FSIS Nationwide Raw Liquid Eggs Microbiological Baseline

Data Collection Program

Study Design and Sampling Frame for Technical Consultation

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FSIS Contact:

Dr. Hans D. Allender, Statistician
Hans.Allender@fsis.usda.gov

202-690-0771

Dr. James Rogers, Branch Chief

James.Rogers@fsis.usda.gov

202-690-6537

Dr. Uday Dessai, Division Director

Uday.Dessai@fsis.usda.gov

202-690-6431

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1. Executive Summary

This document outlines the study design and sampling frame for the national Raw Liquid Egg Baseline Survey (RLEBS) data collection program to be conducted by the Food Safety and Inspection Services (FSIS) of the United States Department of Agriculture (USDA). The liquid egg baseline includes establishments that produce whole eggs, whites, and yolks.

The RLEBS will include 56 plants that produce different forms of liquid eggs in the United States. To achieve representation of all plants, FSIS stratified each sample frame in a 3-strata design according to production volume. The final sample frame includes 54 plants for whole egg production, 46 plants for egg whites production, and 43 plants for egg yolk production. Samples will be collected before pasteurization and any additions of ingredients to the product.

In the RLEBS, samples are allocated to each stratum according to the production volume of the plants in that stratum. Each sample frame oversamples to account for non-response and discarded samples due to various reasons. Each stratum was constructed to minimize within-stratum variability and obtain the maximum number of samples possible within agency resources.

The expected precision (with 50% probability) of *Salmonella* estimates for each product at 90% sample recovery:

Whole eggs: ± 3.2%, with worst-case scenario not to exceed ± 7.0%.

Egg whites: ± 2.7%, with worst-case scenario not to exceed ± 5.9%.

Egg yolks: ± 3.6%, with worst-case scenario not to exceed ± 7.9%.

Final precision of the estimation depends on the actual number of samples analyzed (recovery rate) during the study and the variance among sample results. Before the final analysis, FSIS will use the production volume obtained during the 12-month study to weigh the results. FSIS will use this information to estimate the pathogen prevalence. The sampling frame will be adjusted on an on-going basis to account for newly eligible plants or plants that drop out of the frame.

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

Table 1. RLEBS-Whole eggs stratification for a 12-month study.

Strata	Number of Establishments	Frequency per Month	Percent Volume per Stratum	Percent of Sample	Samples per Establishment per Year	Sample per Year/Stratum
1	4	3	30.7	14.1	36	144
2	23	2	57.2	54.1	24	552
3	27	1	12.1	31.8	12	324
Totals	**54**		**100**	**100**		**1,020**

Table 2. RLEBS-Egg whites stratification for a 12-month study.

Strata	Number of Establishments	Frequency per Month	Percent Volume per Stratum	Percent of Sample	Samples per Establishment per Year	Sample per Year/Stratum
1	6	3	49.5	24.3	36	216
2	16	2	40.0	43.2	24	384
3	24	1	10.5	32.5	12	288
Totals	**46**		**100**	**100**		**888**

Table 3. RLEBS-Egg yolks stratification for a 12-month study.

Strata	Number of Establishments	Frequency per Month	Percent Volume per Stratum	Percent of Sample	Samples per Establishment per Year	Sample per Year/Stratum
1	5	3	46.6	22.0	36	180
2	15	2	42.9	44.1	24	360
3	23	1	10.5	33.9	12	276
Totals	**43**		**100**	**100**		**816**

Study Design and Sampling Frame for Technical Consultation

2. Program Summary:

The Raw Liquid Eggs Baseline Survey (RLEBS) includes whole eggs, whites, and yolks. FSIS will request at least 2,724 samples (1,024 whole eggs, 888 whites, and 816 yolks) from establishments under federal inspection over a 12-month study period. FSIS will analyze the samples to detect and quantify select foodborne pathogens and indicator bacteria. Results of this study will enable the FSIS and the regulated industry to target interventions and effectively work toward reducing the risk of foodborne pathogens associated with raw liquid eggs products.

3. Study Objectives:

Objective 1: Obtain data to develop microbiological risk assessments, risk-based sampling programs, and/or regulatory policy decisions, including the development of future performance guidelines;

Objective 2: Estimate the prevalence and quantitative level of *Salmonella,* Generic *Escherichia coli,* Total Aerobic Bacteria, *Enterobacteriaceae,* and coliforms in unpasteurized, raw liquid egg products;

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

1. Compare prevalence/weighted percentage positives and counts between pathogenic organisms and indicator organisms to determine relationships and associations;
2. Compare the count and prevalence of the selected bacteria if past baseline studies were performed on the same product(where appropriate); and,
3. Assess the effects of various factors on the microbiological profile (e.g., geographic region, inspection system, plant size, etc).

