# Risk-based Sampling for *Escherichia coli* O157:H7 in Ground Beef and Beef Trim

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FSIS

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# Executive Summary

Until January 2003, the Food Safety and Inspection Service (FSIS) tested for the presence of *Escherichia coli* O157:H7 in raw ground beef and beef trim using an approach in which all beef-producing establishments were sampled at approximately the same frequency. Because all eligible establishments had an equal probability of being selected for verification sampling, FSIS resources were allocated uniformly among establishments that may differ greatly in terms of their microbiological controls for *E. coli* O157:H7 in ground beef and trim, and the resulting public health impact of these products.

As part of its effort to provide improved public health assurance of the safety of ground beef, FSIS developed an *E. coli* O157:H7 risk-based verification sampling program. This algorithm was independently peer reviewed in accordance with the Office of Management and Budget guidelines for peer review. The risk-based *E. coli* O157:H7 sampling program provides more comprehensive verification of domestic ground beef servings and allocates more samples to establishments with a higher risk of causing *E. coli* O157:H7 illness.

The risk-based sampling algorithm allocates samples in a random draw where the probability of each establishment being sampled is weighted by FSIS microbiological test results for *E. coli* O157:H7 and production volume. As FSIS collects and analyzes data on establishment *E. coli* O157:H7 interventions and testing programs these will also be used to weight sampling probability. In the simplest terms, the greater an establishment's potential for causing *E. coli* O157:H7 illness, the higher the probability it will be sampled.

## OBJECTIVES

The risk-based sampling algorithm is designed to accomplish three primary objectives:

- To increase the proportion of FSIS samples taken at establishments that are more likely to produce product contaminated with *E. coli* O157:H7.

- To allocate FSIS resources more efficiently by verifying a greater portion of the U.S. trim and ground beef supply with the same number of samples as the current program.

- To verify *all* eligible establishments at a reasonable frequency regardless of an establishment's production volume, interventions, or predicted public health risk associated with their product.

## PRINCIPLES

The risk-based algorithm uses data from FSIS sampling programs for *E. coli* O157:H7 in ground beef and beef trim and from FSIS surveys. The algorithm works on the following principles:

- Every establishment eligible for *E. coli* O157:H7 testing of raw beef is placed in a sampling frame each month – one frame for producers of raw ground beef products and one for suppliers of beef trim.

- Each establishment in the sampling frame is assigned a portion of the probability "space" from 0 to 1. The higher an establishment's potential to cause *E. coli* O157:H7 illness, the larger the space.

- A random number generator selects numbers between 0 and 1. If the number is within an establishment's space, the establishment is selected for sampling. The larger an establishment's probability space, the greater the chance it will be selected.

- The algorithm selection of an establishment ("draw") is random. In each draw, each establishment has a chance of being sampled; but the probability of being selected is dictated by the potential public health risk.

## OUTCOMES

- The monthly probability of selection for *E. coli* O157:H7 sampling in the current program for ground beef producers is the same for every establishment and was estimated at approximately 60% (resulting in an average of about 7 samples per establishment per year). Using the risk-based algorithm to assign samples, 1,443 of the smallest establishments (by production volume) will be sampled slightly less than they are currently, while 92 of the largest producers will be sampled at a slightly higher frequency than they are currently.

- The frequency of sampling will change further as establishment practices become included in the algorithm to account for testing programs and interventions. For example, establishments with interventions and testing programs known to control *E. coli* O157:H7 will be sampled significantly less, while establishments that lack these practices and have little or no testing will show a relative increase in the number of positive samples. In addition, the risk-based sampling program significantly increases the frequency of sampling for establishments that have tested positive for O157:H7 in the past 4 months.

## FUTURE DIRECTION

By summer of 2008, FSIS plans to incorporate establishment practices into the *E. coli* O157:H7 sampling algorithm, including those interventions that reduce *E. coli* O157:H7 contamination and testing programs that effectively detect *E. coli* O157:H7. Accounting for establishment practices such as these will allow FSIS to further target high-risk establishments and provide incentives for establishments to implement the best available practices during the production of ground beef and trim.

## CONCLUSIONS

Compared to FSIS' prior *E. coli* O157:H7 verification sampling program, the risk-based sampling algorithm described in this report (and initiated in January 2008) offers an improved verification testing program for *E. coli* O157:H7 in ground beef and trim. Importantly, because the risk-based sampling algorithm accounts for production volume but does not make it a primary driver, the sampling program will verify the safety of more of the beef supply than an unweighted random program, while still verifying small producers at a reasonable frequency. This is because the algorithm strikes a balance between sampling more of the total beef supply and targeting the sampling of product with the most potential for causing *E. coli* O157:H7 illness. Use of the algorithm to allocate samples for *E. coli* O157:H7 should therefore provide a greater benefit to public health through more efficient allocation of FSIS resources.

# Introduction

As part of its move towards a risk-based inspection system, the Food Safety and Inspection Service (FSIS) has developed and refined a probabilistic algorithm to guide sampling and testing for *Escherichia coli* O157:H7 in ground beef and beef trim. This report describes that algorithm.

First described following a 1982 outbreak of illness associated with consumption of undercooked ground beef,[1;2] *Escherichia coli* O157:H7 is the leading cause of enterohemorrhagic *E. coli* (EHEC) infection in the United States. Cattle are the main reservoir of *E. coli* O157:H7;[3] and ground beef has been the vehicle most often associated with outbreaks of *E. coli* O157:H7 infection. Of the 145 outbreaks of O157:H7 infection reported to the U.S. CDC for the period 1990-1999, ground beef was the confirmed or suspected vehicle in at least 37 (26%).[4]

Those at the extremes of age and those with compromised immune systems are most susceptible to infection with *E. coli* O157:H7.[10;11] Infections typically appear as watery diarrhea accompanied by abdominal pain. Bloody diarrhea may develop. Sequelae include hemorrhagic colitis and the hemolytic uremic syndrome (HUS). About 60% of HUS cases resolve, 30% lead to minor sequelae such as proteinurea, 5% lead to severe sequelae such as stroke and chronic renal failure, and 3-5% lead to death.[12] Treatment involves maintaining fluid and electrolyte balance, control of hypertension, and provision of nutritional support.[18] Progression to HUS requires hospitalization,[12] with further treatment options including dialysis, platelet infusions, and, in cases of utmost severity, renal transestablishmentation.[19]

Since October 1994, FSIS has tested for *E. coli* O157:H7 in raw ground beef produced by the establishments it regulates. The current testing program verifies all eligible establishments at approximately the same frequency. Thus, because all eligible establishments have an equal probability of being tested, FSIS resources are allocated uniformly among establishments that may differ greatly in terms of microbiological controls and the resulting public health impact of their products. However, based on repeated calls for a modern, risk-based approach to inspection, FSIS developed a system of *E. coli* O157:H7 testing based on a probabilistic algorithm.

# The *E. coli* O157:H7 Risk-based Sampling Algorithm

## ALGORITHM PRINCIPLES

The *E. coli* O157:H7 risk-based sampling algorithm works on the following principles:

- Establishments eligible for *E. coli* O157:H7 testing of raw beef are placed in one of two sampling frames each month – one frame for producers of raw ground beef products and one frame for suppliers of beef trim.

- Each establishment in the sampling frame is assigned a portion of the probability "space" from 0 to 1. The higher an establishment's potential to cause *E. coli* O157:H7 illness, the larger the space.

- A random number generator selects numbers between 0 and 1. If the number is within an establishment's space, the establishment is selected for sampling. The larger an establishment's probability space, the greater the chance it will be selected.

- The algorithm selection of an establishment ("draw") is random. In each draw, each establishment has a chance of being sampled; but the probability of being selected is dictated by the potential public health risk.

- Sampling is "without replacement." In other words, if a plant has been selected for sampling it cannot be selected again in the same month.

Currently, the sampling probability of each establishment is based on the average amount of product produced per day and the *E. coli* O157:H7 sample history from the last four months (see *Algorithm design* for details). By Summer 2008, the algorithm is expected to account for

establishment practices and interventions that influence the prevalence and levels of *E. coli* O157:H7 in raw beef products. More samples may be collected during the high prevalence season (see pp 19-22 for more details), though the yearly total of samples will remain approximately the same as in 2006. The algorithm ensures that the amount of beef product verified by FSIS represents a greater portion of the total produced for public consumption. The algorithm accounts for volume but does not make it a primary driver. The risk-based sampling program will be more representative of the beef supply than the current sampling program that provides random sampling by establishment (which does not consider the amount of product produced). In turn, the risk-based sampling program provides greater public health assurance of the safety of ground beef and trim produced for consumption in the U.S.

## ALGORITHM DATA

### Production Volume

As part of its ground beef sampling program, FSIS collects production volume information with each sample. FSIS inspectors are asked to respond to the question: On a typical/average day (all shifts), this establishment produces: (1) >250,000 pounds ground beef; (2) >50,000 to 250,000 pounds ground beef; (3) 1,000 to 50,000 pounds ground beef; or (4) <1,000 pounds ground beef. From December 29, 2003 through May 5, 2006, 24,450 data entries were collected. Collection dates were not recorded for 119 entries and an obviously incorrect date (2008) was recorded for one. There were 24,165 valid entries for production volume code (1, 2, 3, or 4). Of the other 285 entries, 274 were blank and 11 had invalid codes. Entries with blank or invalid codes were discarded. The 24,165 valid entries represented 1,706 unique establishment identifiers. Individual establishments had from 1 to 26 entries for production volume codes Figure 1.

