FSIS Nationwide Market Hogs Microbiological Baseline

Data Collection Program

Study Design for Technical Consultation

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Microbiological Analysis and Data Branch

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Executive Summary

Program summary and study design:
This document outlines the study design and sampling frame for the nationwide Market Hogs Microbiological Baseline Study (MHBS) data collection program conducted by the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA).

Rationale:
To present the most appropriate methods to construct the study design and sample frame for MHBS. This document presents a study design and sample frame that includes 247 plants that process market hogs. To obtain a high representation of all plants in each of the five strata, the sample frame is stratified according to production volume. The final sample frame includes 181 plants.

Design Description:
The MHBS sample frame consists of five strata and 2,508 sampling events or 5,016 samples (divided evenly among pre-evisceration and post chill). The samples were allocated to each stratum according to the volume of the plants in that stratum. The chosen design includes 10% oversampling to account for non-response. Several stratum boundaries were constructed to obtain the design with least within-stratum variability.

Adjustments:
The sample frame was recalculated to consider the most recent volume data available. The frame was used to construct a detailed sample schedule for each plant and was provided to PREP and PHIS. Adjustments to the sample are made on an ongoing basis if plants drop out of the frame or new plants join on e-ADRS.

Conclusion:
The MHBS sample frame presented in this document is the best frame to obtain an accurate prevalence calculation given the constraints of the study. The sample frame is presented below:

Final Stratification of the Market Hogs Baseline Sampling

Stratum	Est./Stratum	Est. Sampled	Sampling Event/Month /Plant	No. Months	Sampling Events/ Establishment/Year	Total Samples/Year /Establishment	Total Samples/ Stratum/Year	Sampling Percent by Stratum
1	**13**	**13**	**6**	**12**	**72**	**144**	**1,872**	**37.32%**
2	**14**	**14**	**5**	**12**	**60**	**120**	**1,680**	**33.49%**
3	**30**	**30**	**1**	**12**	**12**	**24**	**720**	**14.35%**
4	**64**	**64**	**0.25**	**12**	**3**	**6**	**384**	**7.66%**
5	**126**	**60**	**0.25**	**12**	**3**	**6**	**360**	**7.18%**
Total	**247**	**181**					**5,016***	**100.00%**

***Note:** **2,508 samples to be collected at pre-evisceration and 2,508 at post-chill**

Program Summary

The nationwide Market Hogs Microbiological Baseline Study (MHBS) data collection program conducted by the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) will collect samples from the carcasses of market swine slaughtered under federal inspection. Results of this study will enable FSIS and the regulated industry to target interventions that reduce the risk of foodborne pathogens associated with this product class.

Study primary objectives:

(1) Estimate the prevalence and quantitative level of selected bacteria on market swine carcasses at pre-evisceration and post-chill; and
(2) Obtain data for use in the development of microbiological risk assessments, risk-based sampling programs, and/or regulatory policy decisions, including the development of performance guidelines.
(3) Obtain post-hoc statistical analyses of the microbiological data when appropriate

FSIS will schedule the collection of approximately 5,016 sponge samples from market swine carcasses (2,508 at pre-evisceration and 2,508 at post-chill during specified production shifts). Multiple sampling events will occur in each establishment over the twelve-month study period, scheduled to begin in August 2010. Laboratory analysis will detect and quantify selected foodborne pathogens and indicator bacteria.

Literature Review

A. Previous Market Hogs Baselines

The USDA FSIS published the Pathogen Reduction Hazard Analysis and Critical Control Point Systems (PR/HACCP) Final Rule in 1996 with the goal of reducing the prevalence and numbers of pathogenic organisms in meat and poultry products (22). The final rule includes *Salmonella* sp. prevalence criteria for specific meat and poultry products, based on FSIS baseline study data. In addition, the Final Rule mandated that all establishments slaughtering cattle, swine, chicken, or turkey screen products for *Escherichia coli* Biotype 1 (generic *E. coli*) at a certain frequency, based on production volume, in order to track process control over fecal contamination. Since the introduction of the PR/HACCP rule, FSIS has conducted two Market Hog Baseline Studies (MHBS). The first study, April 1995 to March 1996, used tissue excision samples (5). The second study, June 1997 to May 1998, used a sponge sampling method (6).

B. Sampling Techniques and microbiological analysis

Common sampling methods include tissue excision and sponge samples. Performing tissue excision sampling requires that personnel remove carcasses tissue (a surface of 5–10 cm^2 and 2–5 mm deep) from the ham, belly, jowl, and back (17, 18). Excision samples

provide the highest recovery of pathogenic and indicator bacteria (7), but this method raises cross-contamination concerns between samplings and is considered destructive because knife cuts deface the carcass.

Sponge sampling requires that personnel dip a sponge in sterile media and swab a portion of the carcass (i.e. ham, belly, and jowl). This method, also referred to as swabbing, is nondestructive and is the preferred sampling technique. Some studies found that sponge sampling is less effective at detaching bacteria than excision sampling (17, 18). Other studies have found that sponge sampling can be as effective as excision if the sponge materials are more abrasive (11, 24).

To avoid defacing the carcass and cross-contamination, MHBS samples will be collected using the sponge-sampling technique for the 1997–1998 baseline study (6). An entire carcass swab is laborious and bacterial recovery depends on the total surface of the individual carcass. Because carcasses come in different sizes, obtaining a uniform bacterial recovery when swabbing the entire carcass becomes impossible. Sponge sample collection focuses on a pooled sample (i.e., one sponge to swab 100 cm^2 of the ham, belly, jowl, and, at times, the back) (11). This method is practical and yields controlled bacterial recovery because the swabbing surface is the same for all carcasses (17, 18).

The 2010 MHBS will include two sponge samples—one for each side of the carcass. The sample collector will swab the ham, belly, and jowl on each side. The sponge swab technique will allow for direct comparison to the 1997–1998 baseline data and ongoing PR/HACCP verification data. The sampling method in this study is the same as the current PR/HACCP sampling method, which will reduce the need for additional training for the inspection plant personnel (IPP).

Samples collected by IPP will follow the procedures described on computer-generated sample request forms, as well as in Appendices E and F of the PR/HACCP Regulation of July 25, 1996 and subsequently described in FSIS Directive 10,230.5 (21). A single sterile sponge hydrated with 10 ml of cold sterile Buffered Peptone Water (BPW), is used to swab within a sterile 10 × 10 cm, plastic template, covering a 300 cm^2 surface area composite. The composite will include one ham site (100 cm^2), one belly site (100 cm^2), and one jowl site (100 cm^2). The collector will use a second sponge to swab each side of the carcass. One sponge will be analyzed for *Salmonella* sp., and other indicator organisms. The collector will use the second sample to swab the other side of the carcass for *Campylobacter* sp. The individually bagged sponge samples are shipped in an insulated container with chilled gel-ice packs on the same day to the designated laboratory. The container must maintain refrigeration temperatures for an overnight delivery service. Samples collection occurs Monday through Friday during slaughter operation. Samples collected and shipped on Fridays will be labeled specifying "For Saturday Delivery" on the shipping box. Only samples received at the laboratory the day after sample collection with a sample receipt temperature of 0 to 15 ºC (inclusive) will be analyzed. Samples received outside this range will be discarded.

