularity. In this situation, over time, the results could have a bimodal distribution. An hourly sampling plan would take longer to detect the bimodal characterization of the distribution than a 10-min sample plan but could be sufficient for detecting longer-term processing deficiencies. Identifying and investigating a single event of poor short-term processing might not be fruitful because of the lack of in-depth information associated with the event. As these events are identified, more information would be available, thus perhaps making it easier to determine a cause(s) for the bimodal distribution. QC charting procedures, such as an F-chart *(1)* could be used for identifying an excessive frequency of these egregious events.

The full realization of the SPC concept can occur when there is effective action in response to process deviations at various strategic points in the production cycle. Infrequent sampling over a long period could be inadequate for identifying, in a timely manner, inconsistent processing, except in the most egregious and continual processing deficiencies. However, high-frequency sampling, which might lead to process improvements, needs to be balanced with an efficient use of resources. Ultimately, the correct balance of sampling and resources is determined by what is

considered to be important processing deficiencies to identify. Strategies that emphasize flexibility or adaptability should be considered. More frequent sampling might be necessary for new processes in an ongoing validation. Over time, the process should improve and stabilize. Reduced microbiology sampling that is sufficient to detect significant trends of poor processing combined with other types of QC measurements (on process controls) could be implemented to ensure continual good processing.

ROLE OF EMERGING TECHNOLOGIES AND PROCESS CONTROL

Successful use of an indicator organism we believe depends on a sufficient frequency of measurements that would provide power for detecting significant or important changes in the distribution of the measured levels of the indicator organisms. Computer systems that could handle the data, compute appropriate QC charts (e.g., CUSUM-, Shewhart-, or F-charts), and provide rapid feedback would enhance the efficient use of frequently collected data. Thus, implementing cost-effective, on-site testing platforms, with appropriate computer support to identify deviations (trends) from those of a null distribution rapidly could be of great benefit. The merit of introducing new technologies to testing programs has been recognized in two recent FSIS-sponsored expert solicitation recommendations *(18, 28).*

While computerization of data would lead to efficiencies and more rapid feedback than otherwise, the actual measurement process places a bound on how efficient the data handling could become. Traditional culture-based methods require sample collection, shipping, transportation, laboratory analysis, and obtaining results usually in 24 h or more. Emerging technologies, such as nucleic acids, biosensors, and nanotechnology, with the ability to detect multiple organisms or molecular markers simultaneously, may offer new opportunities to lessen the resources needed and expedite making important decisions based on more rapidly returned results. One potential limitation of emerging technologies is that, unlike culture-based methods, some of the emerging technologies are not capable of detecting viable cells. Nonetheless, emerging technologies that can detect viable cells and alleviate this potential defect are being developed. For example, a PCR-based detection system using mRNA (which is unstable and easily degradable) can detect viable bacteria *(28).*

CONCLUSIONS

The term ''indicator'' implies that common causes affect the levels of both indicator microorganisms and pathogens and that these causes can be identified and controlled. The use of measured levels of an indicator organism within SPC is based on the basic premise that the process can be improved over time, by identifying a cause of higher-than-expected indicator organism levels and taking an action that would result in a decrease of levels of the indicator organism, which in turn could also decrease levels and incidence of pathogens on the product.

It is important to explore reasons that would lead to misleading or incorrect decisions when designing SPC monitoring systems for this type of application (e.g., monitoring levels of an indicator with the purpose of controlling levels of pathogens). Further improvements depend upon getting information, within SPC, in an attempt to identify causes of poor processing. We suggest that effective QC using indicator organisms requires many measurements and QC charting procedures that are sensitive to small deviations from a target, such as CUSUM. In addition, more than one type of monitoring activity might be needed (e.g., direct measurement of fecal contamination together with measurements of *E. coli* and *Campylobacter*). Determining the process deficiency characteristic of interest will clarify the correct balance of sampling and resources. Strategies that emphasize flexibility or adaptability should be considered, incorporating other, nonmicrobiological types of QC measurements for process control. As processing becomes stable, the need for microbiological sampling could become less frequent. The use of new technologies could provide greater opportunities to explore the development of reliable, cost-effective, robust, multianalyte, in-establishment testing platforms that can identify out-of-control operations and initiate corrective actions quickly.