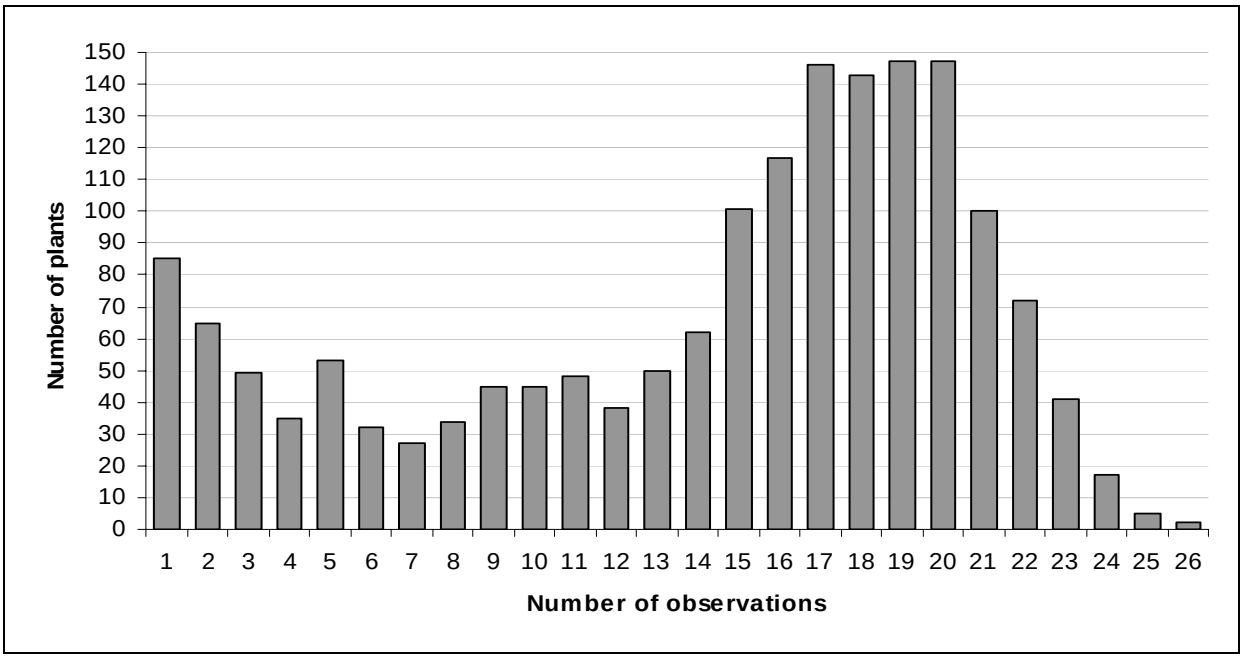


**Figure 1.** Distribution of ground beef volume data points for the 1,706 establishments represented in the analyzed data set.

The algorithm uses production volume codes for the previous 12 months to help assign probability space for the sampling month. In cases where establishments had entries for multiple codes, the mode (most frequently occurring) code is used to represent establishment production volume (ties are assigned to the larger production code). Table 1 shows the number of establishments in different production categories using CY2005 information.

**Table 1.** Number of establishments in different production categories for CY2005.

| Code | Daily Production Amount (lbs) | Number of Establishments |
|---|---|---|
| 1 | >250,000 | 34 |
| 2 | >50,000 to 250,000 | 55 |
| 3 | 1,000 to 50,000 | 323 |
| 4 | <1,000 | 1,124 |

Assuming all ground beef production is represented by the establishments shown in Table 1, it is possible to use the ranges to give bounds to the annual ground beef production in the U.S. Assume a lower bound of 1 pound for category 4. Assume an upper bound of 500,000 pounds for category 1. The greatest possible amount of daily ground beef production is then given by 500,000 x 34 + 250,000 x 55 + 50,000 x 323 + 1,000 x 1124 = 48 million pounds. The least possible amount of daily ground beef production is given by 250,001 x 34 + 50,001 x 55 + 1,001 x 323 + 1 x 1,124 = 11.6 million pounds. Assuming further that there are 250 production days in a year, then the annual production ranges from a low of 2.9 billion pounds to a high of 12 billion pounds. These bounds were compared with another estimate of production volume based on slaughter data. Table 2 shows estimates of beef trim weight based on data from (i) the 2001 FSIS Draft *E. coli* Risk Assessment and (ii) the annual kill for the different products classes for 2005 from the Electronic Animal Disease Reporting System (eADRS). Multiplying the annual number of cattle killed by the estimated pounds of trim for each class of animal results in an estimate of 3.7 billion pounds of beef, which is within the bounds estimated above. Consequently, the reported ranges of production for the ground beef facilities in Table 1 are consistent with the estimated production from Table 2.

**Table 2.** Estimate of annual ground beef production volume.

| Class | Carcass weight (lbs) | % Meat | % of meat used to create trim | Weight of trim (lbs) | Annual number of cattle killed | Trim (lbs) |
|---|---|---|---|---|---|---|
| Steer | 764 | 70% | 18% | 96.26 | 16,868,469 | 1,623,826,300 |
| Heifer | 703 | 70% | 18% | 88.58 | 9,784,554 | 866,696,224 |
| Cow | 539 | 70% | 53% | 199.97 | 4,794,269 | 958,705,178 |
| Bull | 851 | 70% | 90% | 536.13 | 518,293 | 277,872,426 |
| *Total* | | | | | | *3,727,100,128* |

*Production volume and proportion of* E. coli *O157:H7-positive samples*

FSIS testing results for *E. coli* O157:H7 in ground beef were used to analyze the relationship between production volume and *E. coli* O157:H7-positive samples. Because of recent changes in

industry practices only those samples analyzed in 2004 or later were included in the analysis. Sample analysis end dates in the data set ranged from January 01, 2004 through December 31, 2005. There were 18,484 in the data set labeled as MT03 (which refers to a sample randomly collected from a sampling frame consisting of all establishments not weighted by production volume or other characteristics—analysis is discussed in more detail in *Analysis of FSIS E. coli O157:H7 sample data*). The other samples were follow-up or trace-back samples and were not included in the analysis. Establishment production codes were related to the MT03 samples using establishment identification codes. Table 3 shows the MT03 samples collected by year and establishment production codes from 2004 through 2005.

**Table 3.** MT03 samples collected by year and establishment production code from 2004 through 2005.

| | Establishment Production Code | | | | | |
|---|---|---|---|---|---|---|
| **Year** | **1** | **2** | **3** | **4** | **N/A** | ***Total*** |
| 2004 | 203 | 344 | 1,689 | 5,395 | 1 | 7,632 |
| 2005 | 301 | 460 | 2,493 | 7,595 | 3 | 10,852 |
| *Total* | 504 | 804 | 4,182 | 12,990 | 4 | 18,484 |

The samples shown in the column labeled "N/A" represent establishments that did not have production codes provided. The total at the bottom of each of the columns shows the total number of samples collected for the two years for each of the establishment production codes. This can be combined with the number of establishments in each category to show the average number of samples collected for establishments in each production category. Using the mid-range of the production categories as representing the production for all establishments in the category, the proportion of the total production contributed by each of the categories can be compared with the proportion of samples allocated to each of the categories (Table 4).

**Table 4.** Samples per establishment, percent total production volume, and percent total samples in each production volume category from 2004 through 2005.

| Code | Daily production volume (lbs) | Number of establishments | Total number of samples | Samples collected per establishment | % Total production volume | % Total samples |
|---|---|---|---|---|---|---|
| 1 | >250,000 | 34 | 486 | 14.3 | 41.2 | 2.6 |
| 2 | >50,000 to 250,000 | 60 | 804 | 13.4 | 29.1 | 4.3 |
| 3 | 1,000 to 50,000 | 337 | 4,167 | 12.4 | 27.8 | 22.5 |
| 4 | <1,000 | 1,237 | 13,025 | 10.5 | 2.0 | 70.5 |

Although larger plants account for more production it may be that they account for less contamination than smaller plants. Table 5, however, shows that the larger plants tended to have a higher proportion of positive samples than did the smaller plants even when adjusted for production volume.

**Table 5.** Percent positive samples for FSIS *E. coli* O157:H7 sampling in ground beef for different combinations of the years 2004-2005 by establishment production volume category.

| Volume production category | Number in category | Total samples taken for each plant | Positives | Percent positive samples |
|---|---|---|---|---|
| 1 | 34 | 486 | 2 | 0.412% |
| 2 | 60 | 804 | 2 | 0.249% |
| 3 | 337 | 4,167 | 12 | 0.288% |
| 4 | 1,237 | 13,025 | 16 | 0.123% |
| N/A | 3 | 2 | 0 | 0.000% |
| Total | 1,671 | 18,484 | 32 | 0.173% |

*Application of Production Volume in the Algorithm*

A Volume Score (*v*) was calculated for each establishment category based on the average amount of product produced per day and the scaling factor discussed below (Equation 1). For details on how *v* helps determine sampling probability, see "*Putting It All Together*". The algorithm calculates a volume score for each plant according to a simple linear "scaling down" of the actual volume scale. FSIS estimates that the largest plants produce ~750 times more product by weight each day than the smallest plants. Based solely on this data, FSIS would sample the largest producers 750x more than the smallest—and the intermediate producers at proportionate frequencies. However, there are constraints on our sampling program that make this direct relationship of sampling to volume unfeasible. For instance, given the number of samples available for the program, this would mean ~ 1,000 plants would go years without being sampled a single time while the burden on inspectors in large plants as well as the large producers themselves might be unreasonable. The algorithm solves this problem through a "scaling factor" that reduces the 750x difference to a level that risk managers can determine provides a feasible level of sampling for the program. Scaling reduces the actual difference between production categories proportionately according to the relationship described in Equation 1. (For details on how the algorithm executes these criteria, see Appendix, *Visual Basic Source Code*.)

This scaling ensures that the amount of product verified by risk-based sampling is more representative of the total amount produced for consumption than that verified by a random sampling program. At the same time, it allows FSIS risk managers to select a level of scaling that is compatible with both FSIS and industry resources.

$$\text{Volume Score for Establishment } i = S_L + \left( \left(V_i - V_4\right) \Big/ \left( \frac{\left(V_1 - V_4\right)}{\left(S_H - S_L\right)} \right) \right) \qquad \text{(Equation 1)}$$

Where $V_i$ = Production volume of establishment *i*, there are 4 volume categories, 1 produces most and 4 least; $V_1$ = Production volume of establishments in category 1; $V_4$ = Production volume of establishments in category 4; $S_L$ = Lowest score of the scale; and $S_H$ = Highest score in the scale.

## Sample History

FSIS considers an *E. coli O157:H7*-positive verification sample indicative of a public health risk— as evidenced by several FSIS directives and notices. To explore the use of sample history as a determinant of sampling frequency in the algorithm, an analysis was performed to determine whether establishments that have had positive *E. coli* O157:H7 samples are at an increased risk of having future positive samples.

*Analysis of FSIS E. coli O157:H7 sample data*

As part of its ground beef sampling program, FSIS collects several types of samples. The most common sample collected is "MT03," which refers to a sample randomly collected from a sampling frame consisting of all establishments not weighted by production volume or other characteristic. Since 2000, follow-up samples have been collected under codes MT04, MT04A, MT04B, MT04T, and TRACEBAC. Table 6 shows the number of different sample types collected by FSIS from 2000 through 2005.