C. Indicator Organisms

The current FSIS control verification focuses on *E. coli*-based performance criteria. Thus, generic *E. coli* should be included as an indicator organism. When recoverable levels of generic *E. coli* are too low for statistical evaluation, alternative indicators, such as Total Viable Count (TVC), coliforms, and Enterobacteriaceae, may prove useful for process control. The April 1995 to July 1996 baseline estimated prevalence and produced data on levels of aerobic plate count, total coliforms, and *E. coli* as potential indicator organisms for process control. The 2010 MHBS will screen for total aerobic bacteria, Enterobacteriaceae, generic *E. coli*, and total coliforms.

D. Pathogenic Organisms

The 1995 to 1996 MHBS estimated the prevalence and levels of *Salmonella* sp., *Staphylococcus aureus, Clostridium perfringens, E. coli* O157:H7, *Campylobacter jejuni, C. coli*, and *Listeria monocytogenes.* The 2010 MHBS will screen for *Salmonella* sp., *C. jejuni, C. coli*, and *Yersinia enterocolitica*, because the transfer of these pathogens may occur to the carcasses or other surfaces during slaughter (1-4, 8, 9,12-15).

Few studies have attempted direct enumeration of pathogens. Injured or low levels of bacterial cells pose significant challenges, and consequently, there is limited data available on enumeration of *Salmonella* sp., *C. jejuni, C. coli*, and *Y. enterocolitica* on carcasses. In addition, carcass chilling during the swine slaughtering process reduces the incidence of these pathogens (22). Due to the expected low pathogen incidence on the post-chill carcasses, the 2010 MHBS will include an intermediate enrichment step for detecting *Salmonella* sp. *C. jejuni, C. coli*, and *Y. enterocolitica*.

Study Objectives

The Nationwide MHBS has the following primary objectives:

Objective 1: Estimate the prevalence and amount of selected bacteria on market hog carcasses at pre-evisceration and post-chill;

- *Campylobacter*[1]
- *Salmonella* sp
- Generic *E. coli*
- Total Aerobic Bacteria
- Enterobacteriaceae
- Coliforms

[1] During the practice run (shakedown) of the sampling phase, *Campylobacter* could not be recovered at post-chill and it was decided not to test for *Campylobacter* at post-chill during the actual study. The funds allocated to *Campylobacter* testing will be used to increase the overall sample size of the study.

Objective 2: Obtain data to develop microbiological risk assessments, risk-based sampling programs, and/or regulatory policy decisions, including the development of future performance guidelines; and

Objective 3: Obtain post-hoc statistical analyses of the microbiological data when appropriate to explore the following additional issues:

1. Compare the count and prevalence between pairs of selected bacteria to identify important relationships among pathogens and indicator organisms;
2. Compare the count and prevalence of the selected bacteria to similar measures obtained from earlier baseline studies (where appropriate); and
3. Assess the effects of various factors (e.g., production shift, geographic region, season, inspection system, plant size, and specific antimicrobial interventions) on the microbiological profile.

Target Populations

The sampling frame for this baseline study will include all federally-inspected establishments identified in the FSIS e-ADRS that slaughtered at least 500 market hogs during the 12 months prior to the study (May 1, 2009 to April 30, 2010).

Inclusion criteria are defined as:

- Only market hogs are eligible for testing in this program;
- Only market hogs slaughtered under Federal inspection (i.e., receive the mark of inspection and are available for interstate and/or foreign commerce) are eligible for sampling in this study;
- Market hogs classes eligible for sampling include boar/stag, market weight, roaster, and sow; and
- Market hogs excluded from the study include
  (a) Personal exemptions: household slaughter and nonpaying guest.
  (b) Retail exemptions: preparation or processing activities traditionally and usually conducted at retail stores and restaurants where meat and poultry is sold to individual consumers in normal retail quantities; and
  (c) Religious exemptions: hogs slaughtered or processed as required by recognized religious dietary laws and state inspection (i.e., eligible for in-state commerce only).

Study Design

During the MHBS, carcass swabs will be collected from market swine at federally-inspected establishments. Multiple sampling events will occur within each establishment at a frequency determined by the establishment's production volume. Each sampling event will specify the production shift, which will alternate between consecutive sampling events at the establishment. During each sampling event, swabs will be collected from different carcasses at pre-evisceration and post-chill.

E. Establishment Stratification Scheme

FSIS obtains fair representation of all plants in the MHBS study by stratifying the plants by production volume. Stratification— the grouping of establishments by similar production volume— concentrates the sampling resources on the plants that make up a greatest proportion of the national production and at the same time ensures that small plants are adequately represented in the study. If a purely random sample were used instead of stratification, it would likely collect the majority of the samples from establishments with small production volume because these small establishments form a large proportion of the sampling frame. To counter balance for the bias produced by stratification FSIS will "weigh" for production volume the results of national prevalence.

Volume-based stratification was developed using the Electronic Animal Disposition Reporting System (e-ADRS) production data from May 2009 to April 2010. Three stratification-sampling proposals are presented in this document. These stratification proposals were constructed to achieve the best study design given the study constraints. FSIS will not be able to take an unlimited number of samples from each establishment. As such, the study design needs to be adjusted to accommodate this limitation.

To determine the best proposal, three tables were assembled (i.e., Tables 1, 2, and 3) based on data from the first, second, and third proposal respectively. The volume information is based on the total number of hogs slaughtered during the previous 12 months of the study (May 2009 to April 2010).

To determine the impact of small survey design adjustments on the variance, a variance factor (*VF*) is calculated using the equation below for Tables 1, 2, and 3:

$$VF = 1000 \sum_{k=1}^{K} w_k^2 / n_k,$$

Where:

- K is the number of establishments in the stratum.
- w_k is the proportion of production volume per establishment in relation to the total production volume.
- n_k is the number of samples for the k^{th} establishment; and
- 1000 is a factor used to avoid low numbers.

The minimum possible value for the sum of *VF* over all strata is 1000/N, where N is the total number of samples (20). For example, if N = 2508 then the minimum value of *VF* equals 0.398. For calculation purposes, the samples in Tables 1, 2, and 3 refer to swabs collected at one location in the production process (i.e., post-chill).

VF ignores differences in variance contributions due to the between establishment variance. This calculation allows the sampling designers to determine the impact of small adjustments to the survey design on the variance (i.e., low *VF*, better stratification-sampling).

The study is constrained by the minimum number of samples collected in the smallest volume stratum and the maximum number of samples at an establishment per month. Sample collection in the smallest volume stratum aims to include a sufficient number of samples to provide reasonably accurate estimate of the pathogens of interest. Sample collection in the largest volume stratum aims to sample no more than six samples per month from an establishment, leading to a maximum of 72 samples at the establishment per year. Collecting more than six samples could place too much burden on plant inspectors.

