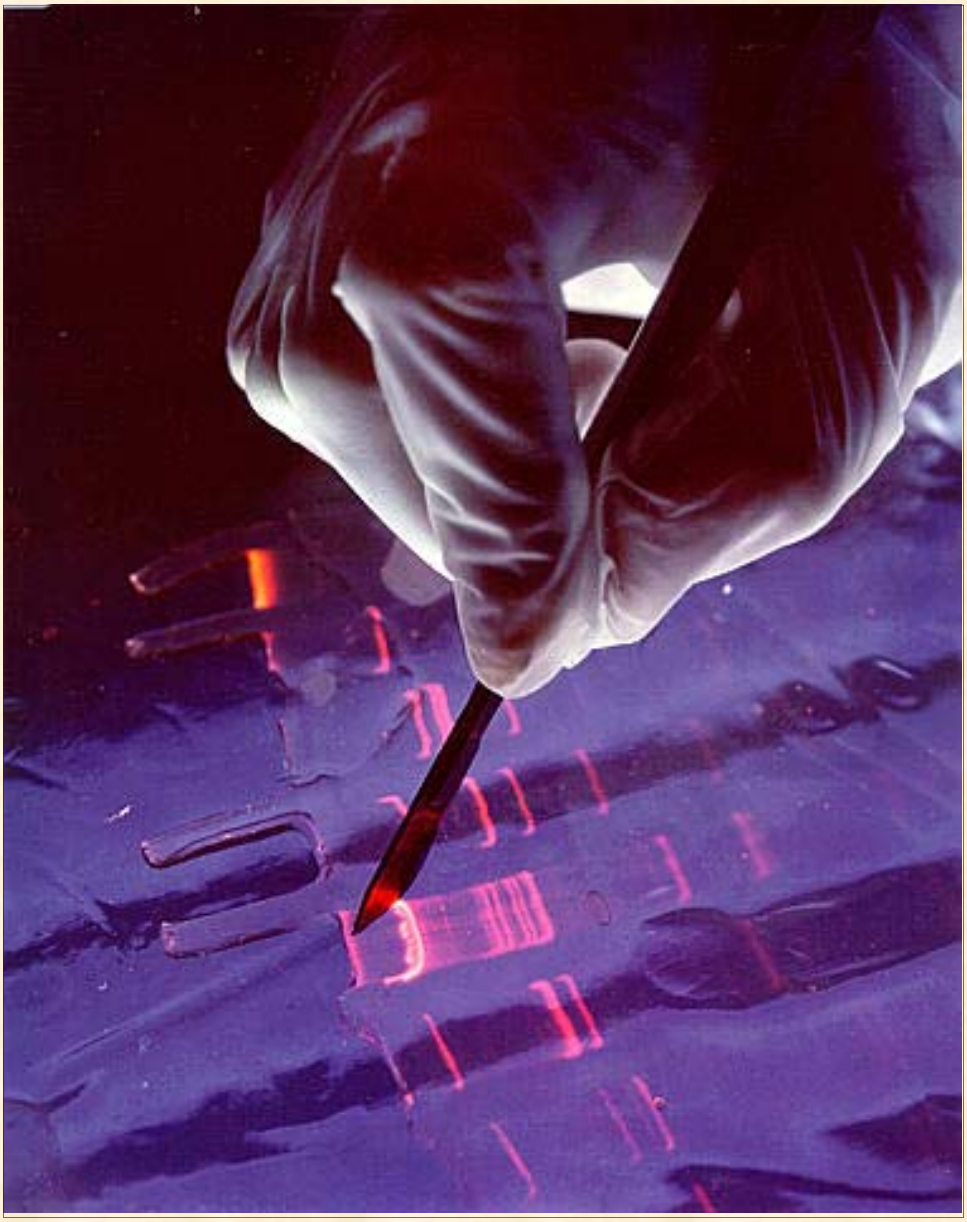
Microbiological Data Program
Progress Update and 2006 Data Summary

United States Department of Agriculture

Agricultural Marketing Service

Science & Technology Programs



Please Visit Our Website at http://www.ams.usda.gov/science/MPO/MDP.htm

United States Department of Agriculture

Marketing and Regulatory Programs

Agricultural Marketing Service

1400 Independence Ave.
Washington, DC
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January 2007

To the Reader:

I am pleased to present the USDA Microbiological Data Program 2006 Data Summary. In 2006, MDP tested six commodities (cantaloupe, leaf and romaine lettuce, tomatoes, green onions, and alfalfa sprouts). Leaf and romaine lettuce were combined as a single commodity with each variety being sampled at half the regular sampling rates. Alfalfa sprouts replaced cilantro and parsley.

MDP is a partnership with cooperating State agencies that are responsible for sample collection and analyses. In 2006, eleven States participated in the program: 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.

This summary is intended to provide the reader with an overview of data collected in 2006 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@usda.gov or visit our Web site at http://www.ams.usda.gov/science/MPO/MDP.htm.

