FSIS Nationwide Young Chicken Microbiological Baseline Data Collection Program

1. Background

To date, FSIS has completed three nationwide studies in broilers and/or young chickens. Beginning in 2006, FSIS intends to conduct a fourth nationwide baseline study designed to estimate the prevalence and quantitative level of selected bacteria, especially *Salmonella* and *Campylobacter*, on broiler carcasses. Ultimately, the microbiological data obtained from these baseline studies will be used in the development of risk assessments, risk-based sampling programs, and/or regulatory policy decisions.

Since 2002, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) formed two subcommittees with work charges regarding future FSIS baseline studies in broilers/young chickens: the Microbiological Performance Standards for Raw Meat and Poultry Subcommittee (2002-2004) and the Analytical Utility of *Campylobacter* Methodologies Subcommittee (2004-2006). Recommendations from both of these NACMCF subcommittee reports were considered during the design of this baseline study.

2. Primary Objectives

This Nationwide Young Chicken Microbiological Baseline Data Collection Program has the following primary objectives:

- To estimate the prevalence and quantitative level of the following bacteria on broiler carcasses at both re-hang and post-chill by testing for:
  - *Campylobacter*
  - Generic *Escherichia coli*
  - *Salmonella*
  - Total Aerobic Bacteria
  - *Enterobacteriaceae*
  - Coliforms

- To obtain microbiological data for use in the development of risk assessments, risk-based sampling programs and/or regulatory policy decisions

- To obtain microbiological data for comparison to findings from earlier baseline studies (where appropriate)

3. Target Population

FSIS will collect carcass rinses at re-hang and post-chill from broilers (representing the same grow-out flock/house) that are slaughtered in federal establishments and available for interstate and/or foreign commerce.

4. Sampling Frame

Those federal establishments identified in FSIS's Electronic Animal Disposition Reporting System (eADRS) that slaughtered a minimum of 100,000 young chickens[1] in fiscal year (FY) 2005 (i.e., the twelve-month period from October 1, 2004 through September 30, 2005) were included in the sampling frame and eligible for selection to participate in this baseline study. We plan to evaluate similar data collected during FY2006 (i.e., the twelve-month period from October 1, 2005 through September 30, 2006) to revise the sampling frame and determine the final assignment of establishments to production volume categories, as described below.

The preliminary sampling frame for this baseline study includes 199 young chicken establishments that represent 89.2% of the 223 federal establishments identified in eADRS as slaughtering young chickens in FY2005. These establishments contributed 99.994% of the total head of young chickens slaughtered in the U.S. under federal inspection during FY2005.

5. Study Design

The Nationwide Young Chicken Microbiological Baseline Data Collection Program will incorporate a multistage cluster design that includes sampling in establishments over time. In all establishments included in the sampling frame, individual broiler carcasses will be selected at intervals defined according to each of three production volume categories. For establishments in certain categories, the production shift during which a sample is to be collected will be specified.

- **Production Volume Category 1** consists of establishments that slaughtered ≥ 90,000,000 head of young chickens in FY2005. Carcass rinses will be collected two times per month (24 sampling events in an establishment per year) in the 13 establishments in this category. After randomly assigning the shift for collection of the first sample in an establishment, subsequent sample requests will alternate between shifts.

- **Production Volume Category 2** consists of establishments that slaughtered ≥ 25,000,000 but < 90,000,000 head of young chickens in

[1] The slaughter totals available in eADRS specify young chicken production and do not differentiate among specific types of young chickens (broilers, roasters, Cornish hens, etc). Sample collection instructions will specify that only broilers are eligible for testing in this program.

FY2005. Carcass rinses will be collected once per month (12 sampling events in an establishment per year) in the 136 establishments in this category. After randomly assigning the shift for collection of the first sample in an establishment, subsequent sample requests will alternate between shifts.

- **Production Volume Category 3** consists of establishments that slaughtered ≥ 100,000 but < 25,000,000 head of young chickens in FY2005. Carcass rinses will be collected one time every two months (6 sampling events in an establishment per year) in the 50 establishments in this category. Establishments in this category typically have a single production shift.

6. Sample Size

This design will result in an approximate total of 4500 carcass rinses collected during 2250 sampling events per year. Rinses will be collected throughout the year from carcasses at both re-hang and post-chill locations and from multiple production shifts in establishments.

7. Technical Consultation

An internal (within USDA) technical consultation was requested from three reviewers within the agency. It was requested that these reviewers consider the statistical and scientific validity of the Agency's intended approach for conducting this baseline study. Many of these suggestions were incorporated into the study design (See Attachments 4 and 5).

8. Study Duration

- A 90-day training period (the "shakedown period") is planned will be conducted prior to full implementation of this study (November 2006 – January 2007).
- The FSIS Nationwide Young Chicken Microbiological Baseline Data Collection Program will tentatively begin in March 2007. The study will continue for a minimum of 12 consecutive months (one year).

9. List of Attachments

- Attachment 1: Summary of National Advisory Committee on Microbiological Criteria for Foods (NACMCF) Recommendations in the Final Report, "Analytical Utility of *Campylobacter* Methodologies" and the FSIS Response
- Attachment 2: Summary of National Advisory Committee on Microbiological Criteria for Foods (NACMCF) Recommendations in the Final Report, "Response to the Questions Posed by FSIS Regarding

Performance Standards with Particular Reference to Broilers (Young Chickens)" and the FSIS Response

- Attachment 3: Expected Precision for Prevalence Estimation in the FSIS Nationwide Young Chicken Microbiological Baseline Data Collection Program
- Attachment 4: USDA Technical Consultation: Charge to Reviewers and Evaluation Criteria
- Attachment 5: USDA Technical Consultation: Reviewers' Comments and the FSIS Response

Attachment 1: Summary of National Advisory Committee on Microbiological Criteria for Foods (NACMCF) Recommendations in the Final Report, "Analytical Utility of *Campylobacter* Methodologies" and the FSIS Response

The Final Report, "Analytical Utility of *Campylobacter* Methodologies" is available at: http://www.fsis.usda.gov/PDF/NACMCF_Campylobacter_092805.pdf.

Here, we paraphrase the recommendations included in this report (and include a page reference), describe how each recommendation was incorporated into the proposed study design, and provide the rationale/justification for this decision.

A. Recommendations Pertaining to the Study Design:

1. **A certain percentage of samples should also be analyzed in a separate surveillance research project to estimate the prevalence of *Campylobacter* species other than *C. jejuni* and *C. coli*. (Pages 6, 12-13)**

   An AOAC-approved method to speciate beyond *C. jejuni* and *C. coli* is not commercially-available at the present time. A repository of *Campylobacter* isolates collected during this study will be created to permit additional characterization in the future. Additionally, we are exploring possible collaborations with various ARS researchers that would permit this additional speciation of isolates.

2. **FSIS should speciate *Campylobacter* spp. to differentiate *C. jejuni* and *C. coli.* (Pages 6, 22)**

   We plan to speciate confirmed isolates collected from this study as *C. jejuni* or *C. coli.*

3. **Clearly state the study objective(s). (Pages 6, 14, 19, and 26)**

   Study objectives were formulated early in the study design process and are formally presented in this proposal.

4. **Consider whether the results of the baseline study will be used to examine multiple points along the poultry processing line. (Pages 14, 26)**

   The proposed study design incorporates sample collection at both re-hang and post-chill.

5. **Consider identifying interventions that the industry can use as "best practices." (Pages 14, 26)**

   During the study design process, many factors that may impact the microbiological profile of young chicken carcasses were considered. Essential information that is required to achieve the primary objectives of the study was prioritized for inclusion on the sample collection form.