**Table 6.** Types and number of samples collected for *E. coli* O157:H7 verification testing from 2000 through 2005. MT03 samples are random samples while MT04 and TRACEBAC samples are taken in response to positive findings within the plant or at retail.

| Year | Project Code | | | | | | *Total* |
|---|---|---|---|---|---|---|---|
| | **MT03** | **MT04** | **MT04A** | **MT04B** | **MT04T** | **TRACEBAC** | |
| 2000 | 4,587 | 339 | 23 | 60 | 11 | 1 | *5,021* |
| 2001 | 5,004 | 506 | | | 4 | | *5,514* |
| 2002 | 5,103 | 604 | | | 34 | 4 | *5,745* |
| 2003 | 5,575 | 141 | | | 19 | | *5,735* |
| 2004 | 7,632 | 47 | | | 4 | | *7,683* |
| 2005 | 10,852 | 13 | | | | | *10,865* |
| *Total* | *38,753* | *1,650* | *23* | *60* | *72* | *5* | *40,563* |

In recent years, fewer follow-up type samples have been collected. For instance, in the years 2000 through 2002, nearly 10% (1,586/16,280) of all samples collected were follow-up type samples. In the years 2003 through 2005, less than 1% (224/24,283) of all samples collected were follow-up type samples. In the year 2005, out of 10,865 samples collected, 13 (0.12%) were follow-up type samples.

Because the last revision to the FSIS sample test protocol occurred in 1999, only samples collected in 2000 or later were analyzed. Sample analysis end dates in the data set ranged from January 01, 2000 through December 31, 2005. There were 40,563 entries in the data set. Of these, 38,753 were random samples (MT03). The proportion of positive MT03 samples was calculated for each of the six years. MT03 samples were analyzed because follow-up samples were taken from establishments that had had positive MT03 samples. (It was thought that including follow-up samples in this analysis would result in over-representation of establishments that had been identified as having contamination in their product.) Table 7 shows the results of MT03 sampling from 2000 through 2005. The proportion of positive samples has dropped markedly since the year 2000.

**Table 7.** Results of *E. coli* O157:H7 verification testing from 2000 through 2005 for sample type MT03.

| Year | Number of negative samples | Number of positive samples | Total number of samples | Proportion positive samples |
|---|---|---|---|---|
| 2000 | 4,564 | 23 | 4,587 | 0.0050 |
| 2001 | 4,964 | 40 | 5,004 | 0.0080 |
| 2002 | 5,075 | 28 | 5,103 | 0.0055 |
| 2003 | 5,557 | 18 | 5,575 | 0.0032 |
| 2004 | 7,618 | 14 | 7,632 | 0.0018 |
| 2005 | 10,834 | 18 | 10,852 | 0.0017 |
| *Total* | *38,612* | *141* | *38,753* | *0.0036* |

*Sample history and sample results*

Table 7 shows that of 38,753 MT03 samples collected from 2000 through 2005, 141 were positive for *E. coli* O157:H7. An Excel spreadsheet containing the sampling results was analyzed to determine the results of subsequent sampling after establishments had had a positive sample result. Results were tabulated every 30 days. Table 8 shows that samples taken relatively soon after a positive result occasionally resulted in positive samples while samples taken later rarely did. Because there appears to be a natural break in positive samples after 120 days, the data for the first four months after a positive sample were accumulated.

**Table 8.** Results of subsequent sampling in establishments after positive *E. coli* O157:H7 test results.

| Days After Positive | Total number of samples | Number of positive samples |
|---|---|---|
| 30 | 522 | 9 |
| 60 | 553 | 11 |
| 90 | 225 | 4 |
| 120 | 134 | 1 |
| 150 | 86 | 0 |
| 180 | 90 | 0 |
| 210 | 73 | 0 |
| 240 | 33 | 0 |
| 270 | 63 | 0 |
| 300 | 44 | 0 |
| 330 | 45 | 1 |
| 360 | 52 | 0 |

Results in Table 8 were compared with results of all MT03 sampling (Table 9). A sample taken within 120 days of a positive MT03 sample is approximately five times more likely to result in a positive sample than a randomly collected sample (odds ratio 4.86— see Table 9). Thus, to maximize the probability of finding *E. coli* O157:H7-contaminated product in a processing establishment, it is wise to collect samples shortly after an establishment has been identified as having had an *E. coli* O157:H7-positive result.

**Table 9.** Comparison of all samples taken within 120 days of an *E. coli* O157:H7-positive MT03 sample with all MT03 samples.

| | Positive | Negative | Total |
|---|---|---|---|
| All samples taken within 120 days of a positive MT03 sample | 25 | 1,409 | 1,434 |
| All MT03 samples taken | 141 | 38,612 | 38,753 |
| Odds ratio: 4.86 (95% confidence interval: 3.17-7.46) | | | |

One point to consider in Table 9 is that establishments that have had an *E. coli* O157:H7-positive sample may have had multiple follow-up samples using the same source material that contributed to the original positive sample. This was especially the case prior to 2003. A possible consequence would be an over-representation of positive grinder loads in the analysis. This point can be addressed by looking only at random (MT03) samples that were collected with 120 days of the original positive (Table 10).

**Table 10.** Comparison of MT03 samples taken within 120 days of a positive MT03 sample with all MT03 samples.

| | Positive | Negative | Total |
|---|---|---|---|
| MT03 samples taken within 120 days of a positive MT03 sample | 4 | 156 | 160 |
| All MT03 samples taken | 141 | 38,612 | 38,753 |
| Odds ratio: 7.02 (95% confidence interval: 2.57-19.20) | | | |

Although Table 10 is based on only 160 random samples, the lower confidence limit for the odds ratio is 2.57. Thus, random samples collected within 120 days of a previous positive sample at the same establishment are more likely to be positive for *E. coli* O157:H7 than samples collected within 120 days of a previous negative sample. In a sense, some samples are counted twice in Table 10; samples taken within 120 days of a positive MT03 sample are also counted in the category of all samples taken. Decreasing the samples in the second category by the number of samples in the first category, however, does not make a noteworthy difference in the analysis (the odds ratio and confidence limits are slightly increased). Because very few follow-up samples have been collected since 2003 (see Table 6), the conclusion that establishments with *E. coli* O157:H7-positive samples are more likely to have another positive sample may not be supportable when using only the most recent data. Table 11 summarizes the results of this analysis when performed on a year by year basis as compared to the aggregation of all six years.

**Table 11.** Year-by-year comparison of all samples taken within 120 days of an *E. coli* O157:H7-positive sample with MT03 samples taken during the year.

| | Year | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|---|---|---|
| All samples taken within 120 days of a positive | Negative | 269 | 458 | 506 | 86 | 47 | 43 | 1,409 |
| | Positive | 6 | 6 | 10 | 2 | | 1 | 25 |
| | Total | 275 | 464 | 516 | 88 | 47 | 44 | 1,434 |
| | % Positive | 2.18% | 1.29% | 1.94% | 2.27% | 0.00% | 2.27% | 1.74% |
| | 5th %ile | 1.20% | 0.71% | 1.20% | 0.92% | 0.11% | 0.80% | 1.27% |
| | 95th %ile | 4.25% | 2.53% | 3.26% | 6.91% | 6.05% | 10.11% | 2.42% |
| MT03 samples taken during the year | Negative | 4,564 | 4,964 | 5,075 | 5,557 | 7,618 | 10,834 | 38,612 |
| | Positive | 23 | 40 | 28 | 18 | 14 | 18 | 141 |
| | Total | 4,587 | 5,004 | 5,103 | 5,575 | 7,632 | 10,852 | 38,753 |
| | % Positive | 0.50% | 0.80% | 0.55% | 0.32% | 0.18% | 0.17% | 0.36% |
| | 5th %ile | 0.36% | 0.62% | 0.41% | 0.22% | 0.12% | 0.11% | 0.32% |
| | 95th %ile | 0.71% | 1.04% | 0.75% | 0.48% | 0.29% | 0.25% | 0.42% |

For example, in 2002 there were 28 positive samples out of 5,103 MT03 samples, for a proportion of 0.0055. The 95th percentile of the uncertainty distribution for this proportion was 0.0075. There were 10 positive samples out of 506 follow-up samples taken within 120 days of a positive, for a proportion of 0.0194. The 5th percentile of the uncertainty distribution for this proportion was 0.0120, an indication that it was significantly different from the proportion of positive MT03 samples for 2002. In 2004, however, there were no positive samples out of 47 samples taken within 120 days following a positive MT03. Eighteen of these were designated as follow-up samples; the other 29 were random samples that fell within the time window. The confidence interval for this proportion is so wide that it is unclear if the true value is closer to 0 or 0.6. These results could have been observed even if the prevalence of *E. coli* O157:H7 in follow-up samples was 20 times higher than in random samples. This is demonstrated graphically in Figure 2.

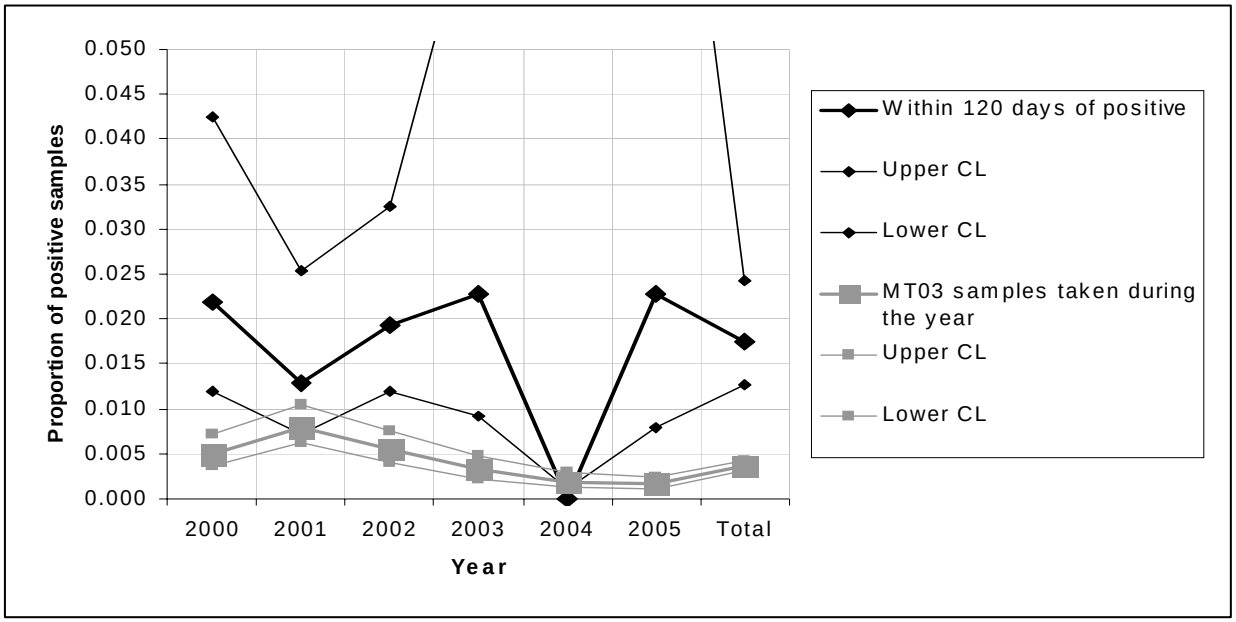


**Figure 2.** Comparison of sample results taken within 120 days of an E. coli O157:H7-positive result with all MT03 samples taken.