Sincerely,

Lloyd C. Day
Administrator

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Acknowledgements

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California Department of Food and Agriculture
California Department of Pesticide Regulation
Colorado Department of Agriculture
Florida Department of Agriculture and Consumer Services
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New York Department of Agriculture and Markets
Ohio 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 Food and Drug Administration (FDA), and the USDA National Agricultural Statistics Service (NASS). The USDA Agricultural Research Service (ARS) and Food Safety and Inspection Service (FSIS) provide consultation as independent research authorities on laboratory methods. 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 2006, 11 States collected fruit and vegetable samples (California, Colorado, Florida, Maryland, Michigan, Minnesota, New York, Ohio, Texas, Washington, and Wisconsin).

The program tested five commodities (cantaloupe, leaf and romaine lettuce, tomatoes, green onions, and alfalfa sprouts) for generic *Escherichia coli (E. coli)*, *E. coli* with pathogenic potential, including *E. coli* O157:H7, and *Salmonella*.

In August 2006, MDP sampling operations were halted due to budget uncertainty for fiscal year (FY) 2007. Laboratory projects initiated earlier in the year were continued using remaining FY 2006 funds. Results presented in this summary cover program operations from January through August 31, 2006.

MDP analyzed a total of 7,646 samples. Sixty-one percent of the samples were from domestic sources, 34 percent were imported, and 5 percent were of unspecified origin. MDP identified 29 samples with potentially pathogenic *E. coli*; however, pathogenic *E. coli* strains were isolated from only 6 samples. These isolates were sent to Pennsylvania State University for further characterization, including serotyping and testing for different virulence-specific genes associated with seven different categories of pathogenic *E. coli*. FDA's Center for Veterinary Medicine (CVM) facility conducted tests on antimicrobial resistance and genomic fingerprinting on these isolates. MDP screening also resulted in three *Salmonella* isolates, one each from alfalfa sprouts, cantaloupe, and green onions.

A number of important benefits are expected from MDP. Microbiological data obtained from this fresh produce screening effort will enhance the understanding of the microbial ecology of fresh fruit and vegetables in the food supply, permit the identification of long-term trends, and over time will contribute significantly to a national produce microbiological baseline. Such baseline data, combined with virulence attributes, serotypes, antimicrobial resistance, and genomic fingerprints will help collaborators such as CDC and FDA in planning public health initiatives.

Microbiological Data Program (MDP) Annual Summary, Calendar Year 2006

This summary consists of the following sections: (I.) Introduction, (II.) Sampling, (III.) Laboratory Operations, (IV.) Database Management, (V.) Summary of 2006 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 is vulnerable to contamination with pathogens. The fact that produce is often consumed raw without any type of intervention that would reduce, control, or eliminate pathogens prior to consumption contributes to its potential as a source of foodborne illness (1, 2). In 2001, Congress authorized funding for a microbiological monitoring program to establish a microbial baseline for fresh produce.

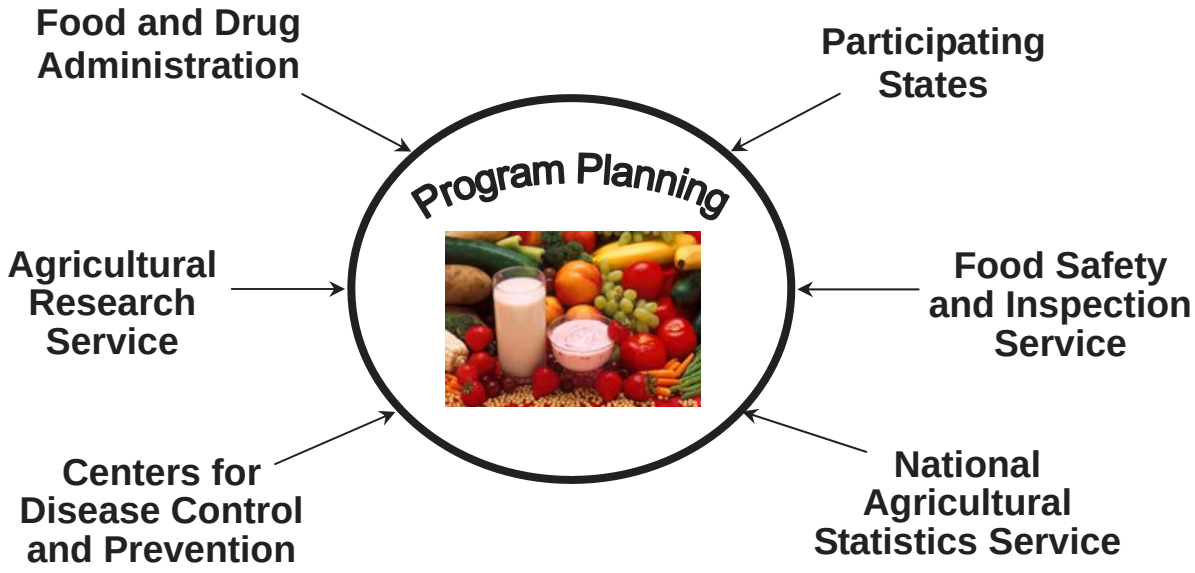
MDP's mission is to collect information regarding the incidence and identification of targeted foodborne pathogens and indicator organisms on fresh fruit and vegetables. This publication provides an overview of data collected in 2006 and summarizes program refinements made during that year. The Agricultural Marketing Service (AMS) Monitoring Programs Office (MPO) manages MDP and is responsible for administrative, sampling, technical, and database activities. This publication is available on the Internet at http://www.ams.usda.gov/science/MPO/MDP.htm.