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REFERENCES

1. AOAC International. 2006. Presidential Task Force on Best Practices for Microbiological Methodology (BPMM), Appendix F. U.S. FDA contract no. 223-01-2464. AOAC International, Gaithersburg, MD.
2. Bank, J. G., and R. G. Board. 1983. The incidence and level of contamination of British fresh sausages and ingredients with salmonellas. *J. Hyg. Camb.* 90:213–223.
3. Berrang, M. E., R. J. Buhr, and J. A. Cason. 2000. *Campylobacter* recovery from external and internal organs of commercial broiler carcass prior to scalding. *Poult. Sci.* 79:286–290.
4. Bilgili, S. F. 2001. Poultry meat inspection and grading, p. 47–72. *In* A. R. Sams (ed.), Poultry meat processing. CRC Press LCC, Boca Raton, FL.
5. Bilgili, S. F., A. L. Waldroup, D. Zelenka, and J. E. Marion. 2002. Visible ingesta on pre-chill carcasses does not affect the microbiological quality of broiler carcasses after immersion. *J. Appl. Poult. Res.* 11:233–238.
6. Brodsky, M. H. 1995. The benefits and limitations of using index and indicator organisms in verifying food safety. Presented at the FSIS Meeting on the Role of Microbiological Testing in Verifying Food Safety, Philadelphia, 1 to 2 May 1995.
7. Buchanan, R. L. 2000. Acquisition of microbiological data to enhance food safety. *J. Food Prot.* 63:832–838.
8. Busta, F. F., T. V. Suslow, M. E. Parish, L. R. Beuchat, J. N. Farber, E. H. Garrett, and L. J. Harris. 2003. The use of indicators and surrogate microorganisms for the evaluation of pathogens in fresh and fresh-cut produce, chap. VII. *In* Analysis and evaluation of pathogens in fresh and fresh-cut measures for the control and reduction/elimination of microbial hazards on fresh and fresh-cut produce. A report of the Institute of Food Technologists for the Food and Drug Administration of the United States. *Compr. Rev. Sci. Food Saf.* 2(Suppl. 1):179–185.
9. Cason, J. A., J. S. Bailey, N. J. Stern, A. D. Whittenmore, and N. A. Cox. 1997. Relationship between aerobic bacteria, salmonellae, and *Campylobacter* on broiler carcasses. *Poult. Sci.* 76:1037–1041.