The confidence intervals for MT03 samples are narrow, while the confidence intervals for samples taken within 120 days of a positive sample can be quite wide, especially for the years 2003 through 2005. The effect of having little sampling information is to give caution that the size of the problem is unknown.

*Limitations*

The conclusion that establishments are more likely to have an *E. coli* O157:H7-positive sample within 120 days of having a positive sample is based on a relatively small data set. Collection of additional follow-up samples over time will greatly improve the ability to draw conclusions about the importance of sample history *E. coli* O157:H7 risk.

*Application of Sample History in the Algorithm*

Establishments that have tested positive for *E. coli* O157:H7 in an FSIS-collected sample within the last 120 days have an increased likelihood of *E. coli* O157:H7 contamination and will be tested more frequently during this time period by FSIS. This is based on the above analysis, which estimates that establishments with a positive sample are five times more likely to test positive again within a 120-day period than those without a positive sample.

## Season

FSIS sample data were analyzed to determine if season impacts the likelihood of *E. coli* O157:H7-positive samples. A total of 38,753 FSIS ground beef tests performed from 2000-2005 were aggregated by month to determine whether establishments were more likely to test positive for *E. coli* O157:H7 during particular months of the year. The aggregated samples showed considerable variation in the percent positive tests from month to month (Figure 3 and Table 12).

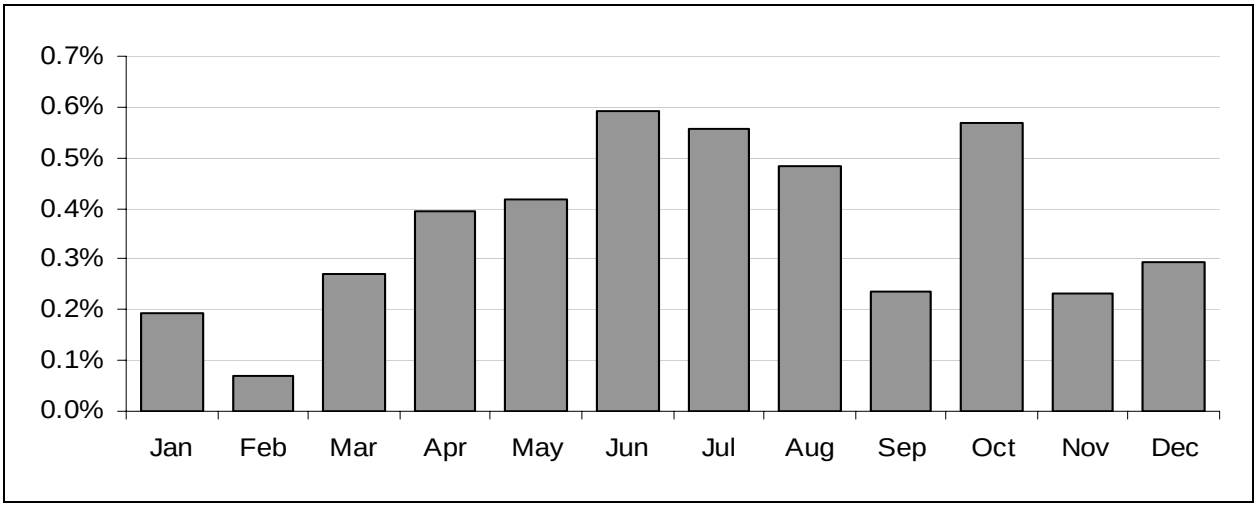


**Figure 3.** The percentage of *E. coli* O157:H7-positive samples by month from 2000-2005. Note the considerable variation between months. The figure is based on 38,753 MT03 samples.

**Table 12.** Results from FSIS *E. coli* O157:H7 MT03 tests on ground beef performed from 2000-2005.

| Month | Number of Negative Samples | Number of Positive Samples | Total Number of Samples | % -Positive Samples |
|---|---|---|---|---|
| January | 3,101 | 6 | 3,107 | 0.193% |
| February | 2,832 | 2 | 2,834 | 0.071% |
| March | 3,317 | 9 | 3,326 | 0.271% |
| April | 3,286 | 13 | 3,299 | 0.394% |
| May | 3,105 | 13 | 3,118 | 0.417% |
| June | 3,196 | 19 | 3,215 | 0.591% |
| July | 2,856 | 16 | 2,872 | 0.557% |
| August | 3,283 | 16 | 3,299 | 0.485% |
| September | 3,395 | 8 | 3,403 | 0.235% |
| October | 3,854 | 22 | 3,876 | 0.568% |
| November | 3,013 | 7 | 3,020 | 0.232% |
| December | 3,374 | 10 | 3,384 | 0.296% |
| *Total* | *38,612* | *141* | *38,753* | *0.364%* |

To determine if there were particular groups of contiguous months (seasons) that correspond to a particularly high rate of *E. coli* O157:H7-positive samples, the number of positive samples for particular month groupings were compared using a Chi-square analysis to establish whether the observed monthly totals reflect seasonal variation or whether they could have occurred by chance (see Table 13 on the following page). This analysis revealed that some months have a significantly higher *E. coli* O157:H7 prevalence relative to the rest of the year that was unlikely to have occurred by chance. In particular, the grouping of test results for the months of April through October showed a significant increase in *E. coli* O157:H7 prevalence relative to the remaining months of November through March (0.46% vs. 0.22% respectively). (Table 13 shows a $p$ value of $10^{-5}$; also see Table 14 for comparison of percentages).

*Application of season in the algorithm*

Analysis of FSIS samples shows that the average number of *E. coli* O157:H7-positive samples from 2000 – 2005 is approximately twice as high during the months of April through October relative to the months of November through March. Therefore, ideally the total number of *E. coli* O157:H7 samples in the monthly draw would be twice as high during the months of April – October as from November - March. Although this would not add to the total number of *E. coli* O157:H7 samples processed each year, this change could create an unreasonable demand on laboratory resources during the high prevalence months. FSIS is still in the process of determining if laboratory resources would be overburdened by the seasonal increase and, if so, what is the maximum seasonal increase that would not exceed available resources.

**Table 13.** A comparison of *E. coli* O157:H7-positive tests for different month groupings vs. the remaining months of the year. For instance, the first row shows that the number of positive tests from June – September observed was 59 out of 12,789 tests performed for these months, while the expected values (assuming the average for the total of all 12 months of 0.364%) are 46.7 out of an adjusted 12,801.2 tests. Comparing the observed and expected values for the remaining months by a chi square analysis shows this breakdown has a p value of 0.026. In other words, the chance that the differences seen in the grouping from June – September occurred by random chance are fairly low. Notice that the differences in the April – October grouping are extremely low (p value 10-5).

| | Observed Positives | | Observed total Test | | Expected Positives | | Expected total Test | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Month Group** | Month Group | Remaining Months | Month Group | Remaining Months | Month Group | Remaining Months | Month Group | Remaining Months | **Chi square** X2 | p |
| Jun-Sep | 59 | 82 | 12789 | 25964 | 46.75 | 94.77 | 12801.25 | 25951.2335 | 4.949806053 | 0.026094 |
| May-Oct | 94 | 47 | 19783 | 18970 | 72.32 | 69.19 | 19804.68 | 18947.808 | 13.66586164 | 0.000218 |
| Apr-Oct | 107 | 34 | 23082 | 15671 | 84.37 | 57.14 | 23104.63 | 15647.8585 | 15.49735067 | 8.26E-05 |
| Jun-Oct | 81 | 60 | 16665 | 22088 | 60.93 | 80.58 | 16685.07 | 22067.4161 | 11.91280571 | 0.000557 |
| Jan | 6 | 135 | 3107 | 35646 | 11.33 | 130.19 | 3101.67 | 35650.8134 | 2.692597643 | 0.100815 |
| Feb | 2 | 139 | 2834 | 35919 | 10.32 | 131.19 | 2825.68 | 35926.8056 | 7.19681801 | 0.007303 |
| Mar | 9 | 132 | 3326 | 35427 | 12.13 | 129.38 | 3322.87 | 35429.6211 | 0.865780985 | 0.352126 |
| Apr | 13 | 128 | 3299 | 35454 | 12.05 | 129.46 | 3299.95 | 35452.5375 | 0.091674676 | 0.762059 |
| May | 13 | 128 | 3118 | 35635 | 11.39 | 130.12 | 3119.61 | 35632.8789 | 0.262527863 | 0.608389 |
| Jun | 19 | 122 | 3215 | 35538 | 11.77 | 129.75 | 3222.23 | 35530.2537 | 4.926950119 | 0.026441 |
| Jul | 16 | 125 | 2872 | 35881 | 10.51 | 131.01 | 2877.49 | 35874.9948 | 3.157446925 | 0.075581 |
| Aug | 16 | 125 | 3299 | 35454 | 12.06 | 129.45 | 3302.94 | 35449.5484 | 1.444481853 | 0.229416 |
| Sep | 8 | 133 | 3403 | 35350 | 12.41 | 129.10 | 3398.59 | 35353.8977 | 1.691353559 | 0.193423 |
| Oct | 22 | 119 | 3876 | 34877 | 14.18 | 127.33 | 3883.82 | 34868.6696 | 4.871667012 | 0.027301 |
| Nov | 7 | 134 | 3020 | 35733 | 11.01 | 130.50 | 3015.99 | 35736.5005 | 1.562179011 | 0.211347 |
| Dec | 10 | 131 | 3384 | 35369 | 12.35 | 129.16 | 3381.65 | 35370.8358 | 0.474580286 | 0.490888 |

**Table 14.** Percent of *E. coli* O157:H7-positive samples from April – October vs. November – March. Samples were collected from 2000 – 2005. Yates corrected X2 = 15.8, p value <0.0001.

| Months | Total Samples | Number Positive | Percent Positive |
|---|---|---|---|
| April – October | 23,082 | 107 | 0.46% |
| November - March | 15,671 | 34 | 0.22% |
| *Total* | *38,753* | *141* | *0.36%* |
| *Odds Ratio = 2.14, 90% confidence interval =2.46 to 1.92* | | | |

## Establishment Practices

By summer of 2008, FSIS plans to incorporate establishment practices into the *E. coli* O157:H7 sampling algorithm, including those interventions that reduce *E. coli* O157:H7 contamination and testing programs that effectively detect *E. coli* O157:H7. Accounting for establishment practices such as these will allow FSIS to more accurately target high-risk establishments and provide incentives for establishments to implement the best available practices during the production of ground beef and trim. A three-step process will be used to incorporate establishment production practices into the sampling algorithm. These steps include:

1. Identify general categories of production practices relevant to *E. coli* O157:H7 control (see *Identification of Relevant Practices* below).

