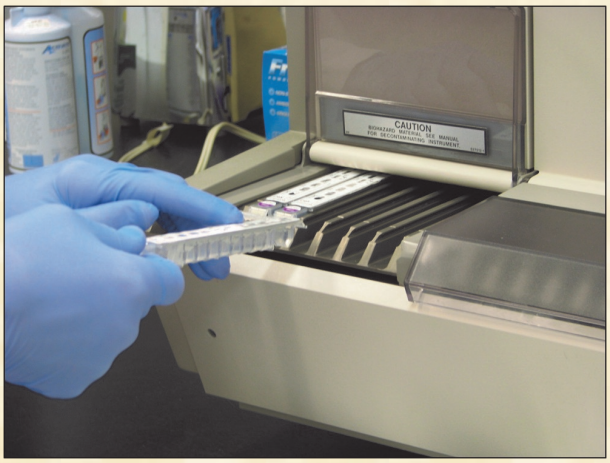
Microbiological Data Program
Progress Update and 2009 Data Summary

United States Department of Agriculture

Agricultural Marketing Service

Science & Technology Programs



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Please Visit Our Website at www.ams.usda.gov/mdp

United States Department of Agriculture

Marketing and Regulatory Programs

Agricultural Marketing Service

1400 Independence Ave.
Washington, DC
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January 2011

To the Reader:

I am pleased to present the USDA Microbiological Data Program (MDP) 2009 Data Summary. In 2009, MDP tested eight commodities (cantaloupe, cilantro, green onions, hot peppers, lettuce, spinach, sprouts, and tomatoes). MDP also performed a special survey of peanut butter to assist the Centers for Disease Control and Prevention and the U.S. Food and Drug Administration during the *Salmonella typhimurium* outbreak investigation.

MDP is a partnership with cooperating State agencies that are responsible for sample collection and analysis. Eleven States participated in the program in 2009: California, Colorado, Florida, Maryland, Michigan, Minnesota, New York, Ohio, Texas, Washington, and Wisconsin. Because together these States represent all regions of the country and more than half the Nation's population, MDP data can be used to develop inferences about the national food supply. With a sampling framework and testing laboratory capability in place, MDP has demonstrated its ability to quickly mobilize and respond to outbreak situations providing data rapidly during local and national outbreaks.

This summary is intended to provide the reader with an overview of data collected in 2009 and summarizes program refinements made during that year. MDP data are important in developing baseline levels of targeted pathogens in the domestic food supply. As a continuous data-gathering program, MDP data can be used to identify microbial trends and to develop risk models.

If you have comments or suggestions on how this summary can be improved, please send electronic-mail to amsmpo.data@ams.usda.gov or visit our Web site at www.ams.usda.gov/mdp.

Sincerely,

Rayne Pegg
Administrator

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Acknowledgements

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State Agencies

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Ohio Department of Agriculture
Texas Department of Agriculture
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Executive Summary

In 2001, the U.S. Department of Agriculture (USDA), Agricultural Marketing Service (AMS) was charged with implementing microbiological testing of fresh fruit and vegetables in the United States. The program's mission is to provide statistically reliable information regarding targeted foodborne pathogens and indicator organisms on fresh fruit and vegetables. The Microbiological Data Program (MDP) is a voluntary data-gathering program, not a regulatory enforcement effort.

AMS coordinates MDP planning and program requirements on a continual basis with the Centers for Disease Control and Prevention (CDC), the U.S. Food and Drug Administration (FDA), and the USDA National Agricultural Statistics Service (NASS). The participating States play a prominent role in program planning, providing sampling and testing support, and technical advice on methods and quality assurance (QA) issues.

MDP collects produce samples from terminal markets and wholesale distribution centers on a year-round basis. The MDP sampling frame is designed to take into account population and consumption on a national scale. In 2009, 11 States collected fruit and vegetable samples (California, Colorado, Florida, Maryland, Michigan, Minnesota, New York, Ohio, Texas, Washington, and Wisconsin).

The program tested eight commodities: cantaloupe, cilantro, green onions, hot peppers, lettuce (conventionally grown and organic), spinach, sprouts (alfalfa or clover), and tomatoes (round and Roma) for non-O157 *Escherichia coli* (*E. coli*) carrying shiga toxin and enterotoxin genes (STEC/ETEC), *E. coli* O157:H7, and *Salmonella*. These samples were also tested for generic *E. coli,* and this testing was discontinued to focus on foodborne pathogens. Per request of CDC, a special survey of peanut butter samples was conducted between February and September in response to the peanut products-related outbreak. Peanut butter samples were screened for the presence of *Salmonella*.

MDP analyzed a total of 16,896 samples during 12 months of sampling and analytical operations. Seventy-four percent of the samples were from domestic sources, 25 percent were imported, and 1 percent was of unspecified origin. MDP identified 51 samples with *E. coli* carrying shiga toxins; however, pathogenic *E. coli* strains were isolated from only 24 samples. These isolates were sent to Pennsylvania State University for further characterization, including serotyping and testing for different virulence-specific genes associated with 13 different categories of pathogenic *E. coli,* and the Ohio Department of Health conducted genomic fingerprinting on these isolates. MDP sample screening for *Salmonella* resulted in 90 preliminary positive samples, and from these, 32 *Salmonella* isolates were reported to the FDA and CDC.

In 2009, three MDP *Salmonella* isolates were matched with past outbreaks. Information from one of the isolates from green onions helped FDA to issue an import alert. In addition, a number of important benefits are being derived from MDP. Coordination with public health agencies has increased, allowing early intervention by regulatory agencies when problem areas are identified, and communication among State and Federal agencies for the reporting and sharing of data on foodborne outbreaks has improved. Microbiological data obtained from MDP's fresh produce screening efforts can be used to enhance the understanding of potential pathogens in fresh fruit and vegetables in the U.S. food supply, permit the identification of long-term trends, and contribute significantly to a national produce microbiological pathogen prevalence baseline. MDP data, which in part reflect changes in

fresh produce group/packaging/shipping practices to meet changing consumer life styles and preferences, will help refine Good Agricultural Practices and Hazard Analysis and Critical Control Points plans used by growers, processors, and food handlers. Furthermore, MDP data, which include antimicrobial resistance, genomic fingerprints, serotypes, and virulence attributes, will assist collaborators such as CDC and FDA in planning public health initiatives and responding to produce-related foodborne outbreaks.

Microbiological Data Program (MDP) Annual Summary, Calendar Year 2009

This summary consists of the following sections: (I.) Introduction, (II.) Sampling, (III.) Laboratory Operations, (IV.) Database Management, (V.) Summary of 2009 Data

I. Introduction

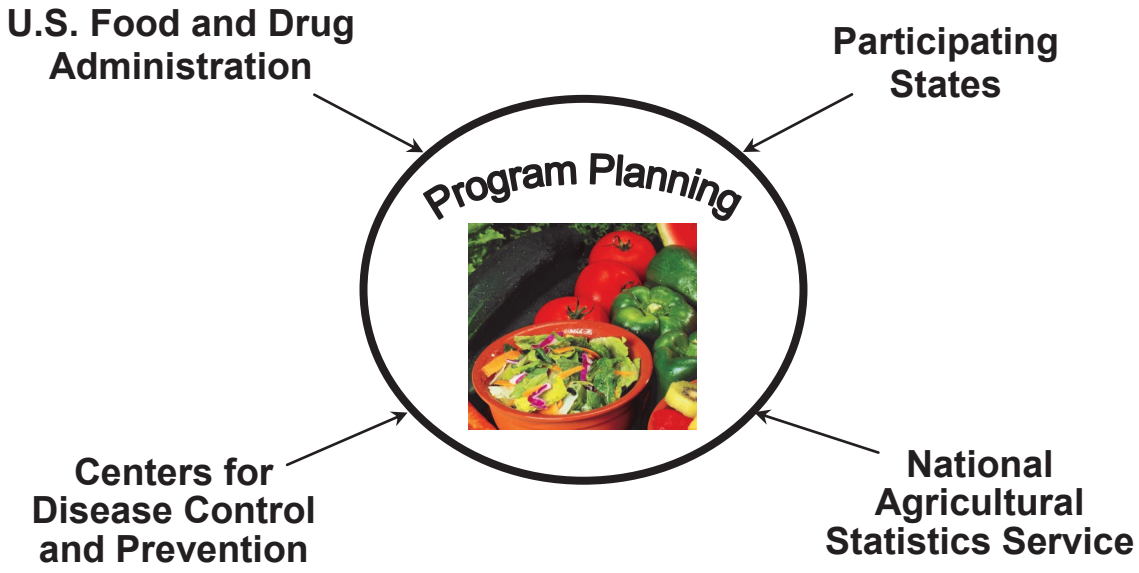
Fresh produce is recognized as an important component of a healthy diet. Because most produce is grown in the environment, it may be vulnerable to contamination with pathogens. Produce is often consumed raw without any type of intervention that would reduce or eliminate pathogens prior to consumption, which contributes to its potential as a source of foodborne illness [*Ref. 1, 2*]. In 2001, Congress authorized funding for a microbiological monitoring program to collect data on fresh fruit and vegetables. The U.S. Department of Agriculture's (USDA) Microbiological Data Program (MDP) plays a key role in support of the Secretary's priority to promote a safe, sufficient, nutritious food supply and his call for a modern food safety system. Fresh produce is recognized as an essential component of everyday healthy eating and in fighting obesity, especially during childhood. It is essential that fresh produce be free from contamination in order to safeguard public health, particularly vulnerable segments of the population, including children and the elderly.

