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Nationwide Microbiological Baseline Data Collection Program for the Raw Ground Beef Component: Domestic Beef Trimmings

December 2005 – January 2007

FOREWORD

This publication is a compilation of data obtained from the Nationwide Microbiological Baseline Data Collection Program for Domestic Beef Trimmings for the thirteen months from December 2005 – January 2007. The program was designed and performed by the Food Safety and Inspection Service (FSIS) to estimate the prevalence of microbiological pathogens and indicator bacteria in beef trim destined to become raw ground beef. The design and implementation of this study was the result of the contribution of many offices and staff members from FSIS in the United States Department of Agriculture. The Microbiological Analysis and Data Branch, Division of Microbiology, Office of Public Health Science conducted this study and prepared this report. The microbiological analyses for this study were conducted by the Field Services Laboratories of FSIS and by the contract laboratory Food Safety Net Service, Ltd., San Antonio, TX. The collection of samples was the responsibility of the FSIS Office of Field Operations (OFO).

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EXECUTIVE SUMMARY

From December 2005 to January 2007, trim and subprimal samples were collected at establishments operating under Federal Inspection. These samples were analyzed to estimate the percent positive and levels of *Salmonella*, generic *Escherichia coli*, Aerobic Plate Count (APC), *Enterobacteriaceae*, total coliforms, and *Escherichia coli* O157:H7. The sampling frame included 250 establishments that slaughtered steers/heifers, cows/bulls, and calves under Federal Inspection and produced trim for use in raw ground beef production. Two sets of trim samples were collected at each sampling event. One set was analyzed at the FSIS Field Services Laboratories for the presence and levels of *E*. *coli* O157:H7. The other set was analyzed at a contract laboratory for the presence and levels of the other listed bacterial targets. This report provides an overview of this baseline study and the microbiological data results derived from domestic beef trimmings sampled during December 2005 through January 2007. The Agency is also conducting a statistical analysis to estimate the National Prevalence for *E. coli* O157:H7 and *Salmonella* in domestic beef trimmings used in raw ground beef production.

INTRODUCTION

The Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) is responsible for the enforcement of the Federal Meat Inspection Act, the Poultry Products Inspection Act and the Egg Products Inspection Act. These Acts empower the Agency to appraise establishments for evidence of unhygienic conditions, to inspect raw and final products for evidence of adulteration, and to review records for the adherence to Hazard Analysis and Critical Control Point (HACCP) principles. In addition, using provisions cited under these Acts, the Secretary of Agriculture is authorized to promote special assessments, such as baseline studies, to determine the presence and number (qualitative and quantitative levels) of pathogens and indicator bacteria in raw products. In contrast to the risk-based format of FSIS HACCP verification programs, baseline studies are statistically designed to assess the industry as a whole by weighting sampling of each establishment according to their relative production volume. Data collected during baseline studies is essential for meeting the mission-critical needs of trend analysis, performance criteria, and risk assessments. Because the data is weighted by production volume, quantitative pathogen data from trim baseline studies provide the scientific basis

for exposure assessment. This is a critical component of risk assessment, establishing microbiological criteria or standards, assessing trim production parameters, and assessing the seasonal and regional variability in prevalence and levels of pathogen and indicator bacteria. Baseline studies are performed outside of the normal regulatory activities of FSIS. However, the Nationwide Microbiological Baseline Data Collection Program for the Raw Ground Beef Component: Domestic Beef Trimmings did include a regulatory component due to the testing for *E. coli* O157:H7. This pathogen is considered an adulterant when found in certain products and a regulatory action by FSIS is thus warranted.

FSIS took several steps to improve the quality of this study:

1. Implemented the use of the "Baseline Study Questions"mailbox where OFO inspection program personnel (IPP) could submit questions about the study.

2. Provided IPP with a training CD titled "Nationwide Raw Ground Beef Component Microbiological Baseline Data Collection Program Trim & Subprimal Sample Collection Training."

3. Used formal FSIS Notices to provide IPP information about the study and instructions for sampling.

4. After the 90-day training phase of the project, it was determined that IPP were not collecting samples as instructed in the FSIS Notice. In response, necessary adjustments were made before the actual study began in December 2005.