Table 1: First Proposed Stratification Scheme

Stratum	Number of Establishments per Stratum	Number of Sampled Establishments per Stratum	Number of Samples per Month per Establishment	Number of Samples at Post-chill per stratum	Percent of Samples	Percent of Volume	Variance Factor (VF)
1	12	12	6	864	34.4	58	0.408
2	15	15	5	900	35.8	35.3	0.143
3	87	87	0.5	522	20.8	6.6	0.043
4	133	75	0.25	225	9	0.1	0.000
	247	189		2,511	100	100	0.594

Table 2: Second Proposed Stratification Scheme

Stratum	Number of Establishments per Stratum	Number of Sampled Establishments per Stratum	Number of Samples per Month per Establishment	Number of Samples at Post-chill per stratum	Percent of Samples	Percent of Volume	Variance Factor (VF)
0	1	1	7	84	3.3	8.1	0.078
1	12	12	6	864	34.3	53.5	0.335
2	14	14	5	840	33.3	31.6	0.121
3	30	30	1	360	14.3	6.1	0.021
4	64	64	0.25	192	7.6	0.5	0.000
5	126	60	0.25	180	7.1	0.1	0.000
	247	181		2,520	100	100	0.555

Table 3: Third Proposed Stratification Scheme

Stratum	Number of Establishments per Stratum	Number of Sampled Establishments per Stratum	Number of Samples per Month per Establishment	Number of Samples at Post-chill per stratum	Percent of Samples	Percent of Volume	Variance Factor (VF)
1	13	13	6	936	37.3	61.6	0.426
2	14	14	5	840	33.5	31.6	0.121
3	30	30	1	360	14.4	6.1	0.021
4	64	64	0.25	192	7.7	0.5	0.000
5	126	60	0.25	180	7.2	0.1	0.000
	247	181		2,508	100	100	0.568

After constructing the tables, FSIS identified the best stratification-sampling design (shown in Table 2), with minimum VF at 0.555; however this design violates the requirement of no more than 6 samples per month per establishment, and it creates a stratum with only one establishment, which is not desirable. Table 1 and Table 3 are within requirements. In addition, Table 3 has a lower VF at 0.568 compared to 0.594 for Table 1. The MHBS will use the third proposal depicted in Table 3.

The stratification boundary defined by the third design is as follows:

- Stratum 1 consists of large establishments that produce more than 3,000,000 hogs per year. This stratum contains 13 plants that produce 61.6% of the total hogs slaughtered in the sampling frame.
- Stratum 2 consists of medium-large establishments that produce more than 1,000,000 hogs per year, but less than 3,000,000 hogs per year. This stratum contains 14 establishments that produce 31.6% of the total hogs slaughtered in the sampling frame.
- Stratum 3 consists of medium establishments that produce more than 30,000 hogs per year, but less than 1,000,000 hogs per year. This stratum contains 30 establishments that produce 6.1% of the total hogs slaughtered in the sampling frame.
- Stratum 4 consists of small establishments that produce more than 1,880 hogs per year, but less than 30,000 hogs. This stratum contains 64 establishments that produce 0.5% of the total hogs slaughtered in the sampling frame.
- Stratum 5 consists of very small establishments that produce more than 500 hogs per year, but less than 1,880 hogs per year. This stratum contains 126 establishments that produce 0.1% of the total hogs slaughtered in the sampling frame.

F. Development of Sampling Frame

FSIS regulates the slaughter and processing of market hogs intended for distribution within, or exported from, the United States. FSIS routinely collects data concerning the number of market hogs slaughtered daily at both the shift and establishment levels. A list of all establishments with active grants of federal inspection for this product class is available, but not included in this document.

This sampling frame shapes the study design process and provides an example of the number and distribution of annual sample requests. The sampling frame for this baseline study will include all federally-inspected establishments identified in the FSIS e-ADRS that slaughtered at least 500 market hogs during the 12 months prior to the study (May 1, 2009 to April 30, 2010). The study anticipates that the day-to-day production at these establishments will vary over time. The final sampling frame will accommodate variability in production, and at the end of the study, FSIS will update the establishment's production with production information obtained during the year of sample collection.

Every month, the establishments in the frame will be randomly assigned to a weekly sampling schedule. Inspection personnel will select the day of sampling (Monday-Friday).

Based on previous baseline studies, schedule flexibility maximizes the number of samples that are analyzed for the study.

G. Frequency of Sampling Events within Establishments

Sampling frequency within an establishment is defined by the stratification category and production volume reported in e-ADRS during the 12 months prior to the initiation of this study. Plants included in this study will be divided in five strata according to volume of production expressed in heads slaughtered per year. Stratum 1 contains plants with high volume of production and stratum 5 contains plants with very low volume. All establishments in stratum 1 to 4 will be sampled. Sixty establishments from stratum 5 will be randomly selected from the 126 establishments in that stratum. In summary, the proposed frame will sample 181 establishments from the 247 eligible production facilities.

Sampling Event Frequency Categories:

- Category 1 Establishments in stratum 1 will be sampled six times per month, totaling 72 sampling events for 144 samples per establishment;
- Category 2 Establishments in stratum 2 will be sampled five times per month, totaling 60 sampling events for 120 samples per establishment;
- Category 3 Establishments in stratum 3 will be sampled once per month, totaling 12 sampling events for 24 samples per establishment;
- Category 4 Establishments in stratum 4 will be sampled once every four months, totaling three sampling events for six samples per establishment; and
- Category 5 Establishments in stratum 5 will be sampled once every four months, totaling three sampling events for six samples per establishment.

With the stratification and sampling allocation defined, Table 4 presents the sampling frame for the market hogs baseline study. This table shows the stratification of plants, frequency of sampling events, and specific plants selected for the study. Appendix 2 describes the selection rules, specific establishment selection, and sampling frequency for the study.

Table 4. Final Stratification of the Market Hogs Baseline Sampling

Stratum	Est./Stratum	Est. Sampled	Sampling Event/Month /Plant	No. Months	Sampling Events/ Establishment/Year	Total Samples/Year /Establishment	Total Samples/ Stratum/Year	Sampling Percent by Stratum
1	**13**	**13**	**6**	**12**	**72**	**144**	**1,872**	**37.32%**
2	**14**	**14**	**5**	**12**	**60**	**120**	**1,680**	**33.49%**
3	**30**	**30**	**1**	**12**	**12**	**24**	**720**	**14.35%**
4	**64**	**64**	**0.25**	**12**	**3**	**6**	**384**	**7.66%**
5	**126**	**60**	**0.25**	**12**	**3**	**6**	**360**	**7.18%**
Total	**247**	**181**					**5,016***	**100.00%**

***Note:** **2,508 samples to be collected at pre-evisceration and 2,508 at post-chill**

H. Sampling Events by Production Shift within Establishments

The study will assess the potential slaughter shift difference by alternating sampling events between shifts. If a plant has an initial sampling event in the first shift, then the following scheduled sampling event will take place during the second shift. The third sampling event will return to the first shift. This method evenly divides sample collection between the two shifts. The shift will be annotated to be consistent the e-ADRS database.

I. Carcass Sample Site

The MHBS will focus on the ham, belly, and jowl; because previous studies suggest these regions face the greatest chance of contamination during the slaughter/dressing procedure (12). The three sites will be swabbed with a single sponge on the right side of the carcass. Another sponge will be used in the same locations on the left side of the carcass. The analysis of each sponge will be used to detect different pathogens and indicator bacteria.