4. Target Populations:

FSIS will composite results from all establishments in the survey to estimate the microorganism concentration in eggs products. More information can be found in the FSIS Notice 16-12.

Establishments producing liquid eggs are included in the study if they:

- Pasteurize or process raw liquid whole eggs, whites, and/or yolks.

Raw liquid egg products are defined as:

- Liquid egg products that are unpasteurized and unprocessed before the addition of any ingredients. The samples are to be collected as close to the pasteurization as possible.

5. Study Specifications and Literature Review:

A. Background:

The FSIS mission ensures the nation's commercial supply of meat, poultry, and egg products are safe to consume, correctly labeled, and properly packaged. Based on previous baseline surveys, FSIS published the Pathogen Reduction Hazard Analysis and Critical Control Point Systems (PR/HACCP) Final Rule to reduce the prevalence and counts of pathogenic organisms in meat and poultry products. The Rule mandates that all establishments slaughtering cattle, swine, chickens, or turkeys screen products for *E. 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 (2), FSIS conducts periodic baseline surveys that analyze various food commodities for foodborne microorganisms.

Egg products do not fall under the PR/HACCP rule. An effort is underway to include egg product establishments in the PR/HACCP rule. The RLEBS will inform future rules and guidelines for these establishments.

B. Sampling Techniques:

The FSIS in-plant personnel (IPP) collect samples following the procedures described in FSIS Notice 16-12 (1), FSIS Directive 10.230.5, and instructions provided on computer-generated sample forms.

Eligible establishments: All federally-inspected liquid egg processing establishments are eligible for inclusion in the baseline survey. Prior to the start of the RLEBS, FSIS requested information about the type of egg product and annual production volume for each establishment during the study's "shakedown", a 90-day trial sampling period. The survey identified 56 establishments that produce liquid egg products. In this document, FSIS proposes a statistical study design and sampling frame for these establishments.

Type of collection: During the RLEBS, IPP will collect liquid egg product following the established sample collection procedure. The samples will be shipped to the lab and analyzed for foodborne pathogenic and indicator bacteria.

Location of collection: IPP will collect samples from the balance tank, silo/tank, or collection pot. Each sampling event will specify the production shift, which will alternate between consecutive sampling events at the establishment.

Sample analysis criteria: The laboratory will analyze samples received the day after sample collection, with a sample receipt temperature of 0 to 10 ºC (inclusive). Laboratory staff will discard samples received outside this temperature range.

Sample collection procedure:

1. Select the location for sample collection, depending on availability (balance tank, silo/tank, and collection pot).
2. Aseptically dip the sterile stainless steel ladle in the product and fill the provided container to 400 ml.
3. Screw the lid tightly onto the container and refrigerate the sample. Do not freeze.
4. Ship all samples to the lab on the day of collection.

C. Indicator Organisms

The RLEBS 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.

D. Pathogenic Organisms

The RLEBS will screen for and provide estimates of the prevalence and levels of *Salmonella.*

6. Study Design:

There are 56 FSIS eligible regulated egg processing plants included in the RLEBS. Not all of these establishments produced the three kinds of products sampled in this survey, for example only 54 produce liquid whole egg, 46 produce whites, and 43 produce yolks. The aggregate of all plants producing some kind of product is 56. Production volume is used for stratification because the baseline survey is intended to reflect the entire federally-regulated supply of the commodity in the survey. The study design is specific to the type of product and the number of establishments.

Plants Producing Liquid Whole Eggs	54
Plants Producing Whites	46
Plants Producing Yolks	43

A. Stratification

The study design includes three strata for each product and the stratification will be based on production volume. To account for bias introduced by stratification, FSIS will adjust/weight the values by production volume to create national prevalence calculation for *Salmonella* (3, 4, 5).

FSIS will define each stratum boundary by comparing several statistical boundary definition techniques. FSIS will 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 offers a sensible approach for distributing error among and between strata.
    ii. Disadvantage: accuracy of frequency distribution depends on the initial quantity of groups/bins included.
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 and the observer's bias may influence the results.

1. Stratification using the Dalenius-Hodge method (3)

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.