MDP's mission is to collect information regarding the incidence and identification of targeted foodborne pathogens on fresh fruit and vegetables. This publication provides an overview of data collected in 2009 and summarizes program refinements made during that year. The Agricultural Marketing Service (AMS) Monitoring Programs Office (MPO) manages MDP and is responsible for the program's administrative, sampling, technical, and database operations.

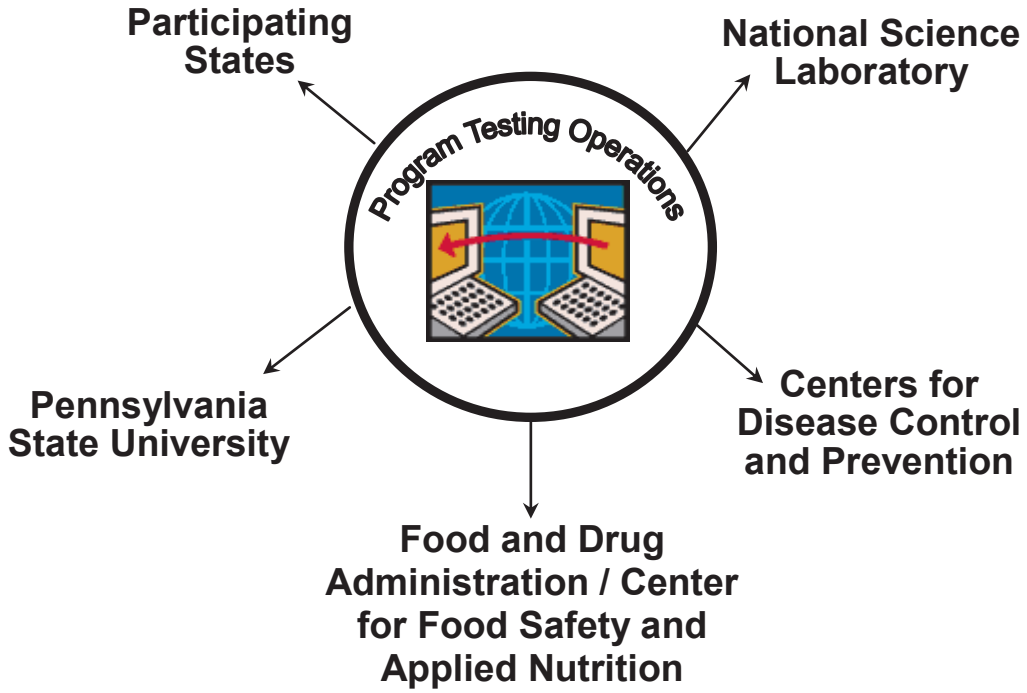
Figure 1 (a) illustrates MDP program planning participants. AMS coordinates MDP planning and program requirements with the Centers for Disease Control and Prevention (CDC), the U.S. Food and Drug Administration (FDA) and participating States and relies on their scientists' expertise for technical direction. The USDA National Agricultural Statistics Service (NASS), in collaboration with AMS, designed the sampling frame taking into account per capita consumption, marketplace availability, and crop production statistics.

Figure 1 (b) depicts MDP program testing operations. The participating State laboratories and AMS National Science Laboratory (NSL) analyze MDP samples collected by trained State sample collectors. Additional testing to characterize positive findings is performed by the Ohio Department of Agriculture (ODA), the Ohio Department of Health (ODH), and the Pennsylvania State University (PSU). Information on MDP data and isolates is shared with CDC and FDA.

Commodities tested were selected in consultation with CDC and FDA and were chosen because they are high-consumption fruit and vegetables in the U.S. diet, are often consumed raw, and have been implicated in foodborne outbreaks. Commodities tested in 2009 included: cantaloupe, cilantro, green onions, hot peppers, lettuce (pre-bagged organic and Romaine), spinach, sprouts (alfalfa or clover), and tomatoes (round and Roma). Commodities were tested for generic *Escherichia coli* (*E. coli*), *E. coli* strains with human pathogenic potential including *E. coli* O157:H7, and *Salmonella*. MDP laboratories also performed multiplex polymerase chain reaction (mPCR) screening for pathogenic *E. coli* on all samples. Isolates of these organisms were sent to specialized laboratories for further characterization including serotyping, testing for antimicrobial susceptibility and virulence attributes, and genomic fingerprinting.



(a) MDP Planning



(b) MDP Program

Figure 1. MDP Program Planning and Program Testing Operations. This figure illustrates (a) agencies/groups that support MDP program policy and planning activities, and (b) agencies/groups that analyze MDP samples, isolates, or results.

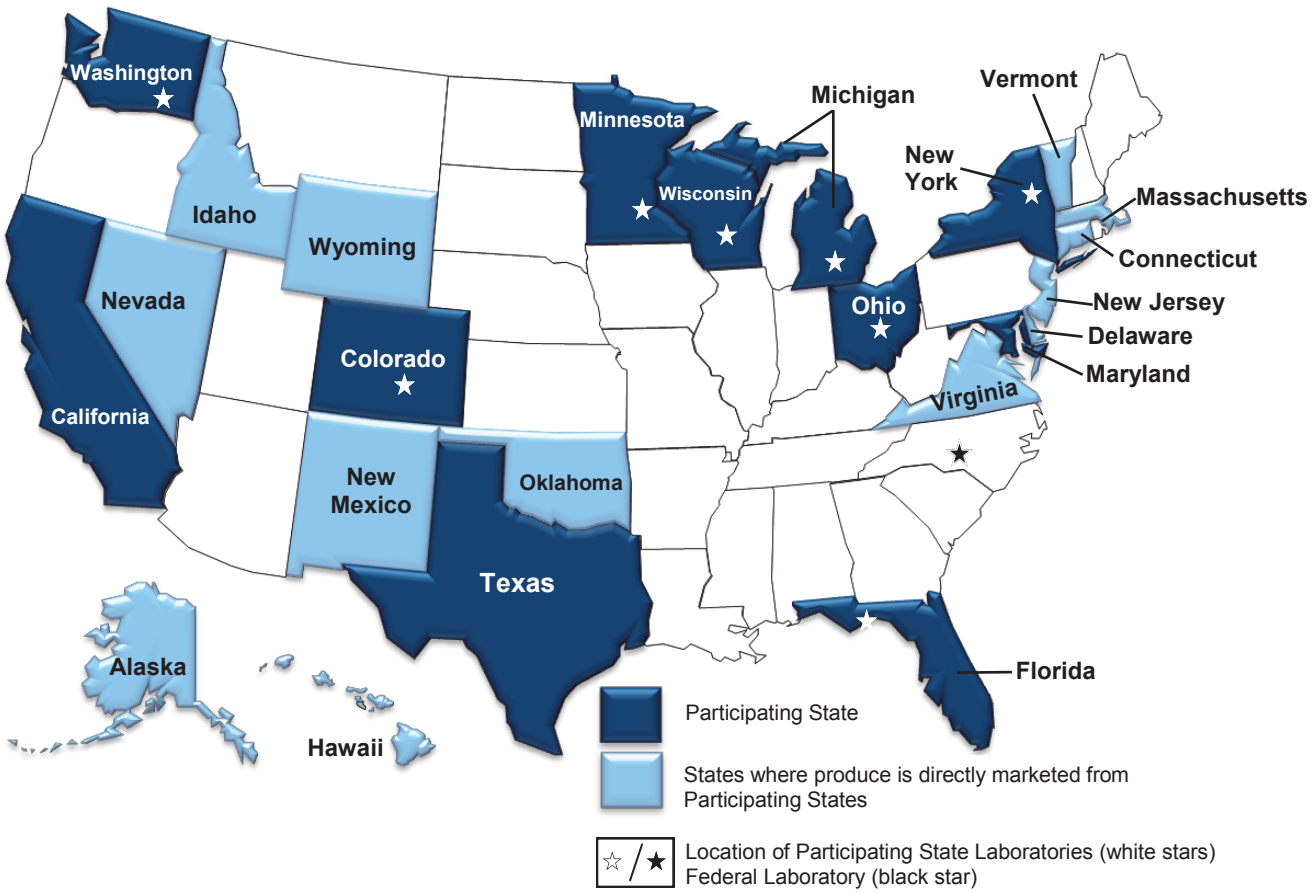


Figure 2. Program Participants. During 2009, AMS established cooperative agreements with 11 States to sample and/or test MDP commodities. Samples collected by California, Maryland and Texas were shipped to one or more MDP laboratories for analysis.

In addition, MDP conducted a special 9-month survey of peanut butter at the request of CDC as a follow up to the peanut paste-related *S. typhimurium* outbreak. Peanut butter samples were tested for *Salmonella* only.

Samples were collected in the 11 participating States through cooperative agreements with their respective agencies (Figure 2). Also shown in Figure 2 are the 13 neighboring States that are in the direct distribution networks for the MDP collection States: Alaska, Connecticut, Delaware, Hawaii, Idaho, Massachusetts, Nevada, New Jersey, New Mexico, Oklahoma, Vermont, Virginia, and Wyoming. Together, these States represent over 50 percent of the Nation's population and all geographic regions of the country, with significant rural-to-urban variability. Therefore, MDP samples are a statistically defensible representation of the country as a whole.