5. When an establishment reported that the requested trim product was not produced, FSIS substituted that establishment with a different establishment from the same production category when possible for the remainder of the study. In categories where all establishments were initially included in the study such substitutions could not be done. Production categories were based on the size of the beef plant and total head slaughtered during a twelve month timeframe (see Appendix 1).

OBJECTIVES

This baseline study had three primary objectives:

1. To collect microbiological data from trim samples in order to determine the presence and levels of specific microbiological targets, including the

important human pathogen *E. coli* O157:H7, and to establish valid prevalence and level estimates from which to measure change over time.

2. To provide data for use in risk assessments, which inform risk management decisions.

3. In addition to the formal risk analysis process, baselines provide public health information to the agency that can be used as guidance when new regulatory programs are being designed.

Program Design Relative to Objectives

The Nationwide Microbiological Baseline Data Collection Program for the Raw Ground Beef Component: Domestic Beef Trimmings establishes the first assessment of the presence and levels of the bacterial targets in trim and subprimals used in the manufacture of raw ground beef. The analytical results of *Salmonella*, *E. coli* O157:H7 and indicator bacteria are expressed as the percentage of positive samples for each bacterial target and as colony forming units (cfu) per gram of trim. The national prevalence estimates for beef trim will be reported in a separate document.

PROGRAM DESIGN

Establishments Included in the Sampling Frame

The sampling frame was derived from a list of plants that reported producing trim and subprimals during a survey conducted by FSIS in 2003. The sample frame originally contained approximately 250 establishments that slaughtered and fabricated carcasses into trim available for use in ground beef production, including those from the large, small and very small plant categories. The frame was designed so that smaller plants were sampled more often (in relation to their production volume) than larger plants (see Appendix 1) as it was hypothesized that smaller plants contributed disproportionately to the incidence of *E. coli* O157:H7 in raw ground beef. The baseline study did not include samples obtained from head meat, organ meat, Advanced Meat Recovery product, or trimmings destined for such products as finely textured beef or partially defatted chopped beef. The term "beef trimmings" included subprimal cuts such as boneless chuck or parts of boneless chuck that are frequently used as components of raw ground beef. The key to whether or not a specific subprimal was included in the beef trimmings was how it was produced and handled in the establishment. Thus, if combo bins of boneless chuck were processed and tested as trimmings, they were to be counted as trimmings.

As discussed, this baseline study included beef trim produced at federally inspected[1] establishments that slaughter cattle and bone out carcasses to produce various parts of carcasses including trimmings that are the primary component of raw ground beef. FSIS was aware that there are processing facilities that purchase parts of carcasses and produce trimmings during the normal course of cutting beef into steaks and roasts for retail and institutional markets. Trimmings are also produced at retail stores and food service facilities.

Deciding where to collect samples was a complex decision. Ideally, the study would cover all trimmings used for raw ground beef production. However, a key element in the design of a microbial baseline is to ensure that samples have been produced and handled in the same manner. Some of the "downstream" trimmings are produced days or even weeks after the carcass was initially fabricated. Without knowing age and temperature history there was no way to account for microbial growth. Therefore, this study focused on the large volume of trimmings produced directly after carcass chilling. All samples were shipped the day they were collected. This helped ensure that all samples had consistent time and temperature histories when they arrived at the laboratory for analysis.

Sample Design

The Nationwide Microbiological Baseline Data Collection Program for Domestic Beef Trimmings analyzed beef trim samples from federally inspected establishments that slaughter and fabricate carcasses into trim available for use in ground beef production. Many factors were considered in the design of the sampling program. Among these were the size and variability of the target sample population, the nature and number of microorganisms to be investigated, the practicality and limitations of sampling, and the specific data to be collected.

Both sampling and non-sampling errors can affect the reliability of results and, thus, had to be considered in designing this program. Sampling errors occur because observations are derived from a portion rather than from the entire population; non-sampling errors can be attributed to many sources inherent in the collection of samples, laboratory analysis, and processing of data. Both types of errors were considered in determining the sample size.

In August 2003, over 300 questionnaires on volume and production details of raw ground beef components were sent to inspection program personnel at slaughter and breaking establishments. This data, in conjunction with fiscal year 2003 slaughter totals from trim production were used in the development of the sampling design of this baseline study.