J. Sampling Location within the Establishment

Slaughter and dressing provide the entry points for microbes onto a carcass. This study aims to evaluate the microbiological profile of market hogs at pre-evisceration and at post-chill before any additional processing occurs. At each sampling event in an establishment, a randomly selected carcass will be sampled during the requested shift—one at pre-evisceration and another at post-chill.

If the plant includes a hot-boning step in the production process, sample collection should occur after the final wash, but before the hot-boning step. When sampling *Salmonella,* the carcasses should not be dripping wet (i.e., FSIS Directive 1023.5). FSIS Notice (Notice 29-10) provides detailed instructions for inspection personnel.

K. Potential Revisions Based on e-ADRS Data

After the Shakedown period, FSIS personnel will incorporate the volume information to update the sample frame to include a more recent set of data of 12 months spanning May 2009 to April 2010. This new data allows for the stratifications associated with this study. An establishment's production volume may require updates to reflect the actual production data during the twelve months of the sampling period of the study. Consequently, the initial production figures and strata assignments are preliminary.

L. Additional Comments on Sample Design

The sample design and the resulting sample size for this study were limited by practical constraints, such as personnel, financial resources, and implementing scientific studies in actual (i.e., uncontrolled) production settings. The design of the MHBS will achieve the stated objectives by collecting and analyzing as many samples as possible to ensure a high level of statistical confidence in the results.

Given the "uncontrolled" nature of the study, FSIS will request more sampling events to ensure that a minimum number of samples are obtained. The final files will record deviations from the actual sample frame and samples discarded at the lab with entries showing non-response.

Expected Statistical Precision and Power

M. Introduction

This study primarily aims to estimate the prevalence of *Salmonella* sp. The discussion will use parameters as they relate to this pathogen.

A confidence interval encases the real population parameter (typically with 95% certainty) when estimating a population parameter. A narrower confidence interval provides greater precision, because the range that encloses the population parameter is tighter. Increasing the sample size achieves a narrower confidence interval. For example, a result of an estimated prevalence of 8% with 95% confidence interval from 7.5% to 8.5% is more accurate than a result of estimated prevalence at 8% with 95% confidence interval from 6% to 10%. The range enclosing the population parameter is narrower in the first case (7.5%–8.5%) than in the second case (6%–10%), so the estimation in the first case is more precise.

The margin of error, defined as the "radius" or half the width of a confidence interval, provides another way to express the precision of the estimation. Using the margin of error in the examples above instead of confidence interval, the precision of the estimation may be expressed as 8% ± 0.5% for the first case and 8% ± 2% for the second case.

N. Expected Precision in Estimating of Pathogen Prevalence

FSIS outlines the relationship between potential precision and the probability to achieve this precision. In addition, it outlines the probability associated with a given margin of error under different outcomes for this sampling design. Statistical power ($P = 1- \beta$) measures the probability of a test to detect a statistically significant difference between two hypothesized point values in a population (i.e., between the estimated mean and a given margin of error).

Statistical power may depend on:

(1) The standard deviation of the error term (i.e., the unexplained random variation about the mean and a contributor to effect size);
(2) Statistical significance (typically fixed at $\alpha = 0.05$ or 95% confidence level); and
(3) Sample size (i.e., more samples produce more accurate estimation and a narrower confidence interval).

The standard deviation of the error term is a characteristic typical of the sampling distribution. According to previous baseline study results, the error for *Salmonella* is assumed to be 0.25 (6) and the statistical significance is 0.05. However, the final sample size of the study may vary. Given the "uncontrolled" nature of the study, not all sample requests will yield results. This potential variation in number of samples collected may influence the precision of the estimate.

This analysis will consider three levels of sampling success:

a) A worst case scenario - approximately 70% of the planned samples (5,016) are analyzable (3,512 or 1,756 pre-evisceration samples and 1,756 post-chill samples);
b) A second case - approximately 80% of the planned samples (5,016) are analyzable (4,012 or 2,006 pre-evisceration samples and 2,006 post-chill samples); and
c) A third case - approximately 90% of the planned samples (5,016) are analyzable (4,514 or 2,257 pre-evisceration samples and 2,257 post-chill samples).

Analysis of the three different recovery rate scenarios provides the relationship between power and sampling error. The sample size for each scenario will be set at post-chill, with values at N_a = 1,756, N_b = 2,006, and N_c = 2,257 with a standard deviation of 0.25 and a significance level of 0.05. With this information, JMP Statistical Software (SAS Institute Inc.) generated the following power versus difference graphs (24).

Figure 1.
The relationship between power and the sampling error for 70% rate of sample recovery produces the following curve. The graph shows that there is a high probability (0.8) of detecting a difference of 0.016 or 1.6% margin of error. With the conditions imposed in this scenario, there is almost certainty (probability 0.987) that the margin of error under these conditions will not surpass 2.5%. The error standard deviation is 0.25, sample size 1,756 at post-chill, and alpha = 0.05.

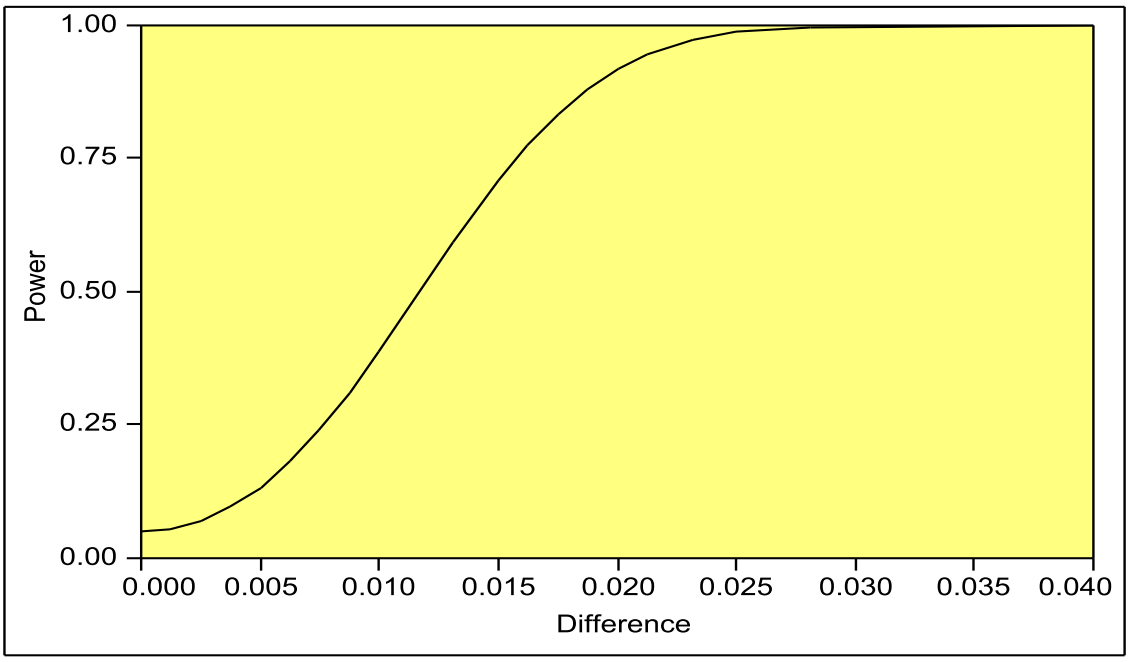


Figure 2.
The relationship between power and the sampling error for 80% sample recovery produces the following curve. The graph shows that there is a high probability (0.8) of detecting a difference of 0.015 or 1.5% margin of error. The error standard deviation is 0.25, sample size is 2,006 at post-chill, and alpha = 0.05.

