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Short communication

Updating a 2-class attributes sampling plan to account for changes in laboratory methods

Michael S. Williams*, Eric D. Ebel, Neal J. Golden

Risk Assessment and Analytics Staff, Office of Public Health Science, Food Safety and Inspection Service, United States Department of Agriculture, 2150 Centre Avenue, Building D, Fort Collins, CO 80526, United States

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ABSTRACT

Advances in microbiological testing methods have led to faster and less expensive assays. Given these advances, it is logical to employ these assays for use in the sampling plan of an existing microbiological criterion. A change in the performance characteristics of the assay can affect the intended effect of the microbiological criterion. This study describes a method for updating a 2-class attributes sampling plan to account for the different test sensitivity and specificity of a new assay and provides an example based on the replacement of a culture-based assay with a real-time polymerase chain reaction assay.

1. Introduction

An attributes sampling plan for a microbiological criterion defines the number of samples tested from a food lot, or across a series of lots. Within-lot sampling can be used to verify the microbiological quality of raw materials for a specific lot. Sampling can also be performed across lots to monitor the proportion of contaminated lots across time. An attributes plan classifies the tested samples as either acceptable or defective (i.e., a 2-class plan) or acceptable, marginally acceptable or defective (i.e., a 3-class plan) (FAO/WHO, 2016; ICMSF, 1974; van Schothorst et al., 2009). A 2-class attributes sampling plan specifies a maximum number of positive samples (c) out of a fixed number of samples (n). Samples may either be enumerated or the theoretical limit of detection (LOD) for a qualitative assay can be used to determine if a microbiological limit (m) has been exceeded.

An example of the application of a 2-class attributes sampling plan are the *Salmonella* performance standards maintained by the Food Safety and Inspection Service (FSIS) in the United States. These sampling plans are part of the Pathogen Reduction; Hazard Analysis and Critical Control Point (PR;HACCP) legislation that has been in operation since the late 1990s (FSIS, 1996b). The performance standard for each commodity consists of a sampling plan with a maximum number of *Salmonella*-positive samples out of a fixed number of samples. Performance standards for *Campylobacter* contamination of poultry products were first introduced in 2011 (FSIS, 2011b). Long running attributes plans, such as the FSIS performance standards, are likely to encounter situations where technological advances lead to new assays with improved performance characteristics. These changes affect the underlying probability of a sample testing positive for a pathogen. If the magnitude of the change in the assay's performance is sufficiently large, the maximum number of positive samples (c) may need to be modified to ensure that the intended objectives of the sampling plan are maintained. In rare cases it may be possible or necessary to modify both the c and n values.

This research note presents a method for updating a 2-class attributes sampling plan to account for the different performance characteristics of a new assay. An example, based on testing comminuted (also known as minced or ground) chicken for the presence of *Campylobacter*, is provided.

2. Data description

FSIS has previously assessed the performance characteristics of two assays using enriched 30 mL aliquots (Ebel et al., 2016). The dataset was derived from 203 samples of comminuted chicken submitted to FSIS laboratories. These samples were collected at a subset of commercial production establishments in the United States under FSIS' performance standards testing program. Each sample was first enriched and then co-analyzed for *Campylobacter* using a culture-based assay (FSIS, 2011a) and the BAX® System real-time polymerase chain reaction (PCR) assay for *Campylobacter* (Dupont, 2013). The testing results for both assays are given as Supplementary material and a detailed description of each assay is provided in Ebel et al. (2016).

Preparation of the sample begins with the addition of 1625 ± 32.5 mL of buffered peptone water (a five-fold dilution) to the 325 g subsample (FSIS, 2013) of comminuted poultry. This diluted

* Corresponding author.
E-mail address: mike.williams@fsis.usda.gov (M.S. Williams).

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mixture of approximately 1950 mL is the starting point for both assays, where a 30 mL aliquot undergoes enrichment by adding an additional 30 mL of 2X Blood-Free Bolton Enrichment Broth and incubating for 48 h (FSIS, 2013). A key assumption for a sample to be declared positive is that one or more viable *Campylobacter* cells are present in the 30 mL aliquot. An average concentration of at least 65 *Campylobacter* in the diluted mixture from a 325 g sample is required to achieve a reasonably high probability (P(+) = 0.63) of one or viable cells in the 30 mL aliquot, which implies a theoretical LOD of 65/325 = 0.2 CFU/g. Another key assumption is that viable organisms in the 30 mL aliquot undergo sufficient exponential growth during the enrichment period for detection by both assays. The 48 h incubation is sufficient to ensure detection of a single viable cell, given the estimated lag period and mean generation time for *Campylobacter* in Bolton broth (Battersby et al., 2016).

After the enrichment period, parallel analyses of samples (and controls) were completed using the culture and PCR assays for *Campylobacter*. For the culture assay, a sample was declared negative if there were no colonies that demonstrated typical *Campylobacter* characteristics. The PCR system reports the species of *Campylobacter* in positive samples and provides an estimate of the apparent concentration of the enriched sample. All samples declared *Campylobacter*-positive by either assay were subjected to additional confirmatory testing on isolated colonies using FSIS' microscopic examination and latex agglutination immunoassay confirmation process (FSIS, 2011a). Note that culture-positive samples denote colonies of actively growing bacteria while PCR-positive samples reflect the presence of DNA whether the bacterial cell is viable or not. Consequently, the likelihood of false-positive results (i.e., test-positive despite the absence of viable bacteria) must be greater for PCR than culture ceteris paribus.