10. Cason, J. A., M. E. Berrang, R. J. Buhr, and N. A. Cox. 2004. Effect of prechill fecal contamination on numbers of bacteria recovered from broiler chicken carcasses before and after immersion chilling. *J. Food Prot.* 67:1829–1833.
11. Cason, J. A., A. Hinton, J. K. Northcutt, R. J. Buhr, K. D. Ingram, D. P. Smith, and N. A. Cox. 2007. Partitioning of external and internal bacteria carried by broiler chickens before processing. *J. Food Prot.* 70:2056–2062.
12. Centers for Disease Control and Prevention. 2009. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 States, 2008. *Morb. Mortal. Wkly. Rep.* 58:333–338.
13. Food and Agriculture Organization of the United Nations and World Health Organization. 1979. Report on a joint FAO/WHO Working Group on Microbiological Criteria for Foods, Geneva, 20 to 26 February 1979. Document WG/Microbiol/79/1. World Health Organization, Geneva.
14. Goepfert, J. M. 1976. The aerobic plate count, coliform and *Escherichia coli* content of raw ground beef at the retail level. *J. Milk Food Technol.* 39:175–178.
15. Gomis, S. M., C. Riddell, A. A. Potter, and B. J. Allan. 2001. Phenotypic and genotypic characterization of virulence factors of *Escherichia coli* isolated from broiler chickens with simultaneous occurrence of cellulites and other colibacillosis lesions. *Can. J. Vet. Res.* 65:1–6.
16. Gordon, D. M., S. Bauer, and J. R. Johnson. 2002. The genetic structure of *Escherichia coli* populations in primary and secondary habitats. *Microbiology* 148:1513–1522.
17. Holland, G. C. 1979. Quality standards for retail meats. *J. Food Prot.* 42:675–678.
18. Institute of Medicine of the National Academies. 2009. Review of the use of process control indicators in the FSIS Public Health Risk-Based Inspection System: a letter report, 13 March 2009, from Sanford Miller to Carol Maczka. The National Academic Press, Washington, DC. Available at: http://books.nap.edu/openbook.php?record_id=12617&page=1. Accessed 4 February 2011.
19. International Commission on Microbiological Specifications for Foods. 2002. Establishment of microbiological criteria for lot acceptance, p. 387–406. *In* Microorganisms in foods, vol. 7. Kluwer Academic/Plenum Publishers, New York.
20. Jimenez, S. M., M. S. Salsi, M. C. Tiburzi, and M. E. Pirovani. 2002. A comparison between broiler chicken carcasses with and without visible fecal contamination during the slaughtering process on hazard identification of *Salmonella* spp. *J. Appl. Microbiol.* 93:593–598.
21. Jimenez, S. M., M. C. Tiburzi, M. E. Salsi, M. E. Pirovani, and M. A. Moguilevsky. 2003. The role of visible faecal material as a vehicle for generic *Escherichia coli*, coliform, and other enterobacteria contaminating poultry carcasses during slaughtering. *J. Appl. Microbiol.* 95: 451–456.
22. Kornacki, J. L., and J. L. Johnson. 2001. Enterobacteriaceae, coliforms, and *Escherichia coli* as quality indicators, p. 69–82. *In* F. P. Downes and K. Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, DC.
23. Lillard, H. S. 1989. Incidence and recovery of *Salmonella* and other bacteria from commercially processed poultry carcasses at selected pre- and post-evisceration steps. *J. Food Prot.* 52:88–91.
24. McCrady, M. H., and E. M. Langevin. 1932. The coli-aerogenes determination in pasteurization control. *J. Dairy Sci.* 15:321–329.
25. Mossel, D. A. A. 1978. Index and indicator organism—a current assessment of their usefulness and significance. *Food Technol. Aust.* June:212–219.
26. Mossel, D. A. A. 1982. Marker (index and indicator) organisms in food and drinking water. Semantics, ecology, taxonomy and enumeration. *Antonie Leeuwenhoek* 48:609–611.
27. Mossel, D. A. A., and P. van Netten. 1991. Microbiological reference values for foods: a European perspective. *J. Assoc. Off. Anal. Chem.* 74:420–432.
28. National Advisory Committee on Microbiological Criteria for Foods. 2010. Response to the questions posed by the FSIS regarding determination of the most appropriate technologies to adopt for FSIS routine and baseline microbiological analyses. *J. Food Prot.* 73: 1160–1200.
29. National Research Council, Food and Nutrition Board, Committee on Food Protection, Subcommittee on the Microbiological Criteria. 1985. An evaluation of the role of microbiological criteria for foods and food ingredients. National Academy Press, Washington, DC.
30. Northcut, J. K., M. E. Berrang, J. A. Dickens, D. L. Fletcher, and N. A. Cox. 2003. Effect of broiler age, feed withdrawal, and transportation on levels of coliforms, *Campylobacter, Escherichia coli* and *Salmonella* on carcasses before and after immersion chilling. *Poult. Sci.* 82:169–173.
31. Pierson, M. D., D. L. Zink, and L. M. Smoot. 2007. Indicator microorganisms and microbiological criteria, chap. 4, p. 69–85. *In* M. P. Doyle and L. R. Beuchat (ed.), Food microbiology, fundamentals and frontiers, 3rd ed. ASM Press, Washington, DC.
32. Rhodes, M. W., and H. Kator. 1988. Survival of *Escherichia coli* and *Salmonella* spp. in estuarine environments. *Appl. Environ. Microbiol.* 54:2902–2907.
33. Russell, S. M. 2003. The effect of airsacculitis on bird weights, uniformity, fecal contamination, processing errors, and populations of *Campylobacter* spp. and *Escherichia coli*. *Poult. Sci.* 82:1326–1331.
34. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M.-A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis.* 17:7–15. Available at: http://www.cdc.gov/EID/content/17/1/pdfs/09-1101p1.pdf. Accessed 9 February 2011.
35. Schardinger, F. 1892. Ueber das Vorkommen Gährung erregender Spaltpilze im Trinkwasser und ihre Bedeutung fűr die hygienische Beurtheilung dessenlben. *Wien Klin. Wochschr.* 5:403–405, 421–423.
36. Smith, S., and D. W. Schaffner. 2004. Indicator organisms in meat, p. 773–779. Encyclopedia of meat science. Elsevier Science, London.
37. Swenarton, J. C. 1927. Can *B. coli* be used as an index of the proper pasteurization of milk? *J. Bacteriol.* 13:419–429.
38. U.S. Department of Agriculture, Food Safety and Inspection Service. 1996. Pathogen reduction; hazard analysis and critical control point (HACCP) systems, final rule. *Fed. Regist.* 61:38805–38989. Available at: http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/93-016F.pdf. Accessed 9 February 2011.
39. U.S. Department of Agriculture, Food Safety and Inspection Service. 1996. Nationwide Broiler Chicken Microbiological Baseline Data Collection Program. Available at: http://www.fsis.usda.gov/OPHS/baseline/broiler1.pdf. Accessed 9 February 2011.
40. U.S. Department of Agriculture, Food Safety and Inspection Service. 2000. Nationwide Young Chicken Microbiological Baseline Data Collection Program. Available at: http://www.fsis.usda.gov/PDF/Baseline_Data_Young_Chicken.pdf. Accessed 9 February 2011.
41. U.S. Department of Agriculture, Food Safety and Inspection Service. 2008. The Nationwide Microbiological Baseline Data Collection Program: Young Chicken Survey. Available at: http://www.fsis.usda.gov/PDF/Baseline_Data_Young_Chicken_2007-2008.pdf. Accessed 9 February 2011.
42. Vargas, V. C. C., L. F. D. Lopes, and A. Mendonça Souza. 2004. Comparative study of the performance of the CuSum and EWMA control charts. *Comput. Ind. Eng.* 46:707–724.
43. Wheeler, D. J., and D. S. Chambers. 1992. Understanding statistical process control, 2nd ed. SPC Press, Knoxville, TN.
44. Winfield, M. D., and E. A. Groisman. 2003. Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Appl. Environ. Microbiol.* 69:3687–3694.
45. Woodward, J. 2003. Making things happen: a theory of causal explanation. Oxford University Press, New York.
46. Zoetendal, E. G. 2004. Molecular ecological analysis of the gastrointestinal microbiodata: a review. Presented at the Experimental Biology Meeting, San Diego, CA, 11 to 15 April 2003.