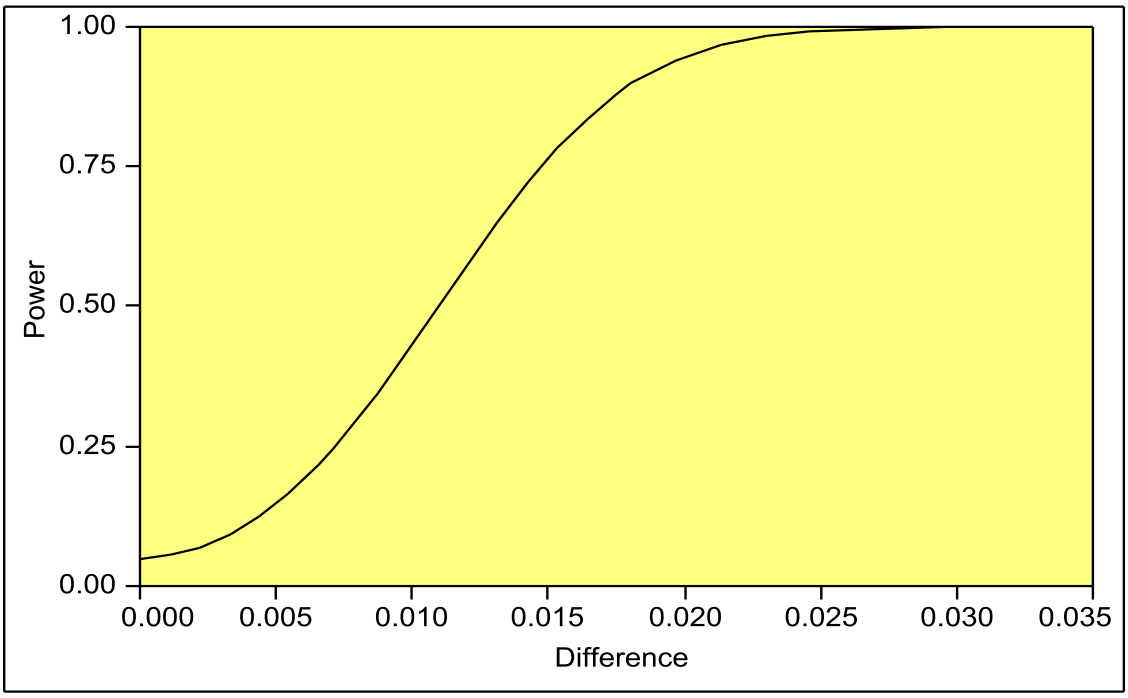
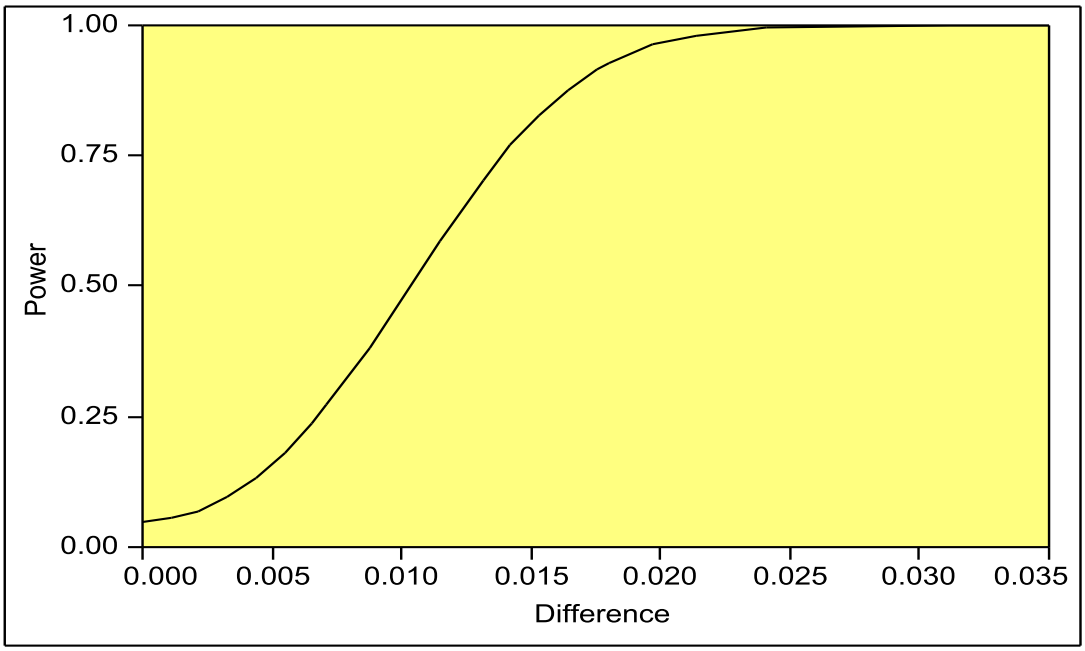


Figure 3.
The relationship between power and the sampling error for 90% sample recovery produces the following curve. The graph shows that there is a high probability (0.8) of detecting a difference of 0.014 or 1.4% margin of error. The error standard deviation is 0.25, sample size is 2,257 at post-chill, and alpha = 0.05.



In summary, assuming an average rate of 80% sample recovery and 0.5 probability of occurrence, the margin of error of the estimated prevalence of *Salmonella* sp. is expected at ± 1.09%. Assuming a prevalence of *Salmonella* sp. at 8%, the confidence interval should be about 6.9%–9.1%. In the worst-case scenario (i.e., scenario 1), parameter estimation should have a maximum margin of error at ± 2.5%.

These calculations are performed for **exploratory purposes** only. It is not possible to predict definitively the precision that will be achieved by the proposed study design.

Potential Sources of Error and Biases

O. Introduction

Sampling or non-sampling error may affect the microbiological results obtained from this study. Once recognized, FSIS personnel will implement statistical procedures to minimize these sources of error.

Sampling error occurs because the sampling process evaluates only a subset of a population. The 2010 MHBS randomly samples market hog carcasses assuming that the

sampled carcasses are representative of the carcasses at the plant on a given day. For example, the heterogeneous nature of the presence and concentration of bacteria on market hog carcasses throughout the slaughter process, within-establishments over time, and among-establishments may result in error when samples are collected infrequently (e.g., collecting a single sample per sampling event with relatively long time intervals between sampling events in establishments).

Sample error may also result from non-response, reducing the effective sample size. Additionally, non-response may introduce bias in the survey estimates when the respondents differ from non-respondents with respect to the measures of interest. This scenario may occur in establishments with relatively low production volumes, but the potential for bias may increase if establishments with high production volumes fail to respond to the survey where samples represent a larger proportion of the population. The study design incorporates "over-scheduling" to ensure that a minimum number of samples are obtained. In addition, sampling events at more than 50 percent of small establishments occurred three times per year to capture reliable data with less error.

