Microbiological Data Program
Progress Update and 2008 Data Summary

United States Department of Agriculture

Agricultural Marketing Service

Science & Technology Programs

September 2009

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United States Department of Agriculture

Marketing and Regulatory Programs

Agricultural Marketing Service

1400 Independence Ave.
Washington, DC
20250

September 2009

To the Reader:

I am pleased to present the USDA Microbiological Data Program (MDP) 2008 Data Summary. In 2008, MDP tested five commodities (alfalfa sprouts, cantaloupe, lettuce, spinach, and tomatoes). MDP also performed a special survey of tomatoes, cilantro, bulb onions, green onions, and hot peppers to assist the Centers for Disease Control and Prevention and the U.S. Food and Drug Administration during the *Salmonella saintpaul* 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 2008: 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 2008 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

Rayne Pegg
Administrator

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Acknowledgements

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State Agencies

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Colorado Department of Agriculture
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Washington State Department of Agriculture
Wisconsin Department of Agriculture, Trade and Consumer Protection

Laboratories

Colorado Department of Agriculture
Inspection & Consumer Services Division
Laboratory Section
2331 West 31st Ave.
Denver, CO 80211-3859

Florida Department of Agriculture and Consumer Services
Bureau of Food Laboratories, Bldg. 9
3125 Conner Blvd.
Tallahassee, FL 32399-1650

Michigan Department of Agriculture
Laboratory Division
1615 South Harrison Rd.
East Lansing, MI 48823-5224

Minnesota Department of Agriculture
Laboratory Services Division
601 Robert Street North
St. Paul, MN 55155-2531

New York Department of Agriculture and Markets
Food Laboratory
1220 Washington Ave.
State Office Campus, Bldg. 7
Albany, NY 12235

Ohio Department of Agriculture
Consumer Analytical Laboratory Bldg. 3
8995 East Main St.
Reynoldsburg, OH 43068

U.S. Department of Agriculture
Agricultural Marketing Service
National Science Laboratory
801 Summit Crossing Pl.
Gastonia, NC 28054

Washington State Department of Agriculture
3939 Cleveland Ave., SE.
Olympia, WA 98501

Wisconsin Department of Agriculture, Trade
and Consumer Protection
Bureau of Laboratory Services
4702 University Ave.
Madison, WI 53705

Program Administration

U.S. Department of Agriculture
Agricultural Marketing Service

Deputy Administrator, Science and Technology Programs:
Robert L. Epstein
1400 Independence Ave., SW., Room 1090-S
Washington, DC 20250

Director:
Martha Lamont
Monitoring Programs Office
8609 Sudley Rd., Ste. 206
Manassas, VA 20110
(703) 330-2300 x 117, Facsimile (703) 369-0678

E-mail: amsmpo.data@ams.usda.gov

Web site: www.ams.usda.gov/mdp

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. The participating States are an important component of MDP program planning activities, particularly those involving technical 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 2008, 11 States collected fruit and vegetable samples (California, Colorado, Florida, Maryland, Michigan, Minnesota, New York, Ohio, Texas, Washington, and Wisconsin). MDP samples are comprised of both domestic and imported produce, as well as conventional and organically produced crops and, for some commodities, pre-washed, ready-to-eat produce.

The program tested five commodities: alfalfa sprouts; cantaloupe; pre-washed, pre-cut, bagged lettuce; fresh (unwashed) and pre-washed, bagged spinach; and tomatoes. Samples were screened for *Salmonella*, *Shigella*, and pathogenic *Escherichia coli (E. coli)* carrying shiga toxins and enterotoxins, including *E. coli* O157:H7. All five commodities were tested for generic *E. Coli*, and bagged lettuce and bagged spinach samples were enumerated for Total Viable Count (TVC) and total coliforms. Any pathogens that were isolated and confirmed were shipped to FDA's Center for Veterinary Medicine (CVM) for additional characterization, including antimicrobial resistance and genomic fingerprinting by pulsed field gel electrophoresis (PFGE); antimicrobial resistance data were included in the National Antimicrobial Resistance Monitoring System (NARMS) database and PFGE patterns were entered into CDC's Pulsenet database. In addition, CVM performed serotyping on all *Salmonella* isolates. All pathogenic *E. coli* isolates were shipped to Pennsylvania State University for serotyping and testing for 17 virulence-specific genes associated with different categories of pathogenic *E. coli*. MDP communicates pathogenic findings to Federal and State agencies that in turn, notify vendors. Affected products may be voluntarily removed from the food distribution system.

During the *Salmonella saintpaul* outbreak, MDP laboratories tested additional samples of tomatoes (601), cilantro (219), bulb onions (150), green onions (141), and hot peppers (225) to aid CDC and FDA in the outbreak investigation. Samples were collected in Colorado, Maryland, New York, Texas, and Washington.

MDP analyzed a total of 11,669 samples during the 11 months of sampling and testing operations. Seventy-nine percent of the samples were from domestic sources, 19 percent were imported, and 2 percent were of unspecified origin. MDP screening resulted in 16 *Salmonella* isolates, and MDP laboratories isolated pathogenic *E. coli* strains from 11 samples. MDP screening for *E. coli* 157:H7 and *Shigella* did not result in any confirmed isolates.

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 reporting and sharing data on foodborne outbreaks has improved. Microbiological data obtained from MDP's fresh produce screening effort can be used to enhance the understanding of indicators and 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 the changes in cultivation, irrigation, harvesting practices, postharvest handling and packaging of fresh produce to meet changing consumer life styles, preferences, and demands, will help refine Good Agricultural Practices (GAP) and Hazard Analysis and Critical Control Points (HACCP) plans used by growers, processors, and food handlers. Furthermore, MDP data which include antimicrobial resistance, genomic fingerprints, serotypes, and virulence attributes, will help 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 2008

This summary consists of the following sections: (I.) Introduction, (II.) Sampling, (III.) Laboratory Operations, (IV.) Database Management, (V.) Summary of 2008 Data

I. Introduction

Fresh produce is recognized as an important component of a healthy diet. Because most produce is grown in a natural 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 (1, 2). In 2001, Congress authorized funding for a microbiological monitoring program to collect data on fresh fruit and vegetables.