2. Identify which of the establishments eligible for *E. coli* O157:H7 testing has practices from one or more of the categories in place (see *Identifying Practices in Eligible Establishments* below).

3. Estimate the impact of each practice on *E. coli* O157:H7 risk as objectively as possible to allow their incorporation into the algorithm (see *Analysis of Production Practices* and *Application of Production Practices in the Algorithm* below).

### *Identification of relevant practices*

The first objective was to identify establishment practices known to reduce the levels and/or prevalence of *E. coli* O157:H7 during production of ground beef and trim. To do this, experts in the fields of microbiology, food sciences and the animal/veterinary sciences were consulted (see Table 15). Tables 16 and 17 show the list of practices identified as important to control of *E. coli* O157:H7 in beef grinding and slaughter respectively. These lists were used to design an industry survey that collects data on establishment practices and testing programs.

**Table 15.** Panel of Experts.*

| Organization | Discipline | Cattle Production | Trans-portation | Slaughter | Grinding | *E. coli* O157:H7 Microbiology | Engineering Processing | Industrial Hygiene |
|---|---|---|---|---|---|---|---|---|
| Texas Tech University | Food science, microbiology | ● | ● | ● | ● | ● | | ● |
| Pennsylvania State University | Food technology, microbiology | | | ● | ● | ● | | ● |
| California Polytechnic State University | Meat science | | | ● | ● | ● | | ● |
| University of Georgia | Microbiology | ● | | ● | ● | ● | | ● |
| University of Nebraska | Veterinary medicine | ● | ● | ● | | | | |
| Better Built Foods | Animal science | | | ● | ● | ● | ● | ● |
| USDA, U.S. Meat Animal Research Center | Meat science, microbiology, food science, and technology | ● | ● | ● | ● | ● | | |
| University of Tennessee, Melton Consultant | Meat science | ● | ● | ● | ● | ● | ● | ● |

*Excerpted from "Expert Elicitation on Risk Factors for *E. coli* O157:H7 Contamination of Ground Beef" October 2006, RTI International (RTI Project Number 08893.004.004, FSIS Contract 53-3A94-03-12)

**Table 16.** Beef grinding practices.*

| |
|---|
| Establishment requires suppliers to have an effective pathogen control intervention program beyond minimum HACCP requirements |
| Establishment has adequate cold chain management of 40°F or lower during storage, processing, packaging, and distribution |
| Establishment conducts monitoring and responds to microbial indicator organisms in raw materials |
| Temperature of incoming beef is below 45°F |
| Establishment does test finished product for *E. coli* O157:H7 |
| Establishment uses an antimicrobial process (e.g., ozone) or additive (e.g., acidified sodium chlorite) on trim |

*Excerpted from "Expert Elicitation on Risk Factors for *E. coli* O157:H7 Contamination of Ground Beef" October 2006, RTI International (RTI Project Number 08893.004.004, FSIS Contract 53-3A94-03-12)

**Table 17.** Cattle slaughter practices.*

| |
|---|
| Documented training and monitoring of employee practices in proper hide removal procedures |
| Hide interventions (before hide removal) |
| Procedures to ensure employees adequately sanitize knives and sharpening steels between contaminated carcasses and surfaces |
| Use of post-harvest carcass interventions |
| Cross-contamination control program (other than hide removal) |
| Carcass internal temperature below 50°F or surface temperature below 45°F at 24 hours |
| Requires suppliers use of pre-harvest, live animal intervention |
| Less than 2 hours elapsed between hide removal and carcass entry into hot box (chiller) |
| Documented training and monitoring of employee practices in evisceration processes |

*Excerpted from "Expert Elicitation on Risk Factors for *E. coli* O157:H7 Contamination of Ground Beef" October 2006, RTI International ((RTI Project Number 08893.004.004, FSIS Contract 53-3A94-03-12).

FSIS will use objective criteria and analysis to determine which practices are relevant to mitigating or increasing the public health risk from *E. coli* O157:H7 and how they will impact sampling frequency (see *Analysis of Production Practices* below). Importantly, this initial list of categories is not all-inclusive. Rather, it is meant to be a starting point that is expected to continually evolve as FSIS receives more information about existing practices and new interventions/testing methodologies arise.

*Identifying ground beef production practices*

A survey has been administered to collect information about the production practices at individual establishments eligible for *E. coli* O157:H7 sampling (the "E. coli raw beef processing checklist"). FSIS inspectors responded for a total of 2,223 establishments that slaughter or produce raw beef products. Of the 1,459 FSIS inspected establishments that produce ground beef, 1,240 responded (app. 85%). The checklist data will serve two important functions. First, the survey was designed to collect data on what specific practices are currently in use by industry. For instance, it asks about the specific types of hide interventions and antimicrobial processes actually in use. Secondly, to account for production practices in the sampling algorithm, FSIS must identify the relevant practices present in each establishment and be able to identify practices that have a significant association with *E. coli* O157:H7 prevelance and levels.

*Analysis of establishment practices*

In order to have scientific support for the use of particular establishment practices in the probabilistic algorithm, FSIS must associate specific establishment practices with either an increase or decrease in *E. coli* O157:H7 in trim and/or ground beef. Practices that are associated

with either an increase or reduction of *E. coli* O157:H7 contamination can then be used in the risk-based sampling algorithm to raise or lower the sampling probability of individual establishments. FSIS proposes two general categories of scientific support for including practices in the sampling algorithm.

- Scientific studies that demonstrate a particular practice reduces *E. coli* O157:H7 in ground beef or trim could provide valid support for their inclusion in the algorithm.

- A comparative analysis of FSIS sample results in establishments that have a particular practice vs. those that do not may lead to a significant association between a given practice and the control of *E. coli* O157:H7. Although this second approach is a useful one, it may not be practical in every case due to the very small number of positive *E. coli* O157:H7 samples. In addition, associative studies such as this suffer from the caveat that they do not provide a cause and effect conclusion to be drawn.

*Application of establishment practices in the algorithm*

It is also difficult to determine exactly how establishment practices will be applied in the risk-based algorithm until all the data discussed above have been analyzed. However, the following methods may prove useful in applying practices validated by one of the two methods described above (see *Analysis of Establishment Practices*).

- Practices for which there are studies that define the effect on *E. coli* O157:H7 could be weighted accordingly. For instance, practices that lead to a 3-$\log_{10}$ reduction could be weighted relative to practices with a 1-$\log_{10}$ reduction.

- Similarly, if there are practices associated with a reduced prevalence of *E. coli* O157:H7-positive test results, they could be weighted accordingly as well. In this second case the weight would be determined by the associated prevalence, i.e. if a particular practice is associated with a four-fold reduction in positive test results, then the practice could be weighted to decrease sampling probability by four-fold.

# Putting It All Together

Having described the general principles of the *E. coli* O157:H7 risk-based sampling algorithm and discussed the data used to inform the algorithm in the above chapter, this chapter focuses on the overall design and function of the algorithm, how the algorithm will be used, and how performance of the risk-based sampling program can be measured. Also included is a description of some caveats associated with implementing and using the algorithm.

## Algorithm Design and Function

The overall design of the *Escherichia coli* O157:H7 risk-based sampling algorithm is shown on the following page in Figure 4. The algorithm computes two scores (Volume Score and Hazard Score), which are then used to calculate the individual sampling probability ($p_i$) of each establishment. Volume Score (*vs*) is calculated for each establishment category based on the average amount of product produced per day (see *Production Volume Data*) and the scaling factor selected by risk managers (see *Application of Production Volume in the Algorithm*). For additional details on how the algorithm fits a volume score to each category according to the scaling factor input, see the Visual Basic source code document and the algorithm itself. The Hazard Score (*hs*) is currently determined by the *E. coli* O157:H7 test results. If an establishment has tested positive within the last four months, the hazard score is 5. Otherwise it is 1. These scores are based on the odds ratio described in the analysis in Table 9.

Because the algorithm is currently designed to be run on the first of each month, it queries for any *E. coli* O157:H7-positive samples that have occurred within 4 months using data starting from the previous month (greater than 150 days ago). By Summer 2008, the Hazard Score will also account for establishment practices such as interventions and testing programs. There are also management decisions that impact the final observed sampling frequency. Most notably, the total number of samples allocated, the chosen scaling factor, and the chosen upper and lower bounds for sample #/plant (see "Estimated Effect on Sampling Frequency" for details). The Sampling Probability (*pi*) is the ratio of the product of *v* and *h* for an individual establishment *i* to the sum of all the *vh* for every eligible establishment (as shown in Figure 4 below). An example of probability ranges calculated by the algorithm is shown in Table 18.