6. **Consider whether FSIS will look at overall numbers of *Campylobacter* spp. on products in the inspected plants to ascertain the success of intervention strategies. (Pages 14, 26)**

   FSIS will enumerate *Campylobacter* from carcass rinses collected at re-hang and post-chill and plans to "compare the count and prevalence … between re-hang and post-chill broiler carcasses to assess the effect of the slaughter process on microbiological contamination."

7. **Consider if data will be used in a future risk assessment. (Pages 14, 26)**

   This potential use is incorporated in the study objectives. Additionally, staff members from the Risk Assessment Division are members of the Statistics Subgroup of the Baseline Studies Committee.

8. **Test the same carcass rinse for *E. coli*, *Salmonella*, and *Campylobacter* to obtain information in relation to the utility of an indicator organism for the poultry industry. (Pages 6, 14, and 26)**

   Each carcass rinse sample collected during this study will be analyzed to identify and enumerate *Salmonella* (including serotyping), *Campylobacter* (including speciation as *C. jejuni* or *C. coli)* generic *E. coli*, coliforms, and *Enterobacteriaceae* (in addition to Aerobic Plate Counts (APC)).

9. **Consult the NACMCF reports entitled: "Response to the Questions Posed by FSIS Regarding Performance Standards with Particular Reference to Broilers (Young Chickens)", "Response to the Questions Posed by FSIS Regarding Performance Standards with Particular Reference to Raw Ground Chicken", and "Response to the Questions Posed by FSIS Regarding Performance Standards with Particular Reference to Raw Ground Turkey". (Pages 17-18)**

   A preliminary draft of the current report and the NACMCF Final Report "Response to the Questions Posed by FSIS Regarding Performance Standards with Particular Reference to Broilers (Young Chickens)" were consulted extensively during the study design process for this baseline study and FSIS provided a formal response to the major recommendations from both reports. The Final Report, "Analytical Utility of *Campylobacter* Methodologies" was not available until late in the study design process.

10. **Charge NACMCF to review the statistical aspects and data collection methodologies of any future baseline study designs. (Page 18)**

    We will make future proposals available to NACMCF members for their review.

11. Identify the population of interest and select a sampling unit that is representative of that population. (Page 19)

The target population and sampling units were defined early in the study design process and are formally presented in this proposal.

12. Account for factors such as seasonal and regional differences as well as inter-flock and inter-plant correlation when developing sampling plans. (Page 19)

The proposed study incorporates a multistage cluster design that includes sampling in establishments over time. Our approach to sampling will ensure that a minimum number of carcass rinses are collected per month and that inter- and intra-plant variability in microbiological profiles over time can be explored using multi-level regression models. Sampling by production shift (in selected production volume categories) will ensure that a minimum number of carcass rinses are collected per shift. Finally, selection of a pair of broiler carcasses representing the same grow out flock/house will both ensure that an equal number of re-hang and post-chill carcass rinses are collected and that inter-flock variability can be explored.

Although stratification by region was not incorporated into the proposed sampling design, the effect of region on microbiological outcomes will be investigated during the statistical analysis of data obtained from this study.

13. Consider statistical power in selecting the number of plants, number of carcasses and frequency of sampling for the baseline study; Create a power calculation matrix to determine the optimal sample size. (Page 19)

Sample allocation for this study was designed to collect and analyze as many carcass rinses as possible given the available personnel and financial resources.

Because the complexity of the study design prohibited traditional sample size and/or power calculations, we provide estimates of the expected level of precision for the estimation of both *Salmonella* prevalence and *Campylobacter* prevalence (i.e., the most conservative of the primary objectives with respect to sample size requirements) for the intended sample allocation. (See Attachment 3.) This approach provides “rough insight” concerning the statistical efficiency of the proposed study design and sample allocation scheme.

14. Define at what point(s) in the process carcasses will be selected for rinsing. (Page 19)

The sample collection protocol instructs inspection personnel to rinse a broiler carcass at the re-hang station and a second broiler carcass (originating from the same grow-out flock/house) at the end of the drip-line (or equivalent in air chill systems) post-chill.

15. Define how carcasses will be randomly chosen at establishments. (Page 19)

At each sampling event in an establishment, a pair of broiler carcasses will be sampled: one broiler carcass will be randomly selected at re-hang and a second broiler carcass, representing the same grow-out flock/house, will be randomly selected at post-chill.

Instructions to inspection personnel regarding random selection of carcasses have been previously provided for *Salmonella* testing to support the Pathogen Reduction/HACCP Regulation. Specifically, a method of random selection (i.e., random number generator, random number table, drawing cards, etc) is used to select a time during the identified shift when carcasses will be available. At the time selected, the inspection personnel will count back or ahead 5 carcasses at the predetermined point for collection of the carcass rinse, and select the next carcass for sampling.

16. Develop a sampling and data collection protocol and provide training to include specific instructions with respect to carcass selection, sampling and data collection methods to ensure consistency. (Page 19)

FSIS Notice 60-06 (Available at: http://www.fsis.usda.gov/OPPDE/rdad/FSISNotices/60-06.pdf) includes specific instructions concerning the sampling, sample collection, and shipping procedures to be used during this study. We plan to distribute a second notice after the completion of the 90-day training period to provide clarification and instructions concerning changes in procedures resulting from observation during the training period.

17. When carcasses are chemically treated as an intervention, there is a need to document this information on the sampling form using standardized language. Information related to such chemical treatments must be collected to ensure sample integrity and would not be used to measure the effect of the treatments; although, the information may be used for generating hypotheses or informing the design of future studies specifically addressing interventions. (Page 20)

Although many factors that may impact the microbiological profile of broiler carcasses were considered during the study design process, inclusion on the sample request form was limited to that information required to achieve the primary objectives of the study. A question pertaining to chemical interventions will be included on the sample collection form, “Does this establishment use an on-line reprocessing system? Yes __ No __.”

18. If FSIS determines that classes of poultry other than broilers will be assessed in the future (e.g., turkeys), FSIS should partner with appropriate researchers to develop methodologies and conduct surveillance studies to sample these products possibly for other *Campylobacter* species in addition to *C*. jejuni and *C*. coli. (Page 23)

FSIS plans to conduct a baseline study in the Young Turkey product class in the near future. In this study, we plan to speciate confirmed isolates as *C. jejuni* or *C. coli.* We are exploring possible collaborations with various ARS researchers that would permit additional speciation of isolates.

19. To ensure the validity, interpretability and generalizability of the study results, sampling and data collection methods should be evaluated, and a document that details the study protocol should be developed and made available. (Page 27)

A formal, written proposal was developed early during the study design process to describe the Agency's approach for designing and conducting the upcoming Nationwide Young Chicken Microbiological Baseline. Technical, written reviews of the study design proposal were conducted internally by three USDA staff from various agencies. Summaries of reviewers' comments and FSIS' subsequent responses are included as Attachments 4 and 5.

B. Recommendations Pertaining to the *Campylobacter* Enumeration Method

1. Develop a standardized protocol with a neutralizing rinse broth for quantitative and qualitative analysis of selected microorganisms (Pages 14 and 19).

A variety of antimicrobial interventions are being used by the chicken slaughter industry. A table listing these chemicals can be found in FSIS Directive 7120.1 amendment 8 (http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1_Amend_8.pdf). An increasing number of chicken slaughter establishments are applying intervention dips or sprays after the chiller tanks.

We are currently using 400 ml of BPW for rinsing chicken carcasses. The advantages of this sampling method are listed below:

1) The BPW provides some buffering capacity and the 400-ml volume provides a dilution effect for residual antimicrobials that might be present on the carcasses at the time of sampling.

2) FSIS laboratory titration experiments determined there is a demonstrable buffering capacity for BPW when challenged with strong acid and base solutions.