[1] The study did not include trimmings produced at State inspected facilities or custom exempt facilities that both slaughter cattle and fabricate carcasses.

For FY 2004, Congress appropriated funds for FSIS to initiate a program of recurring baseline studies that would be conducted by a contract laboratory. During 2004, FSIS awarded a contract to Food Safety Net Services, Ltd., San Antonio, Texas. The beef trim baseline study would be the first baseline study conducted using a contract laboratory. For this study, Food Safety Net Services performed all associated microbiological analyses, with the exception of *E. coli* O157:H7 tests. Since a positive *E. coli* O157:H7 test in beef trim for use in raw ground beef had regulatory consequences, these tests were performed at the three FSIS laboratories. Using both contract and FSIS laboratories required that two samples had to be collected for each sampling event.

It is essential that baseline studies comprise a sufficient number of samples in order that statistically significant differences can be determined. It was estimated that approximately 2,000 analyzed samples would be required to obtain reasonable levels of precision based on the projected prevalence for the bacterial targets included in this baseline study. To achieve this number, the plants were scheduled to be over-sampled by 50%. The plan was to have valid results from 2,000 samples.

Data Limitation

This program was not designed to provide microbiological information on individual establishments. Smaller plants were sampled more often (in relation to their production volume) than larger plants (see Appendix 1). It was proposed that smaller plants contributed to the incidence of *E. coli* O157:H7 in raw ground beef. However, in order to obtain such information from an individual establishment, a large number of samples should be collected from each establishment over a long period of time.

Sampling Location within the Establishment

The unit to be sampled in this baseline was one lot of trim. Furthermore, to determine the proper trim to be sampled, the establishment had to sort its beef trimmings into either (a) lots acceptable to be used in the manufacture of raw ground beef or (b) lots that could only be used in the manufacture of product with a lethality step. This program only sampled from lots that were available or approved to be used to produce raw ground beef. The establishments were directed to determine what composed one lot of trim. If a plant didn't have a "lotting" program and a testing program, the lot would (most likely) have been all product from a given slaughter shift.

Sample Collection and Description

All samples were collected Monday through Friday and shipped on the same day collected.

During the design phase of the study, FSIS was aware that a wide variety of sample collection methods were currently being used for beef trimmings. These included collecting samples of the purge, collecting a sample using a core drilling device, and several variations of collecting various amounts of surface tissue from containers of trim. FSIS had many discussions with scientists from the Department's Agricultural Research Service and scientists from industry. It was decided to use a sample collection method referred to as N60. The N60 method involves collecting 60 separate thin slices of surface tissue from a production lot. The weight of the 60 slices would be two pounds. Of the methods reviewed, the N60 method was considered to be both (1) feasible at the point of trim production, and (2) have the highest probability of detecting *E. coli* O157:H7 if contamination were present. The N60 sampling concept was based on the International Commission on Microbiological Specifications for Foods (ICMCF) [(1)] Case 15 sampling plan, which was the most robust of all sampling plans recommended by ICMSF.

Two separate samples were obtained from each lot. One 2-pound sample was shipped via Federal Express to the contract laboratory, Food Safety Net Services, Ltd. The project code for this sample was MM45. The second sample was shipped via Federal Express to one of the three FSIS Field Service Laboratories for analysis of the presence and levels of *E. coli* O157:H7. The project code for this sample was MM45R.

Selection of Organisms

The recommendations contained within the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) FINAL REPORT: NACMCF Response to USDA/FSIS Request For Guidance On Baseline Study Design and Evaluations For Raw Ground Beef Components[(2)] were used to guide the selection of microorganisms for analysis. In addition, the analyses of specific indicator organisms were included in this study to examine the possibility of using these bacteria as a measure of process control. The organisms chosen for analysis were *Salmonella*, generic *E. coli*, *Enterobacteriaceae*, total coliforms, and *E. coli* O157:H7. In addition, an Aerobic Plate Count (APC) was performed on each sample.

