FSIS Nationwide Raw Chicken Parts Microbiological Baseline

Data Collection Program

Study Design and Sampling Frame for Technical Consultation

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Microbiological Analysis and Data Branch

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1. Executive Summary

This document outlines the study design and sampling frame for the national Raw Chicken Parts Baseline Study (RCPBS) data collection program to be conducted by Food Safety and Inspection Services (FSIS) of the United States Department of Agriculture (USDA).

FSIS presents the study design and sample frame for the RCPBS, which includes 660 plants that process chicken parts in the United States. To obtain a high representation of all plants, the sample frame is stratified according to production volume. The final sample frame includes 572 plants.

The RCPBS sample frame consists of three strata and 5,000 sampling events. The samples are allocated to each stratum according to the volume of the plants in that stratum. The chosen design includes oversampling to account for non-response and discarded samples due to various reasons. Stratum boundaries were constructed to obtain the design with least within-stratum variability and minimum amount of samples.

The expected precision of the design for the estimation of Salmonella is ±0.9%, with worst-case scenario of ±2.3%. Final precision depends on the actual number of samples analyzed during the study; this is the recovery rate and other factors like the variance.

FSIS will weigh the results according to annual production volume to estimate the pathogen prevalence. The Agency will use the production volume obtained during the 6-month study for weighting the results. Adjustments on an ongoing basis to the sample frame are done if plants drop out of the frame or new plants become available for including in the survey.

Given the budgetary and field constraints imposed on this study, the RCPBS sample frame is the best approach to obtain an accurate prevalence calculation. The sample frame is presented below:

Table 1. The stratification for RCPBS conducted during a 6-month study.

Strata	Number of Establishments	Establishments to Sample	Frequency per Month	Percent Volume per Stratum	Percent of Sample	Samples per Establishment during Study	Sample During Study/Stratum
1	106	106	2	93.5	25.4	12	1,272
2	370	282	1.33	6.5	45.1	8	2,256
3	184	184	1.33	-	29.5	8	1,472
Totals	**660**	**572**		**100**	**100**		**5,000**

Note: Stratum Number 3 consists of plants with unknown production volume. Production volume for these plants will be determined during the study.

Study Design and Sampling Frame for Technical Consultation

2. Program Summary:

During the RCPBS, FSIS will collect approximately 5,000 chicken parts rinse samples from establishments under federal inspection. Each establishment will be sampled several times over a 6-month study period. Chicken part rinsastes will be analyzed to detect and quantify selected foodborne pathogens and indicator bacteria. Results of this study will enable FSIS to establish a prevalence estimate and the regulated industry to target interventions and effectively work toward reducing the risk of foodborne pathogens associated with all chicken parts.

3. Study Objectives:

Objective 1: Estimate the prevalence and quantitative level of *Salmonella*, *Campylobacter*, Generic *E. coli,* Total Aerobic Bacteria, *Enterobacteriaceae*,and coliforms on raw chicken parts;

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

1. Compare prevalence and counts between pathogenic organisms and indicator organisms to determine relationships and associations;
2. Compare the count and prevalence of the selected bacteria to similar past baseline studies (where appropriate); and
3. Assess the effects of various factors on the microbiological profile (e.g., geographic region, inspection system, plant size, skin on versus skin off, and specific antimicrobial interventions).

4. Target Populations:

FSIS will estimate the microorganism concentration on chicken parts for the composite of all establishments producing raw chicken parts. More information can be found in the FSIS Notice (28).

Establishments processing chicken parts are included in the study if they:

- Slaughter broilers (chickens raised for meat) and cut the carcasses into chicken parts of the type typically available for purchase by consumers or exports; and/or
- Cut whole chicken carcasses received from other federally-inspected establishments into halves, quarters, and/or individual parts typically available to consumers.

Raw chicken parts are defined as:

- Raw (uncooked) skin-on or skinless, bone-in or boneless chicken breasts, thighs, wings, legs, necks, backs, half- or quarter-carcasses, and internal organs, such as giblets (e.g., liver, heart, or gizzard), typically available to consumers or exports. Other parts may also be included in the study.
- RCPBS will not include whole chicken carcasses or chicken parts that have been ground, otherwise comminuted, marinated, breaded, or further processed before shipping from the establishment. For standards, see 9 CFR 381.170(b), which details requirements for the specified cuts of poultry.

5. Study Specifications and Literature Review:

A. Background:

The FSIS mission is to ensure that the nation's commercial supply of meat, poultry, and egg products are safe to consume, correctly labeled, and properly packaged. Resulting from previous baseline studies, the FSIS published the Pathogen Reduction Hazard Analysis and Critical Control Point Systems (PR/HACCP) Final Rule with the goal of reducing the prevalence and counts of pathogenic organisms in meat and poultry products (23). The rule mandates that all establishments slaughtering cattle, swine, chickens, or turkeys screen products for *Escherichia coli* Biotype 1 (generic *E. coli,* an organism used to track process control of fecal contamination) at a frequency based on production volume. The rule also includes foodborne pathogen prevalence criteria for meat and poultry products. In support of this mission and the PR/HACCP rule, FSIS conducts periodic baseline studies that analyze various food commodities for foodborne microorganisms.