3) The Kemp and Schneider (2000 Poultry Science 79:1857-1860) study indicates that 400 ml of BPW will effectively neutralize acidified sodium chlorite

(Sanova), which appears to be one of the more popular post-chill interventions in use.

4) Bourassa *et al.* (2004 Poultry Science 83:2079-2082) found that rinsates from post-TSP-dipped carcasses offered the same potential for detection of indigenous *Salmonella* whether neutralized or not. However, the data indicate that non-treated 500 ml rinsates had a pH as high as 8.4, which may impact outgrowth potential. It appears that the use of TSP is declining and that its application as a post-chill intervention is less likely than a variety of other options.

In short, it remains unclear whether FSIS should pursue a strategy to neutralize the BPW rinsates, but we will not know for certain until we conduct some additional testing. Therefore, the contract laboratory will conduct a pH analysis on ALL chicken rinsates received during the shakedown phase of the baseline study. Because all establishments will be sampled during shakedown, we will be able to determine any need to refine sampling and/or testing procedures by the end of shakedown phase.

2. **Determine and state the sensitivity of methods used to detect indicator organisms and pathogens. Consider using methods for indicator organisms and pathogens with equal sensitivities (Page 15).**

Campylobacter: The limit of detection (LOD) of the ARS direct plating method recommended by NACMCF for enumeration of *Campylobacter* from chicken rinsates is 1 CFU/ml or approximately 400 CFU/carcass.

Salmonella: The LOD of the most probable number (MPN) method for *Salmonella* quantitative analyses is 0.03 MPN/ml of chicken rinsate or approximately 12 MPN/carcass, based on testing three 10-ml aliquots of undiluted rinsate. The LOD afforded by the enrichment-based MPN method is lower than the direct plating enumeration methods for *Campylobacter* and indicator organisms, and is necessary to maintain consistency with past baseline studies and ongoing PR-HACCP chicken carcass rinsate testing.

Indicator organisms: The LOD for the 3M Petrifilm™ count plate methods for enumeration of generic E*. coli*, coliforms, *Enterobacteriaceae* and total aerobic bacteria (Aerobic plate count-APC) is 1 CFU/ml or approximately 400 CFU/carcass.

3. **Consider a *Campylobacter* method that can be validated and easily used with a high sample throughput (Page 15).**

FSIS will be using the high throughput direct plating enumeration methodology recommended by NACMCF. The contract laboratory that will conduct testing during the study is currently validating the method.

4. **Make chosen method widely available to industry constituents for comparison sample analysis (Page 15).**

   The media and materials required for this method are commercially available and are already in use for industry testing**.**

5. **Use a direct plating method for enumeration of *Campylobacter* (Page 15).**

   FSIS will be using the direct plating enumeration methodology recommended by NACMCF. The detailed method and validation protocols are described as the '*Campylobacter* Enumeration Method for 2006-2007 Young Chicken Baseline Study' and are available for internal use by FSIS and its contract laboratory.

6. **Train technicians to perform the chosen direct plating method (Pages 15, 19).**

   The contract laboratory's technical supervisor and technicians were trained by recognized *Campylobacter* methodology experts from ARS in Athens, GA.

7. **Consult other US Federal agencies and other private and state research institutions to correlate *Campylobacter* methodologies when possible (Page 15).**

   While developing, '*Campylobacter* Enumeration Method for 2006-2007 Young Chicken Baseline Study', FSIS consulted various scientists and experts, including Stanley Bailey, Eric Line and Gregory Siragusa at ARS, Athens GA, and Prof. Omar Oyarzabal at Auburn University, Auburn, AL. FSIS will continue consulting experts in the future.

8. **Use a 1-ml inoculation over four agar plates to achieve plating of a 10^0 dilution (Page 16).**

   For postchill chicken carcass rinsates, 1 ml of undiluted rinsate will be plated over four plates (0.25 ml/plate) to achieve a 10^0 dilution, and 0.1 ml (10^{-1}) will be plated on duplicate plates. For re-hang chicken carcass rinsates, the 10^0 dilution will not be plated because the count is expected to be significantly higher. Instead, 10^{-1}, 10^{-2} and 10^{-3} dilutions will be plated in duplicate. If necessary, dilutions to be tested will be adjusted during the course of the shakedown phase or study.

9. **Back-up enrichment is not necessary (Page 16).**

   FSIS will use a direct plating enumeration method without a back-up enrichment, as recommended by NACMCF. Although it is clear that this non-enrichment-based method will not provide the sensitivity of methods used in the past for determining a national *Campylobacter* prevalence, it will facilitate high throughput enumeration of levels exceeding 400 CFU/carcass to meet FSIS risk assessment needs.

10. Choose Campy-Cefex agar or m-Campy-Cefex, as these would be a sensitive, cost effective choice (Page 17).

We are using the USDA ARS semi-modified (lysed blood instead of laked blood) formulation of Campy-Cefex agar.

11. Use 42 ± 1°C for 48 h incubation time and temperature (Page 17).

We are using 42 ± 1°C for 48 ± 2 h incubation time and temperature.

12. Develop a sample collection protocol that includes, a) sample-handling factors such as rinse methods (i.e. type of neutralizing diluent, rinse solution), b) temperature conditions during shipment, and c) microbiological testing procedures.

a) Protocol for sample-handling: FSIS Notice 60-06 contains specific instructions concerning the sampling, sample collection, and shipping procedures to be used during this study.

b) Temperature conditions during shipment: FSIS Notice 60-06 contains specific instructions concerning the sampling, sample collection, and shipping procedures to be used during this study.

c) Microbiological testing protocol: FSIS has developed detailed method and validation protocols ('*Campylobacter* Enumeration Method for 2006-2007 Young Chicken Baseline Study').

13. Consider training the individuals involved in carrying out the protocol, to assure consistency (Page 19).

a) Protocol for sample-handling: FSIS Notice 60-06 contains specific instructions concerning the sampling, sample collection, and shipping procedures to be used during this study.

b) Temperature conditions during shipment: FSIS Notice 60-06 contains specific instructions concerning the sampling, sample collection, and shipping procedures to be used during this study.

c) Microbiological testing protocol: The contract laboratory's technical supervisor and technicians were trained by ARS laboratories in Athens, GA.

14. Proper carcass draining practices, in addition to using non antimicrobial neutralizing additives, tailored to each chemical treatment, should be developed to maximize *Campylobacter* spp. recovery, as well as generic *E. coli* and *Salmonella* being tested for under the current regulations (Page 20)

Proper carcass draining practices will be addressed in FSIS Notice 60-06.

See response to item number B.1 for antimicrobial neutralizing additives, tailored to each chemical treatment.

15. Consider providing scientific justification for the specific rinse volume chosen.

After careful consideration, FSIS has decided to use 400 ml of BPW to rinse chicken carcasses. This sampling procedure and volume is consistent with past baseline studies and ongoing PR-HACCP testing programs, so additional training is not required for FSIS sample collectors. In addition, the 400 ml rinsate provides sufficient volume to cover interior and exterior surfaces of the carcass to ensure consistency of sampling. The 400 ml rinse should provide sufficient dilution and buffering to neutralize trace amounts of antimicrobials that may be present

16. Ensure that the rinse solutions are at 4 °C before rinsing, and that rinsate is immediately placed on ice (Page 21).

FSIS Notice 60-06 includes specific instructions concerning the sampling, sample collection, and shipping procedures to be used during this study.

17. Use overnight sample shipping, and consider a study to determine the number of ice packs and/or volume of ice needed to maintain temperature, given anticipated ambient temperature extremes (Page 21).

Samples will be shipped by overnight delivery service.

The contract laboratoryhas conducted a validation study to determine the number of ice packs needed to maintain the appropriate temperature during shipping.