2. Stratification using the Geometric Method (6)
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, L is the number of strata;
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 $k_0 = a, ar, ar^2 \ldots\ ar^L = maximum\ k_L$

3. Stratification using the Visual Clustering method

Method Description:

1. The analyst visually assesses the data by plotting it on a scatter plot and uses the natural breaks occurring in the sequence of values as boundaries.
2. For improved visual aid, convert the production volume data to $\log_{10.}$
3. Place data on a graph/scatter plot.
4. Establish natural boundaries.
5. The $\log_{10}$ boundaries are inversed to production volume.

Each of the stratification methods are applied to whole eggs, whites, and yolk to obtain a sampling frame for each product individually. Appendix 1 includes the calculations and selections of the best stratification for each product.

B) Allocation of Samples per Stratum.

FSIS will schedule samples in proportion to each product. There are 54 plants producing whole eggs, 46 plants producing whites and 43 plants producing yolks. A proportional allocation of sampling resources is approximately 38% for whole eggs, 32% for whites and 30% for yolks. Target allocations, not including samples used on the "shakedown" phase is approximately:

1) Whole eggs = 1,020 samples
2) Whites = 888 samples
3) Yolks = 820 samples

C) Determination and Selection of Best Stratum Partition

The selected sampling frame for each product is as follows:

1. Whole Eggs

Table 1: Final RLEBS-Whole Eggs study design and sample frame using the Dalenius-Hodges method. This table covers the entire 12-month study.

Strata	Number of Estab-lishments	Frequency per Month	Percent Volume per Stratum	Percent of Sample	Samples per Establishment per Year	Sample per Year/Stratum
1	4	3	30.7	14.1	36	144
2	23	2	57.2	54.1	24	552
3	27	1	12.1	31.8	12	324
Totals	**54**		**100**	**100**		**1,020**

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

- Stratum 1 – A large establishment is defined as an establishment that produces more than 40 million pounds of liquid whole egg per year. This stratum contains 4 establishments that produce 30.7% of the total annual production in federally-inspected establishments. Sample frequency is set at 3 samples per establishment per month or 36 samples per year per establishment. All establishments in this stratum will be sampled and will receive 14.1% of the total samples scheduled for this study.
- Stratum 2 –A medium size establishment is defined as an establishment that produces less than 40 million pounds of liquid whole egg per year but more than 8.3 million pounds per year. This stratum contains 23 establishments that produce 57.2% of the total annual production in federally-inspected establishments. Sample frequency is set at 2 samples per establishment per month or 24 samples per year per establishment. All establishments in this stratum will be sampled and will receive 54.1% of the total samples scheduled for this study.
- Stratum 3 – A small establishment is defined as an establishment that produces less than 8.3 million pounds per year. This stratum contains 27 establishments that produce 12.1% of the total annual production in federally-inspected establishments. Sample frequency is set at 1 sample per establishment per month or 12 samples per year per establishment. All establishments in this stratum will be sampled and will receive 31.8% of the total samples scheduled for this study.

2. Egg Whites

Table 2: Final RLEBS-Whites study design and sample frame using the Dalenius-Hodges method. This table covers the entire 12-month study.

Strata	Number of Estab-lishments	Frequency per Month	Percent Volume per Stratum	Percent of Sample	Samples per Establishment per Year	Sample per Year/Stratum
1	6	3	49.5	24.3	36	216
2	16	2	40.0	43.2	24	384
3	24	1	10.5	32.5	12	288
Totals	**46**		**100**	**100**		**888**

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

- Stratum 1 – A large establishment is defined as an establishment that produces more than 20 million pounds of liquid egg white per year. This stratum contains 6 establishments that produce 49.5% of the total annual production in federally-inspected establishments. Sample frequency is set at 3 samples per establishment per month or 36 samples per year per establishment. All establishments in this stratum will be sampled and will receive 24.3% of the total samples scheduled for this study.
- Stratum 2 – A medium size establishment is defined as an establishment that produces less than 20 million pound of liquid egg white but more than 6 million pounds per year. This stratum contains 16 establishments that produce 40.0% of the total annual production in federally-inspected establishments. Sample frequency is set at 2 samples per establishment per month or 24 samples per year per establishment. All establishments in this stratum will be sampled and will receive 43.2% of the total samples scheduled for this study.
- Stratum 3 – A small establishment is defined as an establishment that produces less than 6 million pounds of liquid egg white per year. This stratum contains 24 establishments that produce 10.5% of the total annual production in federally-inspected establishments. Sample frequency is set at 1 sample per establishment per month or 12 samples per year per establishment. All establishments in this stratum will be sampled and will receive 32.5% of the total samples scheduled for this study.

3. Egg Yolks

Table 3: Final RLEBS-Yolk study design and sample frame using the Dalenius-Hodges method. This table covers the entire 12-month study.