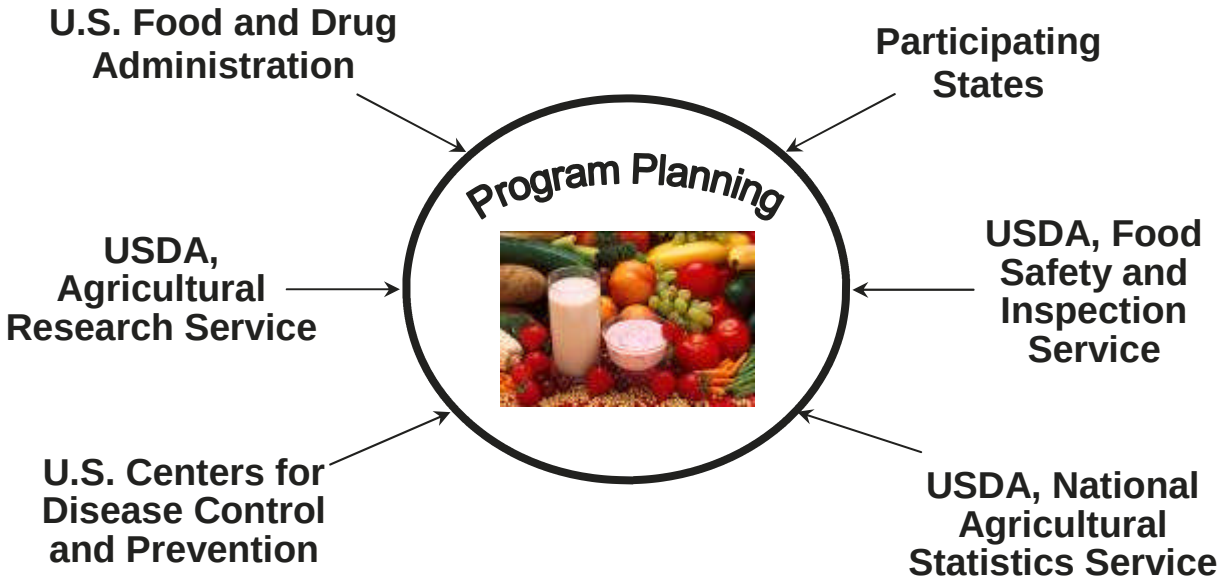
The Microbiological Data Program's (MDP) mission is to collect information regarding the incidence and identification of targeted foodborne pathogens and indicator organisms on fresh fruit and vegetables. This publication provides an overview of data collected in 2008 and summarizes program refinements made during that year. The Agricultural Marketing Service (AMS) Monitoring Programs Office (MPO) manages MDP and is responsible for administrative, sampling, technical, and database activities.

Figure 1 (a) illustrates MDP program planning activities. AMS coordinates its planning and program requirements with the Centers for Disease Control and Prevention (CDC) and the U.S. Food and Drug Administration (FDA). MDP relies on the expertise of scientists from FDA, CDC, and academia. AMS and USDA's National Agricultural Statistics Service (NASS) statisticians designed sampling plans based on per capita consumption, marketplace availability, product origin, and time in transit and storage. The participating States are an important component of MDP program planning activities, including technical and quality assurance (QA) issues.

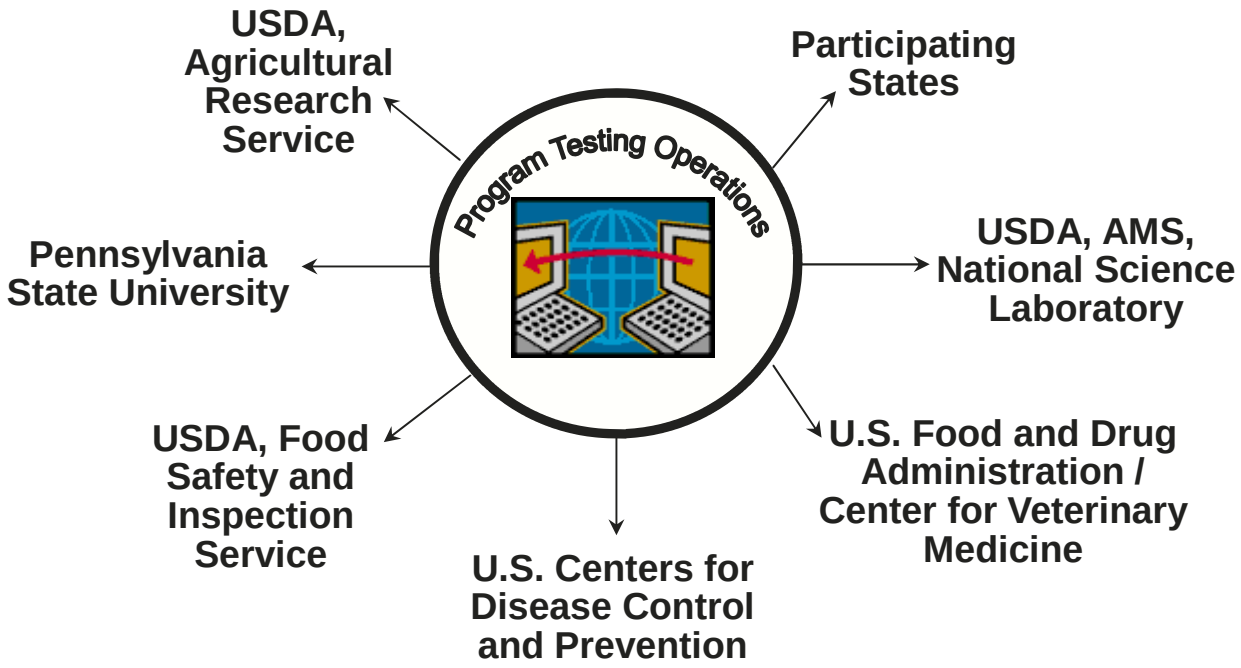
Figure 1 (b) depicts MDP program testing operations. The participating State laboratories and AMS National Science Laboratory (NSL) analyze MDP samples collected by trained State sample collectors. FDA's Center for Veterinary Medicine (CVM) and Pennsylvania State University (PSU) provide additional testing services for isolate characterization. Information on MDP data and isolates is shared with CDC and FDA.

Commodities tested were selected in consultation with 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 2008 included: alfalfa sprouts; cantaloupe; pre-washed, pre-cut, bagged lettuce; fresh (unwashed) and pre-washed, bagged spinach; and tomatoes. Commodities were tested for generic *Escherichia coli* (*E. coli*), *Salmonella*, *Shigella*, and *E. coli* strains with human pathogenic potential, including *E. coli* O157:H7. Isolates of these organisms were sent to specialized laboratories for further characterization including testing for antimicrobial resistance, genomic fingerprinting, serotyping, and virulence attributes. Pre-washed, pre-cut, bagged lettuce and pre-washed, bagged spinach samples were enumerated for Total Viable Count (TVC) and total coliforms. A special survey was conducted on cilantro, bulb onions, green onions, hot peppers, and tomatoes in response to the *Salmonella saintpaul* outbreak of 2008.