18. Develop guidance for alternative ways to achieve microaerobic conditions if a tri-gas incubator is not available. Consider validating the specific methodology for using gas-filled bags (Page 21).

The tri-gas incubator will be used by the testing laboratory. The Microbiology Division has reviewed and commented on written standard operating procedures (SOPs) from the contract laboratory pertaining to the alternative method (e.g., gassed bags) for achieving microaerobic conditions if the tri-gas incubator fails during the course of the study.

19. Consider speciation between *C. jejuni* and *C. coli* and methods such as latex agglutination and multiplex PCR can be used (Page 22).

An AOAC-approved method to differentiate *C. jejuni* and *C. coli* is not commercially available. A repository of *Campylobacter* isolates collected during this study will be created to permit additional characterization in the future. In any event, FSIS has determined that distinguishing these two pathogenic species from each other in the context of this baseline study is not necessary to meet risk assessment and regulatory needs at this time.

20. Consider developing and validating molecular technologies such as microarray for speciation and subtyping of Campylobacter (Page 22).

FSIS will consider new technologies as they are proven effective, but will focus on applying proven testing methodology for this baseline study. ARS will have access to the *Campylobacter* isolates for possible use in the development of new testing methodologies.

21. Confirm each isolate demonstrating typical *Campylobacter* morphology and motility using latex agglutination (Page 22).

FSIS will be using latex agglutination for confirming isolates demonstrating typical *Campylobacter* morphology and motility.

22. Consider picking a minimum of five colonies, up to a total of 10% of the typical colonies on a countable (or lowest dilution) plate, representing each colony morphology, for semi-confirmatory testing by cellular morphology and motility on a wet-mount using phase contrast microscopy (Page 22).

FSIS will be following the recommendations for "picking" a minimum of five colonies, followed by microscopy and latex agglutination for confirmation. Statistical analyses performed by FSIS Risk Assessment Division indicates a diminishing return for reducing the uncertainty of the estimated count when evaluated against the significant additional logistical and resource needs for testing more than 10 colonies per sample. Considering the significant additional testing resources that would be necessary, FSIS has decided that testing 10% of colonies in certain circumstances is not necessary. To best ensure the accuracy and precision of estimated counts, the FSIS instructions state that a maximum of 10 colonies are to be picked under certain circumstances.

23. Use consistent microbiological methods and procedures for a) drying agar plates, b) storage and shelf-life of plates, and c) report enumeration data as CFU/ml rinse when whole carcass rinsates are tested (Page 22).

a) Drying plates: In collaboration with ARS experts and FSIS, the contract laboratory has a protocol for drying Campy-Cefex plates prior to use.

b) Storage and shelf-life of plates: In collaboration with ARS experts and FSIS, the contract laboratory has developed a written SOP for storing Campy-Cefex plates. Plates are stored at 2 to 8 °C for no more than 30 days.

c) Report enumeration data as CFU/ml rinse when whole carcass rinsates are tested: The FSIS protocol for testing has described the procedure for calculating the final result in CFU/ml based on a 400 ml rinsate. This will provide the future option to express results in terms of CFU per carcass as well.

24. **Exploring the feasibility and value of serotyping *Campylobacter* as well as investigate the feasibility of flaA sequence comparisons in subtyping Campylobacter, which has been used at ARS in Athens, GA (Page 24).**

25. **Consider testing a defined subset of *Campylobacter* isolates for antibiotic resistance (Page 24).**

26. **Consider preserving the isolates in storage for further molecular characterization, but such a characterization should not be part of an initial baseline study.**

27. **Perform additional research on subtyping, and consider a combination of two or more subtyping methods as they can often increase discriminatory power (Page 25).**

    These recommendations are beyond the scope of the FSIS Young Chicken Baseline Study**.** However, a repository of the *Campylobacter* isolates collected during this study will be shared with ARS scientists to permit experimental testing for serology, antimicrobial resistance, subtyping, sequencing and other characterization of *Campylobacter* strains in the future.

Attachment 2: Summary of National Advisory Committee on Microbiological Criteria for Foods (NACMCF) Recommendations in the Final Report, Response to the Questions Posed by FSIS Regarding Performance Standards with Particular Reference to Broilers (Young Chickens)" and the FSIS Response

The Final Report, "Response to the Questions Posed by FSIS Regarding Performance Standards with Particular Reference to Broilers (Young Chickens)" is available at:
http://www.fsis.usda.gov/OPHS/NACMCF/2004/NACMCF_broiler_4_13_04.pdf.

Here, we paraphrase the recommendations included in this report (and include a page reference), describe how each recommendation was incorporated into the proposed study design, and provide the rationale/justification for this decision.

A. Recommendations Concerning the Scope of a Baseline Study:

1. **"Collect data on the relationship between the prevalence and cell numbers of Salmonella on broiler carcasses exiting the chill tank and the prevalence and cell numbers of Salmonella on broiler or broiler parts at retail." (Page 7)**

   Sampling poultry at retail is beyond the scope of this baseline study. Further, sampling at retail outlets by FSIS personnel is problematic because: (a) these establishments are not regulated by FSIS; (b) it would require that FSIS purchase the product, thus increasing total study cost; and (c) the complex distribution system would limit our ability to collect samples at retail from the same production lot sampled in the processing plant.

   However, FSIS participates in two FoodNet Working Groups (WG) that involve testing retail poultry for potential pathogens: Campylobacter Regional Differences WG and the NARMS/FoodNet Retail Food Survey WG. These collaborations will facilitate the comparison of data collected by FSIS with retail samples collected as part of these FoodNet projects.

2. **Design study to gain a better understanding of the relationships between contamination present on the exterior or internally in the live bird and the *Salmonella* that is likely to result on processed broilers. (Page 11)**

   Live bird testing is beyond the scope of this baseline study. Carcass rinse samples collected at re-hang are believed to be representative of potential bacterial contamination during the poultry slaughter process, and will serve as a proxy for the pre-harvest microbiological profile in this baseline study.

3. **"The approach applied by certain European countries to identify significant on-farm factors that influence the prevalence of *Salmonella* and *Campylobacter* on broilers should be considered." (Page 11)**

   On-farm epidemiologic investigations are beyond the scope of this baseline study.

4. **Include sampling of both Federal and State-inspected plants. (Page 12)**

   The sampling frame for this baseline study will list establishments identified in FSIS's Electronic Animal Disposition Reporting System (eADRS) that slaughtered a minimum of 100,000 young chickens that received the federal mark of inspection (including Talmadge-Aiken plants) during the twelve month period from October 1, 2004 through September 30, 2005 (FY2005). Further refinement of the sampling frame will occur based on FY2006 eADRS data and broiler production data to be collected during the 90-day training (i.e., "shakedown") period prior to the initiation of this baseline study.

   State-inspected establishments are not included in the sampling frame for this baseline study because FSIS: (a) does not maintain a database that lists all state-inspected establishments or their respective production volumes; (b) does not have regulatory authority in state-inspected establishments; and (c) does not have personnel stationed in state-inspected establishments that could conduct sampling.

B. Recommendations Concerning Statistical Design Issues

5. **Determine sources of variation in *Salmonella* prevalence; Assign variation to a cause; provide for estimates having reasonable precision of variability within and among plants. (Pages 9, 10, 12)**

   Multivariable regression models that consider the hierarchical sampling design (i.e., multiple sampling events in each establishment over time) will be used to analyze these data. Such models will provide estimates for the effect of geographic region, season, and production shift on both prevalence and count of selected bacteria as well as deconstruct the observed variance (i.e., estimate variance components within and between establishments).

6. **Stratify by production volume, month, region. (Page 12)**

   The majority of establishments that produce federally-inspected young chickens (approximately 90%) are included in the study population. Further, the frequency of carcass sampling in establishments is based on production volume categories so that the establishments with the highest annual production volumes are sampled with the highest frequency. However, this does not constitute traditional stratified random sampling based on production volume.