Strata	Number of Estab-lishments	Frequency per Month	Percent Volume per Stratum	Percent of Sample	Samples per Establishment per Year	Sample per Year/Stratum
1	5	3	46.6	22.0	36	180
2	15	2	42.9	44.1	24	360
3	23	1	10.5	33.9	12	276
Totals	**43**		**100**	**100**		**816**

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

- Stratum 1 – A large establishment is defined as an establishment that produces more than 11 million pounds of egg yolk per year. This stratum contains 5 establishments that produce 46.6% of the total annual production in federally-inspected establishments. Sample frequency is set at 3 sample per establishment per month or 36 samples per year per establishment. All establishments in this stratum will be sampled and will receive 22.0% of the total samples scheduled for this study.
- Stratum 2 – A medium size establishment is defined as an establishment that produces less than 11 million pound of egg yolk but more than 2.9 million pounds per year. This stratum contains 15 establishments that produce 42.9% of the total annual production in federally-inspected establishments. Sample frequency is set at 2 samples per establishment per month or 24 samples per year per establishment. All establishments in this stratum will be sampled and will receive 44.1% of the total samples scheduled for this study.
- Stratum 3 – A small establishment is defined as an establishment that produces less than 2.9 million pounds of egg yolk per year. This stratum contains 23 establishments that produce 10.5% of the total annual production in federally-inspected establishments. Sample frequency is set at 1 sample per establishment per month or 12 samples per year per establishment. All establishments in this stratum will be sampled and will receive 33.9% of the total samples scheduled for this study.

D. Expected Statistical Precision and Power

The statistical community believes 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. The precision of the estimation can be adjusted through the margin of error, which is defined as the "radius" or half the width of a confidence interval.

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) (4).

Statistical power depends 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, 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).

The standard deviation of the error term is used to estimate the relationship between the power to detect a specific precision and the sample size needed to achieve it. Because FSIS does not know the standard deviation, it will estimate the standard deviation from the preliminary data obtained in the shakedown phase based on *Salmonella* results. This estimation of standard deviation is:

For whole eggs - 0.50

For egg whites - 0.39, and

For egg yolk - 0.50

It is expected that not all samples requested will yield an outcome and some will be discarded. During the shakedown phase, FSIS obtained a response rate of 90%. This response rate was used in the precision calculations for the actual study. The precision estimate provides assurance that a realistic number of analyzed samples will be collected within the resources allotted for pathogen detection.

Appendix 2 includes input to JMP Statistical Software (Version 8) used to generate graphs of recovery rate with a standard deviation for each product and significance (α) level (7). Appendix 2-A includes the script used to generate the corresponding graphs.

The margin of error of the estimated prevalence of *Salmonella* is expected at (given 90% sample recovery, 0.5 probability of occurrence):

a) Whole eggs: ± 3.2%, with worst-case scenario not to exceed ± 7.0%.
b) Egg whites: ± 2.7%, with worst-case scenario not to exceed ± 5.9%.
c) Egg yolks: ± 3.6%, with worst-case scenario not to exceed ± 7.9%.

Final precision of the estimation depends on the actual number of samples analyzed during the study, the actual sample recovery rate and variance across sample results.

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.

E. Development of Sampling Frame

The sampling frame includes all federally-inspected establishments that produce and further process liquid eggs products. 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, inspection personnel will receive a two-week window to sample the selected establishments randomly. Inspection personnel will select, within the specified 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. Appendix 3 includes information on products, stratification, establishment to be sample, and sampling rules.

F. Sample Collection Method and Sampling Location within Establishments

FSIS will collect liquid egg product samples from all federally-inspected establishments producing raw liquid eggs intended for retail or export that will not undergo further processing in the United States. The sample will be tested for pathogenic and indicator bacteria. The sample collection method can be found in FSIS Notice 16-12 (1).

To collect samples at all shifts available in the processing plant, the IPP will select the location of sampling and then sample a specified type of liquid egg product (liquid whole egg, whites, or yolks). The IPP will dip a sterile stainless steel ladle in the product and pour 400 ml of product into a leak-proof sterile container. They will tightly close the container lid and refrigerate the sample. Collection procedures are outlined in FSIS Notice 16-12 (1).

G. Additional Comments on Sample Design

This study addresses two distinct objectives:

- To estimate the national prevalence and quantitative levels of selected foodborne microorganisms; and

- To obtain data for use in the development of the programs throughout the agency.