To minimize non-response bias and the associated error, inspectors will receive detailed instructions to follow during sample collection. In addition, the study includes a dedicated e-mail address. The inspector can submit questions and receive clarification if questions arise. During the 90-day training period (shakedown), FSIS personnel will initiate non-response monitoring and establishment follow-up to maximize the response rate. Monthly preliminary reports should improve the response rate during the actual study and initiate follow-up with individual establishments as needed. This enhanced communication enables FSIS to minimize potential non-response error that may jeopardize the integrity of data obtained from the sampling events.

Non-sampling error occurs when either the sampling frame does not represent the population or the sample size does not represent the frame properly. The 2010 MHBS utilizes the data from the "Shakedown" period to improve the sampling frame with the aim to minimize non-sampling error.

A. *Sampling Technique Error*

Sampling techniques present inherent error because the 300 cm^2 surface area may not represent the microbiological status of the entire carcass surface area, especially when the expected bacterial counts are low.

The process of swabbing the market hog carcass may also introduce error. Micro-crevices on the carcass surface and the carcass temperature affect bacterial recovery. Inconsistent application of magnitude, direction, and force on the surface of the carcass by each collector during sampling may affect bacterial recovery and introduce error. The sponge material (e.g., surface pore structure and water content) may alter efficacy of bacterial removal during swabbing. Different sponge materials have different friction coefficients, altering removal of bacteria from the carcass surface. Other complications to consider include sponge pore size. A large pore size may not efficiently release bacteria in the diluents. Re-suspension of the pathogen from the surface of sponge requires optimum

diluents volume to generate enough hydrodynamic force to remove and suspend the pathogen. Several procedures to standardize the sampling technique should minimize the potential for this error. Instructions to inspectors provide details concerning the sponging process (e.g., the number and direction of passes on the carcass surface). All establishments receive the same brand of sponge and consistent volume of Buffered Peptone Water (BPW) to moisten the sponge prior to sample collection.

Variability in sponge sample storage and shipment due to geographic and climate diversity may introduce error. Sponges are refrigerated shipped overnight in a temperature-controlled container. Sponge processing occurs on the day of receipt at the laboratory.

P. Laboratory Error

Inconsistency and variability in laboratory procedures can create measurement error in the data. Such errors include media preparation and storage, sample preparation and processing, sample dilution, plating, incubating, counting, and data entry. The process of obtaining total bacterial counts is a critical source of error for studies that seek to estimate bacterial prevalence or concentrations. Manual plate counts for highly concentrated samples are challenging. On a typical plate, inherent variability exists in the distribution and, in some cases, the morphology of colonies. This requires subjective judgment by the technician possibly resulting in error. Counting error may occur when a partial count from a small area of the plate with a high bacterial count is extrapolated for a full count.

To add consistency to the process, one laboratory that is ISO-17025-Accredited will analyze the samples. The laboratory has standard operating procedures for media preparation and storage and detailed sample preparation instructions and microbiological methods. The laboratory technicians received training and conducted analyses following a similar study in young turkey and young chicken carcasses. Preliminary reports of the microbiological data generated by the laboratory will identify data entry errors to ensure data quality.

Statistical Analysis Plan

Q. Analytical Approach

Given the size, scope, and duration of the upcoming MHBS data collection program, FSIS anticipates that the data collected during the study would serve several purposes, which may require several types of statistical analyses. Despite these challenges, FSIS plans to maintain certain consistencies among the various types of statistical analyses. All analyses to compute population-based estimates will use the final weight assigned to each observation. All models will have the same hierarchical structure resulting from the complex survey design.

R. Regular Reporting of Microbiological Test Results

Project management will receive monthly reports of microbiological data (e.g., timeliness of submission, accuracy, and completeness) during the course of this baseline study. The number of individual samples requested, discarded, and analyzed will be summarized for this study for each month organized by shift-of-collection. A summary table will be prepared with respect to the number of establishments contributing samples during the month. The preliminary reports will yield the response rate to sample requests and the crude (unweighted) rates of positive samples for pathogens. The monthly reports are for internal use and will not be distributed to a wider audience.

A quarterly report will contain the results for three consecutive months, including monthly tables and the findings from preliminary descriptive analyses of the microbiological test results (e.g., crude (unweighted) rate of positive samples, CFU/cm^2 or MPN/cm^2 for each selected bacterium, carcass type, and shift-of-collection). Quarterly reports are for internal use and will not be distributed to a wider audience.

S. Estimation of Prevalence and Quantitative Levels

The qualitative results, expressed as the detection (positive result) or non-detection (negative result) of each bacterium, provide an estimate of the percent positive of the unweighted sample. The quantitative results (i.e., number of colony forming units per 100 cm^2) provide an estimate of the geometric mean of the observed contamination levels. Additional variables in the dataset indicate the establishment, the shift, and the date of collection for each sponge sample.

The National Prevalence is equivalent to an average of weighted positive sample results according to individual plant production volume. FSIS expects that the results of the sample percent positive for pathogens will differ slightly from the national prevalence. The anticipated variation is due to the influence of the production volume of individual plants and other potential adjustments introduced in the calculation of the national prevalence. The study will monitor indicators such as total aerobic bacteria, Enterobacteriaceae, generic *E. coli*, and total coliforms to link them to process control and effectiveness of interventions.

During and at the end of the collection phase, FSIS will check the results for accuracy and quality. In addition, FSIS will capture market hog production from e-ADRS to determine the production volume of each establishment during the twelve-month period of sample collection. Statisticians at FSIS will use this establishment production volume for weighting the samples, which will account for the variability in slaughter totals associated with the establishment's production at the time of sample collection.

Prior to final analysis, FSIS may adjust the sampling weights to account for non-response. FSIS plans to calculate estimates of prevalence using commercially available statistical software package developed for the design of complex surveys (30). Based on sampling replication methods, the statistical package will calculate the variance estimates of the point estimates and if necessary adjust for non-responses. Developing estimates of prevalence using models is another option.

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Appendix 1

Rules for Selection of Establishments and Sampling Plan

Rules for Selection of Establishments and Sampling Plan for Stratum 5

A) Sixty plants were selected at random from126 available plants in this stratum, see Table 5.

B) The selected 60 plants were divided into four groups (1, 2, 3, and 4) of 15 plants each, see Table 5 (i.e., Group 1: plants 1 to 15; Group 2: plants 16 to 30; Group 3: plants 31 to 45; and Group 4: plants 46 to 60).

C) Each group contains four sub-groups, three sub-groups of four plants and one sub-group of thee plants.

D) During the first month of the study, collect samples from group 1. Sub-group A will be sampled on week one, sub-group B will be sampled on week two, sub-group C will be sampled on week three, and sub-group D will be sampled on week four.

E) The procedure will be repeated each subsequent month for groups 2, 3 and 4.

F) Repeat the process from the beginning on month 5 and 9 to complete the 12 month of data collection for the 60 plants. Following this procedure, each plant will be sampled 3 times during the study.

Table 5. Sampling scheme for Stratum 5 (60 randomly selected plants).