Analytical services were provided by microbiology laboratories in eight States (Colorado, Florida, Michigan, Minnesota, New York, Ohio, Washington, and Wisconsin) and the AMS NSL. The States of California, Maryland and Texas did not provide testing services. Their samples were transshipped overnight to be analyzed by one or more State laboratories or the AMS NSL.

MDP employed DNA-based screening for *Salmonella* and for pathogenic *E. coli*, including *E. coli* O157:H7. All produce samples were screened for the presence of pathogenic *E. coli* that carry shiga toxin (STEC) and/or enterotoxin (ETEC) genes,

using mPCR technology. STEC and ETEC are two groups of *E. coli* that cause the majority of enteric diseases and therefore are important to human health. Additionally, samples were tested for generic *E. coli* using an automated assay system during the first quarter of the year. Generic *E. coli* testing was discontinued in April to focus on foodborne pathogens.

MDP laboratories are members of the Food Emergency Response Network (FERN), a Government initiative that is co-managed by FDA and USDA's Food Safety and Inspection Service (FSIS). FERN's mission is to integrate the Nation's food testing laboratories to detect, identify, respond, and recover from a bioterrorism or public health emergency/outbreak involving the food supply. MDP laboratories have taken a very active role in FERN activities, including participation in validation of realtime polymerase chain reaction (rt-PCR) based detection methods for *E. coli* O157:H7 and *Salmonella*, and in proficiency testing rounds. MDP laboratories have also responded to outbreak emergencies by sampling specific commodities and testing for the pathogens implicated in outbreaks.

USDA is a member of the interagency Task Force on Antimicrobial Resistance established in 1999 to address antimicrobial resistance, which has been identified as a priority food safety and public health issue. As such, isolates from positive MDP samples were sent to ODA for antimicrobial resistance testing. These data were added to the CDC National Antimicrobial Resistance Monitoring System (NARMS) database. Additionally, ODH performs genomic fingerprinting on MDP isolates by Pulsed Field Gel Electrophoresis (PFGE) for inclusion in the CDC PulseNet database. With the assistance of CDC, MDP isolates were matched to pathogens from past outbreaks and/or identified as unique to the database.

As the program evolves, procedures and methods are being refined to provide information necessary for making science-based food safety decisions. MDP continues to adopt faster, more sensitive analytical tools for improved microbial detection and reporting. Working with FDA and FERN, MDP is harmonizing methodologies for testing foodborne pathogens among the agencies. MDP periodically upgrades data collection systems for better database management.

II. Sampling

The goal of the MDP sampling program is to obtain a statistical representation of selected commodities in the U.S. food supply by randomly selecting samples from the national food distribution system. The MDP sampling frame is designed to take into account regional diversity, marketplace availability, population, and consumption on a national scale [*Ref. 3*]. The samples include both domestic and imported fresh produce that are either conventionally or organically grown. The sampling rationale was developed by MPO in consultation with NASS, FDA, and CDC.

Collecting data over time from a range of sources permits statistical statements to be made about the distribution of targeted pathogens within the target population. The target population is all units of a commodity available at the wholesale level in a participating State during a defined timeframe (e.g., 1 year). The extension of statistical statements to the distribution of microorganisms within the inferential population (the entire amount of the commodity actually consumed by the U.S. public during the same timeframe) requires that strong assumptions be made about the relationship between the participating States and the United States as a whole, and between the wholesale and point-of-consumption levels. Nevertheless, because the States that participate in MDP fully represent the U.S. inferential population, and many microorganisms may enter the food supply at or before the wholesale level, the MDP data are useful and defensible.

Cantaloupe, cilantro, green onions, hot peppers, lettuce (pre-bagged organic and Romaine), spinach, sprouts (alfalfa or clover), and tomatoes (round and Roma) were collected by the Program in 2009. Based on consultations with FDA, cilantro was reintroduced during 2009, replacing green onions. These commodities were selected because they are often consumed raw and have been implicated in outbreaks. Peanut butter samples were also collected in 2009 to assure consumers that this product was not implicated on the peanut paste-related *Salmonella* outbreak.

The peanut butter special survey lasted 8 months (February to September), and during this timeframe, cantaloupe was replaced with peanut butter. MDP collected 1,542 peanut butter samples and tested them for presence of *Salmonella.* None of the samples tested positive for *Salmonella;* therefore, MDP resumed its normal sample collection schedule in October.

All samples in a State are collected on the same day or within a 2-day interval. Sample size is specific to each commodity and is based on the analytical method requirements. A description of sample sizes is given in Table 1. More detailed information on sample size is posted on the MDP website at www.ams.usda.gov/mdp.

Inferences cannot reasonably be made from the sample units to the lots from which they originate because the units do not provide enough information to generate statistically reliable lot estimates. However, statistical methods can be applied to make whole-target-population inferences from the data and to compare these inferences over time.

MDP benefited from the well established sampling framework of the Pesticide Data Program (PDP), a program administered by MPO since 1991. States that were already providing sampling services for PDP also began collecting samples for MDP in 2001 and continue, to date, through annual cooperative agreements with AMS. All sample collectors receive training and are provided with factsheets on the commodities they collect. The information in each factsheet includes acceptable and unacceptable products, availability, sample size, and instructions for data entry, packaging, and shipping.

The sampling of commodities is conducted at distribution centers and terminal (wholesale) markets from which food commodities are released to supermarkets and grocery stores, and include domestic and imported commodities. Samples are collected on a year-round basis. Sampling is apportioned according to

Commodity	Code	Sample Size	Number of Samples Collected per Lot
Alfalfa sprouts	SR	3 oz./85 g	3
Cantaloupe	CN	1 cantaloupe	3
Cilantro	CL	8 oz./225 g	3
Green Onions	GO	8 oz./225 g	3
Hot Peppers	HP	8 oz./225 g	3
Lettuce, Organic	LT	16 oz./450 g	3
Lettuce, Romaine	LT	16 oz./450 g	3
Peanut Butter	PB	14 oz./397 g	3
Spinach	SP	16 oz./450 g	3
Tomatoes, Round	TO	3 tomatoes	3
Tomatoes, Roma	TR	5 tomatoes	3

Table 1. Sample Sizes. This tables shows sample quantities by commodity.

population of the participating State, therefore, the higher the population of the State, the greater the number of samples taken. The monthly population-based collection numbers are as follows: California, 14 (January-September) and 13 (October-December); Colorado, 2; Florida, 7; Maryland, 4; Michigan, 6; Minnesota, 2; New York, 9; Ohio, 6; Texas, 8 (January-September) and 9 (October-December); Washington, 4; and Wisconsin, 2. This schedule results in a monthly target of 64 sites sampled per commodity. At each site, 3 samples are collected from the same lot in each facility for a total of 192 samples collected every month for each commodity.

Distribution centers and terminal markets in each State are selected at random based on probability proportional to the site's distribution volume (i.e., the amount of produce that moves through the site). Therefore, the larger the site, the greater the chance it will be sampled. If the commodity of interest is not available at the designated primary site, an alternate site may be chosen. MDP does not allow samples to be taken from public markets or retail stores because of the potential for contamination by the consumer and because commodity handling practices at this level in the distribution chain may vary widely. During 2009, 16,896 samples were collected from over 390 sites across the country and analyzed by MDP laboratories. Table 2 shows the distribution of Sample Origin by State or Country. Approximately 74 percent of all MDP samples were grown in the United States, 25 percent were imports, and 1 percent was of mixed national origin and unknown origin. The largest number of samples came from California (30%) and Mexico (20%). Figure 3 illustrates the proportion of samples that were domestic, imported, and of unknown origin for each commodity. Table 3 shows the number of samples collected by commodity by collection State.

All samples are collected using aseptic techniques (i.e., sample collectors wear sterile latex gloves and place samples in sterile sample bags). Once bagged, samples must be properly identified and tamper-proofed to ensure that chain-of-custody requirements are met. Sufficient frozen ice packs and packing materials for cushioning and insulation are required to maintain refrigerated temperatures during transport. The condition of each sample is checked and recorded upon receipt at each laboratory. If the integrity of a sample is in question, the laboratory will request that the particular commodity be sampled again. All samples are shipped on the same day as sample collection by overnight delivery so that laboratory analysis can begin the following day.

Unlike PDP operations, where specific commodities are sent to laboratories specializing in the analysis of a particular commodity, MDP laboratory analyses are performed in the same State from which the sample was collected. Exceptions to this are California, Maryland, and Texas. California samples were shipped to Colorado, Florida, Michigan, Ohio, Washington, and AMS NSL for analysis; Maryland samples were shipped to Ohio; and Texas samples were shipped to AMS NSL in Gastonia, North Carolina.