   Stratified random sampling based on month was not incorporated into the proposed sampling design. However, sample requests will be distributed

across the study period so that a minimum number of samples will be collected during each month/season.

Stratified random sampling based on geographic region was not incorporated into the proposed sampling design. By including the majority of establishments that produce young chickens in the study population, all geographic regions[1] in the U.S. will be represented. Assessing the effect of region on microbiological outcomes is a secondary aim that will be investigated during the analysis of data obtained from this study.

7. Ensure the "number of samples are sufficient to meet agency specified discriminatory power for comparisons of interest." (Page 12)

Sample allocation for this study was designed to collect and analyze as many carcass rinses as possible given the available personnel and financial resources.

Because the complexity of the study design prohibited traditional sample size and/or power calculations, we provide estimates of the expected level of accuracy for the estimation of both *Salmonella* prevalence and *Campylobacter* prevalence (i.e., the most conservative of the primary objectives with respect to sample size requirements) for the intended sample allocation under increasingly complex study design scenarios. (See Attachment 3). This approach provides "rough insight" concerning the statistical efficiency of the proposed study design and sample allocation scheme.

8. Commission pilot studies "to determine the feasibility of the sampling programs and to gain preliminary knowledge about variability to better define appropriate sampling plans." (Pages 9 and 16)

Although a recent study conducted in collaboration between USDA's Agricultural Research Service (ARS) and FSIS has been completed, preliminary data concerning within- and between-plant variability in microbiological outcomes was unavailable to inform sample size and/or power calculations during the design phase of this baseline study.

9. "To understand the impact of seasonality, data must be collected for at least one year (12 consecutive months)." (Pages 10, 12)

Assessing the effect of season on microbiological outcomes is a secondary aim that will be investigated during the analysis of data obtained from this study. The collection period for the upcoming baseline study will span a minimum period of 12 consecutive months. Sample requests will be distributed across this period so that a minimum number of samples will be collected during each season.

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[1] Regions were defined as follows for the purpose of data exploration to support study design efforts: Northcentral (IL, IN, IA, MI, MN, OH, WI); Northeast (CT, ME, MA, NH, NY, RI, VT, NJ, PA, DE, DC, MD);
Southwest (AR, KS, LA, MO, NE, NM, OK, TX); Southeast (FL, GA, PR, VI, AL, MS, TN, KY, NC, SC, VA, WV);
West (AK, AS, AZ, CA,CO, GU, HI, ID, NV, MP, OR, UT, WA, MT, ND, SD, WY)

C. Recommendations Concerning Factors Associated with Prevalence and/or Cell Number[2]

10. Collect data that "relates to (sic) specific process steps to changes in prevalence and/or cell number." (Page 7)

Intensive sampling at multiple points in an establishment is beyond the scope of this baseline study.

11. The main focus of new baseline studies for *Salmonella* prevalence on broilers should allow for discrimination between controllable and non-controllable factors affecting the prevalence and/or cell numbers including:

- **Pre-slaughter practices**
- **Regionality**
- **Seasonality**
- **Climatic variations**
- **Line speeds**
- **Volume of production**
- **In-plant interventions for reduction of *Salmonell*a (e.g., washing, antimicrobial treatments, etc.)" (Pages 10, 11)**

Collect the following information

- **Date of slaughter**
- **Date of sampling**
- **Type of establishment and production volume**
- **Location of facility**
- **Location within establishment where the samples are collected**
- **Types of interventions applied**
- **Sample transportation and holding conditions prior to analysis**
- **Also refers to above list from Page 10. (Page 15)**

During the study design process, many factors that may impact the microbiological profile of broiler carcasses were considered. Essential information that is required to achieve the primary objectives of the study were included on the sample request form.

12. Understand product manufacturing steps and their effect on quantitative data. (Page 16)

A complete description of these steps and intensive sampling at multiple points in each selected establishment is beyond the scope of this study.

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[2] Cell number refers to a quantitative count for bacteria.

D. Recommendations Concerning Sample Collection and Laboratory Analyses

13. Examine for index organisms and other pathogens in addition to *Salmonella.* (Page 12)

This baseline study will include analyses for the identification and enumeration of *Salmonella* (including serotyping), *Campylobacter*, generic *E. coli*, coliforms and *Enterobacteriaceae* (in addition to Aerobic Plate Counts (APC)).

14. Standardize and validate methods for sample collection, shipment and laboratory analyses to ensure consistency; Document appropriate implementation in the field. (Page 15)

FSIS Notice 60-06 includes specific instructions concerning the sampling, sample collection, and shipping procedures to be used during this study.

A single contract laboratory will be performing microbiological analyses for this study as described by FSIS. The laboratory method used to culture *Campylobacter* is based on the draft report of the NACMCF Subcommittee on the Analytical Utility of *Campylobacter* Methodologies. Training on this method has been provided to contract laboratory personnel. A validation study concerning this method is being conducted prior to full implementation of the study.

15. Conduct operational readiness reviews prior to full implementation. (Page 16)

Historically, FSIS Nationwide Baseline Data Collection Programs have included a 90-day training period (internally known as the “shakedown period”) prior to full implementation of each study. One purpose of this training period is to ensure that personnel involved in the program are familiar with the sample collection, shipping, analytical, and reporting procedures for each baseline study. A similar training period is planned for this upcoming baseline study.

E. Recommendations Proposing Ongoing Sampling be Conducted

16. Establish a statistically-based sampling plan for ongoing yearly measurements of change; Consider enumeration of *Salmonella* and other enteric pathogens for some of the samples in its verification sampling and testing program. (Pages 7, 12)

The establishment and implementation of an ongoing surveillance program for foodborne pathogens (either in conjunction with or separate from verification sampling) is beyond the scope of this baseline study.

Attachment 3: Expected Precision for Prevalence Estimation in the FSIS Nationwide Young Chicken Microbiological Baseline Data Collection Program

As for all surveys, three criteria were considered during the design and sample allocation process for this study: accuracy and precision of the desired estimate; cost of the study; and feasibility of the execution of the design (*3*). The sample allocation plan for this study design is expected to permit a precision of between ±2 and ±5% for the estimation of pathogen prevalence levels in post-chill carcasses.

The complexity of the proposed study design makes it difficult to perform traditional *a priori* precision and/or power calculations. The primary objectives and secondary aims for this study identify many estimates and comparisons of interest -- each with a unique power and/or sample size requirement. Preliminary data regarding the expected within- and between-establishment variance for the prevalence of *Salmonella* and *Campylobacter* and the quantitative levels of indicator organisms on broiler carcasses, which is necessary to inform such calculations, is limited. Finally, FSIS is not aware of commercially available software that is capable of performing sample size and/or power calculations for study designs that incorporate all of the features of the proposed design concurrently.

Consequently, we will demonstrate the precision we expect to achieve for the estimates of pathogen prevalence on post-chill carcasses. Prevalence estimation is a primary objective for this baseline study. Further, the sample size required to achieve a desired level of precision is typically more conservative for prevalence estimation than for the estimation of the quantitative count of a selected pathogen. Thus, the precision that would be expected when estimating both *Salmonella* prevalence and *Campylobacter* prevalence in post-chill broiler carcass rinses was explored for seven sample allocation options under four study design scenarios.

Here, we consider four study design scenarios: simple random sampling (SRS), stratified random sampling (STRS), longitudinal sampling (LS) assuming a moderate within-establishment correlation ($\rho = 0.25$), and longitudinal sampling (LS) assuming a strong within-establishment correlation ($\rho = 0.50$). For each scenario, we evaluate seven possible sample allocations (i.e., sample sizes) for a range of expected pathogen prevalence values (Table 1). By reducing the complex study design to its simplest form and stating certain statistical assumptions, we can provide “rough insight” concerning the

statistical efficiency of the alternative study designs and sample allocation schemes considered for this baseline study.