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



(a) MDP Planning



(b) MDP Program Operations

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.*

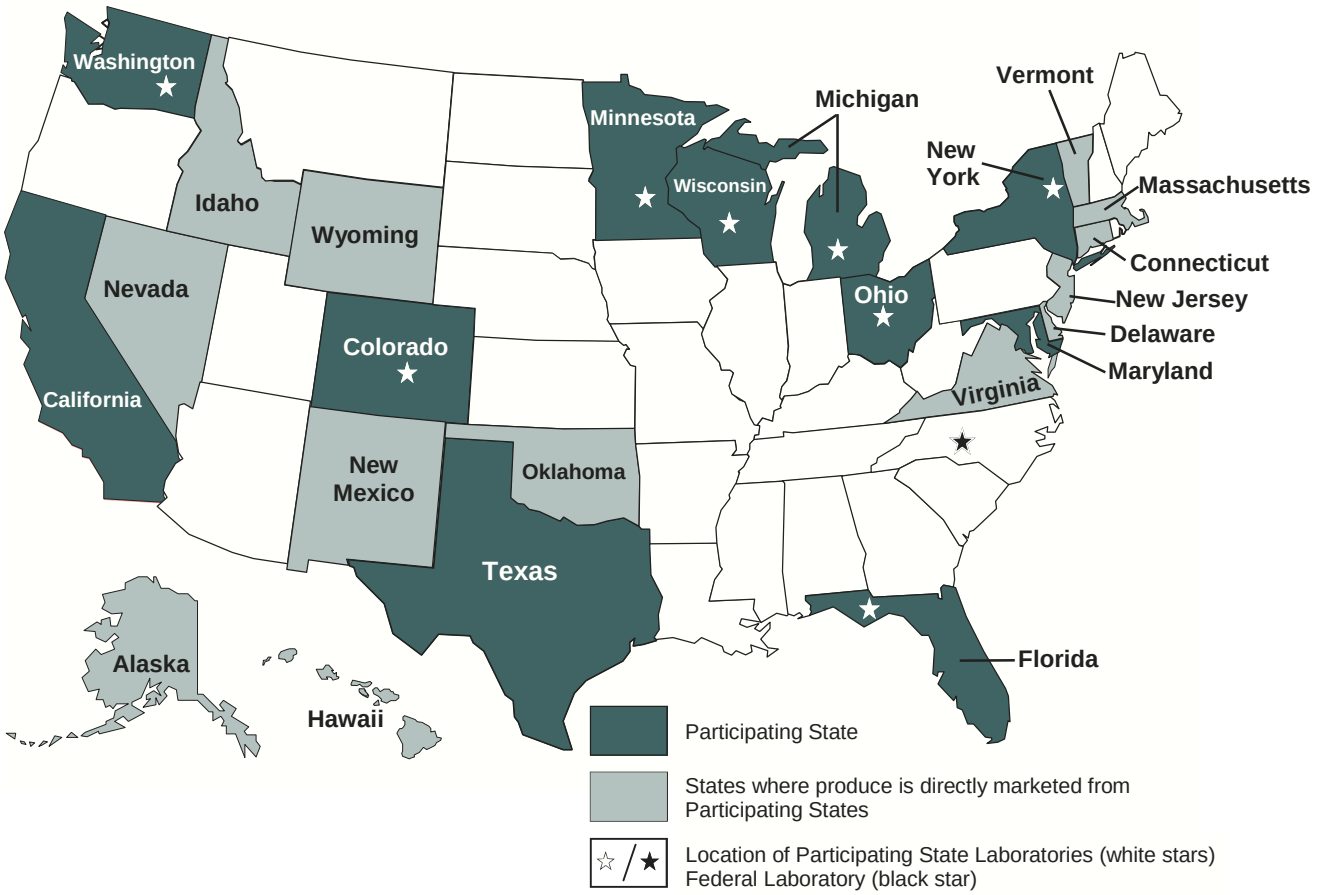


Figure 2. Program Participants. *During 2008, AMS established cooperative agreements with 11 States to sample and/or test MDP commodities. Samples collected by California, Maryland and Texas are analyzed by the Ohio laboratory and the National Science Laboratory in Gastonia, NC. In addition, the Colorado laboratory analyzed cantaloupes and green onions collected by Florida. States that do not participate in MDP's sampling program but are in the direct distribution networks of the participating States are also shown.*

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 more than 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 one USDA AMS facility, NSL. Samples collected by Maryland were analyzed by the Ohio laboratory. Samples collected by Texas were analyzed by AMS NSL. The Colorado laboratory analyzed spinach collected by California; the Ohio laboratory analyzed alfalfa sprouts and lettuce collected by California; and the AMS NSL analyzed cantaloupes and tomatoes collected by California.

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

FDA/CVM for antimicrobial resistance testing. These data are being added to the National Antimicrobial Resistance Monitoring System (NARMS) database. Additionally, CVM performs genomic fingerprinting on MDP isolates for inclusion in the PulseNet system.

In 2007, MDP laboratories replaced the enzyme-based assay for enumeration of generic *E. coli* and the Most Probable Number (MPN) analysis with an automated system. This new system is based on the same unique enzyme assay; however, the setup, assay, and the capture and analysis of data have been automated. The automated system allowed the laboratories to reduce staff time by streamlining this labor-intensive assay while at the same time removing the bias in subjective interpretation of results. The automated system was also used for screening bagged lettuce and bagged spinach samples for total coliforms; however, the assay for detecting coliform bacteria is based on acid production due to fermentation and is different from the one used for detecting *E. coli*. The automated system was also used to provide Total Viable Count (TVC) data for bagged lettuce and bagged spinach samples.

AMS employed DNA-based screening for *Salmonella*, *Shigella*, and pathogenic *E. coli*, including *E. coli* O157:H7. All samples were screened for the presence of pathogenic *E. coli* that harbor shiga toxins (STECs) and enterotoxins (ETECs) using mPCR technology. STEC and ETEC are two groups of *E. coli* that cause the majority of enteric diseases and are therefore important to human health.

As the program evolves, procedures and methods are being refined to provide information necessary for making science-based food safety decisions. AMS continues to use quicker, more reliable and more sensitive technologies for improved microbial detection and improve 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, population, and consumption on a national scale. 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 U.S. 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 is a useful and defensible baseline survey.

Alfalfa sprouts, cantaloupes, lettuce, and tomatoes remained in the program at 2007 levels. Based on consultations with FDA, spinach was introduced in 2008 replacing green onions. The five commodities were selected because they are high-consumption fruit and vegetables in the U.S. diet, are often consumed raw, and have been implicated in outbreaks.

For 2½ months, mid July through the end of September, MDP implemented a temporary

program change to focus on the *Salmonella saintpaul* foodborne outbreak. During that timeframe, five States (Colorado, Maryland, New York, Texas, and Washington) added cilantro, bulb onions, green onions, hot peppers, and additional tomatoes to their normal collection scheme to aid CDC and FDA in their search for the source of the *Salmonella* contamination. Table 1 shows the number of extra samples collected and tested for each specially added commodity. MDP resumed its normal collection schedule in October.