For bagged lettuce, all lettuce varieties were acceptable and included conventionally grown and organic samples. Bagged lettuce samples consisted of a single lettuce variety or mixtures of more than one variety. Bags containing lettuce mixed with spinach were not acceptable. These commodities are harvested primarily by hand, although some mechanical harvesting does occur. Alfalfa and clover sprouts are most often grown in drums and packaged in controlled environments. Other produce may be packaged in the field or taken to a packinghouse (e.g., tomatoes require classification for color and/or size). At the packing-house, the produce is cleaned, trimmed, sized, sorted, chopped into small pieces for ready-to-eat purposes, bagged, wrapped, and chilled for preservation until arrival at distribution centers and terminal markets. Cleaning is typically accomplished

Part 1. Domestic Samples

States	Commodity Codes											# of Samples	% of Total
	CL	CN	GO	HP	LT-C	LT-O	PB	SP	SR	TO	TR		
Alabama							2			3	9	14	0.1
Arizona	42			3	3			6		27	6	87	0.5
Arkansas							39			15	6	60	0.4
California	165	294	120	201	896	799	74	1634	420	329	147	5,079	30.1
Colorado		3	6		6	6		27	57	6		111	0.7
Connecticut							3	3	57	3		66	0.4
Delaware										3		3	<0.1
Florida	105	36		240	42	6	27	27	261	584	246	1,574	9.3
Georgia				45			6			6		57	0.3
Idaho							16		36			52	0.3
Illinois				12		15	94	6	27		6	160	0.9
Indiana	3									6		9	0.1
Iowa							1					1	<0.1
Kansas							6					6	<0.1
Kentucky				3	3		1	18	18		3	46	0.3
Maine							12	9				21	0.1
Maryland		6	12	33	45	6	13	57	216	42	33	463	2.7
Massachusetts					3	21	5	90	48			167	1.0
Michigan		3	18	33			57	72	198	30	60	471	2.8
Minnesota	6		3	15	12	21	61	4	27	9		158	0.9
Mississippi				6								6	<0.1
Missouri							13			3	12	28	0.2
Nebraska							134			9		143	0.8
New Hampshire							2					2	<0.1
New Jersey		30	15	30			285	15		9	15	399	2.4
New York			3	39	3	6	58	18	189	18	9	343	2.0
North Carolina				144	3	3	9		9	9	27	204	1.2
Ohio	48	6	39	54	12	30	418	15	84	36	27	769	4.6
Oregon			3			6	21	8		3		41	0.2
Pennsylvania		3		12	18	9	48	30	57	18		195	1.2
South Carolina		3	12						3	21	6	45	0.3
Tennessee							2			16	12	30	0.2
Texas	18	57	15	78	45	27	19	63	258	84	45	709	4.2
Vermont							7					7	<0.1
Virginia	3			6			1	24	105	39	21	199	1.2
Washington		12		15	15	3	3	27	45	3		123	0.7
Wisconsin		3		3			8	15	105	3		137	0.8
Unknown State	12	33	12	126	21	12	2	44	51	75	60	448	2.7
No. of Domestic	402	489	258	1,098	1,127	970	1,447	2,212	2,271	1,409	750	**12,433**	
% of Total	49.3	84.2	22.1	62.9	95.8	83.7	93.8	95.0	99.7	60.0	42.7		**73.6**

Part 2. Imported Samples

Country	Commodity Codes											# of Samples	% of Total
	CL	CN	GO	HP	LT-C	LT-O	PB	SP	SR	TO	TR		
Argentina							3					3	<0.1
Canada		6	30		15		92	21		162	27	353	2.1
Chile			6									6	<0.1
Dominican Republic				3								3	<0.1
Ecuador				6								6	<0.1
Guatemala	327		18									345	2.0
Honduras	51											51	0.3
Mexico	24	74	845	575	21	108		66		728	954	3,395	20.1
No. of Imports	402	80	899	584	36	108	95	87	0	890	981	**4,162**	
% of Total	49.3	13.8	77.1	33.5	3.1	9.3	6.2	3.7	0	37.9	55.9		**24.6**

Part 3. Mixed National Origin Samples

Countries	Commodity Codes											# of Samples	% of Total
	CL	CN	GO	HP	LT-C	LT-O	PB	SP	SR	TO	TR		
Canada / USA					3							3	<0.1
Mexico / USA					6	78		26		3	6	119	0.7
No. of Mixed Origin					9	78		26		3	6	**122**	
% of Total					0.8	6.7		1.1		0.1	0.3		**0.7**

Part 4. Unknown Origin Samples

	Commodity Codes											# of Samples	% of Total
	CL	CN	GO	HP	LT-C	LT-O	PB	SP	SR	TO	TR		
Unknown Origin	12	12	9	63	5	3		3	6	48	18	**179**	
% of Total	1.5	2.1	0.8	3.6	0.4	0.3		0.1	0.3	2.0	1.0		**1.1**

Sample Totals:	**816**	**581**	**1,166**	**1,745**	**1,177**	**1,159**	**1,542**	**2,328**	**2,277**	**2,350**	**1,755**	**16,896**

Commodity Legend	
CL = Cilantro	PB = Peanut Butter
CN = Cantaloupe	SP = Spinach
GO = Green Onions	SR = Sprouts (Alfalfa)
HP = Hot Peppers	TO = Tomatoes, Round
LT-C = Lettuce, Conventional	TR = Tomatoes, Roma/Plum
LT-O = Lettuce, Organic	

Table 2. Sample Origin by State or Country. This table shows the number of samples per State or Country of origin as determined by the reported grower, packer, or distributor information.

State	Cantaloupe	Cilantro	Green Onions	Hot Peppers	Lettuce (Conventional)	Lettuce (Organic)	Peanut Butter	Spinach	Sprouts (Alfalfa)	Tomatoes (Round)	Tomatoes (Roma)	Total
California	159	117	243	360	168	318	327	486	486	502	360	3,526
Colorado	24	18	36	54	33	39	48	72	69	72	54	519
Florida	93	69	143	216	177	108	186	281	279	279	216	2,047
Maryland	60	36	72	108	51	90	84	144	141	143	108	1,037
Michigan	72	54	108	162	120	96	144	216	207	216	162	1,557
Minnesota	27	18	36	54	24	48	48	72	72	72	54	525
New York	108	81	162	243	144	180	216	324	324	324	243	2,349
Ohio	72	54	108	162	168	48	144	216	213	216	162	1,563
Texas	129	80	153	227	157	151	201	301	285	310	234	2,228
Washington	48	36	69	105	87	57	96	144	132	144	108	1026
Wisconsin	24	18	36	54	48	24	48	72	69	72	54	519
Totals	816	581	1,166	1,745	1,177	1,159	1,542	2,328	2,277	2,350	1,755	16,896

Table 3. Samples Collected by State. This table shows the number of samples collected by each State by commodity.

with chlorinated water, although other disinfecting agents, such as ozone, may be used. Some commodities may have a food-grade wax applied to replace natural waxes removed during washing to help prevent water loss. Fungicides may be added to the wax or applied separately to retard spoilage. Chilling may be accomplished by various means such as vacuum cooling, hydrovac cooling, room-chilling, or forced air cooling. After initial chilling, the produce is stored under chilled conditions (avoiding freezing) and, depending on the commodity, under low-oxygen atmospheric conditions (primarily carbon dioxide). Except for leafy greens and sprouts, the produce is often harvested before reaching full ripeness to minimize spoilage and bruising. Prior to shipment to distribution centers and terminal markets, some commodities are often artificially ripened using techniques such as ethylene oxide gassing. Some shipping companies transport produce in refrigerated trucks or rail cars; others use ice; still others use no method of cooling, depending on the commodity. Therefore, MDP data reflect not only agricultural practices but also handling practices occurring during harvesting, storage (including postharvest treatment), bagging, and shipping operations.

MDP uses electronic Sample Information Forms (e-SIFs) to capture information needed to characterize the sample. Sample collectors use laptop or handheld computers in the field to record sample identification information such as: (1) State of sample collection, (2) collection date, (3) sampling site code, (4) commodity code, and (5) testing laboratory code. Other available information about the sample is also recorded, such as the country of origin, any production claims (such as organic), and any postharvest treatments. The e-SIFs are electronically mailed the same day as sample collection or, at the latest, by the following morning after collection to ensure that sample information is received at each laboratory by the time the samples arrive for analysis.