B. Sampling Techniques:

FSIS inspection program personnel (IPP) collect samples following the procedures described in FSIS Directive 10,230.5 (2/4/98) and instructions provided on computer-generated sample forms and FSIS Notice 10,230.5 (17, 18).

Eligible establishments: All federally-inspected establishments according to the current PR/HACCP sampling method are eligible for inclusion in the baseline study (23). Prior to the start of RCPBS, information about the type of part and chicken parts annual production volume for each establishment was unknown. During the study's "shakedown" (a 90-day trial sampling period), FSIS sent out surveys requesting this information. The survey identified 660 establishments that provided samples; however, only 476 establishments provided production volume information. In this document, FSIS proposes a statistical study design and sampling frame, which will include 572 federally-inspected chicken parts processing establishments. Eighty-eight establishments will not be included in the study, because these establishments produce less than 15,000 pounds of chicken parts annually and have a combined production volume of only 0.002% of the reported production volume.

Type of collection: During the RCPBS, IPP will collect rinsate samples from chicken parts following established procedures. The rinsates will be shipped to the labs and analyzed for foodborne pathogenic and indicator bacteria (5, 9, 15).

Location of collection: IPP will collect samples at the end of the production line, before packaging and shipping to customers. Each sampling event will specify the production shift, which will alternate between consecutive sampling events at the establishment.

Sample analysis criteria: The laboratories will analyze samples received the day after sample collection, with a sample receipt temperature of 0 to 10 ºC (inclusive). Samples received outside this temperature range will be discarded.

Sample collection procedure:

1. Randomly select a production line.
2. Use a random number generator to select a type of chicken part.
3. At the end of the production line before packing, aseptically place 4 lbs. of the selected chicken part in a sterile bag provided by FSIS. Only one type of chicken part will go to the bag (e.g., only wings).
4. Pour 400 ml buffered peptone water (BPW) into the sterile bag containing chicken parts and shake vigorously following instructions provided.
5. Pour the shaken BPW rinsate into the sterile screw-top container provided by FSIS and secure the top tightly.
6. Cool samples (on ice or in refrigerator) within five minutes of collection and keep sample temperature at approximately 4ºC, until shipping. Do not freeze.
7. Ship all samples to the lab on the day of collection.

C. Indicator Organisms

RCPBS samples will be analyzed for coliforms, *Enterobacteriaceae,* generic *E. coli,* and aerobic plate count (APC). Analysis of indicator organisms may be useful in identifying process control efficiency (5, 9).

D. Pathogenic Organisms

RCPBS will screen for and provide estimates of the prevalence and levels of the foodborne pathogens *Salmonella* and *Campylobacter*, similar to previous poultry baselines (6, 8, 9, 10, 12, 13, 16, 19, 20, 21, 22).

6. Study Design:

FSIS will work with 660 chicken parts processing plants that were identified during the survey phase according to their yearly production volume (4, 7). Production volume is used for stratification because the survey is intended to reflect the entire federally-regulated supply of the commodity in the study.

A. Stratification

The 184 establishments that did not provide information on annual production volume were placed into a separate stratum and sampled at the same frequency as the low volume establishments. FSIS assumed that establishments that did not respond to the survey during shakedown were small volume producers, so sampling them at low volume frequency is appropriate. During the study, FSIS will attempt to obtain the missing information and place these 184 establishments in their respective stratum when possible. Of the remaining 476 establishments (660–184), 88 establishments with production volume under 15,000 pounds per year will not be included in the study. This leaves 388 establishments (476–88) to be divided in two strata. The study design will have three strata; two with known production volume and one with unknown production volume. The two strata design rationale for known volume establishments is because collection restrictions permit only two samples per month per establishment. A larger stratum for high production volume establishments allows more establishments to receive the maximum allocation of sample per month. To account for bias introduced by stratification, FSIS will adjust/weight by production volume the values that will be used in the national prevalence calculations (1, 4, 7, 14).

FSIS will define each stratum boundary by comparing several statistical boundary definition techniques. FSIS will compare the error associated with each method and choose the method that minimizes error within the design.

Three stratification methods are considered in this analysis:

1. Dalenius-Hodge or Cumulative Root Frequency method
    i. Advantage: most commonly used and is known for the most sensible way of distributing the error among and between strata.
    ii. Disadvantage: accuracy depends on the initial quantity of groups on which the frequency distribution is based (in this analysis 41 bins were used),
2. Geometric
    i. Advantage: works well for skewed distributions.
    ii. Disadvantage: the lowest ranking value of production volume influences the rest of the boundary partitions.
3. Visual clustering
    i. Advantage: works well to show natural breaks in the data.

ii. Disadvantage: the particular distribution of production volume as well as the observer's bias may influence the results.

1. Stratification using the Dalenius-Hodge method

Method Description:

1. Arrange the stratification variable *X* in ascending order;
2. Group the *X* into a number of classes, *J*;
3. Determine the frequency for each class f_i *(i=1, 2, ...J)*;
4. Determine the square root of the frequencies in each class;
5. Cumulate the square root of the frequencies $\sum_{i=1}^{J}\sqrt{f_i}$
6. Divide the sum of the square root of the frequencies by the number of strata:

$$Q = \frac{1}{L}\sum_{i=1}^{J}\sqrt{f_i}$$

7. Take the upper boundaries of each stratum to be the *X* values corresponding to

Q, 2Q, 3Q,……(L-1)Q, LQ.