It is important to recognize that these calculations are performed for exploratory purposes only. There is a lack of available data concerning the expected within- and between-establishment variance for the prevalence of *Salmonella* and *Campylobacter* and the quantitative levels of indicator organisms on broiler carcasses. Thus, the following results can not definitively predict the precision that will be achieved by the proposed design.

When calculating the expected standard errors and precision for each alternative, we made the following assumptions:

- 8.3 billion head of broilers are slaughtered annually (based on FY2005 data).
- The normal approximation to the binomial distribution is appropriate.
- A 95% confidence level was desired for the prevalence estimates ($z_{1-(a/2)} = 1.96$).

The standard error (SE) of the prevalence estimate was calculated for each design scenario as follows:

- Simple random sample (SRS)
  $SE = SQRT((N-n)/N) \times SQRT((p(1-p))/(n-1))$ Equation 3.11 (*3*)
- Stratified random sample (STRS)
  $SE = SQRT\{ \sum^{L}_{h=1}((N_h^*/N)^2) \times ((p_h(1-p_h))/(n_h-1)) \times ((N_h^*-n_h)/N_h^*))\}$
  Equations 5.8 and 6.3 (*3*)
- Longitudinal sample (LS)
  $SE = SQRT(((p^*(1-p))/N)^*(1+(s^2/n_a+(n_a-1))^*\rho))$ Equation 15.3 (*1*)

Where N = number of head slaughtered annually; n = the number of post-chill rinses analyzed; p = the expected prevalence; L=the number of stratum; N_h*= the number of head slaughtered annually in stratum h; p_h= the expected prevalence in stratum h; n_h= number of post-chill rinses in stratum h; n_a=mean cluster size (i.e., mean number of samples per establishment); s= variance of cluster size; and ρ=intraclass correlation coefficient = 0.25 or 0.5 depending on the scenario. SQRT signifies the square root function.

The precision was calculated as: $\pm z_{1-(a/2)} *SE.$

Table 1: Combinations of simplified design scenarios and sample allocations evaluated while exploring the statistical efficiency and precision of alternative study designs.

<table>
<tr><th>Sample Allocation Option (number of post-chill samples; number of establishments)</th><th>Simple Random Sample Design</th><th>Stratified Random Sample Design</th><th>Longitudinal Design (ρ=0.25)</th><th>Longitudinal Design (ρ=0.50)</th></tr>
<tr><td>Option 1
(840 samples;
60 establishments)</td><td rowspan="8">No additional assumptions</td><td rowspan="4">Assumes that there are three strata where L=1 represents 23% of the population, L=2 represents 72% of the population, and L=3 represents 5% of the population; Assumes that prevalence increases from Stratum 1 to Stratum 3 by 5% increments</td><td rowspan="8">Establishment is assumed to be the cluster; Cluster size is the number of samples to be requested within an establishment; Moderate correlation coefficient assumed</td><td rowspan="8">Establishment is assumed to be the cluster; Cluster size is the number of samples to be requested within an establishment; Strong correlation coefficient assumed</td></tr>
<tr><td>Option 2
(1200 samples;
60 establishments)</td></tr>
<tr><td>Option 3
(1680 samples;
80 establishments)</td></tr>
<tr><td>Option 4
(2160 samples;
80 establishments)</td></tr>
<tr><td>Option 5
(2322 samples;
198 establishments)</td><td rowspan="4">Not Applicable</td></tr>
<tr><td>Option 6 (4140 samples;
198 establishments)</td></tr>
<tr><td>Option 7 (4644 samples;
198 establishments)</td></tr>
</table>

The design effect (DEFF) compares the expected variance of a study design scenario to the variance that would be obtained from a simple random sample (SRS), where the variance was calculated as SE^2.

$$DEFF = Variance_{\text{Alternative design}} / Variance_{\text{Simple random sampling}}$$

Design effects equal to 1 indicate that the alternative sampling design is as statistically efficient as SRS; design effects <1 indicate the alternative design is more efficient than SRS; and design effects >1 indicate that the alternative design is less efficient than SRS (*2*).

Figures 1 and 2 illustrate the expected precision as calculated for each applicable allocation option * study design scenario combination assuming a 10% expected prevalence for *Salmonella* and a 65% expected prevalence for *Campylobacter*, respectively. These figures suggest that sampling in all establishments that meet the eligibility criteria is expected to result in greater precision than randomly selecting establishments from those that meet the eligibility requirements to participate in the study. Additionally, they demonstrate the loss in precision that is expected when introducing the effect of clustering into the study design (as occurs with the longitudinal sampling design scenarios). Finally, these figures suggest that increasing the sample size beyond that proposed for Option 5 (n=2,322 post-chill carcass rinses) is not expected to yield significant gains in the expected precision of these prevalence estimates. Similar trends were observed when the expected precision was calculated for other prevalence levels, but only these two figures are provided as examples.

The sample allocation that is proposed for this study is best represented as Option 5 in these tables and figures. Our selected study design and sample allocation is expected to permit a precision of between ±2 and ±5% for the estimation of pathogen prevalence levels in post-chill carcasses. Table 3 presents the expected precision for this proposed sample allocation as calculated for longitudinal sampling assuming a 10% expected prevalence for *Salmonella* and a 65% expected prevalence for *Campylobacter*. Additionally, this proposed allocation plan is expected to result in precision similar to that expected for

several alternative risk-based sampling plans where risk is defined according to production volume. These additional data are not shown.

Figure 1. Expected Precision for Post-Chill Broiler Carcass when *Salmonella* Prevalence is 10%.

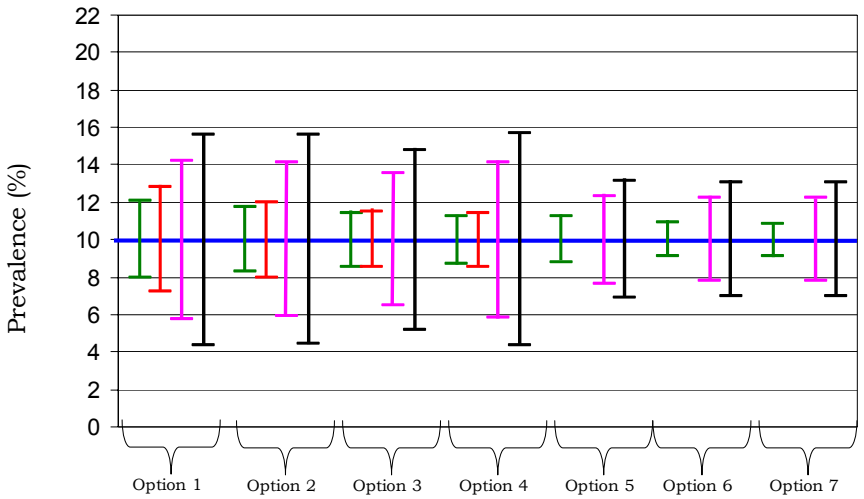
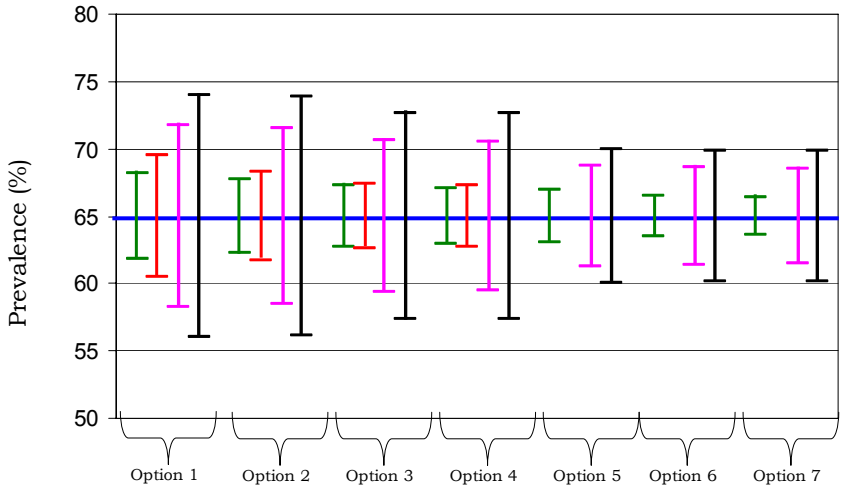


Figure 2. Expected Precision for Post-Chill Broiler Carcass when *Campylobacter* Prevalence is 65%.