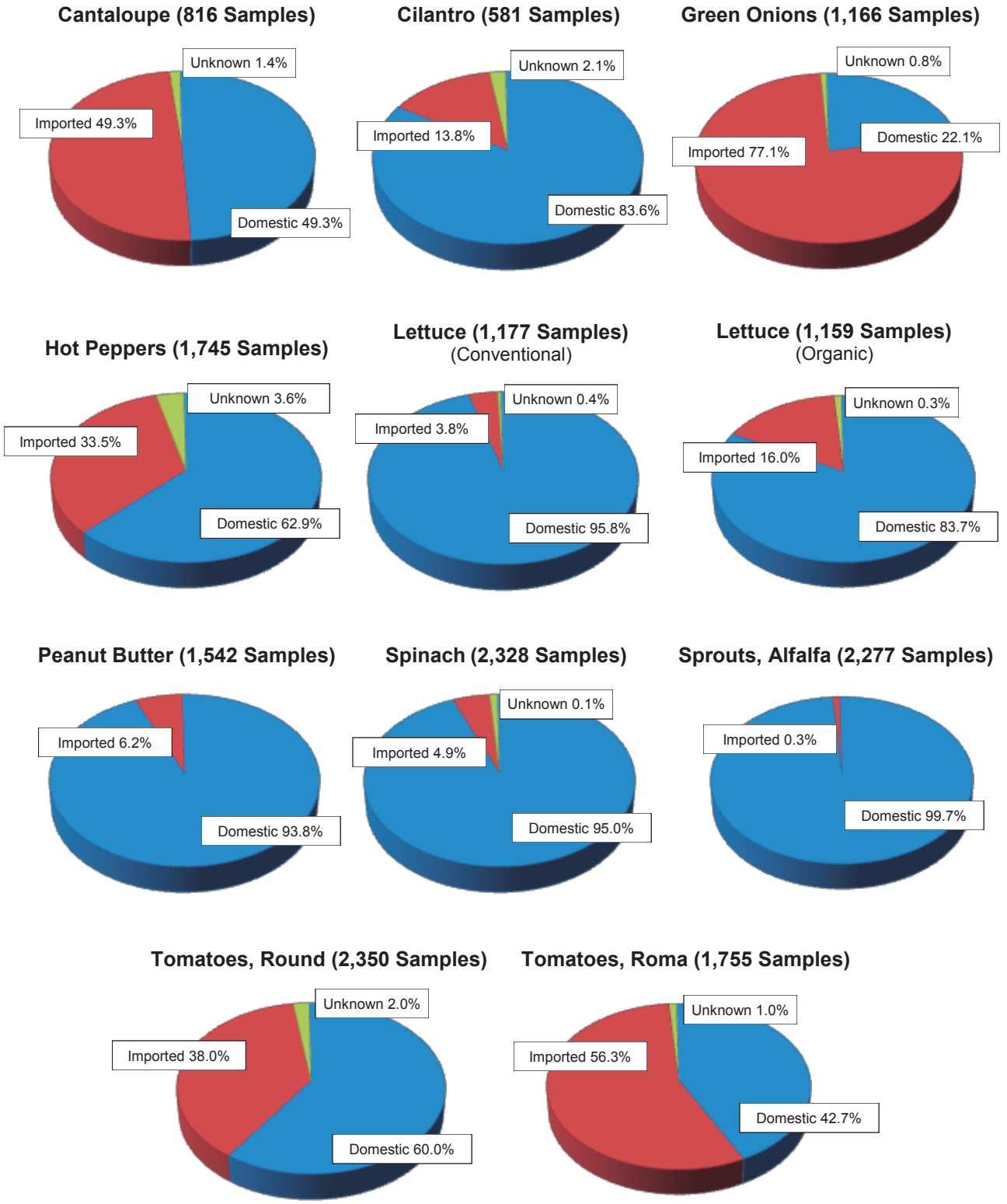


Figure 3. Commodity Origin. The proportion of domestic, imported or unknown origin for each commodity is depicted for samples tested in 2009.

MDP sampling operations are conducted following Standard Operating Procedures (SOPs) designed to provide consistency across the program and ensure the integrity of the analytical data. SOPs also contain specific instructions for sample selection, shipping and handling, and chain-of-custody. SOPs are updated as needed and serve as a technical reference for conducting program sampling reviews to ensure that program goals and objectives are met. All program SOPs are available on the Internet at www.ams.usda.gov/mdp.

III. Laboratory Operations

Participating microbiology laboratories tested MDP samples for generic *E. coli* and screened MDP samples for *Salmonella* and *E. coli* strains carrying shiga toxins and enterotoxins (STECs and ETECs, respectively), including *E. coli* O157:H7. Laboratories used mPCR technology to screen all samples for pathogenic *E. coli* (STEC and ETEC), based on the presence of gene sequences that code for shiga toxins and enterotoxins. From January through March 2009, MDP laboratories also performed generic *E. coli* testing for all commodities.

For all pathogenic *E. coli* isolates, the Gastroenteric Disease Center at PSU performed serotyping using specific antisera and tested for additional virulence-related genes using polymerase chain reaction (PCR). The ODA laboratory performed antimicrobial susceptibility testing for all pathogenic isolates. The ODH laboratory performed serotyping on *Salmonella* isolates and genomic fingerprinting using PFGE on all pathogenic isolates from Colorado, Florida, Michigan, Ohio, Washington, and the AMS NSL. MDP laboratories in New York, Minnesota, and Wisconsin performed PFGE on all pathogenic isolates obtained by their laboratories.

Upon arrival at the testing facility, samples were logged, visually examined for acceptability, and discarded if determined to be damaged (decayed, extensively bruised, or spoiled). Samples were refrigerated until analysis commenced. Laboratories were permitted to refrigerate commodities for up to 48 hours to allow for different sample arrival times from the various collection sites.

Prior to the analytical tests, all samples were washed in Universal Pre-enrichment Broth (UPB) in order to streamline the screening process for all target bacteria. Peanut butter and alfalfa sprouts were blended. To improve pathogen detection, washing was followed by a soaking step for all commodities except green onions and sprouts. For green onions and sprouts, the plant materials were removed prior to incubation. Genomic DNA was extracted from each enriched sample using an automated system. Next, the extracted DNA was cleaned for use in detecting pathogens by PCR. The BAX® automated PCR system was used for screening samples for the presence of *Salmonella* and enterohemorrhagic *E. coli* O157:H7. Similarly, an appropriate aliquot of extracted DNA for each sample was used in screening for the presence of non-O157 pathogenic *E. coli* strains by mPCR. Table 4 shows the number of samples tested for each organism by commodity.

MDP laboratories tested 2,531 samples for the presence of generic *E. coli* using an automated assay system. This assay uses the presence of a unique enzyme in *E. coli* for detection. It enumerates the number of *E. coli* cells per gram of sample using Most Probable Number (MPN) based statistical analysis. Generic *E. coli* testing was discontinued in April to focus on foodborne pathogens.

In order to improve pathogen detection, cantaloupe, cilantro, hot peppers, lettuce, peanut butter, spinach, and tomato samples were soaked overnight. For green onions and sprouts, any debris/plant material was removed from the wash prior to overnight incubation.

Commodity	*E. coli*	Pathogenic *E. coli*	*E.coli O157:H7*	*Salmonella*	Total Number of Tests
Cantaloupe	231	816	231	816	2,094
Cilantro		581	581	581	1,743
Green Onions		1,166	1,166	1,166	3,498
Hot Peppers		1,745		1,745	3,490
Lettuce, Conventional	261	1,177	1,177	1,177	3,792
Lettuce, Organic	315	1,159	1,159	1,159	3,792
Peanut Butter				1,542	1,542
Spinach	575	2,328	2,328	2,328	7,559
Sprouts (Alfalfa)	573	2,277	2,277	2,277	7,404
Tomatoes, Round	576	2,350	576	2,350	5,852
Tomatoes, Roma		1,755		1,755	3,510
Total	**2,531**	**15,354**	**9,495**	**16,896**	**44,276**

Table 4. Number of Samples Analyzed. This table shows the number of samples tested for each organism.

MDP laboratories utilized automated systems for the extraction and purification of genomic DNA from enriched bacterial cultures in order to streamline the labor intensive preparation of DNA samples for PCR assays. MDP laboratories used automated PCR systems for the detection of *Salmonella* and enterohemorrhagic *E. coli* O157:H7 in samples, with the exception of peanut butter samples, which were analyzed only for *Salmonella* using the VIDAS system.

All samples (except peanut butter) were screened by mPCR procedures for STECs and ETECs. Cultural methods involving selective growth media to permit the enrichment of pathogens were used in isolation procedures. When necessary, pathogenic cells were concentrated from mixed cultures using an Immunomagnetic Separation (IMS) technique, which isolates target bacteria using specific antigen-antibody reactions. In addition to cultural methods, identification based on automated biochemical tests and serotyping of surface antigens was used in the verification of isolates for the target pathogens.

The main objectives of the Quality Assurance/ Quality Control (QA/QC) program were to ensure the reliability of MDP data and to ensure performance equivalency of participating laboratories. Direction for the MDP QA program was provided through written SOPs based on FDA's 8th edition Bacterial Analytical Methods (BAM) [*Ref. 4*], AOAC® methods, the USDA FSIS Microbiological Laboratory Guide, and the U.S. Environmental Protection Agency's Good Laboratory Practices. SOPs provide uniform administrative, sampling, and laboratory procedures. MDP analytical methods are published at www.ams.usda.gov/mdp.

Several MDP laboratories have been accredited or are in the process of International Organization for Standardization (ISO) accreditation. MDP laboratories participate in an annual proficiency testing round, administered by MPO, for selected target organisms in produce samples. Several MDP laboratories also participated in microbiological proficiency testing for foods, including produce, administered by FERN.

Positive and negative controls and a sterile media blank were required for each sample set. MDP laboratories use positive control strains of *E. coli* O157:H7 and *S. typhimurium* that carry a gene coding for Green Fluorescent Protein (GFP). Expression of the GFP, detected by exposing the cultures to ultraviolet light, indicates the presence of the control cultures without the need for performing lengthy biochemical tests. All controls and blanks were processed in conjunction with sample cultures from the pre-enrichment step to isolation and identification of target isolates using cultural, immunological, biochemical, and serological methods.

A technical advisory group, comprised of microbiologists from each MDP-participating laboratory, provided technical feedback on program SOP revisions and addressed technical and QA issues. Additionally, MDP consulted with scientists from other Federal agencies (FDA, CDC, USDA Agricultural Research Service, and FSIS) and academia on technical issues. For day-to-day QA oversight, each participating facility was required to have a Quality Assurance Unit (QAU) that operated independently from the laboratory staff. Preliminary QA/QC review procedures were performed onsite by each laboratory's QAU. Final review procedures were performed by MDP staff responsible for collating and reviewing data for conformance with SOPs.

Laboratory performance was monitored through onsite reviews by MDP staff to determine compliance with MDP SOPs. Corrective actions, if necessary, were performed as a result of onsite reviews.