Commodity	Number of Samples Collected and Analyzed
Cilantro	219
Green Onions	141
Hot Peppers	225
Onions, Bulb	150
Tomatoes	601
TOTAL	1,336

Table 1. Number of Samples Tested during* Salmonella saintpaul *Outbreak. *This table shows the number of samples collected and analyzed in response to the* Salmonella saintpaul *outbreak. The 601 tomatoes are in additional to the regularly scheduled tomatoes.*

All samples in a State are collected on the same day of each week. The samples of cantaloupes, hot peppers, and tomatoes collected from a site consist of three individual units or groups of units of produce collected from the same box/ container. Fresh, pre-sealed bags or clamshells of alfalfa sprouts, lettuce, and spinach samples are from the same lot number. Other unbagged fresh commodities, such as loose spinach or green onion bunches, are collected in groups of three as specified weights from the same box/ container. 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. Nevertheless, 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. Additional information is provided on specific requirements for packaging samples that are sensitive to ethylene.

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 (refer to Table 2 and Figure 3 for sample origin information). Except for national emergency situations, such as special collections in response to a foodborne outbreak, samples are collected on a year-round basis and typically over at least two growing seasons to accommodate differences in growing conditions. Sampling is apportioned according to population of the participating State. That is, 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; Colorado, 2; Florida, 7; Maryland, 4; Michigan, 6; Minnesota, 2; New York, 9; Ohio, 6; Texas, 8; 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.

Commodity	Country		Number of Samples
Cilantro	Mexico		15
		TOTAL	15
Cantaloupe	Canada		3
	Costa Rica		162
	Guatemala		324
	Guatemala/Honduras		12
	Honduras		171
	Mexico		87
	Nicaragua		12
		TOTAL	771
Green Onions	Canada		15
	Mexico		63
	Mexico/USA		3
		TOTAL	81
Hot Peppers	Mexico		9
		TOTAL	9
Lettuce, Bagged	Canada		3
	Mexico/USA		21
		TOTAL	24
Onions	Mexico		12
		TOTAL	12
Spinach	Canada		24
	Mexico		60
	Mexico/USA		18
		TOTAL	102
Tomatoes	Canada		175
	Mexico		1,072
	Mexico/USA		6
		TOTAL	1,253

Table 2. Distribution of Imported Samples. *This table details the number of imported samples by country of origin and by commodity. None of the alfalfa sprouts were reported as imported.*

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. Between February and December 2008, 11,669 samples were collected from 376 sites across the country and analyzed by MDP laboratories. Table 3 provides a detailed breakdown of sample numbers collected by commodity.

All samples are selected and bagged using aseptic techniques (i.e., sterile latex gloves and 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 the use of adequate packing materials for cushioning and insulation are required to maintain refrigerated temperatures during transport. Sample temperatures and the condition of each sample are observed and recorded upon receipt at each laboratory. If the integrity of a sample is in question, the laboratory requests that the particular commodity be sampled again. All samples are shipped on the same day as sample collection by overnight delivery so that the laboratory can begin analysis 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 include California, Maryland, and Texas; these State samples are shipped to the Colorado and Ohio laboratories and AMS NSL in Gastonia, NC, for analysis.

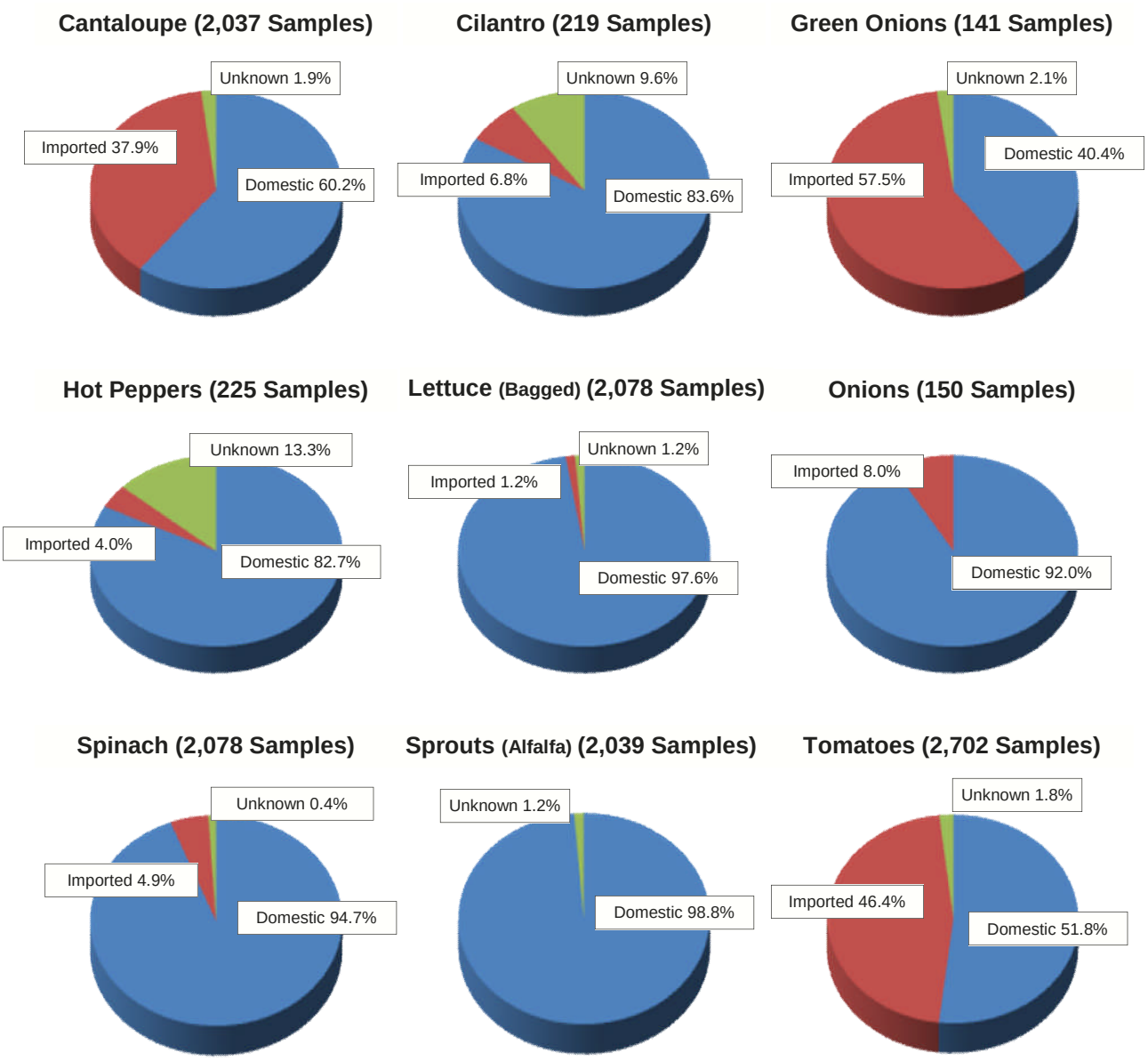


Figure 3. Commodity Origin. *The proportion of domestic, imported or unknown origin for each commodity is depicted for samples tested in 2008.*