Legend for Figures

__ Simple Random Sampling (SRS)
__ Stratified Random Sampling (STRS)
__ Longitudinal Sampling (ρ = 0.25)
__ Longitudinal Sampling (ρ = 0.50)

Table 3: Expected Precision and Design Effect for the Estimation of Pathogen Prevalence in Post-Chill Broiler Carcass Rinses Using Three Study Design Scenarios.

Study Design Scenario	Precision (%) for estimation of *Salmonella* prevalence (10% prevalence expected)	Precision (%) for estimation of *Campylobacter* prevalence (65% prevalence expected)	Design Effect
Simple Random Sampling	±1.22	±1.94	Reference
Longitudinal Sampling (ρ= 0.25)	±2.35	±3.74	1.9
Longitudinal Sampling (ρ= 0.50)	±3.10	±4.92	2.5

References

1. Fleiss, J., B. Levin, and M. Paik. 2003. Statistical Methods for Rates and Proportions, Third ed. John Wiley and Sons, Inc, Hoboken.
2. Lehtonen, R., and E. Pahkinen. 2004. Practical Methods for Design and Analysis of Complex Surveys, Second ed. John Wiley and Sons, Ltd, Chichester.
3. Levy, P., and S. Lemeshow. 1999. Sampling of Populations: Methods and Applications, Third ed. John Wiley and Sons, Inc, New York.

Attachment 4: Internal USDA Technical Consultation: Charge to Reviewers and Evaluation Criteria

Charge to Reviewers:

FSIS requested an internal (within USDA) technical consultation concerning the statistical and scientific validity of the Agency's intended approach for conducting the upcoming Nationwide Young Chicken Microbiological Baseline Data Collection Program.

Reviewers provided a written report that described the findings and conclusions of their review with respect to each of the following evaluation criteria. Reviewers also suggested alternative approaches and/or solutions for areas that they criticized.

Evaluation Criteria:

In addition to evaluating the overall study design, FSIS is requesting a review of the following specific issues:

- Are the objectives and secondary aims clearly defined? Can they be achieved by the proposed study design?
- Is the sampling frame adequate?
- Is the process for the selection of establishments and broiler carcasses for inclusion in the study population clear? Is this approach likely to yield the data required to meet the stated objectives and aims?
- Will the information to be collected at the time of sample collection allow the stated objectives and goals to be achieved?
- Is sufficient detail regarding the estimated precision and the statistical analysis of the data provided to evaluate the study design? If not, what additional information should be provided at this stage of study design?
- Does FSIS adequately respond to recommendations from the National Advisory Committee on Microbiological Criteria for Foods (NACMCF)?

Internal USDA Reviewers:

- Supervisory Microbiologist, Agricultural Research Service
- Statistician, National Agricultural Statistical Service
- Food Safety Program Leader, Cooperative State Research, Education and Extension Service

Attachment 5: Internal USDA Technical Consultation: Reviewers' Comments and the FSIS Response

Here, we paraphrase the reviewers' recommendations, describe how each recommendation was incorporated into the revised study design, and provide the rationale/justification for this decision.

1. **Regarding secondary objective #1, the proposal never explains (except in a response to one of the NACMCF recommendations) why these two particular points in the processing chain (re-hang and post-chill) were selected for sampling.**

   To address this concern, we have revised this objective to read, *"1. Compare the count and prevalence for selected bacteria between re-hang and post-chill broiler carcasses to assess the effect of the slaughter process on microbiological contamination."* Additionally, we have added text that more fully describes our interpretation of the microbiological profile at each of these points in the slaughter process. Re-hang samples *"are believed to be representative of the potential bacterial contamination during the poultry slaughter process, and will serve as a proxy for the pre-harvest microbiological profile."* Post-chill samples *"represent the microbiological profile at the conclusion of the slaughter process, prior to further processing."*

2. **Regarding secondary objective #2, the phrase "multiple bacteria" is not clear in this context. Different phrasing for the sake of clarity is desirable here.**

   This objective was revised to read, *"2. Compare the counts and prevalences of the selected bacteria in a pair-wise fashion to identify important relationships among pathogens and indicator organisms."*

3. **As I see it, the sampling design is a variant of stratified random sampling.**

   Our description of this study design is based on the definition of cluster sampling as stated by Levy and Lemeshow. To paraphrase, cluster sampling occurs when the sampling plan uses a sampling frame consisting of clusters of individual enumeration units and sample selection occurs in a step-wise fashion. Specifically, the sampling frame used for this study identified establishments that slaughtered at least 100,000 head of young chickens in FY2005. It is not possible to enumerate the individual carcasses that will be produced on future dates. Thus, we assign the week and shift (where appropriate) for sample collection within selected establishments. Finally, individual carcasses are selected according to a randomly selected time during which eligible carcasses will be available. We believe the nature of the sampling frame and the step-wise approach to selecting individual carcasses for rinsing is best described as a clustered sampling design.

4. **The mechanism for selection of carcasses for sampling at re-hang and post-chill is unclear. Some protocols need to be set up to be sure that bias doesn't creep into the selection process.**

   This section has been revised as follows to provide greater detail regarding instructions for selection of carcasses for this study:

   "Selection of Broiler Carcasses. *At each sampling event in an establishment, a pair of broiler carcasses will be selected: one broiler carcass will be selected at re-hang and a second broiler carcass, representing the same grow-out flock/house, will be selected at post-chill.*

   Instructions for carcass selection are modeled after those described for the Salmonella testing that supports the Pathogen Reduction/HACCP Regulation. Section Three of this document instructs inspection personnel on the random selection of chicken carcasses using either random number tables, drawing cards, computer- or calculator-generated random numbers, or other methods as previously trained by the Agency. After randomly selecting a chiller for carcass selection (if more than one is in operation), the inspector is instructed to "randomly select a time from (the times that carcasses will be on hand) for collecting the sample….At the random time selected, go to the predetermined point for carcass selection. Count back or ahead 5 carcasses and select the next carcass for sampling."

   FSIS Notice 60-06 provides carcass selection instructions for this specific to this baseline study. Changes indicated in this Notice include, "Sample from the specified production shift. Sample pairs must be selected from the SAME GROW OUT FLOCK/HOUSE. Sample only "BROILERS" for this baseline study.""

   Note: A draft of the FSIS Notice that provides specific instructions concerning the selection of carcasses for this study was unavailable at the time this proposal was distributed for internal review.

5. **How does FSIS intend to summarize the data?**

   We agree that greater detail should have been provided concerning our intended analytical approach. For future study design proposals, we intend to provide a greater level of detail to peer reviewers.