Table 2. RCBS stratification scheme calculated using the Dalenius-Hodges method for boundary definition. This table shows the number of strata based on annual volume, the number of establishments in each stratum, the production volume per stratum, the standard deviation, and the coefficient of variation of each stratum.

Strata	Number of Establishments	Stratum Volume	Standard Dev	CV
1 (≥70,000,000 lbs)	106	21,2440,465	102,475,156	48
2 (<70,000,000 lbs)	370	4,248,991	11,063,468	260
3 (unknown volume)	184	.	.	.

2. Stratification using the Geometric Method
Method Description:

1. Arrange the stratification variable *X* in ascending order;

2. Take the minimum value as the first term and the maximum value as the last term of the geometric series with L+1 terms;

3. Calculate the common ratio: $r = (max/min)^{1/L}$;

4. Take the boundaries of each stratum to be the X values corresponding to the terms in the geometric progression with this common ratio:

Minimum $k_0 = a, ar, ar^2 \ldots\ldots ar^L$ = maximum k_L

Table 3. RCPBS stratification scheme calculated using the Geometric method for boundary definition. This table shows the number of strata based on annual volume, the number of establishments in each stratum, the production volume per stratum, the standard deviation, and the coefficient of variation of each stratum.

Strata	Number of Establishments	Stratum Volume	Standard Dev	CV
1 (≥370,000 lbs)	244	98,663,430	121,091,293	123
2 (<370,000 lbs)	232	73,013	9,1092	125
3 (unknown volume)	184	.	.	.

3. Stratification using the Visual Clustering method

Method Description:

In the visual clustering method, the analyst visually assesses the data by plotting it on a scatter plot (Fig.1) and uses the natural breaks occurring in the sequence of values as potential boundaries. For improved visual aid, the production volume data is converted to $\log_{10}$ and observed on a graph in Figure 1. Once the natural boundaries are established, the $\log_{10}$ boundaries are reverse to production volume.

Figure 1. Scatter plot of log10 of production volume for raw chicken parts establishments. The x-axis shows log 10 of production volumes for the chicken parts plants with a known volume.

The line shows the strata breaks based on natural data fracture. The strata (1 and 2) are labeled in the red boxes. Between strata 1-2 $\log_{10} = 7.4 \sim 25{,}000{,}000$ pounds.

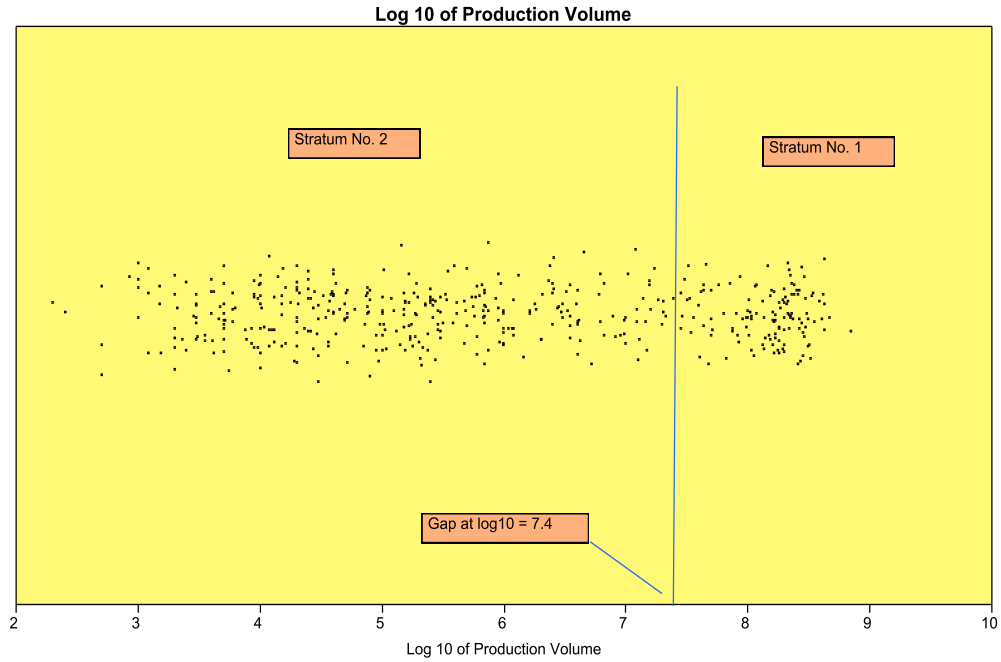


Table 4. RCBS stratification scheme calculated using the Visual Clustering method for boundary definition. This table shows the number of strata based on annual volume, the number of establishments in each stratum, the production volume per stratum, the standard deviation, and the coefficient of variation of each stratum.

Strata	Number of Plants	Stratum Volume	Standard Dev	CV
1 (≥25,000,000 lbs.)	130	181,026,007	113,844,797	63
2 (<25,000,000 lbs.)	346	1,611,084	3,946,643	245
3 (unknown volume)	184	.	.	.