Alfalfa sprouts, cantaloupe, lettuce, spinach, and tomatoes were collected and tested as routine commodities for 2008. Alfalfa sprouts are most often grown in drums and packaged in controlled environments. Cantaloupe and tomatoes are shipped and sold to distribution centers and terminal markets as whole, individual units. For pre-washed, pre-cut bagged lettuce, all lettuce varieties were acceptable, whether packaged as a single variety or mixed. For fresh, unwashed and pre-washed, bagged spinach, all spinach varieties were acceptable; pre-washed bagged spinach was acceptable either as a single variety or mixed. Bags containing lettuce or spinach mixed with other greens were not acceptable. Most of these commodities are harvested primarily by hand although some mechanical harvesting does occur.

The produce may be packaged in the field or taken to a packinghouse (e.g., tomatoes require

State	Cantaloupe	Cilantro	Green Onions	Hot Peppers	Lettuce (Bagged)	Onions	Spinach	Sprouts (Alfalfa)	Tomatoes	Total
California	420				455		462	438	423	2,198
Colorado	66				66		66	66	314	578
Florida	231				231		231	231	231	1,155
Maryland	132	33	24	36	129		131	123	132	740
Michigan	198				198		198	195	198	987
Minnesota	66				66		66	66	65	329
New York	297	81	81	81	297		297	297	300	1,731
Ohio	198				198		198	198	198	990
Texas	231	72		72	240	150	231	239	643	1,878
Washington	132	33	36	36	132		132	120	132	753
Wisconsin	66				66		66	66	66	330
Totals	2,037	219	141	225	2,078	150	2,078	2,039	2,702	11,669

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

classification for color and/or size). At the packinghouse, some types of produce are 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 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 alfalfa 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 Sample Information Forms (SIFs) to document information required for chain-of-custody and to capture other information needed to characterize the sample. Sample collectors use the forms to record information such as: (1) State of sample collection, (2) collection date, (3)

commodity code, (4) testing laboratory code, and (5) sample collector name. Other information collected includes the country of origin of the sample, any production claims (such as organic), and any postharvest treatments.

An electronic SIF (e-SIF) capturing system was implemented in 2003 and continues to be used to record relevant sample information. A customized software application allows States to capture SIFs electronically using laptop or handheld computers. Sample information is captured in the MDP database files on the same day as sample collection or early the next day.

MDP sampling operations are conducted with the use of 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*, *Shigella*, and *E. coli* strains carrying shiga toxins and enterotoxins (STECs and ETECs, respectively), including *E. coli* O157:H7. MDP laboratories performed mPCR screening of all samples for pathogenic *E. coli*, based on the presence of genes coding for shiga toxins and enterotoxins. Isolates of these organisms were sent to FDA's CVM and to the Gastroenteric Disease Center at PSU for further characterization. Tests performed by FDA/CVM and PSU included testing for antimicrobial resistance, genomic fingerprinting, serotyping, and testing for virulence attributes.

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 from time of receipt in the laboratory to allow for different sample arrival times from the various collection sites. Only excess soil was removed prior to testing.

All samples were washed in Universal Pre-enrichment Broth (UPB) with 0.1 percent Tween® (Polysorbate 80). Cantaloupes, bulb onions, green onions, lettuce, hot peppers, spinach, and tomatoes were manually shaken, followed by overnight soaking. Alfalfa sprouts were blended using a Stomacher® blender, the plant material removed, and the remaining wash incubated overnight. Soaking followed by overnight incubation enhances recovery of pathogens that may be trapped in cracks, crevices, and biofilms.

For *E. coli* enumeration, TVC, and total coliform assays, an AOAC®-certified enzyme-based automated TEMPO® system was used for detection and enumeration (pre-washed, pre-cut, bagged lettuce and pre-washed, bagged spinach samples only). This system reports enumeration in colony-forming units per gram of sample test (cfu/g).

Genomic DNA was extracted from each enriched sample and purified for use in detecting pathogens via DNA-based PCR assays. During 2008, the laboratories assessed and began employing automated systems for the extraction and purification of genomic DNA from bacterial cultures in order to streamline the labor-intensive preparation of DNA samples for PCR assays.

All samples were screened by mPCR procedures for STECs and ETECs. MDP laboratories used PCR assays and automated instruments for the detection of *Salmonella*,

Shigella, and enterohemorrhagic *E. coli* O157:H7 in produce samples. Cultural methods involving selective growth media and Immunomagnetic Separation (IMS) technology were employed for isolation of target bacteria. In addition to cultural methods, automated identifications based on biochemical tests and serotyping of surface antigens were used in the confirmation of isolates for the target pathogens.

The main objectives of the QA/Quality Control (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 2001 Bacteriological Analytical Manual (3), AOAC® methods, USDA's Food Safety and Inspection Service (FSIS) Microbiological Laboratory Guide, the U.S. Environmental Protection Agency's Good Laboratory Practices, and International Standards Organization (ISO) 17025 guidelines. MDP analytical methods are published at www.ams.usda.gov/mdp. SOPs provide uniform administrative, sampling, and laboratory procedures. MDP laboratories participated in the proficiency testing of an unknown pathogen (*Salmonella*, *Shigella*, or *E. coli* O157:H7) that was spiked into produce samples. Proficiency testing rounds are administered by MPO.

Positive and negative controls and a sterile media blank were required for each sample set. MDP laboratories use positive control strains of *Salmonella typhimurium* and *E. coli* O157:H7 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 taken along with the sample cultures from the preenrichment step to isolation and identification of target isolates using cultural, immunological, and serological methods. MDP laboratories also used automated instrumentation for confirmation of isolates based on biochemical reactions.

A Technical Advisory Group, comprised of microbiologists from each 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's Agricultural Research Service (ARS), 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 are performed by MPO staff responsible for collating and reviewing data for conformance with SOPs.