Practical constraints, such as finite personnel, financial resources, and implementing scientific studies in real-life production settings, constrained the sample design and the resulting sample size for this study. Considering these constraints, the RLEBS 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, the FSIS will request more samples than will actually yield results. This "over-sampling" ensures that enough analytes are collected and analyzed to provide appropriate statistical power for the study. The results 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. In short, the RLEBS sampling error may result from

- Samples are taken from a few specimens of the population of liquid eggs;
- Uneven distribution of target organisms throughout the sample; and
- Some samples will not yield a result.

The FSIS assumes that the raw liquid egg processing plants in the study are representative of the entire raw liquid egg 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 "over-schedules" sampling to guarantee that a minimum number of samples are obtained. In addition, small establishments are sampled to capture more reliable data on these establishments. During the study, FSIS will closely monitor the reasons for non-response and will follow-up with inspection personnel to maximize the response rate. Moreover, FSIS will receive monthly preliminary reports from the laboratory 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 RLEBS 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 liquid product collected during sampling may not represent the microbiological status of all liquid egg processed by the plant, especially when the expected bacterial counts are low. The process of collecting liquid egg for sampling may also introduce error. The IPP collect a specified liquid egg product prior to pasteurization/processing or addition of ingredients.

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. Inspectors follow specific directions on the collection process. All establishments receive the same brand of materials and a consistent volume of liquid egg is collected. Improper refrigeration may introduce error. Samples not refrigerated prior to shipment to the lab may be temperature abused. To prevent this error, inspectors should refrigerate analytes prior to shipment 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 liquid egg products baseline study. Analysis will occur at one laboratory that is ISO-17025-Accredited, and A2LA-Accredited (9,10). 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 consistently analyze the RLEBS data during several 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, discarded, and shift-of-collection. 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. FSIS expects 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.

Data obtained from LQAD will provide daily processing totals for auxiliary information to assign sampling weights to the individual observations in the dataset. The sampling weights account for the variability in processing totals associated with establishment production at the time of collection. It also affects the establishment's stratum and amount of product collected during the survey.

Prior to final analysis, FSIS will adjust the described 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 (8). 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

Determination of Stratification

Sampling Frequency

Stratum size and field restrictions constrain the frequency of sampling.

1. Stratum 1: Three samples per establishment per month or 36 samples per year per establishment. All plants in this stratum will be sampled.
2. Stratum 2: Two samples per establishment per month or 24 samples per year per establishment. All plants in this stratum will be sampled.
3. Stratum 3: One sample per establishment per month or 12 samples per year per establishment. All plants in this stratum will be sampled.

Determination and Selection of Best Stratum Partition

Once the strata have been determined using different methodologies, FSIS will use a stratum boundary definition method that aims to minimize the variance within each stratum (V_{min}) (3). 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, 2 and 3)

S_h is the standard error of the stratum h (h = 1, 2 and 3) and

n is the total amount of samples for that partition.

This formula and the provided sampling frequency will be used to determine the best stratification on each of the following products.

A) Stratification of establishments producing whole eggs.

Table A. RLEBS-Whole Egg sample allocation 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	**36**	**4**	**144**	**37**	**1,332**	**19**	**684**
2	**24**	**23**	**552**	**14**	**336**	**18**	**432**
3	**12**	**27**	**324**	**3**	**36**	**17**	**204**
Totals	**72**	**54**	**1,020**	**54**	**1,704**	**54**	**1,320**

Minimum variance calculations for whole egg product:

V_{min} Dalenius = $6.23 \times 10^{13} / 1{,}020 \sim 6.17 \times 10^{10}$ (minimum variance)

V_{min} Geometric = $2.76 \times 10^{14} / 1{,}704 \sim 1.6 \times 10^{11}$

V_{min} Visual = $1.92 \times 10^{14} / 1{,}320 \sim 1.46 \times 10^{11}$

The Dalenius method offers the minimum variance as well as the minimum sample size among the three partitions. FSIS selected this stratification because it allows FSIS to collect an appropriate number of samples to obtain best minimum variance within the given budget restrictions. Table 1 shows the selected partition and final sample allocation for liquid whole eggs.

Table 1: Final RLEBS-Whole Eggs study design and sample frame using the Dalenius-Hodges method. This table covers the entire 12-month study.

Strata	Number of Establishments	Frequency per Month	Percent Volume per Stratum	Percent of Sample	Samples per Establishment per Year	Sample per Year/Stratum
1	4	3	30.7	14.1	36	144
2	23	2	57.2	54.1	24	552
3	27	1	12.1	31.8	12	324
Totals	**54**	**-**	**100**	**100**	**-**	**1,020**

B) Stratification of establishments producing egg whites.