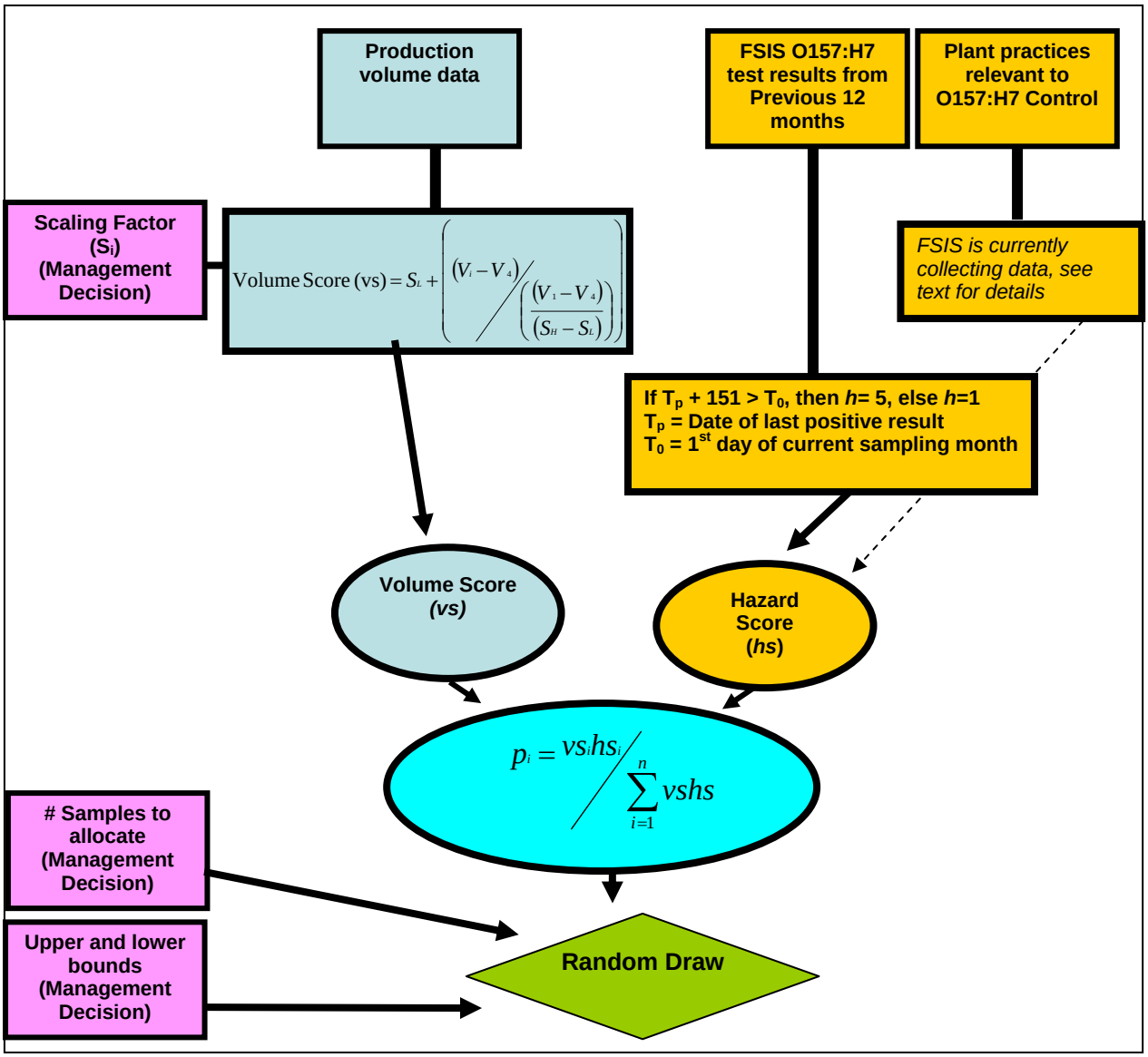


**Figure 4.** A schematic of the probabilistic algorithm design for FSIS' risk-based *E. coli* O157:H7 verification sampling program. See text for details.

In the final step, all the establishments are placed in a random draw without replacement using their individual $p_i$ to weight their chance of being selected. Briefly, each establishment's $p_i$ is equal to a unique range between 0 and 1. A random number generator then picks a single number between 0 and 1 for each sample. If a number lands within the range of an establishment, then it is selected for sampling and removed from the list before the next number is drawn.

**Table 18.** An example of probability ranges calculated by the algorithm. These ranges were calculated by running the algorithm with data from FSIS-inspected beef grinders from 2005. Note that the ranges shown are only for 13 establishments out of a total of approximately 1,500 in this algorithm simulation. Thus, the sum of all probability ranges is 1.0.

| Establishment Probability Points | Bottom of Sample Probability Range | Top of Sample Probability Range |
|---|---|---|
| 4 | 0 | 0.001084893 |
| 5 | 0.001084893 | 0.002441009 |
| 4 | 0.002441009 | 0.003525902 |
| 4 | 0.003525902 | 0.004610795 |
| 2 | 0.004610795 | 0.005153241 |
| 2 | 0.005153241 | 0.005695688 |
| 2 | 0.005695688 | 0.006238134 |
| 2 | 0.006238134 | 0.00678058 |
| 3 | 0.00678058 | 0.00759425 |
| 5 | 0.00759425 | 0.008950366 |
| 4 | 0.008950366 | 0.010035259 |
| 4 | 0.010035259 | 0.011120152 |
| 5 | 0.011120152 | 0.012476268 |

## Algorithm User Interface

The algorithm was programmed in Visual Basic (Microsoft Corp., Redmond, WA) to be user friendly and adaptable. The interface allows the user to run the algorithm simply by clicking on a small number of action buttons. The program then downloads the required data, processes the data, and displays the list of establishments selected from the random draw for sampling, as well as a report on establishment statistics and other useful information. The entire process requires less than five minutes. Because the probability of sampling is dependent on the (production) Volume Score and the Hazard Score, the algorithm can be readily adapted to accept any type of data that can be compiled into the risk score without making any changes to the core program. This will become important over time as more data are collected on *E. coli* O157:H7 risk factors.

## Measuring Performance

The overarching goal of FSIS' *E. coli* O157:H7 testing program is to help ensure that industry is producing raw ground beef that is as free from *E. coli* O157:H7 contamination as possible. Two questions, therefore, can be asked to evaluate the testing program:

### How effectively are FSIS *E. coli* O157:H7 testing resources being used to verify the safety of the trim and raw ground beef supply?

- One measure of how effectively testing resources are utilized is the percentage of the U.S. trim and ground beef supply verified each month. Assuming each verification sample is a measure of an establishment's *E. coli* O157:H7 controls, the monthly production volume of all verified establishments can be used to

estimate the percentage of supply verified each month by dividing total pounds produced by pounds of product verified.

- A second measure of effectiveness is the proportion of total contaminated lots produced that are identified by the testing program. The more effective a risk-based sampling program, the greater the percentage of total contaminated lots produced it will identify. One metric for this measure is the ratio of the prevalence of positives in the risk-based sample pool to the prevalence of positives in an unweighted random pool. Ideally, FSIS could run a random, unweighted sampling program side by side with the risk-based algorithm and directly compare the results of the two programs. However, it is not feasible to run an entire sampling program solely to measure the performance of a risk based one. One alternative is to take the ratio of the proportion of positives from the risk-based program over a "frequency adjusted" proportion. In this case, the frequency adjusted proportion would unweight the sample results from each establishment in the sampling frame so that the results are comparable to a sampling program where every establishment was sampled at the same frequency. In a hypothetical example of 100 establishments the results from each establishment would be adjusted so that they each accounted for exactly $1/100^{th}$ of the total samples taken. A third option is to use a "bootstrap" approach to simulate the results of a simple, random draw from the risk-based sampling program results and compare the two. FSIS has developed a bootstrap model that accomplishes this and allows us to model the results of a simple random program for comparison. Unfortunately, due to the exceedingly low prevalence of *E. coli* O157:H7 in FSIS samples, measuring these kinds of differences with statistical confidence is difficult. One advantage to the bootstrap approach is that it is an iterative method where the sample results are essentially "resampled" thousands of times. The results from thousands of iterations can then be displayed as a distribution of outcomes allowing a measure of the uncertainty.

## What is the public health impact of the sampling program?

To a large extent, if the program is verifying the safety of (i) a greater portion of product and (ii) the riskiest portion of product, then it is reducing the exposure of consumers to *E. coli* O157:H7-contaminated ground beef.

## Adjusted proportion of positive samples

This method of calculating proportion of positive samples weights plants by their production volume and adjusts for the difference in sampling frequency between plants by using the proportion of positive samples reported for each plant. Reporting program results as an adjusted proportion is important for at least two reasons. First, an unadjusted proportion gives no information about the risk/serving of the ground beef supply since samples representing 500lbs of production are weighted equally with those that represent 500,000lbs. Secondly, the risk-based sampling program does not sample all plants equally; therefore, adjusting for the difference in sampling frequency between plants will allow a better comparison of the results from year to year.

*Unit of interest*

The unit of interest for this metric is an adjusted proportion of adulterated 325g sample units. Because we do not currently have access to enumeration data, it is not possible to transform the proportion of positive samples to the proportion of contaminated servings or pounds. However, the proportion of adulterated 325g samples is a measure of the *proportion* of the supply contaminated with O157:H7— which is a more useful risk metric than the unadjusted proportion currently used. In addition, 325 grams is a convenient unit because it is between a usual serving size of about 100 grams and a pound (454 grams).

*Calculation*

The basic calculation (Equation 2) looks very similar to the equation for calculating the proportion of positive samples.

$$\text{Proportion of adulterated sample units} = \frac{\text{Number of adulterated sample units}}{\text{Total number of sample units}} \qquad \text{(Equation 2)}$$

The number of adulterated sample units is weighted by volume and adjusted for over sampling (Equation 3).

$$\text{Number of adulterated sample units} = \frac{453.59\text{ g}}{\text{lb}} \times \frac{\text{sample unit}}{325\text{ g}} \times \sum_{i=1}^{4} \left( \left( \frac{\text{Production lbs}}{\text{Day}} \right)_i \times \text{Days}_i \times n_i \times \frac{\sum_{j=1}^{n_i} \frac{\text{Positives}_j}{\text{Samples}_j}}{n_i} \right) \qquad \text{(Equation 3)}$$

Note that the number of adulterated sample units is dependent on the weighted proportion of establishments with positive results in each production category. An establishment that has 3 out of 6 positive samples will have the same effect on the metric as an establishment that produces the same amount of product and has 6 out of 12 positive samples. In the risk based verification sampling program it is expected that each establishment will be sampled at least once per year with high risk plants being sampled the most frequently. Thus, all establishments will be represented. Although the proportion of positive samples in high risk plants will affect the number of adulterated sample units, the number of positive samples will not.

The total number of sample units is weighted by volume (Equation 4).

$$\text{Total number of sample units} = \frac{453.59\text{ g}}{\text{lb}} \times \frac{\text{sample unit}}{325\text{ g}} \times \sum_{i=1}^{4}\left(\left(\frac{\text{Production lbs}}{\text{Day}}\right)_i \times \text{Days}_i \times n_i\right) \quad \text{(Equation 4)}$$

Equation 2 can be re-expressed as Equation 5.

$$\text{Proportion of adulterated sample units} = \frac{\frac{453.59\text{ g}}{\text{lb}} \times \frac{\text{sample unit}}{325\text{ g}} \times \sum_{i=1}^{4}\left(\left(\frac{\text{Production lbs}}{\text{Day}}\right)_i \times \text{Days}_i \times n_i \times \frac{\sum_{j=1}^{n_i}\frac{\text{Positives}_j}{\text{Samples}_j}}{n_i}\right)}{\frac{453.59\text{ g}}{\text{lb}} \times \frac{\text{sample unit}}{325\text{ g}} \times \sum_{i=1}^{4}\left(\left(\frac{\text{Production lbs}}{\text{Day}}\right)_i \times \text{Days}_i \times n_i\right)}$$

(Equation 5)

The constants in Equation 5 cancel out resulting in Equation 6.

$$\text{Proportion of adulterated sample units} = \frac{\sum_{i=1}^{4}\left(\left(\frac{\text{Production lbs}}{\text{Day}}\right)_i \times \text{Days}_i \times n_i \times \frac{\sum_{j=1}^{n_i}\frac{\text{Positives}_j}{\text{Samples}_j}}{n_i}\right)}{\sum_{i=1}^{4}\left(\left(\frac{\text{Production lbs}}{\text{Day}}\right)_i \times \text{Days}_i \times n_i\right)} \quad \text{(Equation 6)}$$