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

IV. Database Management

MDP maintains an electronic database that serves as a central data repository. The central database resides at MPO in Manassas, VA. 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/

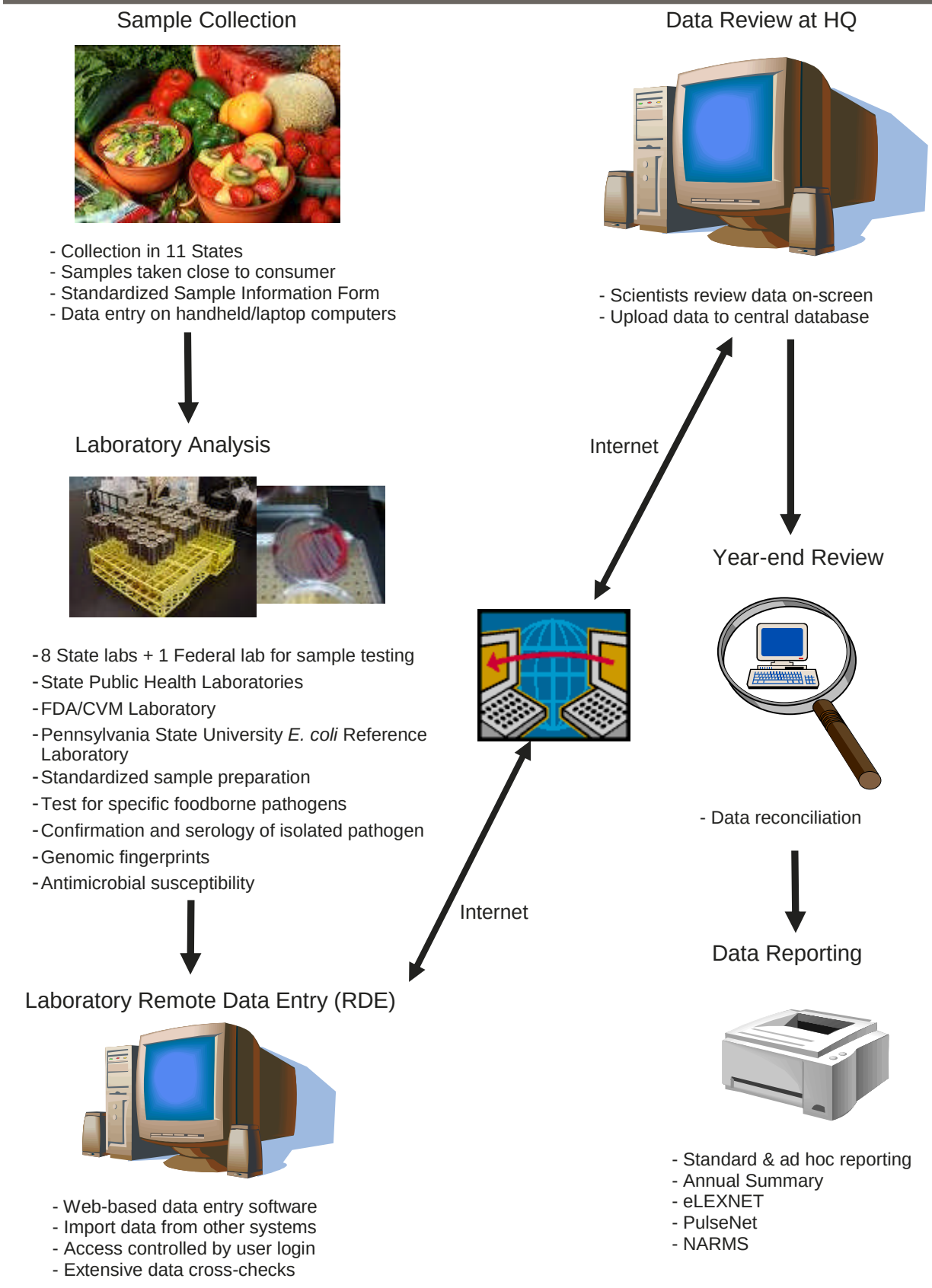


Figure 4. MDP Data Pathway. *An illustration of MDP data path from sample collection, through laboratory analysis and reporting.*

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 SIF on handheld or laptop computers. The electronic SIF (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® 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 2008 Data

Table 2 specifies the distribution of imported samples by commodity and country of origin. Figure 3 illustrates the proportion of samples that were domestic, imported, and of unknown origin for each commodity. Seventy-nine percent of the samples were from domestic sources, 19 percent were imported, and 2 percent were of unspecified origin. Table 3 shows the distribution of samples among each commodity and collection State.

All samples were washed or blended (alfalfa sprouts) in UPB in order to streamline the screening process for all target bacteria. *E. coli*, TVC, and total coliform enumerations were performed using the automated TEMPO® system – TVC and total coliform testing was performed only on pre-washed, pre-cut, bagged lettuce and pre-washed, bagged spinach samples. A soaking step was implemented for cantaloupe, lettuce, spinach, and tomatoes to improve pathogen detection. Alfalfa sprouts were blended, the plant material removed, and the remaining wash incubated overnight.

Genomic DNA was extracted from each enriched sample and purified for use in detecting pathogens. The BAX® instrument, an 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 pathogenic *E. coli* by mPCR or *Shigella* by conventional PCR. Table 4 shows the number of samples tested for each organism.

Positive individual samples were cultured for isolation and identification of the organism. Identification of isolates was confirmed using a conventional biochemical testing system, an AOAC® performance-tested kit, or a commercial biochemical kit or system, approved by MDP. In addition to biochemical identification of an isolate, all MDP participating State laboratories were required to confirm the identification by serotyping. Isolates were then sent to FDA/CVM for expanded serotyping (except pathogenic *E. coli* isolates which were

State	*E. coli*	Pathogenic *E. coli*	*E. coli O157:H7*	*Salmonella* by BAX	*Salmonella* by VIDAS	Shigella	Total Coliform	Total Viable Count	Total Number of Tests
California	2,198	2,198	2,198	2,198		2,198	917	622	12,529
Colorado	330	330	330	578	90	330	132	102	2,222
Florida	1,155	1,155	1,155	1,155		1,155	462	449	6,686
Maryland	647	647	647	740		644	258	260	3,843
Michigan	987	987	987	987		984	382	378	5,692
Minnesota	329	329	329	329		329	132	126	1,903
New York	1,485	1,485	1,485	1,731		1,484	588	488	8,746
Ohio	990	990	990	990		990	395	396	5,741
Texas	1,231	1,231	1,231	1,878	330	1,230	387	365	7,883
Washington	648	648	648	753		648	264	201	3,810
Wisconsin	330	330	330	330		330	117	117	1,884
Totals	10,330	10,330	10,330	11,669	420	10,322	4,034	3,504	60,939

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

sent to PSU for serotyping and virulence testing), antimicrobial resistance testing, and genomic fingerprinting.