Analytical Methods

All of the methods of analysis were derived from the FSIS Microbiology Laboratory Guidebook and the Official Methods of Analysis of AOAC International. To analyze the samples for the indicator bacteria, 25 gm of trim was added to 225 ml of buffered peptone water (BPW) and stomached for two minutes. Dilutions from 10^{-1} to 10^{-4} were made and plated onto Petri film to enumerate *Enterobacteriaceae*, generic *E. coli*, total coliforms, and to perform the APC [(3)(4)(5)].

To analyze the samples for *Salmonella*, another 25-gm sample was added to 225 ml of BPW and stomached for two minutes. An aliquot of the homogenate was screened for *Salmonella* using the DuPont BAX system [(6)(7)]. Samples that were screen positive were then analyzed using a 9-tube Most Probable Number (MPN) procedure to estimate the levels of *Salmonella* in the sample [(6)(7)]. *Salmonella* isolates were shipped to the USDA National Veterinary Services Laboratory for serotype determination.

To analyze the trim samples for *E. coli* O157:H7, five individual 65-gm portions were prepared using the methods as outlined in the MLG [(8)]. Samples that were BAX screen positive were reported as potential positive samples and were further analyzed per MLG 5. For enumeration by MPN, the procedure is described below:

Enumeration of *E. coli* O157:H7 in Trim by MPN

1. Aseptically weigh out a 325-gm test portion from the potential positive sample into a sterile Stomacher bag.

2. Add 650 ml of mEC+n Broth, pre-warmed as per MLG 5 for this and all subsequent dilutions, and stomach for two minutes.

3. Transfer 30 ml into each of 3 sterile bottles or bags to represent a 10-gm sample dilution.

4. Transfer 3 ml into each of 3 sterile tubes containing 7 ml of mEC+n Broth to represent a 1-gm sample dilution.

5. Transfer 0.3 ml into each of 3 sterile tubes containing 9.7 ml of mEC+n Broth to represent a 0.1-gm sample dilution.

6. The above steps provide for a 9-tube 3-dilution MPN (i.e., 9 individual dilutions for each potentially positive sample).

7. Analyze each dilution per MLG 5A and MLG 5.

8. MPN dilutions that screen negative will be recorded as negative.

9. MPN dilutions that screen positive will be confirmed using MLG 5.

10. Interpret the pattern of confirmed positive dilutions according to the MPN table for 10-1-0.1 gm analysis [(9)].

RESULTS

Nineteen hundred samples were analyzed for the presence of *E. coli* O157:H7. Thirteen samples were positive, for a percent positive rate of 0.68. This is a raw number and should not be considered as the national prevalence. Of the thirteen positive samples, twelve were enumerated. Six were below the limit of detection (LOD) and the range per gram of the remaining samples above the LOD was 0.036 to 1.5 cfu per gram of trim. The average number of *E. coli* O157:H7 colony forming units (cfu) per gram was 0.56 for the six samples with MPN values.

The relationship of the percent of samples that tested above the LOD for non-target microorganisms was as expected. More than 99% of the samples were above the LOD for Aerobic Plate Count (35ºC) microorganisms. Fifty-nine percent of the samples were above the LOD for *Enterobacteriaceae*. Total coliforms were detected above the LOD 41.9% of the time and generic *E. coli* was detected above the LOD in 15.7% of the samples.

Seventeen hundred nineteen samples were analyzed for the presence of *Salmonella* sp. Of these, 22 tested positive, for a percent positive rate of 1.28. There was an average of 12.6 cfu of *Salmonella* per gram of trim for the 9 samples with MPN values. Thirteen of the 22 positive samples were below the LOD for the MPN assay. For those samples above the LOD, the range was 0.4 to 46 cfu per gram of trim.

The *Salmonella* serotypes isolated most often from trim samples were Cerro (3) and Montevideo (3). *S.* Heidelberg, *S.* Infantis and *S.* Kentucky were each found 2 times. And the remaining *Salmonella* serotypes were each found once: Agona, Bredeney, Dublin, Fresno, Lille, Meleagridis, Oranienburg, Schwarzengrund (4,12), Typhimurium and Typhimurium (4,12:i:-).