Table B. RLEBS- Egg White sample allocation 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	**36**	**6**	**216**	**29**	**1,044**	**8**	**288**
2	**24**	**16**	**384**	**14**	**336**	**21**	**504**
3	**12**	**24**	**288**	**3**	**36**	**17**	**204**
Totals	**72**	**46**	**888**	**46**	**1,416**	**46**	**996**

Minimum variance calculations for egg whites product:

Using the same procedure and formulas as above, the results are as follow:

V_{min} Dalenius = $6.15 \text{ X } 10^{13} / 888 \sim 6.92 \text{ X } 10^{10}$ (minimum variance)

V_{min} Geometric = $1.40 \text{ X } 10^{14} / 1{,}416 \sim 9.88 \text{ X } 10^{10}$

V_{min} Visual = $7.62 \text{ X } 10^{13} / 996 \sim 7.65 \text{ X } 10^{10}$

The Dalenius-Hodges method offers the minimum variance as well as the minimum sample size among the three partitions. FSIS selected this stratification because it allows collection of the number of samples within the given budget restrictions and offers the best minimum variance. Table 2 shows the selected partition and final sample allocation for liquid whites.

Table 2: Final RLEBS-Whites study design and sample frame using the Dalenius-Hodges method. This table covers the entire 12-month study.

Strata	Number of Estab-lishments	Frequency per Month	Percent Volume per Stratum	Percent of Sample	Samples per Establishment per Year	Sample per Year/Stratum
1	6	3	49.5	24.3	36	216
2	16	2	40.0	43.2	24	384
3	24	1	10.5	32.5	12	288
Totals	**46**	**-**	**100**	**100**	**-**	**888**

C) Stratification of establishments for egg yolk.

Table C. RLEBS- Egg yolk sample allocation 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	**36**	**5**	**180**	**7**	**252**	**17**	**612**
2	**24**	**15**	**360**	**25**	**600**	**14**	**336**
3	**12**	**23**	**276**	**11**	**132**	**12**	**144**
Totals	**72**	**43**	**816**	**43**	**984**	**43**	**1,092**

Minimum variance calculations for egg whites product:

Using the same procedure and formulas as above, the results are as follow:

V_{min} Dalenius = $9.34 \times 10^{12} / 816 \sim 1.14 \times 10^{10}$ (minimum variance)

V_{min} Geometric = $1.43 \times 10^{13} / 984 \sim 1.45 \times 10^{10}$

V_{min} Visual = $2.97 \times 10^{13} / 1{,}092 \sim 2.72 \times 10^{10}$

The Dalenius method offers the minimum variance as well as the minimum sample size among the three partitions. FSIS selected this stratification because it allows collection of the number of samples within the given budget restrictions and offers the best minimum variance. Table 3 shows the selected partition and final sample allocation for liquid whites.

Table 3: Final RLEBS-Yolk study design and sample frame using the Dalenius-Hodges method. This table covers the entire 12-month study.

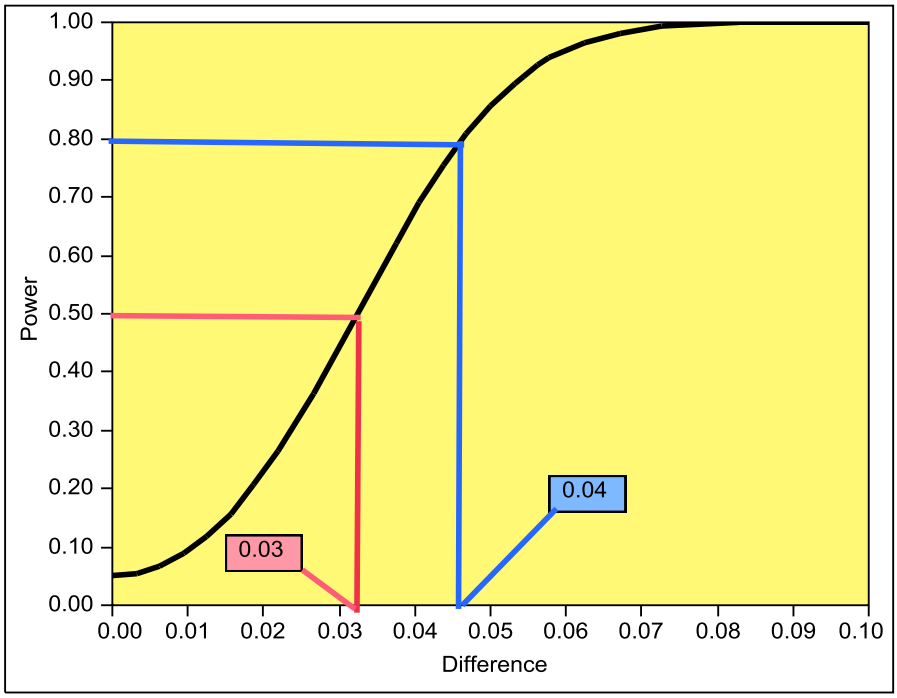
Strata	Number of Estab-lishments	Frequency per Month	Percent Volume per Stratum	Percent of Sample	Samples per Establishment per Year	Sample per Year/Stratum
1	5	3	46.6	22.0	36	180
2	15	2	42.9	44.1	24	360
3	23	1	10.5	33.9	12	276
Totals	**43**		**100**	**100**		**816**