B. Allocation of samples per stratum

The following sample allocation is based on field restrictions.

1. Stratum 1: Two samples per establishment per month (12 times per establishment per 6 months). All plants in this stratum will be sampled.
2. Stratum 2: Eight samples per establishment per 6 months. Eighty-eight establishments producing < 15,000 pounds of chicken parts per year will not be sampled.
3. Stratum 3: Eight samples per establishment per 6 months. All establishments with unknown production volume in this stratum will be sampled.

Table 5. Sample allocation for RCPBS using the Dalenius-Hodges, Geometric, and Visual Clustering Methods.

Stratum	Samples/Year Per Plant	Dalenius Method # Plants	Total Samples per Stratum	Geometric Method # Plants	Total Samples per Stratum	Visual Clustering Method # Plants	Total Samples per Stratum
1	12	106	1,272	244	2,928	130	1,560
2	8	282	2,256	144	1,152	258	2,064
3	8	184	1,472	184	1,472	184	1,472
Totals		572	5,000	572	5,552	572	5,096

C. Determination and Selection of Best Stratum Partition

To determine the best stratum boundary definition method, FSIS used a formula detailed by Cochran (1) to determine the minimum variance (V_{min}). Formula 1 describes the minimum within-stratum variance (V_{min}) using a fixed total sample size (n) for each method. In this equation, the term representing the finite population correction (fpc) is not included.

Formula (1) for the minimum variance for the partition:

$$V_{min} = [\Sigma(W_h S_h)^2]/n$$

Where:

$W_h = N_h/N$ is the weight of the stratum h (h = 1 and 2)

S_h is the standard error of the stratum h (h = 1 and 2) and

n is the total amount of samples for that partition.

Minimum Variance Calculations:

Chicken Parts Comparison for 2 strata with known production volume

V_{min} Dalenius = $(9.3 \text{ X } 10^6)^2 / 5{,}000 = 8.7 \text{ X } 10^{13} / 5{,}000 \sim 1.74 \text{ X } 10^{10}$

V_{min} Geometric = $(7.7 \text{ X } 10^6)^2 / 5{,}552 = 6 \text{ X } 10^{13} / 5{,}552 \sim 1.1 \text{ X } 10^{10}$ (minimum variance)

V_{min} Visual = $(9.7 \text{ X } 10^6)^2 / 5{,}096 = 9.5 \text{ X } 10^{13} / 5{,}096 \sim 1.86 \text{ X } 10^{10}$

The Geometric method offers the minimum variance; however, the sample size of this partition is above the resources allocated to this baseline. The Dalenius-Hodges boundary definition method offers the second best minimum variance and has a sample size that matches the baseline budget. FSIS selected this stratification because it allows collection of the number of samples within the given budget restrictions and offers a reasonable amount of variance. Table 6 shows the selected partition and final sample allocation.

Table 6: Final RCPBS study design and sample frame using the Dalenius-Hodges method. This table shows the number of strata, the number of establishments in each stratum, the number of establishments that will be sampled in each stratum, frequency of sampling per month, percent volume per stratum (the percentage that the stratum volume is out of all known production), the percent of sample (the percentage that each stratum will contribute to the overall number of samples), samples per establishment per year, and total samples per year. This table considers 6-month study.

Strata	Number of Estab-lishments	Estab-lishments to Sample	Frequency per Month	Percent Volume per Stratum	Percent of Sample	Samples per Establishment per Year	Sample per Year/Stratum
1	106	106	2	93.5	25.4	12	1,272
2	370	282	1.33	6.5	45.1	8	2,256
3	184	184	1.33	-	29.5	8	1,472
Totals	**660**	**572**		**100**	**100**		**5,000**

In Summary, the strata and sampling frequency are defined as follows:

- Stratum 1 –Large establishments that produce more than 70 million pounds of chicken parts per year. This stratum contains 106 establishments that produce 93.5% of the total known annual production in federally-inspected establishments. Sample frequency is set at 2 sample per establishment per month or 12 samples per 6-month per establishment. All establishments in this stratum will be sampled. Establishments in this stratum will receive 25.4% of the total samples scheduled for this study.

- Stratum 2 –The rest of establishments with known production volume that produce less than 70 million pounds of chicken parts per year. This stratum contains 370 establishments that produce 6.5% of the total known annual production volume. Sample frequency is set at 8 samples per establishment per 6-month. Establishments in this stratum will receive 45.1% of the total samples scheduled for this study. The 88 establishments that produce less than 15,000 pounds of chicken parts per year are excluded. The combined production volume of these 88 establishments is under 0.002% of the know production volume.
- Stratum 3 – Contains 184 establishments with unknown production volume of chicken parts. Sample frequency is set at 8 samples per establishment per 6-month and all establishments in this stratum will be sampled. Establishments in this stratum will receive 29.5% of the total samples scheduled for this study.

NOTE: Total known annual production is the sum of the production volumes provided in the survey at Shakedown, not including the 184 establishments with missing volume information.