IV. Database Management

MDP maintains an electronic database that serves as a central data repository. The central database resides at MPO in Manassas, Virginia. The data captured and stored in the MDP database include product information and analytical findings for each sample collected along with QA/QC results for each set of samples. The MDP data pathway is depicted in Figure 4.

MDP uses a Web-based Remote Data Entry (RDE) system to capture and report MDP data. The RDE system is centralized, with all user interface software and database files residing in Washington, DC. The laboratory users need only a Web browser to interface with the RDE system. Access to the RDE system is controlled through separate user login/password accounts and user access rights for the various system functions based on position requirements. The RDE system utilizes Secure Sockets Layer (SSL) technology to encrypt all data passed between users' computers and the central Web server.

A separate Windows®-based system allows sample collectors to electronically capture the standardized Sample Information Form (SIF) on handheld or laptop computers. The e-SIF system generates formatted text files containing sample information that are e-mailed to MDP headquarters and then imported into the Web-based RDE system.

The RDE data entry screens have extensive edits and cross-checks built in to ensure that acceptable values are entered for all critical data elements. This task is made easier by the practice of capturing and storing standardized codes for all critical alphanumeric data elements rather than their complete names, meanings, or descriptions. This coding scheme allows for faster and more accurate data entry, saves disk storage space, and makes it easy to perform queries on the database. The data entry screens also perform edits on numeric fields, dates, and other character fields to ensure that entries are within prescribed boundaries.

At MDP headquarters, the RDE system allows scientists to review and approve the data for inclusion in the central database. The central MDP database is maintained using Microsoft®

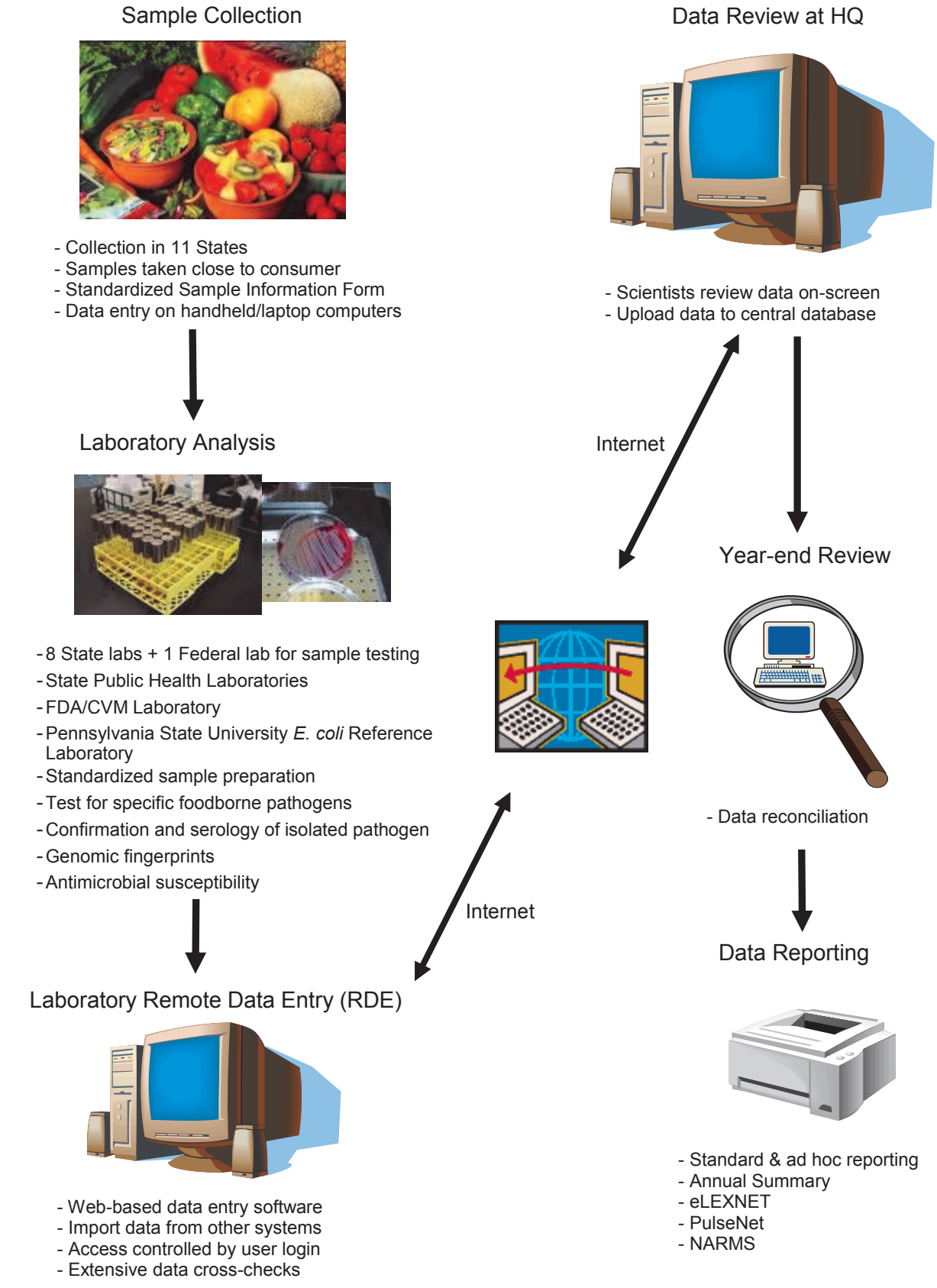


Figure 4. MDP Data Pathway. An illustration of MDP data path from sample collection, through laboratory analysis and reporting.

Access and SQL Server database tools. Access to the central MDP database is limited to MDP headquarters personnel and is controlled through password protection and user access rights.

V. Summary of 2009 Data

MDP collected a total of 16,896 samples. In addition, MDP tested 1,542 peanut butter samples as a special survey conducted from February through September 2009. This survey was initiated at CDC's request to assure consumers that peanut butter for retail sale was not implicated in a *Salmonella* outbreak traced to other peanut products. Peanut butter samples were tested only for *Salmonella.*

The 2009 MDP testing program was streamlined to target priority organism-commodity combinations. Consequently, all commodities were screened for *Salmonella*; all commodities, except peanut butter, were screened for non-O157 STEC and ETEC; and only eight commodities were screened for *E. coli* O157:H7. Peanut butter, hot peppers, and tomatoes (both round and Roma) were not tested for *E. coli* O157:H7.

Enumeration of *E. coli:* Testing of generic *E. coli* was performed on a total of 2,531 fresh produce commodities using an automated detection and enumeration system on samples collected from January through March. Table 5 shows that MPN values of 1,000 *E. coli* cells/gram of sample were found in 6 samples and 5 of those samples were alfalfa sprouts.

Pathogenic *E. coli:* A total of 15,354 samples were screened for STECs and ETECs using an mPCR assay developed by FDA. Table 6 shows that 51 samples were identified as positive pathogenic *E. coli* by mPCR. The laboratories were successful in isolating 24 pathogenic *E. coli* (13 STECs and 11 ETECs). This represents a 47-percent rate of successful isolation, higher than the 30 percent reported in 2008. In addition to the technological differences between detection by PCR and isolation by cultural means,

MPN Range	Number of Samples
< 10	2,442
10 - 99	73
100 - 999	10
1,000 - 10,000	4
> 10,000	2
Total Number of Samples Tested	2,531

Table 5. Number of Samples Tested for *E. coli.* This table shows the results of *E. coli* enumeration by Most Probable Number (MPN).

several other factors influence the rate of successful isolation. The overwhelming amount of background microflora when compared to the small number of target bacterial cells and the diverse physiology of bacteria present in produce wash cultures pose a challenge in identification and isolation by selective enrichment of targets.

The 24 isolates were sent to other laboratories for further characterization. PSU conducted tests that included 13 virulence-specific genes associated with different classes of pathogenic *E. coli* and serotyping for somatic O antigens and flagellar H antigens. ODA conducted tests on antimicrobial resistance for 15 different antibiotics for all isolates. ODH and selected State laboratories performed genomic fingerprinting by PFGE for isolates. The PFGE gel images were loaded onto the PulseNet database by the laboratories. All PFGE information on MDP isolates was sent to CDC and FDA.

Table 7 shows results of additional testing on isolates of non-O157 pathogenic *E. coli*. Non-O157 pathogenic *E. coli* was isolated from 23 samples, 16 of which were spinach. A single spinach sample was found to contain one isolate each of STEC and ETEC. All but one of the STEC isolates contained the shiga toxin 2 (Stx-2) gene. The other STEC isolate is under further investigation. Seven out of 13 STEC isolates contained an additional virulence factor for the gene coding for

Commodity	Number of Samples Screened by mPCR	Number of Pathogenic *E. coli*-Positive Samples	Number of Positive Isolates Obtained
Cantaloupe	816	1	0
Cilantro	581	9	4
Green Onions	1,166	3	0
Hot Peppers	1,745	1	1
Lettuce, Conventional	1,177	2	1
Lettuce, Organic	1,159	3	2
Spinach	2,328	29	16
Sprouts (Alfalfa)	2,277	2	0
Tomatoes, Round	2,350	1	0
Tomatoes, Roma	1,755	0	0
Total	15,354	51	24

Table 6. Summary of Sample Analysis for Pathogenic *E. coli*. This table summarizes the number of samples initially screened for *E. coli* and further tested for pathogenic *E. coli* and the number of samples that tested positive for pathogenic *E. coli*.

enterohemolysin (*hlyA*), which causes lysis of erythrocytes. None of the isolates contained the *eae* gene coding intimin, a virulence-related gene required for attachment to epithelial cells. All of the ETEC isolates contained either the heat-labile or heat-stable toxins or both. Results from antimicrobial resistance testing revealed that an ETEC isolate from a spinach sample was resistant to the antibiotic kanamycin and a STEC isolate also from spinach was resistant to tetracycline. Based on PulseNet information provided by CDC, three STEC isolates from spinach matched isolates from humans and one STEC isolate from spinach was shown to be a new strain with O172 and H2 antigens. To characterize a non-O157 pathogenic *E. coli* isolate as a human pathogen capable of causing disease, there must be interactions among several proteins including toxins, encoded by respective genes. MDP only identified toxin genes; the additional testing required for determining the disease-causing potential of these isolates is beyond the scope of MDP.