The available data describe a situation where two imperfect tests are applied to a single population when the true contamination status of each sample is unknown. These data were used in conjunction with a Bayesian "no gold standard" analysis approach (Branscum et al., 2005) to estimate the true underlying prevalence (π) of 30 mL *Campylobacter* contaminated samples, the test sensitivity (*Se*) and specificity (*Sp*) of both assays, and the covariance between the assays for both contaminated (Cov_c) and uncontaminated samples (Cov_{nc}) (Ebel et al., 2016). The inclusion of the covariance terms is necessary in applications where the outcomes of each test for a given sample are not independent. Dependence occurs when the two tests measure the same biological processes. In this application, both the PCR and culture assay rely on the detection of viable organisms from a single enriched sample, so it is likely that a sample with high levels of contamination will have a higher probability of testing positive on both the PCR and culture assays. The typical example of when it is reasonable to assume the independence of test results is disease detection applications where one test is designed to detect an antigen while the other test is designed to detect an antibody. The use of a model that ignores these covariance terms, when in fact the test results are dependent, typically leads to an over-estimation of the sensitivity and specificity of the assays (Spencer, 2012; Torrance-Rynard and Walter, 1997; van Smeden et al., 2014). The relationship between these 7 parameters and their estimated values are summarized in Tables 1 and 2.

Table 2
Performance characteristics (95% credibility intervals) for 30 mL enriched comminuted chicken samples using both a PCR and culture assay to determine the presence/absence of *Campylobacter* (Ebel et al., 2016).

	Culture	PCR
Se	0.9144 (0.7640, 0.9969)	0.9285 (0.7916, 0.9974)
Sp	0.9616 (0.8784, 0.9981)	0.9529 (0.8694, 0.9967)

Note: Covariance between the methods was $Cov_c = 0.020$ $(-0.003, 0.073)$ for contaminated samples and $Cov_{nc} = 0.022$ $(0.000, 0.076)$ for non-contaminated samples.

3. Methods

Suppose there is an existing sampling plan and interest lies in changing to a new assay with a different sensitivity and specificity. As an example, assume the assay used for the existing performance standard is FSIS' 30 mL enrichment and culture method used for testing comminuted poultry, with test sensitivity and specificity of Se_{Cult} and Sp_{Cult}, respectively. While FSIS currently has no performance standard based on a 30 mL aliquot for this product and pathogen, for the purposes of illustration assume an existing performance standard of $c_{Cult} = 3$ positive samples out of $n = 52$ samples (i.e., weekly sample collection) is in effect and that interest lies in replacing the enrichment and culture assay with the enrichment and PCR BAX assay with Se_{PCR} and Sp_{PCR}. The effects of the FSIS confirmatory assay for presumptive positive samples are ignored for now to simplify the discussion. If the changes in the performance characteristics of the new assay are sufficiently different from the existing assay, then the number of allowable positive samples may need to be updated to equilibrate the existing and new performance standards.

Note that c_{Cult} is the maximum allowable number of samples positive using the culture method; therefore, the implied apparent prevalence of the performance standard is c_{Cult}/n. Our task is to determine if c_{PCR} should be different from c_{Cult}. If the number of samples collected (n) is the same for both assays, then the answer depends on the magnitude of difference in the sensitivities and specificities of the assays.

The expected value of c_{PCR} is determined by considering the relationships in Table 1 and using a two-step process. The first step entails calculating the true prevalence of 30 mL aliquots, π, implied by the current performance standard of $c_{Cult} = 3$, $n = 52$. This is determined by setting the sum of the left column of Table 1 (i.e., the expected number of positive samples on both assays plus the expected number of samples that are culture-positive and PCR-negative) equal to the maximum number of allowable positives using the culture method (i.e., $c_{Cult} = 3$) and solving for π. This yields an implied true prevalence of

$$\pi = \left(\frac{c_{Cult}}{n} - ((1 - Sp_{PCR})(1 - Sp_{Cult}) + Sp_{PCR}(1 - Sp_{Cult}))\right) / \begin{bmatrix} (Se_{PCR}Se_{Cult} + Cov_c) - ((1 - Sp_{PCR})(1 - Sp_{Cult}) + Cov_{nc}) + \\ (1 - Se_{PCR})Se_{Cult} - Cov_c - (Sp_{PCR}(1 - Sp_{Cult}) - Cov_{nc}) \end{bmatrix}.$$

Note that following algebraic simplification, the conditional covariances between the assays no longer appear in the estimator of the

Table 1
The relationship between the estimated parameters describing the performance characteristics of each assay and the sample counts for each assay in a cross-classification table.

Existing laboratory assay			
		Culture positive	Culture negative
New laboratory assay	PCR-positive	$n\pi(Se_{PCR}Se_{Cult} + Cov_c) + n(1 - \pi)\times$ $((1 - Sp_{PCR})(1 - Sp_{Cult}) + Cov_{nc})$	$n\pi(Se_{PCR}(1 - Se_{Cult}) - Cov_c) +$ $n(1 - \pi)((1 - Sp_{PCR})Sp_{Cult} - Cov_{nc})$
	PCR-negative	$n\pi((1 - Se_{PCR})Se_{Cult} - Cov_c) +$ $n(1 - \pi)(Sp_{PCR}(1 - Sp_{Cult}) - Cov_{nc})$	$n\pi((1 - Se_{PCR})(1 - Se_{Cult}) + Cov_c) +$ $n(1 - \pi)(Sp_{PCR}Sp_{Cult} + Cov_{nc})$