D. Expected Statistical Precision and Power

This section explores the expected precision that the FSIS will achieve in estimating pathogen prevalence on chicken parts using the selected sample size (5,000). It is generally accepted that the true population parameter exists within a confidence interval (typically with 95% certainty). A narrower confidence interval provides greater precision, because the range that encloses the population parameter is tighter. A narrower confidence interval can be achieved by increasing the sample size. Another way to express the precision of the estimation is through the margin of error, which is defined as the "radius" or half the width of a confidence interval. Below are power calculations to determine the sample size and precision of the study.

FSIS outlines the relationship between a potential precision and the probability to achieve this precision when calculating pathogen prevalence. In addition, it defines the probability associated with a given margin of error under different outcomes for this sampling design. Statistical power measures the probability of a test detecting a statistically significant difference between two hypothesized point values in a population (i.e., between the estimated mean and a given margin of error) (26).

Statistical power is dependent upon the following:

(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, which is typically fixed at $\alpha = 0.05$ or 95% confidence level; and
(3) Sample size (i.e., the more samples are taken the more accurate is the estimation producing a narrower confidence interval).

To estimate the relationship between the power to detect a specific precision and the sample size needed to achieve it, the standard deviation of the error term is typically used. Because this is the first chicken parts baseline ever conducted, the standard deviation is unknown. FSIS will use 0.30, the standard error from the young chicken baseline study for *Salmonella* conducted in 2007.

It is expected that not all samples requested will yield an outcome and some will be discarded. Three levels of sample recovery (70%, 80%, and 90% recovery of the 5,000 scheduled samples) are considered in Figures 2, 3, and 4 (Appendix No 2). This ensures that a realistic number of analyzed samples will be collected within the budget allotted and guarantees that the pathogens will be detected. Analysis of three different recovery rate scenarios provides the relationship between power, sampling size, and precision achieved. Each scenario uses the following number of analyzed samples (N_i):

a) 70% recovery; $N_a = 3{,}500$,
b) 80% recovery; $N_b = 4{,}000$, and
c) 90% recovery; $N_c = 4{,}500$

These recovery rates with a standard deviation of 0.30 and significance (α level) of 0.05 form the input for JMP Statistical Software (SAS Institute Inc.) to generate the graphs (power versus difference to detect) shown on Appendix 2 (27). Appendix 2-A shows the scrip detail of the graphs.

Assuming an average rate of sample recovery of 80% as shown in Figure 2, Appendix 1 and fifty-fifty (0.5) probability of occurrence, the margin of error of the estimated prevalence of *Salmonella* is expected at approximately ± 0.9%. In the worst-case scenario, parameter estimation should have a maximum margin of error at ± 2.3%. If the study produces a medium rate of sample recovery at 80%, about 4,000 samples should suffice to yield a power level of at least 80% to detect a level of precision of ± 1.3%. With the proposed sampling frame and depending on the level or recovery, the precision of the estimation of pathogens should be somewhere between ± 0.9% and ± 2.3%

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

E. Development of Sampling Frame

The sampling frame includes all federally-inspected establishments identified in the FSIS Electronic Animal Disposition Reporting System (e-ADRS) that slaughtered or processed chickens. The study anticipates that the day-to-day production at these establishments will vary over time, and FSIS aims to create a final sampling frame that will accommodate variability in production volume. As such, each stratum contains a range of production volumes, and each

establishment's production volume will be updated at the end of the study to reflect actual production quantities during the year of sample collection.

Each month, the selected establishments will be assigned randomly two collection weeks. Inspection personnel will select, within the specific weeks, the collection day (Monday–Friday). Assigning this flexibility to inspectors maximizes the collection rate. Previous baseline experience suggests that scheduling the specific day of the week to collect samples reduces the collection rate to an unacceptable level. General sampling rules are given in appendix 1.

F. Sample Collection Method and Sampling Location within Establishments

FSIS will collect rinses of raw chicken parts from all federally-inspected establishments producing raw, cut-up chicken parts intended for retail or export that will not undergo further processing in the United States. The rinses will be tested for pathogenic and indicator bacteria. The sample collection method can be found in the current PR/HACCP sampling method document (10, 11 23).

To collect samples at all shifts available in the processing plant, the IPP will randomly select a location along the production lines with one type of chicken part prior to freezing or packaging for distribution. The IPP will select several pieces of the same chicken part (i.e., all legs, wings, etc.) available at the time of collection (approximately 4 lbs of chicken parts) randomly and aseptically and will follow the sampling procedure described in the FSIS Draft Notice 08-10. For example, if chicken breasts are selected, the IPP will collect 4 lbs of chicken breasts. This is detailed in the FSIS Notice (28)

G. Additional Comments on Sample Design

This study addresses two distinct objectives: to estimate the prevalence and quantitative levels of selected foodborne microorganisms and to obtain data for use in the development of the programs throughout the agency. The sample design and the resulting sample size for this study were limited by practical constraints, such as finite personnel, financial resources, and implementing scientific studies in real-life production settings. Considering these constraints, RCPBS will achieve the stated objectives by collecting and analyzing as many samples as possible to ensure a high level of statistical confidence.

Some of the sample requests will not yield a result. Recognizing this limitation, the described sample design incorporates "over-sampling." As such, FSIS will request more samples than will actually yield results. This "over-sampling" ensures that enough analytes are collected and analyzed so that the study has an appropriate level of statistical power. The final files will record deviations from the actual sample frame with entries showing non-response.