Enumeration of *E. coli*

In 2008, a total of 10,330 samples were enumerated for *E. coli* using the automated TEMPO® system, an AOAC-certified method. As shown in Table 5, only 525 samples displayed MPNs greater than 10 (approximately 5% of samples).

E. coli is typically found as a commensal organism in the intestinal tracts of warm-blooded animals and humans. As such, it is often used as an indicator organism and as a measure of cleanliness for irrigation waters, produce wash waters, and other surfaces with which the produce may be in contact. A detection of *E. coli* does not mean or imply the presence of a pathogen but indicates that there is a possibility of pathogen presence and that further testing may be warranted if the number of *E. coli* cells is above a certain threshold. Use of *E. coli* enumeration is most useful for Hazard Analysis Critical Control Points (HACCP) analysis.

MPN Range	Number of Samples
< 10	9,805
10 - 99	389
100 - 999	108
1,000 - 10,000	13
> 10,000	15
Total Number of Samples Tested	10,330

***Table 5. Number of Samples Tested for* E. coli.** *This table shows the results of* E. coli *enumeration.*

Pathogenic *E. coli*

In 2008, a total of 10,330 samples were screened for pathogenic *E. coli* that harbor shiga toxins (STECs) and enterotoxins (ETECs) using an mPCR assay developed by FDA. Table 6 displays the number of samples initially screened for *E. coli* and further tested for pathogenic *E. coli*, as well as the number of samples that tested positive for pathogenic *E. coli*.

Thirty-five samples were identified as positive for pathogenic *E. coli* and 11 isolates were obtained. Seven of these isolates were identified as carrying shiga toxins (STECs) and four as carrying enterotoxins (ETECs). In addition to the technological differences between detection by PCR and isolation by cultural means, several other factors influence the rate of successful isolation, including: an overwhelming amount of background micro-flora in comparison to the small number of target bacterial cells, differential growth rates of various bacteria, and additional growth requirements.

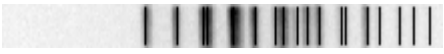
The 11 STEC and ETEC isolates were sent to PSU for serotyping and further characterization and to FDA/CVM for antimicrobial resistance testing. For each isolate, PSU conducted 17 tests that included identifying virulence-specific genes associated with different classes of pathogenic *E. coli* and serotyping for somatic O antigens and flagellar H antigens. FDA/CVM conducted tests on resistance to 15 different antibiotics and genomic finger-printing on these isolates. The results of PSU and FDA/CVM testing are shown in Table 7. Two of the STEC strains were isolated from pre-washed, pre-cut bagged lettuce and five from spinach (either fresh, unwashed spinach or pre-washed, pre-bagged spinach); three ETEC strains were isolated from spinach and one from alfalfa sprouts. To characterize an isolate as a human pathogen capable of causing disease, there must be an interplay of several proteins, including toxins, encoded by respective genes. MDP only identified toxin genes; the additional testing required to determine the disease-causing potential of these isolates is not within the scope of MDP.

Total Viable Count

Total Viable Count (TVC) provides an indication of the presence of microorganisms such as bacteria in a sample, more specifically, the total number of colony-forming units per gram (cfu/g) or milliliter of sample. TVC

Commodity	Number of Samples Tested	Number of Samples Screened by mPCR	Number of Pathogenic *E. coli*-Positive Samples	Number of Positive Isolates Obtained
Cantaloupe	2,037	2,037	0	0
Lettuce, Bagged	2,078	2,078	8	2
Spinach	2,078	2,078	22	8
Sprouts (Alfalfa)	2,039	2,039	4	1
Tomatoes	2,098	2,098	1	0
Total	10,330	10,330	35	11

***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.

Commodity	Pathogenic Class	Toxic Genes Identified	Serotyping O Antigen	Serotyping H Antigen	Pulsed-Field Gel Electrophoresis
Lettuce, Bagged	STEC	Stx-2, HlyA	8	28	
Lettuce, Bagged	STEC	STa, Stx-1, HlyA	136	16	
Spinach	ETEC	LT, STb	8	15	
Spinach	ETEC	LT	163	19	
Spinach	ETEC	STa, STb	42	37	
Spinach	STEC	Stx-1, Stx-2, HlyA	73	12	
Spinach	STEC	Stx-2, HlyA	130	11/47	
Spinach	STEC	Stx-2	Neg	38	
Spinach	STEC	Stx-1, Cnf-2	Neg	16	
Spinach	STEC	Stx-1, Stx-2	Neg	16	
Sprouts (Alfalfa)	ETEC	STa	Neg	4	

Cnf-2 = Cytotoxic necrotizing factor 2

HlyA = hemolysin A

LT = heat-labile toxin

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

Neg - no serological reaction; did not react with standard antisera

Table 7. Characterization of Pathogenic* E. coli *Isolates Screened by mPCR. *This table provides data obtained from additional testing of pathogenic* E. coli *isolates initially screened by MDP laboratories. Information includes: pathogenic class, identified toxin genes, and serotyping results.*

MPNs were performed via the TEMPO® system. Table 8 portrays the TVC MPNs for bagged lettuce and spinach samples, which were washed prior to commercial packaging.

Coliform Bacteria

Coliform, or enteric, bacterial count is a subset of TVC and is often used as an indicator of direct or indirect fecal contamination. Table 9 shows the total coliform MPNs for bagged lettuce and spinach samples, which were washed prior to commercial packaging. Testing was performed via the automated TEMPO® system. In this assay, bacteria that produce acid from lactose fermentation are enumerated.

Salmonella

As depicted in Table 10, all 11,669 samples were screened for *Salmonella* by BAX-PCR. Of these samples, 86 were positive via the preliminary screening method. Of these 86 samples, 16 *Salmonella* isolates were confirmed: 9 from alfalfa sprouts, 3 from cantaloupe, 1 from cilantro, 1 from bagged lettuce, 1 from hot peppers, and 1 from spinach.