*Result*

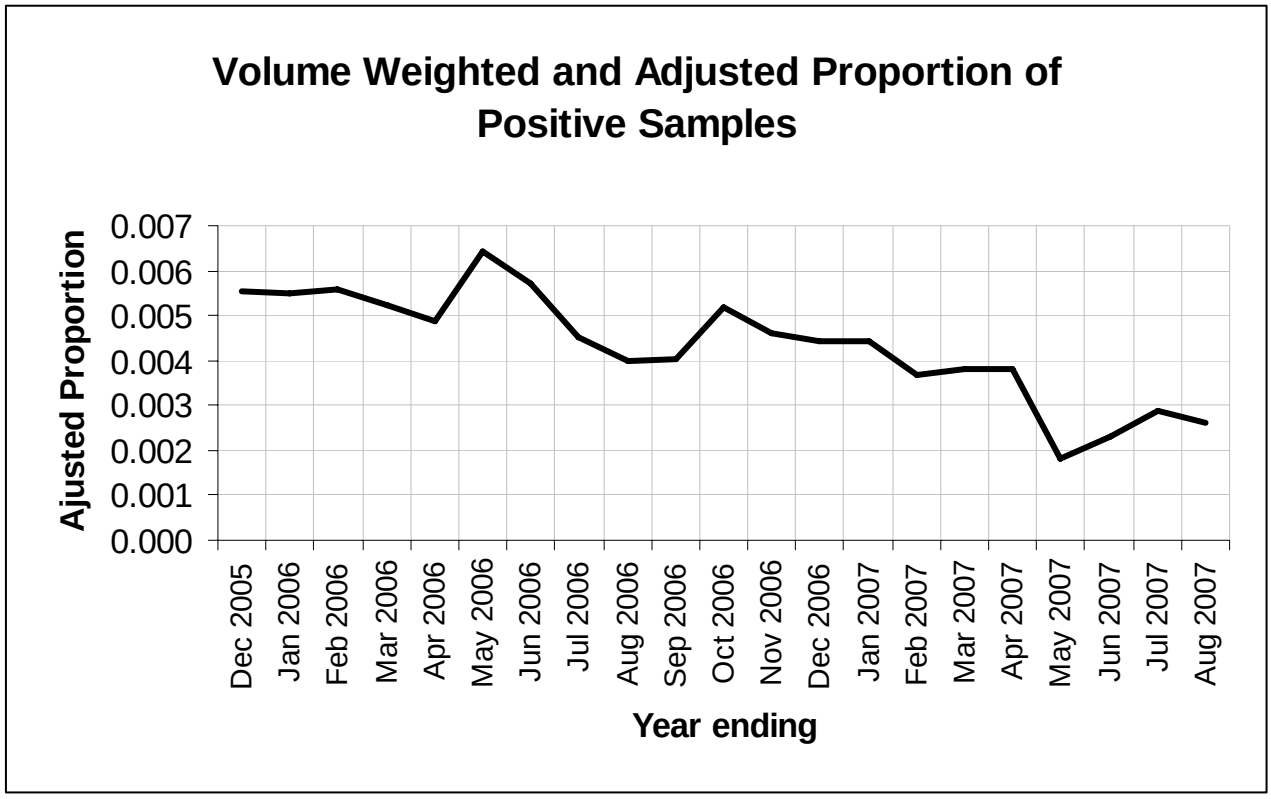


**Figure 5.** Proportion of adulterated ground beef. The proportion of adulterated beef is calculated as a volume adjusted proportion of positive samples (chart displays a rolling annual average ending on the indicated month.)

## Estimated human illnesses

Human illness is another common metric, which is often an output of risk assessments.

*Unit of interest*

The unit of interest here is the absolute number of human illnesses. This unit has the advantage of being able to directly inform economic analyses. Its primary disadvantage is that it is population dependent. In other words, the risk of illness could decrease while the estimated human illnesses increase due to population growth.

*Calculation*

Human illnesses for any year other than the base years is calculated as

$$\text{Human illnesses} = \begin{matrix}\text{Number of}\\ \text{adulterated}\\ \text{sample units}\end{matrix} \times \text{Multiplier} \qquad \text{(Equation 7)}$$

where the number of adulterated sample units is calculated as in Equation 3; and the multiplier is calculated as shown in Equation 8.

$$\text{Multiplier} = \frac{\begin{matrix}\text{Total}\\ \text{human}\\ \text{illnesses}_{\text{Base years}}\end{matrix}}{\begin{matrix}\text{Total number of}\\ \text{adulterated}\\ \text{sample units}_{\text{Base years}}\end{matrix}} \qquad \text{(Equation 8)}$$

Total human illnesses for the base years are calculated by using reported illnesses in the FoodNet catchment area, adjusting them for the United States population, and then further adjusting the by an underreporting multiplier (Equation 9).

$$\begin{matrix}\text{Total}\\ \text{human}\\ \text{illnesses}_{\text{Base years}}\end{matrix} = \sum_{i=1}^{m}\left(\begin{matrix}\text{Reported}\\ \text{FoodNet}\\ \text{Illnesses}_i\end{matrix} \times \frac{\text{U.S. population}_i}{\text{FoodNet catchment population}_i} \times \begin{matrix}\text{Underreporting}\\ \text{factor}\end{matrix}\right) \qquad \text{(Equation 9)}$$

where $m$ = the number of base years.

The total number of adulterated sample units for the base years (Equation 10) is calculated by summing the results from  for each of the base years.

$$\begin{matrix}\text{Total number of}\\ \text{adulterated}\\ \text{sample units}_{\text{Base years}}\end{matrix} = \sum_{i=1}^{m}\begin{matrix}\text{Number of}\\ \text{adulterated}\\ \text{sample units}_i\end{matrix} \qquad \text{(Equation 10)}$$

where $m$ = the number of base years.

*Result*

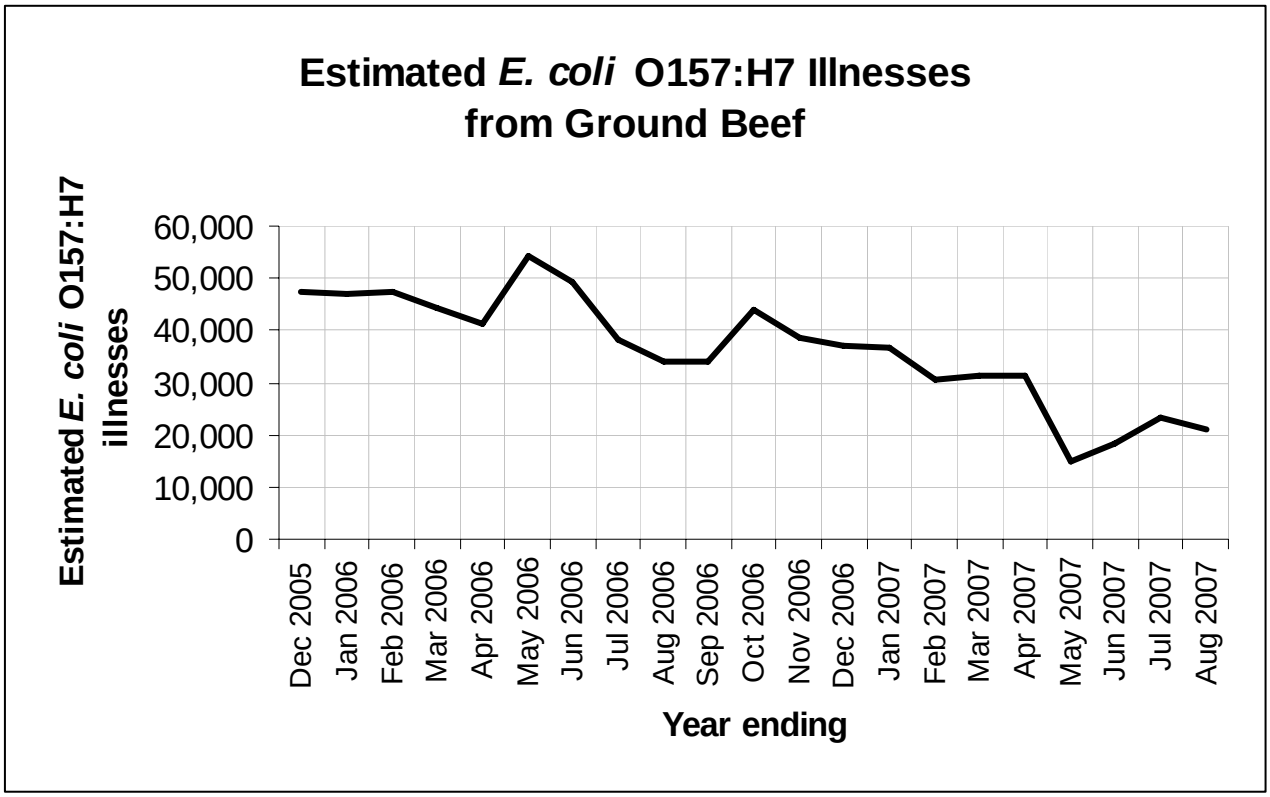


**Figure 6.** Estimated number of annual *E. coli* O157:H7 illnesses resulting from beef. The number of *E. coli* O157:H7 illnesses attributed to ground beef was estimated from analyses of CDC outbreak data, FoodNet data and the volume adjusted proportion of positives. (Chart displays a rolling annual average ending on the indicated month.)

**Estimated human illnesses per 100,000 population**

Estimating disease incidence allows for direct comparison with the CDC Health People 2010 goals.

*Unit of interest*

Although commonly expressed as a proportion, human illnesses per 100,000, this metric is actually meant to express a rate, human illnesses per 100,000 per year. It is population independent and, as noted, allows for direct comparison with CDC metrics.

*Calculation*

The calculation is the same as for Equation 7 with the addition of a population adjustment.

$$\frac{\text{Human illnesses}}{100{,}000} = \text{Number of adulterated sample units} \times \text{Multiplier} \times \frac{100{,}000}{\text{U.S. population}} \quad \text{(Equation 11)}$$

FoodNet reported values do not use the underreporting factor noted in Equation 9. Therefore, to make this metric directly comparable with FoodNet metrics a multiplier without the underreporting factor is used (Equation 12).

$$\frac{\text{Human illnesses}}{100{,}000} = \text{Number of adulterated sample units} \times \text{MultiplierNoUR} \times \frac{100{,}000}{\text{U.S. population}} \quad \text{(Equation 12)}$$

where MultiplierNoUR is calculated the same as the multiplier in Equation 8, Equation 9, and Equation 10, but without the underreporting factor in Equation 9.