Appendix 2

Figures Showing Power and Sampling Error

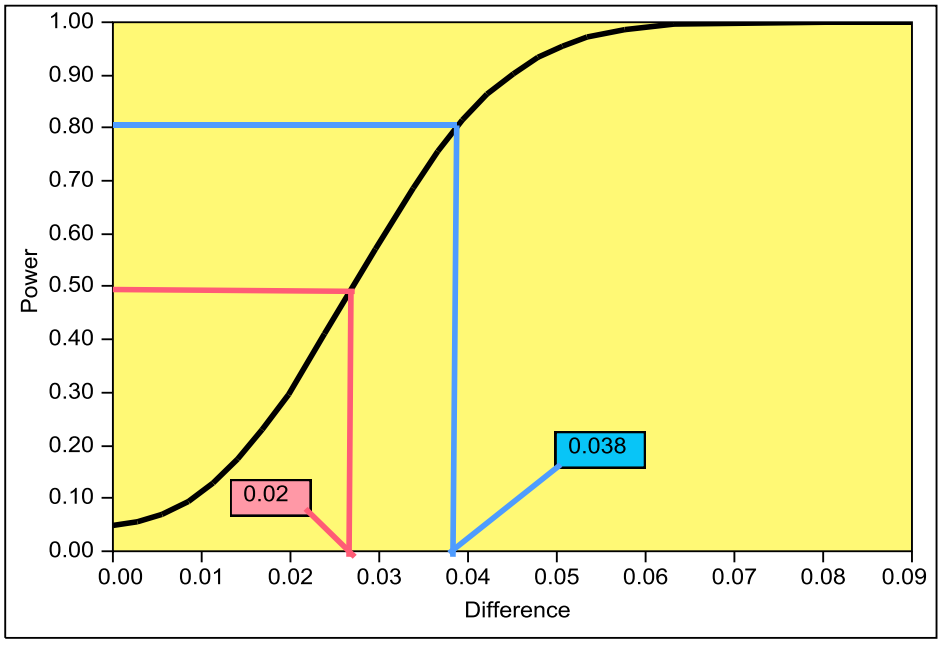
A. Whole eggs -Relationship between statistical power and sampling error

Figure 1. This figure shows the relationship between power and the sampling error for 90% rate of sample recovery, i.e., a sample size of 0.9 × 1,020 = 918. The curve shows that there is a high probability (0.8) of detecting a difference of 0.046 or 4.6% margin of error. With the conditions imposed in this scenario, there is a 99% probability that the margin of error will not surpass 7%. This approach offers a 50% probability of occurrence, which sets the margin of error at 0.032 or 3.2%. The error standard deviation is 0.50, sample size 918, and alpha = 0.05.