Salmonella: As depicted in Table 8, a total of 16,896 samples were screened for *Salmonella* by BAX® PCR. Of these samples, 90 were reported as presumptive positives via the preliminary screening method and 32 were successfully isolated and confirmed: 8 from cilantro, 2 from cantaloupes, 4 from green onions, 4 from hot peppers, 1 from lettuce, 6 from spinach, 6 from alfalfa sprouts and 1 from tomato (Roma). These isolates were sent to ODH or to State health department laboratories for serotyping and genomic fingerprinting by PFGE and to ODA for antimicrobial resistance. Results were uploaded onto the CDC PulseNet database.

Table 9 provides the compiled information on characteristics of MDP *Salmonella* isolates. About one-third of the isolates (10) belonged to serogroup C. Six of the *S. arizonae* or *diarizonae* demonstrated variable serogroups. *S. litchfield* isolates from Florida cilantro samples were identified as having a unique PFGE pattern not previously reported in 1,285 Litchfield PFGE patterns already in the PulseNet database.

Collection State	Commodity	*E. coli* Class	Toxin Genes	O Antigen	H Antigen	Antimicrobial Resistance	Comments Based on PulseNet/CDC
Maryland	Spinach	STEC	Stx-2, Hy1A	X25	-		
New York	Spinach	ETEC	STa	61w	34		
Florida	Spinach	ETEC	STa	153	21		
Wisconsin	Spinach	STEC	Stx-2	-	2		New Strain; O172:H2 in *E. coli* Reference Center and PulseNet per CDC.
Colorado	Spinach	ETEC	STa, STb	-	10	Kanamycin	
California	Lettuce	ETEC (2)	LT, STb	83w	15		Organic Lettuce
Michigan	Spinach	STEC	Stx-2	73	18		
California	Spinach	STEC	Stx-2, Hy1A	116	21	Tetracycline	
California	Spinach	STEC	Stx-2	116	21		
Florida	Spinach	STEC	Stx-2, Hy1A	113	36		All other sources for this organism are human per CDC.
Texas	Spinach	STEC	Stx-2, Hy1A	8	-		All other sources for this organism are human per CDC
Michigan	Spinach	STEC	Stx-2, Hy1A	-	19		
Michigan	Cilantro	ETEC	LT, STb	175	15		
Florida	Spinach	ETEC	STa	-	36		Isolated from the same sample.
Florida	Spinach	STEC	Stx-2, Hy1A	168	8		
Michigan	Cilantro	ETEC	LT, STb	83w	15		
New York	Spinach	STEC	Stx-2	-	21		
Maryland	Spinach	STEC	Stx-2	113w	21		All other sources for this organism are human per CDC.
Michigan	Lettuce	STEC	STa, Stx-2, Hy1A	168	-		Conventionally grown lettuce.
California	Cilantro	ETEC (2)	LT, STb	175w	15		
California	Hot Pepper	ETEC	STa	-	5		
Minnesota	Spinach	STEC	Stx	11	15		
TOTAL		24					

STa and STb - heat-stable toxins A and B, respectively

HlyA = hemolysin A

LT = heat-labile toxin

Stx-1 and Stx-2 - shiga toxins 1 and 2, respectively

Under heading of E. coli Class, numbers with ( ) denote multiple isolates.

Table 7. Characterization of 2009 non-O157 Pathogenic *E. coli* Isolates. This table provides data obtained from additional testing of pathogenic *E. coli* isolates initially screened by MDP laboratories.

Commodity	Number of Samples Tested	Number of Presumptive Positive Samples	Number of Positive Isolates Obtained
Cantaloupe	816	2	2
Cilantro	581	9	8
Green Onions	1,166	4	4
Hot Peppers	1,745	7	4
Lettuce, Conventional	1,177	1	1
Lettuce, Organic	1,159	0	0
Peanut Butter	1,542	14	0
Spinach	2,328	15	6
Sprouts (Alfalfa)	2,277	23	6
Tomatoes, Round	2,350	14	0
Tomatoes, Roma	1,755	1	1
TOTALS	16,896	90	32

Table 8. Summary of Analysis for *Salmonella*. This table shows the number of samples tested for *Salmonella* and the number of presumptive positives and isolates obtained.

Three *Salmonella* isolates demonstrated resistance to antimicrobial compounds tested: *S. kentucky* isolated from a Wisconsin lettuce sample was resistant to streptomycin and tetracycline; *S. oranienberg* isolated from a California cantaloupe sample carried resistance to chloramphenicol; and a *S. havana* isolated from a Texas hot pepper sample was found resistant to trimethoprim and sulfamethoxazole. Information on *Salmonella* antimicrobial resistance has been provided to the CDC NARMS. MDP data, including the characteristics of pathogenic isolates, were provided to FDA and CDC.

***E. coli* 0157:H7:** No enterohemorrhagic *E. coli* O157:H7 strain was isolated from the 9,495 samples screened, although there were 16 presumptive PCR positives reported. These presumptive positives suggested that the target bacteria might be present in extremely low numbers. As with pathogenic *E. coli* analysis, several factors contribute to successful detection and isolation, including the level of naturally occurring background microflora present in produce compared to the small number of target bacterial cells, and the diverse physiology of the complex mixtures of bacteria, which can pose a challenge in selective enrichment of targets.

Foodborne Outbreaks: Tables 9 and 10 show results of testing done by MDP in support of FDA and CDC outbreak investigations. MDP initiated a special survey in response to an outbreak investigation implicating peanut paste and peanut paste byproducts. Although peanut butter for retail sale was not implicated in the outbreak, consumers' fears led to a decline in consumption of this product. The MDP peanut butter special survey was implemented to provide data to reassure consumers that this product was not implicated in the outbreak. MDP tested 1,542 samples of peanut butter for *Salmonella* using the VIDAS method. In the course of screening, 14 samples were found to be positive by VIDAS. Although selective media were used in *Salmonella* isolation procedures, no *Salmonella* was isolated from

Collection State	Commodity	*Species*	Sero-group	Antigenic Formula	Antimicrobial Resistance	Comments Provided by FDA or CDC
Minnesota	Sprouts	*S. diarizonae* (2)	Z	S(IIIa) 50:z4,z23:-		
California	Sprouts	*S. oranienberg* (2)	C1	SI 7:m,t:-		Outbreak associated per CDC
Wisconsin	Spinach	*S. mbandaka*	C1	SI 6,7:z10,e,n,z15		
Texas	Plum Tomatoes	*S. bareilly*	C1	SI 6,7,14:y:1,5		
Michigan	Spinach	*S. anatum*	E1	SI 3,10:e,h:1,6		
California	Sprouts	*S. poona* (2)	G1	SI 22:z:1,6		
New York	Hot Peppers	*S. saintpaul*	B	SI,4,5],12:e,h:1,2		Outbreak associated per CDC
Wisconsin	Lettuce	*S. kentucky*	C3	SI 8,20:i:z6	Streptomycin, Tetracycline	
Michigan	Green Onions	*S. javiana* (3)	D1	SI 9,12:l,z28:1,5		Outbreak associated per CDC– Import Alert per FDA
Florida	Hot Peppers	*S. arizonae*	Z	S(IIIa) 50:z4,z23:-		
New York	Green Onions	*S. javiana*	D1	SI 9,12:l,z28:1,5		
Michigan	Spinach	*S. paratyphi B*	B	SI 4,5,12:b:1,2		
California	Cantaloupe	*S. oranienburg*	C1	SI 7:m,t:-	Chloramphenicol	
Texas	Cantaloupe	*S. newport*	C2	SI 6,8,20:e,h:1,2		
Texas	Hot Peppers	*S. havana*	G	SI 13,23:f,g:-		
Florida	Cilantro	*S. saintpaul* (3)	B	SI 1,4,[5],12:e,h:1,2		
California	Spinach	*S. arizonae* (2)	L	S(IIIa) 21:g,z51:-		
California	Spinach	*S. arizonae*	Y	S(IIIa) 48:z29:-		
Maryland	Cilantro	*S. assen*	L	SI 21:a:5		
Florida	Cilantro	*S. lomalinda*	D1	SI 1,9,12:a:e,n,x		
Texas	Hot Peppers	*S.havana*	G	SI 1,13,23:f,g:-	Trimethoprim, Sulfamethoxazole	
Texas	Cilantro	*S. montevideo*	C1	SI 6,7,14:g,m,s:-		
Florida	Cilantro	*S. litchfield* (2)	C2	SI 6,8:l,v:1,2		Unique out of 1,285 litchfields already in PulseNet database per CDC
TOTALS		32				

Table 9. Characterization of 2009 *Salmonella* Isolates. This table provides data obtained from additional testing of *Salmonella* isolates initially screened by MDP laboratories.