*Result*

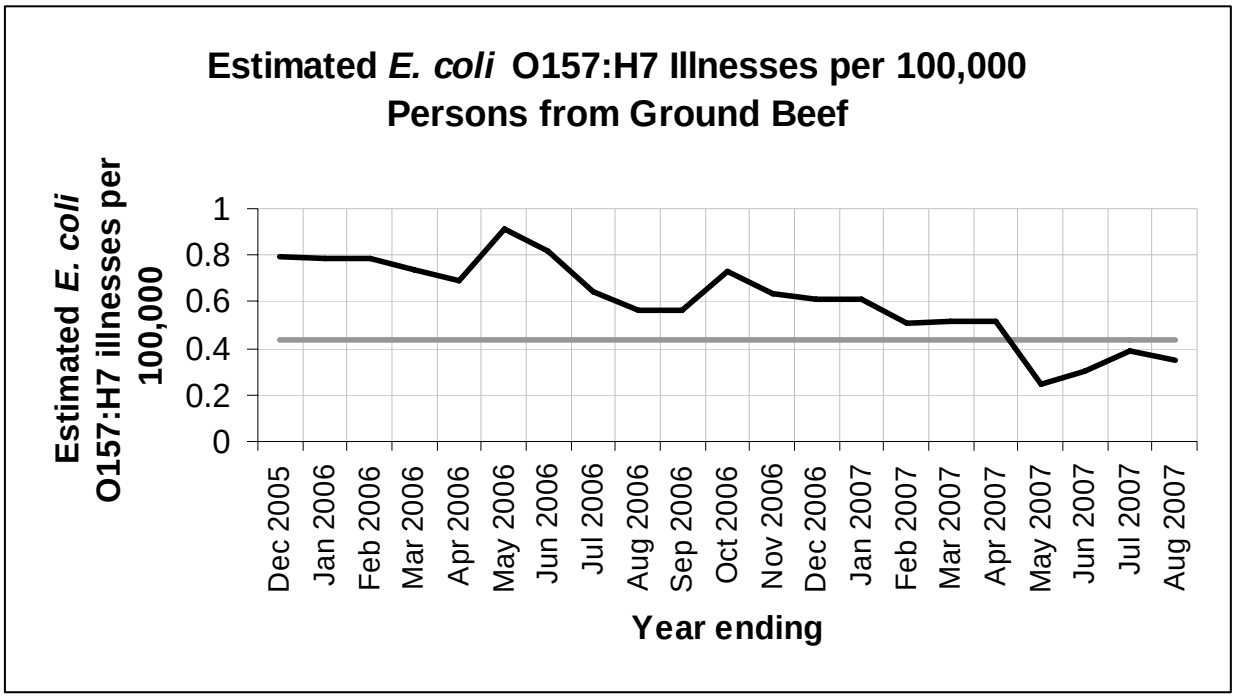


**Figure 7.** *E. coli* O157:H7 illnesses incidence. The gray line shows the CDC healthy people 2010 objective for *E. coli* O157:H7 adjusted for attribution to beef. The black line shows the rolling average of the estimated incidence in ground beef. The incidence was calculated based on CDC outbreak data, FoodNet data and the volume adjusted proportion of positives.

## Estimated Effect on Sampling Frequency

The prior FSIS verification sampling program for ground beef producers had a goal of sampling every establishment with the same monthly probability of selection. The average frequency of sampling by production category ranged from 69% to 81% for establishments with no positive samples in CY2007 (see Table 19).

**Table 19.** The sampling frequency each month under the prior FSIS *E. coli* O157:H7 verification sampling program in CY2007. See "Data and Assumptions" (below) for details of analysis.

| Sample History | Production Category | Number of Establishments in Category (CY2007) | Average # Monthly Samples/plant (CY2007) |
|---|---|---|---|
| *No positive samples* | *< 1,000 pounds* | 1004 | 0.69 |
| | *1,000 to 50K pounds* | 305 | 0.77 |
| | *>50K to 250K pounds* | 52 | 0.81 |
| | *> 250K pounds* | 35 | 0.75 |
| *One positive sample* | *< 1,000 pounds* | 17 | 0.74 |
| | *1,000 to 50K pounds* | 6 | 0.78 |
| | *>50K to 250K pounds* | 2 | 0.96 |
| | *> 250K pounds* | 1 | 0.83 |

The prior FSIS *E. coli* O157:H7 verification sampling program has a ceiling of one sample per month. In other words, no establishment can be sampled more than once per month. Furthermore, there is no requirement that the program ensure assignment of all eligible establishments each year. Without this floor it is possible for some establishments to miss sampling for an entire year due to chance. The current risk-based algorithm incorporates both a floor to ensure minimal sampling and a ceiling to limit the amount of sampling. Both of these parameters have been set by risk managers. The proposed floor requires collection of at least three samples per year per establishment. The proposed ceiling limits samples to two per month per establishment or twenty-four per year. Incorporating these limits into the algorithm to assign samples, FSIS found that the smallest establishments would be sampled slightly less than they had been and the largest establishments would be sampled at a slightly higher frequency than they were previously (see Table 20).

**Table 20.** Expected sampling frequency each month under the current risk-based *E. coli* O157:H7 verification sampling program. See "Data and Assumptions" (below) for details.

| Sample History | Production Category | Estimated Average # Monthly Samples per plant (Risk-based Program) |
|---|---|---|
| *No positive samples in last 4 months* | *< 1,000 pounds* | 0.63 |
| | *1,000 to 50K pounds* | 0.99 |
| | *>50K to 250K pounds* | 1.98 |
| | *> 250K pounds* | 1.94 |
| *One positive sample* | *< 1,000 pounds* | 2.0 |
| | *1,000 to 50K pounds* | 4.0 |
| | *>50K to 250K pounds* | 4.0 |
| | *> 250K pounds* | 4.0 |

### Data and Assumptions

- For the analysis of the prior FSIS *E. coli* O157:H7 verification sampling program, a data set from CY2007 of 1,396 ground beef producers and 11,979 annual beef samples tested for *E. coli* O157:H7 was used. The average monthly frequency was calculated for producers with and without an *E. coli* O157:H7-positive FSIS sample for the same time period (CY2007) and reported as the average monthly sample number per plant. Actual number of samples for any given plant in a single month varies.

- To initiate FSIS' current risk-based *E. coli* O157:H7 sampling verification in January 2008, CY2007 FSIS *E. coli* O157:H7 verification sampling program was used to scheduled samples. Plants with an *E. coli* O157:H7-positive sample will receive either 16 or 8 samples (depending on production volume) over four months.

### Important Caveats

- If significantly more or fewer samples are diverted to or from FSIS' risk-based *E. coli* O157:H7 verification sampling program, the estimated sampling frequencies could change significantly for all categories of grinders.

- FSIS is currently collecting data on interventions and testing practices of ground beef and trim producers. Once these data are collected and analyzed, they will be incorporated into the risk-based *E. coli* O157:H7 verification sampling algorithm. The frequencies of *E. coli* O157:H7-positive samples are expected to shift significantly for establishments with effective testing programs and interventions.

## CONCLUSIONS

In January 2008, the risk-based sampling algorithm described in this report was used to direct FSIS resources among establishments to test for *E. coli* O157:H7 in ground beef and beef trim. This algorithm provides a sound scientific basis for directly FSIS resources and improve FSIS' verification sampling program to further protect public health.

## REFERENCES


1. Riley LW, Remis RS, Helgerson SD, et al. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N Engl J Med* 1983;308:681-5.

2. Wells JG, Davis BR, Wachsmuth IK, et al. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. *J Clin Microbiol* 1983;18:512-20.

3. Zhao T, Doyle MP, Shere J, et al. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. *Appl Environ Microbiol* 1995;61:1290-3.

4. Ge B, Zhao S, Hall R, et al. A PCR-ELISA for detecting Shiga toxin-producing *Escherichia coli*. *Microbes Infect* 2002;4:285-90.

5. Besser RE, Lett SM, Weber JT, et al. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *JAMA* 1993;269:2217-20.

6. Centers for Disease Control and Prevention. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider--Connecticut and New York, October 1996. *MMWR Morb Mortal Wkly Rep* 1997;46:4-8.

7. Day WA, Maurelli AT. *Shigella* and enteroinvasive *Escherichia coli*: paradigms for pathogen evolution and host-parasite interactions, in Escherichia coli: *Virulence Mechanisms of a Versatile Pathogen* (Donnenberg, M.S., ed.), Academic Press, San Diego, CA, pp. 209-237. In: Anonymous2002;

8. Centers for Disease Control and Prevention. Outbreak of *Escherichia coli* O157:H7 and *Campylobacter* among attendees of the Washington County Fair-New York, 1999. *MMWR Morb Mortal Wkly Rep* 1999;48:803-5.

9. Barrett TJ, Kaper JB, Jerse AE, et al. Virulence factors in Shiga-like toxin-producing *Escherichia coli* isolated from humans and cattle. *J Infect Dis* 1992;165:979-80.

10. Blanco J, Blanco M, Garabal JI, et al. Enterotoxins, colonization factors and serotypes of enterotoxigenic *Escherichia coli* from humans and animals. *Microbiologia* 1991;7:57-73.

11. Mead PS, Griffin PM. *Escherichia coli* O157:H7. *Lancet* 1998;352:1207-12.

12. Karmali MA, Steele BT, Petric M, et al. Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. *Lancet* 1983;1:619-20.

13. Proulx F, Seidman EG, Karpman D. Pathogenesis of Shiga toxin-associated hemolytic uremic syndrome. *Pediatr Res* 2001;50:163-71.

14. Le Saux N, Spika JS, Friesen B, et al. Ground beef consumption in noncommercial settings is a risk factor for sporadic *Escherichia coli* O157:H7 infection in Canada. *J Infect Dis*

1993;167:500-2.

15. Wall PG, McDonnell RJ, Adak GK, et al. General outbreaks of vero cytotoxin producing *Escherichia coli* O157 in England and Wales from 1992 to 1994. *Commun Dis Rep CDR Rev* 1996;6 :R26-33.

16. Carter AO, Borczyk AA, Carlson JA, et al. A severe outbreak of *Escherichia coli* O157:H7--associated hemorrhagic colitis in a nursing home. *N Engl J Med* 1987;317:1496-500.

17. Siegler RL. The hemolytic uremic syndrome. *Pediatr Clin North Am* 1995;42:1505-29.

18. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* 1998;11:142-201.

19. Ebel ED, David MJ, Mason J. Occurrence of *Salmonella enteritidis* in the U.S. commercial egg industry: report on a national spent hen survey. *Avian Dis* 1992;36:646-54.

20. http://www.fsis.usda.gov/PDF/RBVS_Risk_Assess_Jun07.pdf