DISCUSSION

The Nationwide Microbiological Baseline Data Collection Program for the Raw Ground Beef Component: Domestic Beef Trimmings was designed to determine the presence and the levels of selected bacteria in beef trim and subprimals that were produced in federally inspected plants to produce raw ground beef. Although FSIS has conducted baselines on other beef products and with beef carcasses (Cows & Bulls, Raw Ground Beef, Steers & Heifers; http://www.fsis.usda.gov/Science/Baseline_Data/index.asp), this is the first baseline study that has examined one of the components that make up raw ground beef. Thus, the data reporting the percent positive and levels for *Salmonella*, generic *Escherichia coli*, *Enterobacteriaceae*, coliforms, and *E. coli* O157:H7 and the determination of aerobic bacteria provides new insight as to the microbiological profile of this component of raw ground beef.

This report presents the overall findings of this study. A separate report will describe the estimation of the National Prevalence of *E. coli* O157:H7 and *Salmonella* in trim and subprimals. In addition, information regarding sanitation interventions performed in each plant was collected during this study, so that an association between interventions and the presence and levels of each bacterial pathogen can be determined. Also, data collected on the indicator bacteria will be used to examine the relationship between these bacteria and the effectiveness of process control within each plant.

The presence and levels of the pathogen *E. coli* O157:H7 found in trim and subprimals implies that these components are a source of the pathogen in raw ground beef. The examination of the other four raw ground beef components, as recommended by NACMCF[(2)], will give FSIS a broader picture of the proportion of each component's contribution to *E. coli* O157:H7 in raw ground beef and thus suggest whether additional preventative measures are necessary to control this pathogen from entering the food supply.

FSIS has recently implemented regulatory testing programs for trim and other raw ground beef components. The Agency is currently evaluating the need and priority for additional baseline studies that will analyze the other components.

TABLES

Table 1. Percent Positives of Selected Microorganisms on Raw Ground Beef Components (Trim)

Microorganisms	Sample Positive			
	Samples Analyzed	Number Positive	Percent Positive	SE[3]
Indicator Organisms[1]				
Aerobic Plate Count @ 35°C	1719	1707	99.30	0.20
Enterobacteriaceae	1719	1015	59.05	1.19
Total Coliforms	1719	721	41.94	1.19
Generic *Escherichia coli*	1719	270	15.71	0.88
Pathogenic Organisms[2]				
Escherichia coli O157:H7[4]	1900	13	0.68	0.19
Salmonella	1719	22	1.28	0.27

[1] Equal to or above the detection limit
[2] Qualitative results
[3] Standard Error using binomial distribution
[4] *E. coli* O157:H7 has a higher acceptance temperature for analysis than *Salmonella*

Table 2. Mean Level of Selected Microorganisms per Gram on Raw Ground Beef Components (Trim)

Microorganisms	Number of Samples Quantified	Number of Samples Positive[1]	Level of Positives			
			Log_{10} Mean		Geometric Mean[6,7]	
			Mean	SE	Mean	95% CL
Indicator Organisms[2]						
Aerobic Plate Count @ 35°C	1,719	1,707	4.71	3.79	1,209.45	(1,074.2, 1,361.6)
Enterobacteriaceae	1,719	1,015	3.39	2.98	11.11	(9.88, 12.49)
Total Coliforms	1,719	721	3.2	2.97	5.12	(4.60, 5.69)
Generic *Escherichia coli*	1,719	270	1.9	1.7	1.69	(1.59, 1.80)
Pathogenic Organisms[3]						
Escherichia coli O157:H7	12[4]	6	-0.54	-0.86	0.07	(-)[5]
Salmonella	22	9	0.72	0.39	0.66	(0.9, 30.2)

[1] Positive by quantitative method
[2] Equal to or above the limit of detection.
[3] Mean and range by MPN method
[4] One quantitative positive sample not enumerated
[5] Insufficient numbers of positive results to calculate valid CL

[6] $Geometric\ mean = \mu_g = \exp(\frac{1}{n}\sum_{i=1}^{n}\ln x_i)$, [7] $Geometric\ standard\ deviation = \sigma_g = \exp\{\sqrt{\sum_{i=1}^{n}\frac{(\ln x_i - \ln \mu_g)^2}{n}}\}$, and

$$SE_{geometric} \approx \frac{standard\ deviation\ of\ Log(x_i) \times Geometric\ Mean}{\sqrt{n-1}}$$