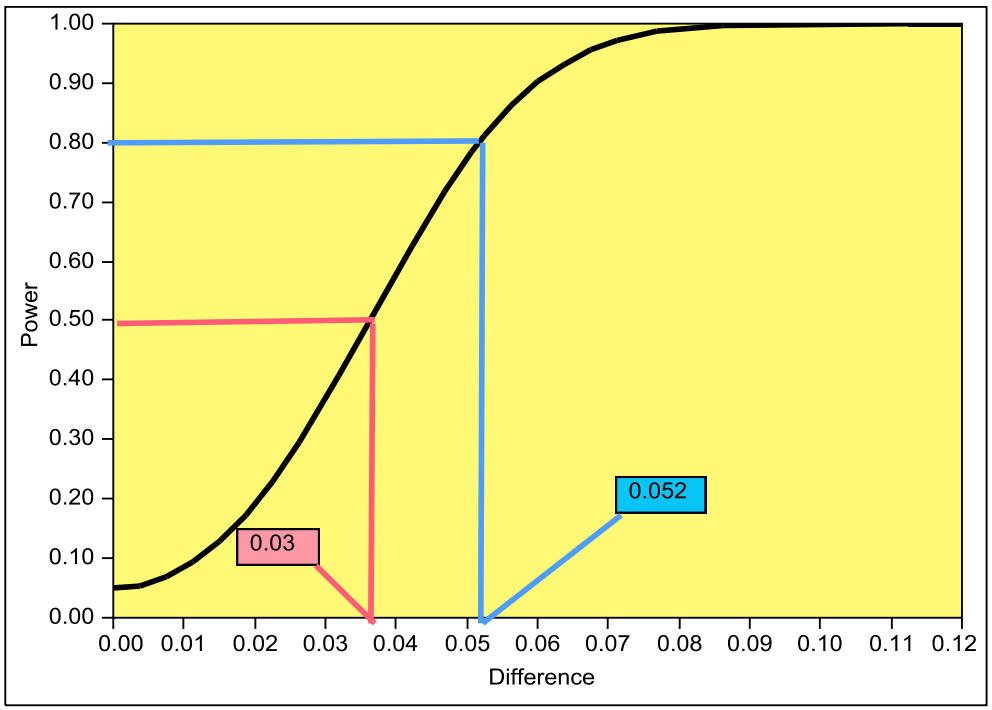
Whites-Relationship between statistical power and sampling error

Figure 2. This figure shows the relationship between power and the sampling error for 90% rate of sample recovery, i.e., a sample size of 0.9 × 888 = 800. The curve shows that there is a high probability (0.8) of detecting a difference of 0.038 or 3.8% margin of error. With the conditions imposed in this scenario, there is a 99% probability that the margin of error will not surpass 5.9%. This approach offers a 50% probability of occurrence, which sets the margin of error at 0.027 or 2.7%.The error standard deviation is 0.39, sample size 800, and alpha = 0.05.



B. Yolks- Relationship between statistical power and sampling error

Figure 3. This figure shows the relationship between power and the sampling error for 90% rate of sample recovery, i.e., a sample size of 0.9 × 816 = 735. The curve shows that there is a high probability (0.8) of detecting a difference of 0.036 or 3.6% margin of error. With the conditions imposed in this scenario, there is a 99% probability that the margin of error will not surpass 7.9%. This approach offers a 50% probability of occurrence, which sets the margin of error at 0.036 or 3.6%. The error standard deviation is 0.50, sample size 735, 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.

Appendix 3

Whole Egg, 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 three times every month or 36 times during the year of the study. An algorithm designed by FSIS will assign at random a week for each sample within each month for each of the establishments in this group.

Rules for Selection of Establishments and Sampling Plan for Stratum 2

Establishments in stratum '2' will be sampled two times per month or 24 times during the year of the study. An algorithm designed by FSIS will assign at random a week for each sample within each month for each of the establishments in this group.

Rules for Selection of Establishments and Sampling Plan for Stratum 3

Establishments at stratum '3' will be sampled one time per month or 12 times during the year of the study. An algorithm designed by FSIS will assign at random a week for each sample within each month for each of the establishments in this group.

Egg White, 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 three times every month or 36 times during the year of the study. An algorithm designed by FSIS will assign at random a week for each sample within each month for each of the establishments in this group.

Rules for Selection of Establishments and Sampling Plan for Stratum 2

Establishments in stratum '2' will be sampled two times per month or 24 times during the year of the study. An algorithm designed by FSIS will assign at random a week for each sample within each month for each of the establishments in this group.

Rules for Selection of Establishments and Sampling Plan for Stratum 3

Establishments at stratum '3' will be sampled one time per month or 12 times during the year of the study. An algorithm designed by FSIS will assign at random a week for each sample within each month for each of the establishments in this group.

Egg Yolk, 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 three times every month or 36 times during the year of the study. An algorithm designed by FSIS will assign at random a week for each sample within each month for each of the establishments in this group.

Rules for Selection of Establishments and Sampling Plan for Stratum 2

Establishments in stratum ‘2’ will be sampled two times per month or 24 times during the year of the study. An algorithm designed by FSIS will assign at random a week for each sample within each month for each of the establishments in this group.

Rules for Selection of Establishments and Sampling Plan for Stratum 3

Establishments at stratum ‘3’ will be sampled one time per month or 12 times during the year of the study. An algorithm designed by FSIS will assign at random a week for each sample within each month for each of the establishments in this group.

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