7. Potential Sources of Error

It is important to identify potential sources of error that may affect the results obtained from the proposed study and attempt to minimize such error prior to the beginning of the study. Below are possible sources of error that may occur during this study and procedures that will be implemented to minimize the error.

In this study design and sample frame, errors will be classified as sampling and non-sampling. Sampling errors develop from sampling a subset of a population and not the entire population. Testing the entire population is not possible during RCPBS because of budget constraints. In short, the RCPBS includes three causes of sampling error: samples are taken from a majority of the chicken parts processing plants but not all; the target organisms are unevenly distributed throughout the sample; and some establishments will not respond to the survey. FSIS assumes that the chicken parts processing plants that will be sampled during the study are representative of the entire chicken parts processing industry. To adjust for the uneven distribution of microorganisms in each sample, FSIS aims to sample each establishment at frequent and evenly spaced intervals to assure that the probability of isolating the target microorganisms is equal for each sample. Non-response may introduce bias and may occur in establishments with low production volumes. To help prevent non-response, FSIS will create a special e-mail account to provide further clarification as questions arise and to ensure that the forms and instructions are understood. The sample frame incorporates "over-scheduling" where more samples than needed are requested to guarantee that a minimum number of samples are obtained. In addition, approximately 76% of small establishments were selected for a sampling frequency of 8 times per 6-month in order to capture more reliable data in small production units. During the study, FSIS will closely monitor non-response and will follow-up with inspection personnel to maximize the response rate. Moreover, FSIS will require monthly preliminary reports to improve the response rate. This enhanced communication enables FSIS to minimize potential non-response error that may jeopardize the integrity of data obtained from the sampling results.

Non-sampling error biases survey studies and occurs when either the sampling frame does not represent the population or the sample size does not represent the frame properly. The 2012 RCPBS utilizes the data from the Shakedown to improve the sampling frame with the aim to minimize non-sampling error.

A. Sampling Technique Error

Sampling techniques present inherent error because the 4 lbs of raw chicken parts collected and rinsed during sampling may not represent the microbiological status of all chicken parts processed by the plant, especially when the expected bacterial counts are low.

The process of collecting chicken parts for sampling may also introduce error. The IPP are instructed to collect several pieces of the same chicken parts per sample and to change the selection of parts in subsequent collections; however, IPP may be limited to select the same

chicken part (e.g., chicken breasts), because of lack of production of other chicken parts at the time of collection.

Variability in analyte storage and shipment due to geographic and climate diversity may introduce error. Several procedures to standardize the collection technique should minimize the potential for this error. Instructions to inspectors provide details concerning the collection process. All establishments receive the same brand of materials and a consistent volume of Buffered Peptone Water (BPW) prior to sample collection. Error may be introduced when samples are not refrigerated prior to sending them to the lab and therefore may be temperature abused. Analytes should be refrigerated prior to shipment and should be shipped in a temperature-controlled container. Analyte processing occurs on the day of receipt at the laboratory.

B. Laboratory Error

Inconsistency and variability in laboratory procedures can create measurement error in the data. Such errors include media preparation and storage, analyte preparation and processing, analyte 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 analyte 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 bacteria count is extrapolated for a full count.

Laboratory technicians received training and conducted similar analyses for the young chicken carcass baseline study. Analysis will occur at one laboratory that is ISO-17025-Accredited, USDA-Accredited, and A2LA-Accredited. The laboratory has standard operating procedures for media preparation and storage, detailed analyte preparation instructions, and microbiological methods. Preliminary reports of the microbiological data generated by the laboratory will identify data entry errors to ensure data quality.

8. **Data Analysis Plan**

A. Analytical Approach

FSIS will maintain certain consistencies when analyzing RCPBS data during several types of statistical analyses. First, the final weight assigned to each observation will remain consistent for all analyses used to compute population-based estimates. Second, the same hierarchical structure resulting from the complex survey design will apply to all models.

B. 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 reports summarize the number of individual samples requested and discarded. A report will include a summary table illustrating 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. Project managers will also receive quarterly reports that 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/ml, or MPN/ml for each selected bacterium, part type, and shift-of-collection). The reports are for internal use, and FSIS will not distribute the reports to a wider audience.

C. Estimation of Prevalence and Quantitative Levels

The qualitative results, expressed as the detection (positive result) or non-detection (negative result) of each bacterium using the microbiological analyses, provide an estimate of the percent positive of the unweighted sample. The quantitative results 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 sample collection.

The national prevalence is equivalent to an average of positive sample results that have been weighted according to individual plant production volume. It is expected that the results of the percent positive for pathogens will differ slightly from the national prevalence due to the influence of the production volume of individual plants and other potential adjustments introduced in the calculation of the national prevalence.

e-ADRS daily processing totals provide auxiliary information to assign sampling weights to the individual observations in the dataset. The sampling weights will account for the variability in processing totals associated with establishment production at the time of collection, which can affect the establishment's stratum and amount of product collected during the survey.

Prior to final analysis, the described sampling weights will be adjusted 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 (27). Based on sampling replication methods, the statistical package will calculate the variance estimates of the point estimates and if necessary adjust for non-response. Developing estimates of prevalence using models is another option.