Table 3. Distribution of APC @ 35ºC

Range, cfu/g	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
<10[(1)]	12	0.7	12	0.7
10-100	206	12.0	218	12.7
101-1,000	740	43.0	958	55.7
1,001-10,000	443	25.8	1401	81.5
10,001-100,000	210	12.2	1611	93.7
100,001-1,000,000	77	4.5	1688	98.2
1,000,001-10,000,000	31	1.8	1719	100.0
Total	1719	100	-	-

[(1)]Below the level of detection

Table 4. Distribution of *Enterobacteriaceae*

Range, cfu/g	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
<10[(1)]	704	40.95	704	41.0
10-100	731	42.52	1435	83.5
101-1,000	206	11.98	1641	95.5
1,001-10,000	51	2.97	1692	98.4
10,001-100,000	20	1.16	1712	99.6
100,001-1,000,000	6	0.35	1718	99.9
1,000,001-10,000,000	1	0.06	1719	100.0
Total	1719	100	-	-

[(1)]Below the level of detection

Table 5. Distribution of Coliforms

Range, cfu/g	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
<10[(1)]	998	58.06	998	58.1
10-100	543	31.59	1541	89.6
101-1,000	130	7.56	1671	97.2
1,001-10,000	33	1.92	1704	99.1
10,001-100,000	12	0.70	1716	99.8
100,001-1,000,000	2	0.12	1718	99.9
1,000,001-10,000,000	1	0.06	1719	100.0
Total	1719	100	-	-

[(1)]Below the level of detection

Table 6. Distribution of Generic *Escherichia coli*

Range, cfu/g	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
<10[(1)]	1449	84.29	1449	84.3
10-100	239	13.90	1688	98.2
101-1,000	20	1.16	1708	99.4
1,001-10,000	10	0.58	1718	99.9
10,001-100,000	1	0.06	1719	100.0
Total	1719	100	-	-

[(1)]Below the level of detection

Table 7. Distribution of *Escherichia coli* O157:H7

Range, MPN/g	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
<0.03[1]	6	50.0	6	50.0
0.031-0.30	3	25.0	9	75.0
0.31-3.0	3	25.0	12	100.0
Total[2]	12	100	-	-

[1]Below the level of detection

[2]One positive sample not enumerated

Table 8. Distribution of *Salmonella*

Range, MPN/g	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
<0.3[(1)]	13	59.1	13	59.1
0.31-3.0	2	9.1	15	68.2
3.01-30.0	6	27.3	21	95.5
31.0-300.0	1	4.5	22	100.0
Total	22	100	-	-

[(1)]Below the level of detection

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APPENDIX 1

Domestic Trim Baseline Sample Design Modification

The following describes the stratification of the samples based on production volume information obtained during a survey submitted to selected slaughter and breaking establishments.

Stratified Design

Plants in the sampling frame were grouped into four strata, primarily based on plant size, using Fiscal Year 2003 Electronic Animal Disposition Reporting System (eADRS) slaughter data.

Stratum 1 consisted of all beef plants having at least 100,000 head FY03 slaughter (approximately 55 plants). Each plant in this stratum was scheduled to receive two sample requests each month for the duration of the study.

Stratum 2 consisted of beef plants having at least 1,000 but less than 100,000 head FY03 slaughter. Approximately 110 plants were selected from this stratum. Each selected plant in this stratum was scheduled to receive one sample request each month for the duration of the study.

Stratum 3 consisted of all beef plants having at least 100 but less than 1,000 head FY03 slaughter and that were identified in the RGBC survey as producing domestic trim product. A subset of approximately 30 plants was randomly selected from this stratum. Each selected plant in this stratum was scheduled to receive a total of six sample requests during the period of the study.

Stratum 4 consisted of all beef plants having at least 100 but less than 1,000 head FY03 slaughter and that were not included in the RGBC survey. A subset of approximately 30 plants was randomly selected from this stratum. Each selected plant was scheduled to receive a total of six sample requests during the period of the study.

This design resulted in 3000 planned sample requests and provided for samples being collected throughout all seasons of the year and representing all districts.