Order	Plant ID	Production	Stratum	Month Worked	Group	Sub-Group
1	04477 M	820	5	12	Group 1	A
2	06354 M	1,016	5	12	Group 1	A
3	17965 M	946	5	12	Group 1	A
4	31776 M	1,590	5	12	Group 1	A
5	10624 M	1,565	5	11	Group 1	B
6	09760 M	632	5	12	Group 1	B
7	08078 M	1,720	5	12	Group 1	B
8	09581 M	702	5	12	Group 1	B
9	09264 M	549	5	12	Group 1	C
10	21207 M	974	5	12	Group 1	C
11	08609 M	1,011	5	12	Group 1	C
12	06066 M	633	5	12	Group 1	C
13	02580 M	663	5	12	Group 1	D
14	05648 M	1,713	5	12	Group 1	D
15	22035 M	1,348	5	12	Group 1	D

Order	Plant ID	Production	Stratum	Month Worked	Group	Sub-Group
16	09173 M	769	5	12	Group 2	A
17	33928 M	803	5	12	Group 2	A
18	05659 M	925	5	12	Group 2	A
19	33971 M	603	5	12	Group 2	A
20	08562 M	889	5	12	Group 2	B
21	27426 M	1,388	5	12	Group 2	B
22	33940 M	575	5	12	Group 2	B
23	10061 M	600	5	11	Group 2	B
24	10804 M	1,735	5	12	Group 2	C
25	27467 M	521	5	12	Group 2	C
26	11111 M	988	5	12	Group 2	C
27	27449 M	515	5	12	Group 2	C
28	34713 M	945	5	12	Group 2	D
29	06161 M	615	5	12	Group 2	D
30	12448 M	1,757	5	12	Group 2	D
31	08636 M	1,338	5	12	Group 3	A
32	08559 M	555	5	12	Group 3	A
33	20321 M	764	5	12	Group 3	A
34	13276 M	730	5	12	Group 3	A
35	20856 M	1,066	5	12	Group 3	B
36	08131 M	809	5	12	Group 3	B
37	08915 M	516	5	12	Group 3	B
38	21572 M	1,813	5	12	Group 3	B
39	08850 M	1,295	5	12	Group 3	C
40	10176 M	1,116	5	12	Group 3	C
41	05633 M	580	5	12	Group 3	C
42	19252 M	886	5	12	Group 3	C
43	32062 M	640	5	12	Group 3	D
44	08498 M	875	5	12	Group 3	D
45	10808 M	1,068	5	12	Group 3	D
46	09701 M	710	5	12	Group 4	A
47	10692 M	768	5	12	Group 4	A
48	08728 M	511	5	12	Group 4	A
49	09423 M	911	5	12	Group 4	A
50	07420 M	1,202	5	12	Group 4	B
51	34319 M	736	5	12	Group 4	B
52	10038 M	1,025	5	12	Group 4	B
53	05766 M	1,096	5	12	Group 4	B
54	34699 M	1,019	5	12	Group 4	C
55	21156 M	544	5	12	Group 4	C
56	21938 M	656	5	12	Group 4	C
57	09784 M	793	5	12	Group 4	C
58	19562 M	636	5	12	Group 4	D
59	08868 M	1,010	5	12	Group 4	D
60	10147 M	698	5	12	Group 4	D

Rules for Selection of Establishments and Sampling Plan for Stratum 4

A) Sixty-four plants in this stratum were divided into four groups (1, 2, 3, and 4), each containing 16 plants, see Table 6 (i.e., Group 1: plants 1 to 16; Group 2: plants 17 to 32; Group 3: plants 33 to 48; and Group 4: plants 49 to 64).

B) Each group was divided in 4 sub-groups (A, B, C, and D) containing four plants each.

C) During the first month of the study, collect samples from group 1; sub-group A will be sampled on week one, sub-group B will be sampled on week two, sub-group C will be sampled on week three, and sub-group D will be sampled on week four.

D) Repeat the procedure for group 2 in the second month, and continue this process each subsequent month with the next group until completing the first 4 months.

E) Repeat the rotation from the beginning starting on month 5 and 9 to complete the 12 months of data collection for the 60 plants. Following this procedure, each plant will be sampled 3 times during the study.

Table 6. Sampling Scheme for Stratum 4 plants (64 plants).

Order	Plant ID	Production	Stratum	Month Worked	Group	Sub-Group
1	00325 M	24,496	4	12	Group 1	A
2	01628 M	13,092	4	12	Group 1	A
3	01775 M	21,510	4	12	Group 1	A
4	02522 M	8,066	4	12	Group 1	A
5	02875 M	5,701	4	12	Group 1	B
6	04005 M	2,325	4	12	Group 1	B
7	05497 M	1,979	4	12	Group 1	B
8	06208 M	10,675	4	12	Group 1	B
9	06270 M	2,016	4	12	Group 1	C
10	06518 M	26,394	4	12	Group 1	C
11	06677 M	4,560	4	12	Group 1	C
12	06678 M	5,833	4	12	Group 1	C
13	06682 M	7,657	4	12	Group 1	D
14	07882 M	5,076	4	12	Group 1	D
15	07883 M	7,958	4	12	Group 1	D
16	08404 M	3,515	4	12	Group 1	D
17	08633 M	2,847	4	12	Group 2	A
18	09166 M	29,933	4	12	Group 2	A
19	09199 M	27,948	4	12	Group 2	A
20	09230 M	5,330	4	12	Group 2	A

Order	Plant ID	Production	Stratum	Month Worked	Group	Sub-Group
21	09410 M	2,038	4	12	Group 2	B
22	09442 M	8,392	4	12	Group 2	B
23	09704 M	3,018	4	12	Group 2	B
24	09792 M	2,603	4	7	Group 2	B
25	09880 M	26,312	4	12	Group 2	C
26	10131 M	3,117	4	12	Group 2	C
27	10226 M	7,090	4	10	Group 2	C
28	10269 M	2,586	4	12	Group 2	C
29	10757 M	10,134	4	12	Group 2	D
30	11011 M	1,949	4	12	Group 2	D
31	11116 M	9,750	4	12	Group 2	D
32	11159 M	2,793	4	12	Group 2	D
33	12441 M	2,170	4	12	Group 3	A
34	13324 M	23,101	4	12	Group 3	A
35	17419 M	4,694	4	12	Group 3	A
36	18691 M	2,222	4	12	Group 3	A
37	19002 M	8,569	4	12	Group 3	B
38	19741 M	6,917	4	12	Group 3	B
39	19904 M	3,342	4	12	Group 3	B
40	19922 M	3,953	4	12	Group 3	B
41	20017 M	4,708	4	12	Group 3	C
42	20129 M	27,377	4	12	Group 3	C
43	20855 M	1,903	4	12	Group 3	C
44	20917 M	11,837	4	12	Group 3	C
45	20981 M	4,282	4	12	Group 3	D
46	21108 M	6,749	4	12	Group 3	D
47	21188 M	12,916	4	12	Group 3	D
48	21285 M	15,249	4	12	Group 3	D
49	21747 M	21,765	4	12	Group 4	A
50	22064 M	6,379	4	12	Group 4	A
51	27279 M	1,881	4	12	Group 4	A
52	27488 M	2,105	4	12	Group 4	A
53	27499 M	6,356	4	12	Group 4	B
54	31578 M	3,155	4	12	Group 4	B
55	31644 M	5,809	4	12	Group 4	B
56	31647 M	2,646	4	12	Group 4	B
57	31865 M	7,822	4	12	Group 4	C
58	32170 M	6,298	4	12	Group 4	C
59	33860 M	3,247	4	12	Group 4	C
60	33916 M	3,133	4	12	Group 4	C
61	34078 P	3,154	4	2	Group 4	D
62	34114 M	3,967	4	12	Group 4	D
63	34181 M	12,315	4	12	Group 4	D
64	39876 M	11,430	4	10	Group 4	D

Rules for Selection of Establishments and Sampling Plan for Stratum 3

A) Thirty plants in this stratum were divided in four sub-groups—two sub-groups (A and B) with eight establishments each and two sub-groups (C and D) with seven plants each, see Table 7.