MPN Range	Number of Lettuce Samples	Number of Spinach Samples
Blank (<250)	21	17
250 - 999	23	2
1,000 - 9,999	125	6
10,000 - 99,999	411	50
100,000 - 999,999	662	233
>1,000,000	782	1,172
Total Number of Samples Tested	2,024	1,480

Table 8. Number of Samples Tested for Total Viable Count. *This table depicts the most probable number (MPN) ranges for total viable count (TVC) in bagged lettuce and spinach.*

MPN Range	Number of Lettuce Samples	Number of Spinach Samples
< 3	927	603
3 - 9	338	263
10 - 99	378	496
100 - 999	245	371
1,000 - 9,999	175	204
10,000 - 99,999	6	27
100,000 - 999,999	0	0
> 1,000,000	0	1
Total Number of Samples Tested	2,069	1,965

Table 9. Number of Samples Tested for Coliforms. *This table depicts the most probable number (MPN) ranges for total coliforms in bagged lettuce and spinach samples.*

Commodity	Number of Samples Tested by BAX	Number of Samples Tested by VIDAS	Number of Presumptive Positive Samples	Number of Positive Isolates Obtained
Cilantro	219	0	2	1
Cantaloupe	2,037	0	13	3
Green Onions	141	0	0	0
Hot Peppers	225	0	1	1
Lettuce, Bagged	2,078	0	25	1
Onions	150	0	1	0
Spinach	2,078	0	4	1
Sprouts (Alfalfa)	2,039	0	16	9
Tomatoes	2,702	420	24	0
TOTAL	11,669	420	86	16

***Table 10. Summary of Analysis for* Salmonella.** *This table shows the number of samples screened for* Salmonella *using BAX and VIDAS, the number of positive individual samples, and the number of isolates obtained.*

These 16 isolates were sent to FDA/CVM or State health department laboratories for identification by serotyping, antimicrobial resistance for 15 antibiotics, and genomic fingerprinting. Table 11 identifies each isolate and the associated serogroup. The isolates from alfalfa sprouts were all *Salmonella cubana*. While all the same species, they were not all genetically identical based on genomic fingerprinting and hence originate from different sources.

***E. coli* 0157:H7**

No enterohemorrhagic *E. coli* O157:H7 strain was isolated from the 10,330 samples screened (refer to Table 12). In this case, as with pathogenic *E. coli* analysis, several factors contribute to successful isolation, including the level of background microflora versus the number of target bacterial cells, differential bacterial growth rates, and additional growth requirements.

	Serotype/Identification			
Commodity	**Genus**	**Species**	**Serogroup**	**Pulsed-Field Gel Electrophoresis**
Cantaloupe	*Salmonella*	*S. Javiana*	D1	
Cantaloupe	*Salmonella*	*S. Javiana*	D1	
Cantaloupe	*Salmonella*	*S. Luciana*	F	
Cilantro	*Salmonella*	*S. Meleagridis*	E1	
Hot Peppers	*Salmonella*	*S. Cerro*	K	
Lettuce, Bagged	*Salmonella*	*S. Typhimurium*	B	
Spinach	*Salmonella*	*S. Newport*	C2	
Sprouts (Alfalfa)	*Salmonella*	*S. Cubana*	G2	
Sprouts (Alfalfa)	*Salmonella*	*S. Cubana*	G2	
Sprouts (Alfalfa)	*Salmonella*	*S. Cubana*	G2	
Sprouts (Alfalfa)	*Salmonella*	*S. Cubana*	G2	
Sprouts (Alfalfa)	*Salmonella*	*S. Cubana*	G2	
Sprouts (Alfalfa)	*Salmonella*	*S. Cubana*	G2	
Sprouts (Alfalfa)	*Salmonella*	*S. Cubana*	G2	
Sprouts (Alfalfa)	*Salmonella*	*S. Cubana*	G2	
Sprouts (Alfalfa)	*Salmonella*	*S. Cubana*	G2	

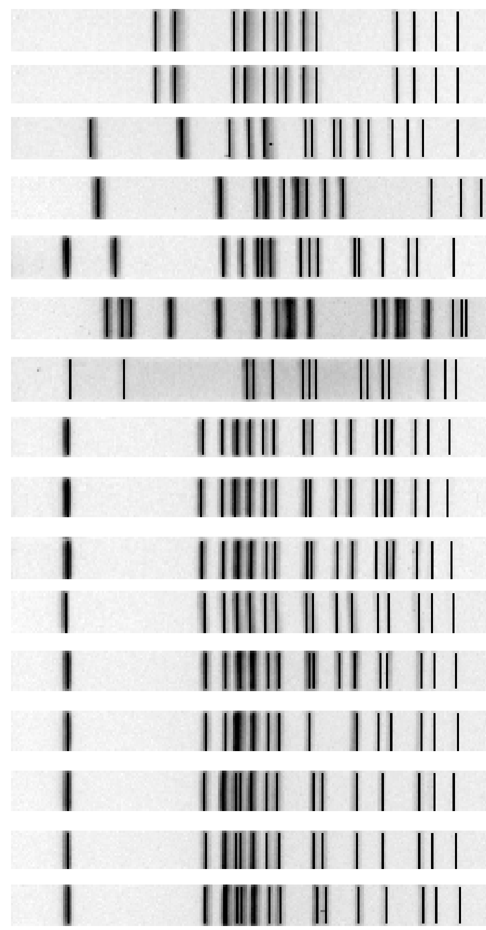


Table 11.* Salmonella *Identification and Serogroup. *This table summarizes the genus, species, and serogroup for each of the 16* Salmonella *isolates obtained in 2008.*

Shigella

No *Shigella* species were isolated from 10,322 samples screened (refer to Table 13).

Foodborne Outbreaks

In late May 2008, reports of a national foodborne outbreak were in the press headlines. At the request of CDC and FDA, MDP quickly initiated sampling and testing of additional tomatoes in Colorado and Texas – MDP sampling States where there were initially human illnesses – as well as in Maryland, New York, and Washington. The additional tomatoes sampled were only tested for *Salmonella spp*. As the investigation continued, MDP was asked to test cilantro, bulb onions, green onions, and hot peppers. MDP's existing sampling framework allows rapid response, so sampling and testing of these commodities was initiated within a week of the request. MDP's electronic, standardized reporting system allows for immediate capture of sample origin information as well as analytical results and associated QA data. Over the course of the outbreak, MDP laboratories tested 1,336 additional samples for *Salmonella*, demonstrating the quick response and adaptability of the program to foodborne produce-related outbreaks.

Commodity	Number of Samples Tested	Number of Presumptive Positive Samples	Number of Positive Isolates Obtained
Cantaloupe	2,037	0	0
Lettuce, Bagged	2,078	0	0
Spinach	2,078	0	0
Sprouts (Alfalfa)	2,039	0	0
Tomatoes	2,098	0	0
Total	10,330	0	0

Table 12. Summary of Analysis for* E. coli *O157:H7. *This table shows the number of samples tested for* E. coli *O157:H7 and the number of presumptive positives and isolates obtained.*

Commodity	Number of Samples Tested	Number of Presumptive Positive Samples	Number of Positive Isolates Obtained
Cantaloupe	2,037	0	0
Lettuce, Bagged	2,077	0	0
Spinach	2,072	0	0
Sprouts (Alfalfa)	2,039	0	0
Tomatoes	2,097	0	0
Total	10,322	0	0

***Table 13. Summary of Analysis for* Shigella.** *This table shows the number of samples tested for* Shigella *and the number of presumptive positives and isolates obtained.*

References:

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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 jelly fish 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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