Appendix 1

Rules for Selection and Sampling of Establishments

Rules for Selection of Establishments and Sampling Plan for Stratum 1

Large establishments making up stratum „1' will be sampled twice every month or 12 times during the 6 months. An algorithm designed by FSIS will assign at random a week for sampling within each month for each of the 106 establishments in this group.

Rules for Selection of Establishments and Sampling Plan for Stratum 2

Establishments in stratum „2' will be sampled eight times during the 6 month study. An algorithm designed by FSIS will assign at random the week for sampling within every months and two extra months to complete 8 samples for each of the 282 establishments in this group.

Rules for Selection of Establishments and Sampling Plan for Stratum 3

Establishments at stratum „3' will be sampled eight times during the 6 month study. An algorithm designed by FSIS will assign at random a week for sampling within every month and two additional months to complete 8 samples for to each of the 184 establishments in this group.

Appendix 2

Figures showing the relationship between statistical power and sampling error for different rates of recovery

Figure 2. This figure shows the relationship between power and the sampling error for 70% rate of sample recovery. The curve shows that there is a high probability (0.8) of detecting a difference of 0.014 or 1.4% margin of error. With the conditions imposed in this scenario, there is almost certainty (probability 0.99) that the margin of error will not surpass 2.3%. The error standard deviation is 0.30, sample size 3,500, and alpha = 0.05.

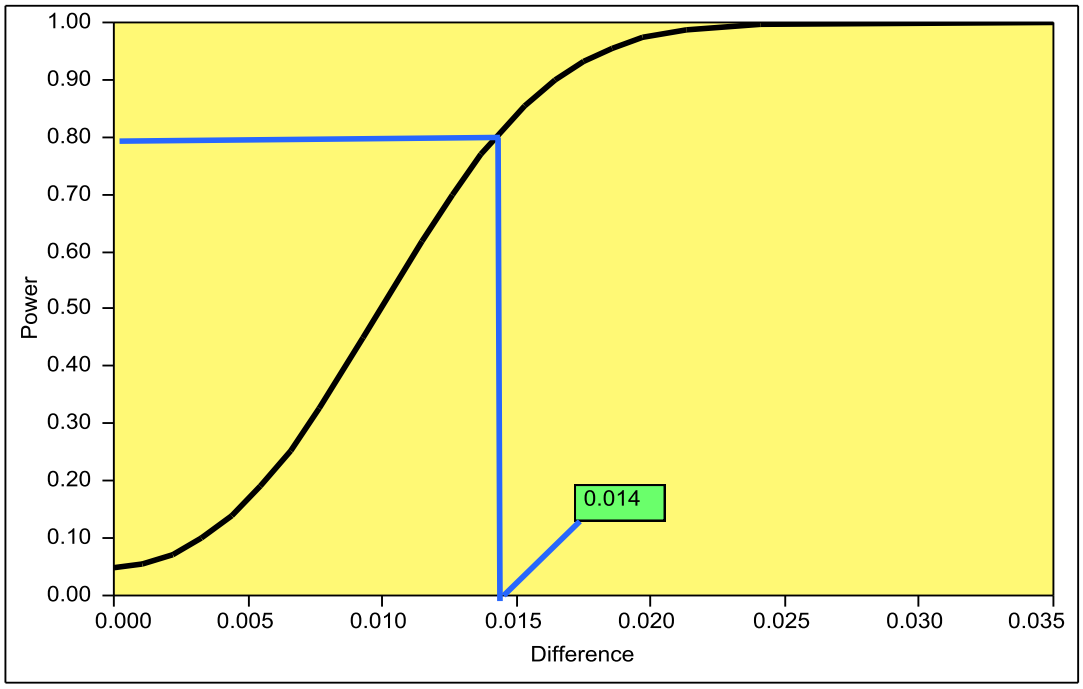


Figure 3. This figure shows the relationship between power and the sampling error for 80% sample recovery. The curve shows that there is a high probability (0.8) of detecting a difference of 0.013 or 1.3% margin of error. The error standard deviation is 0.30, sample size is 4,000, and alpha = 0.05.