B) During the first month of the study, sub-group A will be sampled on week one, sub-group B will be sampled on week two, sub-group C will be sampled on week three, and sub-group D will be sampled on week four.

C) Repeat the operation every month, but rotate the sub-groups to prevent sampling the same establishment on the same week as the previous month. For example for month two, schedule sub-group B on week 1, sub-group C on week 2, sub-group D on week 3, and sub-group A on week 4. Complete the 12 month of data collection for the 30 plants. Following this procedure, each plant will be sampled once a month or 12 times during the study.

Table 7. Sampling Scheme for Stratum 3 (30 plants).

Order	Plant ID	Production	Stratum	Month Worked	Group	Sub-Group
1	00226 M	105,025	3	12	Group 1	A
2	00242 M	715,091	3	12	Group 1	A
3	00363 M	260,132	3	12	Group 1	A
4	00548 M	288,129	3	12	Group 1	A
5	00818 M	815,292	3	12	Group 1	A
6	01737 M	45,380	3	12	Group 1	A
7	02926 M	290,832	3	12	Group 1	A
8	05502 M	46,217	3	12	Group 1	A
9	05537 M	914,482	3	12	Group 1	B
10	06113 M	80,663	3	12	Group 1	B
11	06173 M	85,038	3	12	Group 1	B
12	06720 M	304,882	3	12	Group 1	B
13	07237 M	81,374	3	12	Group 1	B
14	07636 M	100,370	3	12	Group 1	B
15	09228 M	62,658	3	12	Group 1	B
16	09520 M	288,187	3	12	Group 1	B
17	13189 M	62,867	3	12	Group 1	C
18	17496 M	242,564	3	12	Group 1	C
19	18229 M	63,718	3	12	Group 1	C
20	19185 M	316,599	3	12	Group 1	C
21	20608 M	260,865	3	12	Group 1	C
22	20748 M	54,146	3	12	Group 1	C
23	20760 M	50,087	3	12	Group 1	C
24	21069 M	403,584	3	12	Group 1	D
25	21179 M	88,441	3	12	Group 1	D
26	21651 M	125,063	3	12	Group 1	D
27	21687 M	52,102	3	10	Group 1	D
28	21799 M	85,279	3	12	Group 1	D

Order	Plant ID	Production	Stratum	Month Worked	Group	Sub-Group
29	21898 M	139,341	3	12	Group 1	D
30	33844 M	48,340	3	10	Group 1	D

Rules for Selection of Establishments and Sampling Plan for Stratum 2

A) The 14 plants in this stratum were divided in four sub-groups—two sub-groups (A, and B) with four establishments each and two sub-groups (C and D) with three plants each, see Table 8.

B) Sample all 14 plants in the group on the first, second, third and fourth week of every month of the study. This will ensure four sample events per month per plant.

C) Making sure not to sample on the same day of the sampling event described in B, schedule one extra sampling event the first week of the month for group A, the second week of the month for group B, the third week for group C, and the fourth week for group D.

D) On the second month have the extra sampling event starting the first week of the month with group B, then C, D and A. On the third month, conduct an extra sampling event with group C on the first week, followed by D, A and B. On the fourth month start the extra sampling event with group D on the first week followed by A, B, and C.

E) Repeat the extra sample event cycle starting on month 5 and 9 of the study as described above until completing the 12 months of the study. This way all plants will have five sampling events per month or 60 sampling events per year.

Table 8. Sampling scheme for Stratum 2 (14 plants).

Order	Plant ID	Production	Stratum	Month Worked		
1	00199N M	2,619,535	2	12	Group 1	A
2	00221A M	2,257,047	2	12	Group 1	A
3	00244L M	2,429,824	2	12	Group 1	A
4	00244M M	2,114,102	2	12	Group 1	A
5	00244P M	1,946,733	2	12	Group 1	B
6	00320M M	2,370,226	2	12	Group 1	B
7	00360 M	1,849,164	2	12	Group 1	B
8	00413 M	2,715,022	2	12	Group 1	B
9	00717 M	2,372,345	2	12	Group 1	C
10	00717CRM	2,786,818	2	12	Group 1	C
11	00717M M	2,443,410	2	12	Group 1	C
12	00791 M	2,369,061	2	12	Group 1	D
13	00995 M	2,606,188	2	12	Group 1	D
14	05804 M	2,856,802	2	12	Group 1	D

Rules for Selection of Establishments and Sampling Plan for Stratum 1

A) The 13 plants in this stratum were divided in four sub-groups; sub-group A has four plants, while sub-groups B, C and D have three plants each; see Table 9.

B) Schedule one sampling event every week for each plant to secure four sampling events per month.

C) Making sure not so sample on the same day of the sampling event described in B, schedule an extra sampling event for group A and B on the first month and another on the third week of the moth. Schedule an extra sampling event for group C and D on the second week of the month and another on the fourth week of the month. This procedure will result in two extra sampling events per plant per month.

D) On the second month schedule the two extra sampling events for groups A and B on the second and fourth week and for groups C and D on the first and third weeks of the month.

F) Repeat the extra sample schedule following step C for months 3, 5, 7, 9 and 11 and step D for months 4, 6, 8, 10, and 12 to complete the 12 months of the study. At the end of the study all plants in this stratum will have six sampling events per month or 72 sampling events per year.

Table 9. Sampling scheme for Stratum 1 (13 plants).

Order	Plant ID	Production	Stratum	Month Worked	Group	Sub-Group
1	00003S M	5,147,783	1	12	Group 1	A
2	00003W M	4,908,023	1	12	Group 1	A
3	00017D M	4,056,258	1	12	Group 1	A
4	00085B M	5,177,708	1	12	Group 1	A
5	00085O M	4,569,473	1	12	Group 1	B
6	00244 M	4,526,635	1	12	Group 1	B
7	00244I M	3,848,565	1	12	Group 1	B
8	00244W M	5,066,554	1	12	Group 1	C
9	01620 M	4,869,291	1	12	Group 1	C
10	13597 M	5,252,526	1	12	Group 1	C
11	17564 M	4,279,451	1	12	Group 1	D
12	18079 M	8,624,342	1	12	Group 1	D
13	31965 M	5,369,251	1	12	Group 1	D