Commodity	Species	Sero-group	Antigenic Formulae	PulseNet Number	PulseNet Match-Up Comment	Anitmicrobial Resistance to 15 Antibiotics Tested
Peanut Butter	None					
Green Onions	*S. javiana*	D1	SI 9,12:1,z28:1,5	MDP-09-00018	Associated with outbreak 0908MIJGG-1	None
Hot Peppers	*S. saintpaul*	B	SI 1,4,[5],12:e,h:1,2	MDP-09-00013	Associated with outbreak 0905MIJN6-1	None
Sprouts (Alfalfa)	*S. oranienberg*	C1	SI17:m,t:-	MDP-09-00003 MDP-09-00004	Associated with outbreak 0904MLIJX-1	None

Table 10. *Salmonella* Species Isolated from Commodities Implicated in Outbreaks. This table illustrates additional test results that provide further characterization of MDP isolates.

any sample. However, four of the samples yielded *Enterobacter sakazakii*, which is considered a pathogen, especially for children. It is of interest to note that due to antigenic cross-reactivity, other organisms may yield positive results on the VIDAS screening method. Testing of this large number of peanut butter samples expanded CDC and FDA's investigation capabilities during the outbreak.

S. javiana was isolated by the Michigan laboratory in green onions and was also found by FDA in a follow up sample collected after MDP notification. In August of 2009, FDA detected *S. javiana* in green onions on the Southwestern Import District border. In addition, a second positive sample finding was detected by FDA's Detroit District office. These positive sample findings were further strengthened by a *Salmonella* positive sample finding reported by MDP. The overwhelming strength of these three sample findings from the same lot of product spurred FDA into action by placing the firm on import alert to cease entry of the violative product until corrective action was taken by the firm. MDP's positive sample finding supported FDA's ability to take regulatory action quickly to ensure protection of the public's health. Further investigation by CDC linked this *S. javiana* isolate to a previous outbreak. The PFGE pattern for *S. javiana* isolate reveals that it matches nine human cases of Salmonellosis linked to green onions imported from Mexico in an outbreak investigation that included seven States (Figure 5A).

S. saintpaul was isolated from a New York hot pepper sample. This information was shared with CDC during the *Salmonella*-hot pepper outbreak, and CDC matched this isolate with a multi-State outbreak of 30 cases of human Salmonellosis (Figure 5B).

S. oranienberg was isolated from a California alfalfa sprout sample and was matched by CDC to another multi-State outbreak of Salmonellosis involving 26 cases of illness. In addition, the PFGE pattern of the MDP isolate is shown to be identical to *Salmonella* isolated from spent irrigation water samples collected in Arizona (Figure 5C).

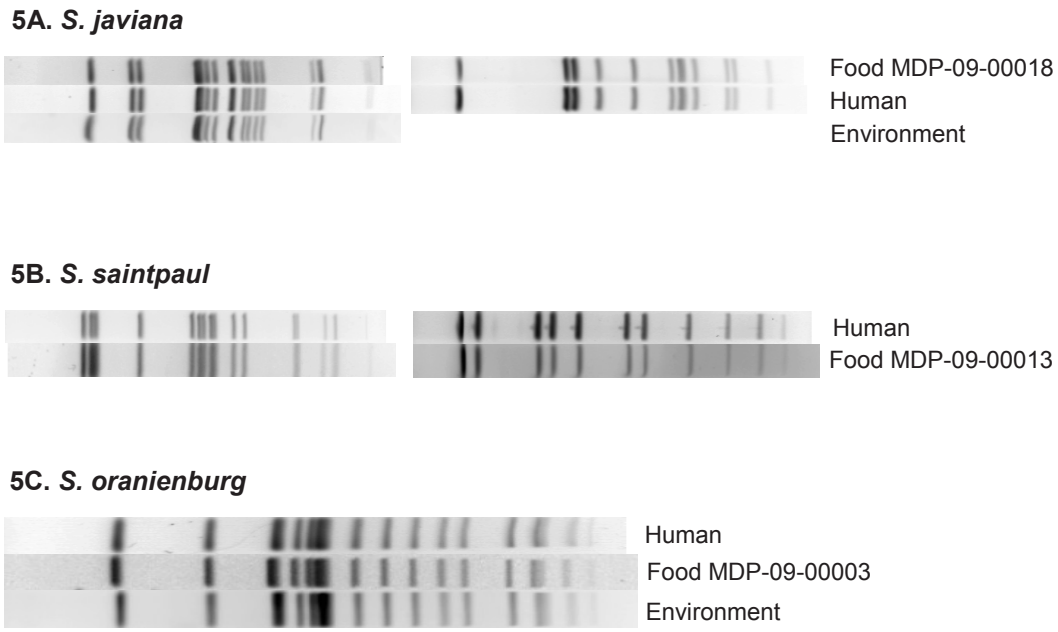


Figure 5. *Salmonella* Isolates PFGE Patterns. This figure compares PFGE patterns of MDP food isolates to human or environmental isolates submitted by other agencies to the PusleNet Database. Matching PFGE patterns helps epidemiological investigators trace the source of foodborne illnesses.

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Definitions:

Antimicrobial susceptibility: The result of microbes changing in ways that reduce or eliminate the effectiveness of drugs, chemicals, or other agents to cure or prevent infections.

AOAC® INTERNATIONAL: An internationally recognized organization that validates and approves analytical methods for foods and agriculture.

Aseptic: Free of microbial contamination.

Cultural Methods: Use of rich or selective media for the growth and identification of target bacteria.

Deoxyribonucleic acid (DNA): The molecule that encodes genetic information required to constitute a living and reproducing organism. DNA-based technologies exploit the uniqueness in the DNA sequences of a given organism in detection and identification methods.

eLEXNET: The electronic laboratory exchange network (eLEXNET) is an electronic system administered by the Food Emergency Response Network (FERN) that allows the exchange of laboratory analytical data among over 100 public health laboratories at the Federal, State and local levels. eLEXNET is FERN's data capture mechanism.

Enterohemorrhagic *E. coli* (EHEC): Strains of *E. coli* that are the primary cause of hemorrhagic colitis or bloody diarrhea, which can progress to the potentially fatal hemolytic uremic syndrome. EHEC are typified by the production of verotoxin or Shiga toxins (Stx). *E. coli* O157:H7 is the prototypic EHEC.

Enterotoxigenic *E. coli* (ETEC): Strains of *E. coli* that are the causative agent of travelers' diarrhea and illness characterized by watery diarrhea with little or no fever. Pathogenesis of ETEC is due to the production of any of several enterotoxins, including heat-labile enterotoxin and heat-stable toxin.

Genomic fingerprinting: Techniques used in the identification and/or classification of organisms exploiting the differences in the DNA sequence.

Green Fluorescent Protein (GFP): Expression of the gene from jellyfish in bacterial control cultures is used as a marker.

Indicator organism: A microorganism or group of microorganisms whose presence indicates unsanitary condition or fecal contamination.

Isolate: Target bacterial strain isolated as a pure culture and identified.

Most Probable Number (MPN): Most Probable Number (MPN) is a statistical expression for estimating the microbial density in a culture or per unit volume of water.

National Antimicrobial Resistance Monitoring System (NARMS): A collaborative effort among the Centers for Disease Control and Prevention, the Food and Drug Administration, and the U.S. Department of Agriculture to monitor antimicrobial resistance of human enteric bacteria, including *Campylobacter, Salmonella, Escherichia coli* O157:H7, and *Shigella*.

Pathogen: Specific causative agent (e.g., a bacterium or virus) of disease.

Polymerase Chain Reaction (PCR): A technique used to amplify a specific region of DNA into a large number of copies in order to produce enough DNA to be adequately tested. PCR can be used to identify, with a very high probability, disease-causing viruses and/or bacteria.

Multiplex PCR (mPCR) involves simultaneous amplification of more than one specific region of DNA or specific genes for various analytes.

Proficiency test sample: Any matrix sample prepared for the purpose of determining biases, accuracy, and/or precision among analysts and/or laboratories or of a single analyst or laboratory.

PulseNet: A national network of local, State, and Federal public health and food laboratories coordinated by the Centers for Disease Control and Prevention (CDC) to detect foodborne disease case clusters and outbreaks and facilitate identification of the source by standardized genomic fingerprinting (molecular subtyping) of various pathogenic bacteria using pulsed-field gel electrophoresis (PFGE) technology.

Pulsed field gel electrophoresis: (PFGE) is designed to separate DNA too large to be separated by conventional gel electrophoresis and is a highly discriminatory method for the differentiation of bacterial isolates based on differences in DNA content.

Serotyping: An antigen and antibody reaction technique that is used to differentiate strains of microorganisms based on differences in the antigenic composition of a certain structure such as the cell wall components or flagella.

Shiga toxin: A family of toxins produced by *Shigella dysenteriae* type I and shiga toxin-producing *E. coli.* These toxins have a cytotoxic effect on intestinal epithelial cells that causes characteristic bloody diarrhea.

Virulence attributes/factors: A bacterial product, usually a protein or carbohydrate (polysaccharide) that contributes to virulence or pathogenicity.

Virulence: The degree or intensity of pathogenicity of an organism as indicated by case fatality rates and/or ability to invade host tissues and cause disease.

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