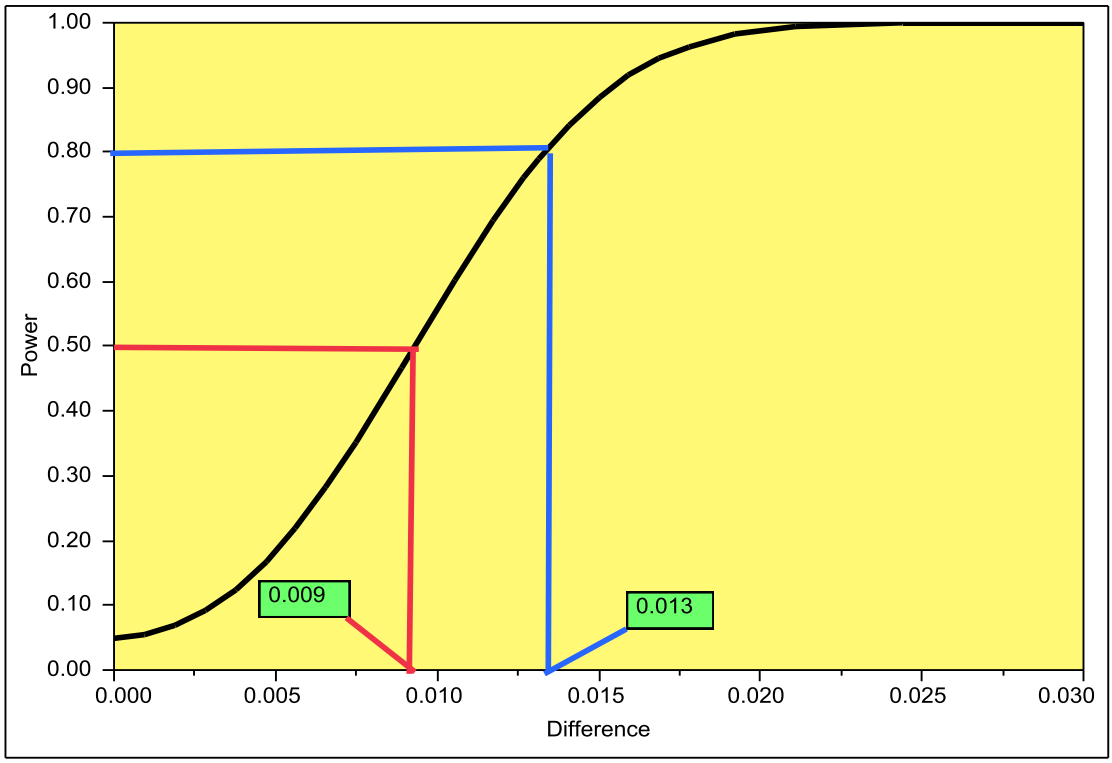
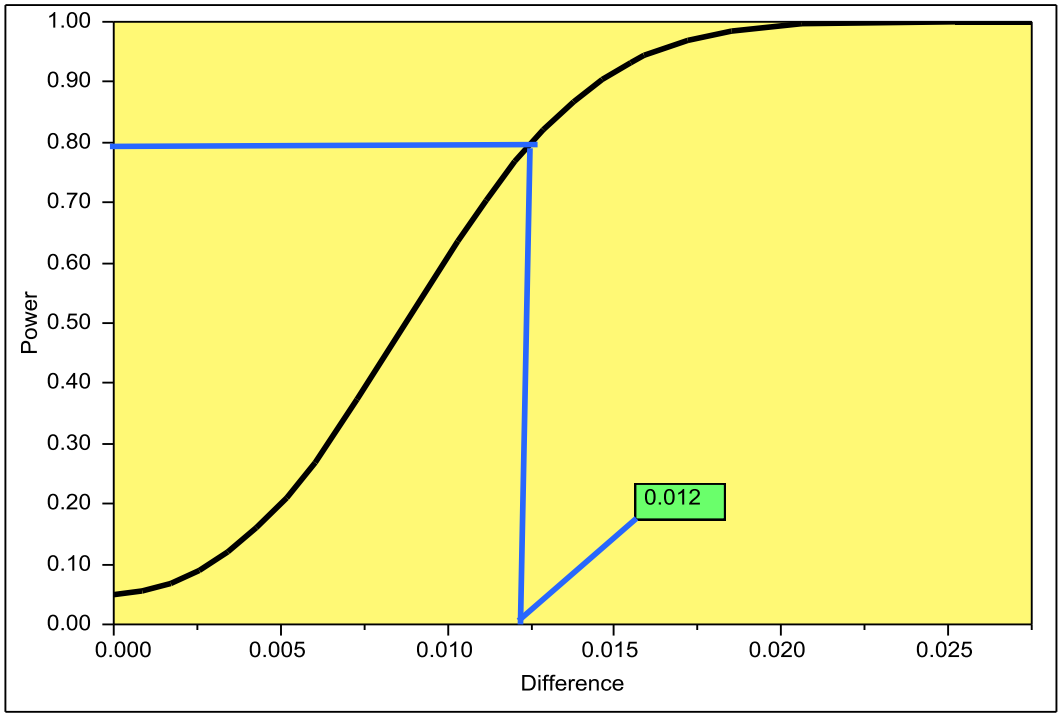


Figure 4. This figure shows the relationship between power and the sampling error for 90% sample recovery. The curve shows that there is a high probability (0.8) of detecting a difference of 0.012 or 1.2% margin of error. The error standard deviation is 0.30, sample size is 4,500, and alpha = 0.05.



Appendix 2A

Script for Power Graphs

```
Y Function(
        1 - F Distribution(
                F Quantile( 0.95, 1, 2101 ),
                1,
                2101,
                2102 * ::_Dif_ ^ 2 / 0.09
        ),
        ::_Dif_
)
```

In the above script:

Y Function (Y Exp, xName, <properties>)

Draws function Y Exp in the Y dimension as xName varies across the range of the x axis of the graph. Additional named property arguments: min(lower x), max(upper x), fill(patter, value to fill to), Inc(upper bound of increment).

y = F Distribution (q, dfnum, dfden, <nonCentrality=0>)

Returns the probability that an F distributed random variable is less than q.

q = F Quantile (p, dfnun, dfden, <nonCentratlity=0.)

Returns quantile, the value for which the probability is p that a random value would be lower.

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