   This section for the current study design has been revised as follows:

   "A design-based approach (as implemented in a commercially available statistical software package developed for complex surveys) will be used to analyze the microbiological data obtained during this study. This approach will account for complexities associated with the sampling design and incorporate auxiliary information necessary to estimate the prevalence and level of the selected bacteria of interest for broiler carcasses at both re-hang and post-chill, a primary objective of this study. Specifically, we plan to weight results according to the number of individual carcasses each represents in order to estimate the prevalence or level. Variance estimation for these outcomes will incorporate information about the sampling design (e.g., clustering, effects of non-response, etc.) as well as utilize techniques such as post-stratification according to production volume category. The

design effect will be calculated to allow a comparison of the efficiency of parameter estimation achieved by the complex design that was implemented relative to simple random sampling.

To address the secondary aims of this study, a series of multilevel (hierarchical) regression models (both univariate and multivariate) that account for the effects of correlated (i.e., clustered) outcomes will be constructed. These models will be used to: compare the prevalence and count of selected bacteria on broiler carcasses selected at re-hang relative to those collected at post-chill; compare the counts and prevalences of the selected bacteria in a pair-wise fashion to identify important relationships among pathogens and indicator organisms; estimate the effect of geographic region, season, and production shift on both prevalence and count. Additionally, these models will permit the deconstruction of the observed variance of these outcomes so that variation can be assigned to the establishment-level, the flock-level, or the carcass-level."

6. I do not think you are able to compare this baseline study with previous studies since the populations and design are different.

The identification of temporal changes since the implementation of the PR/HACCP Final Rule (especially for *Salmonella* prevalence) is of great interest to the Agency. However, we agree that there are several limitations associated with comparing the prevalence estimates obtained from this upcoming baseline study to those of previous baseline studies. Because of our concerns, we specifically included the terms *"where possible"* and *"where appropriate"* in objective 3 to acknowledge that these comparisons may not be appropriate.

As for any evaluation of temporal trends, we plan to identify differences in study design and microbiological methods and assess the impact of these factors on the interpretation of results from these comparisons. The discussion of these limitations would be a necessary component of reporting of these results.

7. Does this study really provide a quantitative level of the foodborne organisms? Are you doing MPN? Or another quantitative measure?

Although the evaluation of the microbiological methods to be used for this baseline study was outside the scope of this review, we do recognize the impact of these methods on the data to be obtained during the course of this study. Variability and uncertainty are inherent in any microbiological method. However, the microbiological methods used for this study will permit us to estimate a quantitative level for the selected bacteria and to describe the variability associated with these estimates.

Briefly, AOAC-approved methods will be used to enumerate selected bacteria during this study. 3M™ Petrifilm™ Plates will be used to enumerate generic *Escherichia coli*, total aerobic bacteria, *Enterobacteriaceae*, and coliforms. To enumerate *Salmonella,* the validated Most Probable Number (MPN) method as described in "4.03. Isolation And Identification Of *Salmonella* From Meat, Poultry And Egg Products" in the FSIS Microbiology Laboratory Guidebook will be conducted (http://www.fsis.usda.gov/PDF/MLG_4_03.pdf). Although currently there is not an AOAC-approved method for the enumeration of *Campylobacter* species, a direct plating technique will facilitate quantitation in this study.

8. **Why are you only doing the antimicrobial resistance pattern in Salmonella and not in *Campylobacter*? Antimicrobial resistance issues are a significant problem in *Campylobacter* (e.g. fluoroquinolone).**

   We are aware of the importance of antimicrobial resistance in *Campylobacter* species. We do not plan to conduct these analyses during the course of the study. However, the repository of *Campylobacter* isolates collected during this study could permit this additional characterization in the future.

9. **How does the sampling schemes take into effect the "time in shift" or position in line of the bird?"**

   We do not plan to collect information concerning either the time of sample collection within a shift or the position/order of the carcass on the line.

10. **The description involving the categories and production volume is very good, but the justification for the difference in sampling frequencies (e.g. 2 vs 1) is unclear. Is it just economics?**

    The available personnel and financial resources were an important consideration when determining the design and sample allocation plan for this baseline study. However, the total number of head slaughtered within an establishment also influenced our decision to sample more frequently in establishments in Production Volume Category 1 relative to Category 2. In addition to slaughtering a greater total number of head in FY2005, establishments in Production Volume Category 1 are more homogeneous with respect to the number of head slaughtered by month and shift than those in Category 2. Thus, we believe that it will be feasible to conduct twice monthly sampling in Category 1 establishments. Variability in the production volume (and possibly product availability) may not facilitate this frequency of testing in all Category 2 establishments. Additionally, the crude sampling weight (total head $\text{slaughtered}_{\text{category}}$/total samples $\text{allocated}_{\text{category}}$) is similar between these categories.

    The paragraph that describes the rationale for the sampling frequencies was revised to read,

    "Several factors were considered when determining the sampling intervals for this study. Available personnel and financial resources were an important consideration. Sample request forms will specify the week during which the carcass rinses should be collected. Inspection personnel will select the day (Monday-Friday) on which the rinses will be collected based on workload. By specifying the week of rinse collection, we control the laboratory workload while ensuring that a minimum number of carcass rinses are analyzed per month. An additional consideration was the differences in the production volume among the categories. The highest production volume category is sampled with the highest frequency. This approach considers the availability of broiler carcasses, the workload of in-plant personnel, and issues associated with statistical weighting of samples."

11. Although you sample a pair from the same flock/house (this is good), it would be useful to follow ONE bird from re-hang to post-chill.

We agree. Repeated microbiological testing on the same carcass could be a more definitive measure of process control. However, there are several limitations to this approach. It was believed to be too restrictive for inspection personnel to implement given their other regulatory activities. Secondly, rinsing a carcass at re-hang would alter the microbiological profile (perhaps both prevalence and level) of that individual carcass. Obtaining a lower count at post-chill may be an effect of the previous rinsing rather than an effect of the process. It would not be possible to attribute this effect without also collecting a "control" sample from the same grow out flock/house that was not rinsed at re-hang for analysis. Available personnel and financial resources do not permit this additional testing. Thus, we believe that collecting rinses at re-hang and post-chill on different carcasses originating from the same flock/house will allow us to evaluate the effectiveness of the slaughter process in reducing the counts of selected bacteria and the flock-to-flock variability associated with these counts.

12. The term systematic allocation (to shift) is unclear.

We removed the word "systematic" in the section heading, and focus on the concept of alternating shifts in the remainder of the section. We also added an example in order to describe this process more clearly.

This section has been revised as follows:

*"**Sampling by Production Shift.** For Production Volume Categories 1 and 2, sample collection forms will specify the shift during which a sample is to be collected. (Establishments in Production Volume Category 3 typically have a single production shift.) Production shifts will be defined to be consistent with data entry for shift slaughter totals in eADRS.*

After randomly assigning the shift for collection of the first sample in an establishment, subsequent sample requests will alternate between shifts. For example, an establishment might receive a series of sample collection forms that specify the shifts as follows: 1st form specifies Shift 2, 2nd form specifies Shift 1, 3rd form specifies Shift 2, 4th form specifies Shift 1, etc. Specifying the shift for sample collection and alternating the sample requests between shifts will ensure that a minimum number of carcass rinses are collected per shift."

13. The questionnaire/form to collect data should be included as an appendix to the proposal.

We agree. The sample request forms were unavailable at the time this proposal was distributed for internal review. For future study design proposals, we plan to include a "mock-up" of the form(s) to be used in the study. Alternatively, we may provide the wording for questions in future study design proposals.

FSIS Notice 60-06 contains a detailed description of the questions included on these forms.

14. “Randomly selected carcasses within establishments”- Is this done with a random numbers table? Is there consideration of the carcass position in the line or production shift? This is still confusing.

Based on concerns regarding carcass selection expressed by two reviewers, we have made considerable revisions to this section as previously described. As previously indicated, we do not plan to collect information concerning either the time of sample collection within a shift or the position/order of the carcass on the line.