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 Food and Drug Administration (FDA). The USDA Agricultural Research Service (ARS) and Food Safety and Inspection Service (FSIS) provide consultation as independent research authorities on laboratory methods. MDP relies on the expertise of scientists from FDA 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, particularly those involving technical and quality assurance (QA) issues.

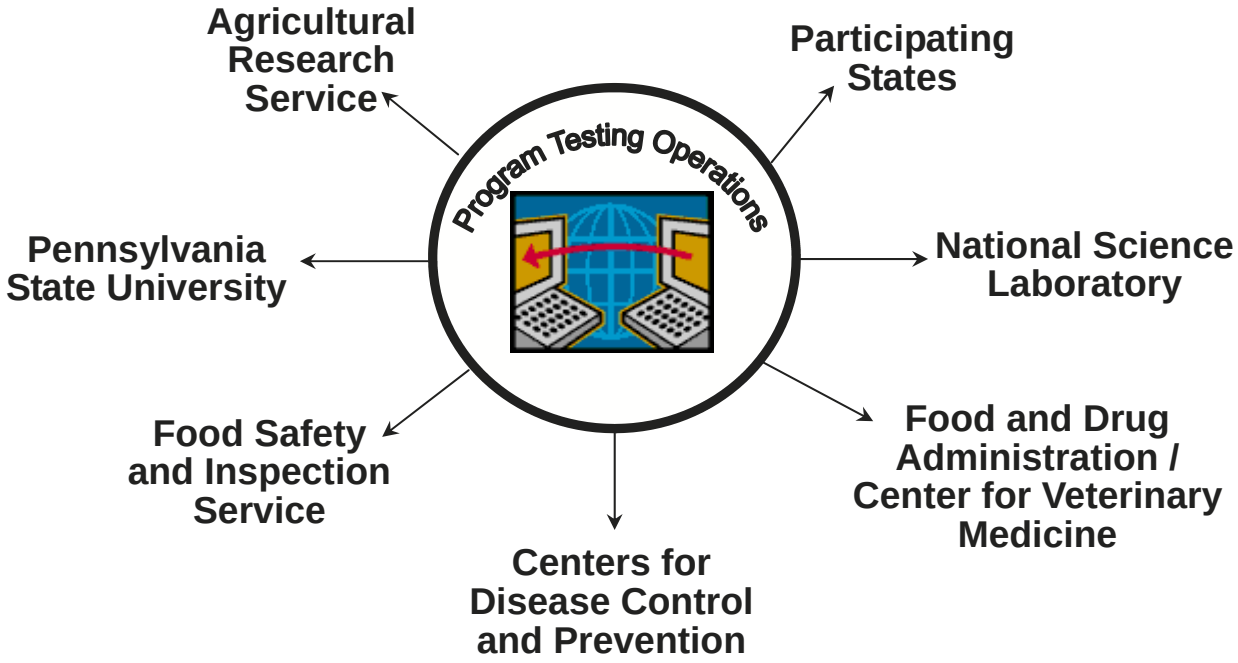
Figure 1 (b) depicts MDP program testing operations. The participating State laboratories and AMS National Science Laboratory (NSL) analyze MDP samples collected by trained State sample collectors. FDA's Center for Veterinary Medicine (CVM) and Pennsylvania State University (PSU) provide additional testing services for isolate characterization. Information on MDP data and isolates is shared with USDA's ARS and FSIS, 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 2006 included: cantaloupe, leaf and romaine lettuce, tomatoes, green onions, and alfalfa sprouts. Commodities were tested for generic *Escherichia coli* (*E. coli*), *E. coli* strains with human pathogenic potential including *E. coli* O157:H7, and *Salmonella*. Isolates of these organisms were sent to specialized laboratories for further characterization including serotyping, testing for antimicrobial resistance and virulence attributes, and genomic fingerprinting. Each MDP laboratory also performed multiplex polymerase chain reaction (mPCR) screening for pathogenic *E. coli* on samples that tested positive for the presence of *E. coli*.

Samples were collected in the 11 participating States through cooperative agreements with their respective agencies (Figure 2). Also



(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.*

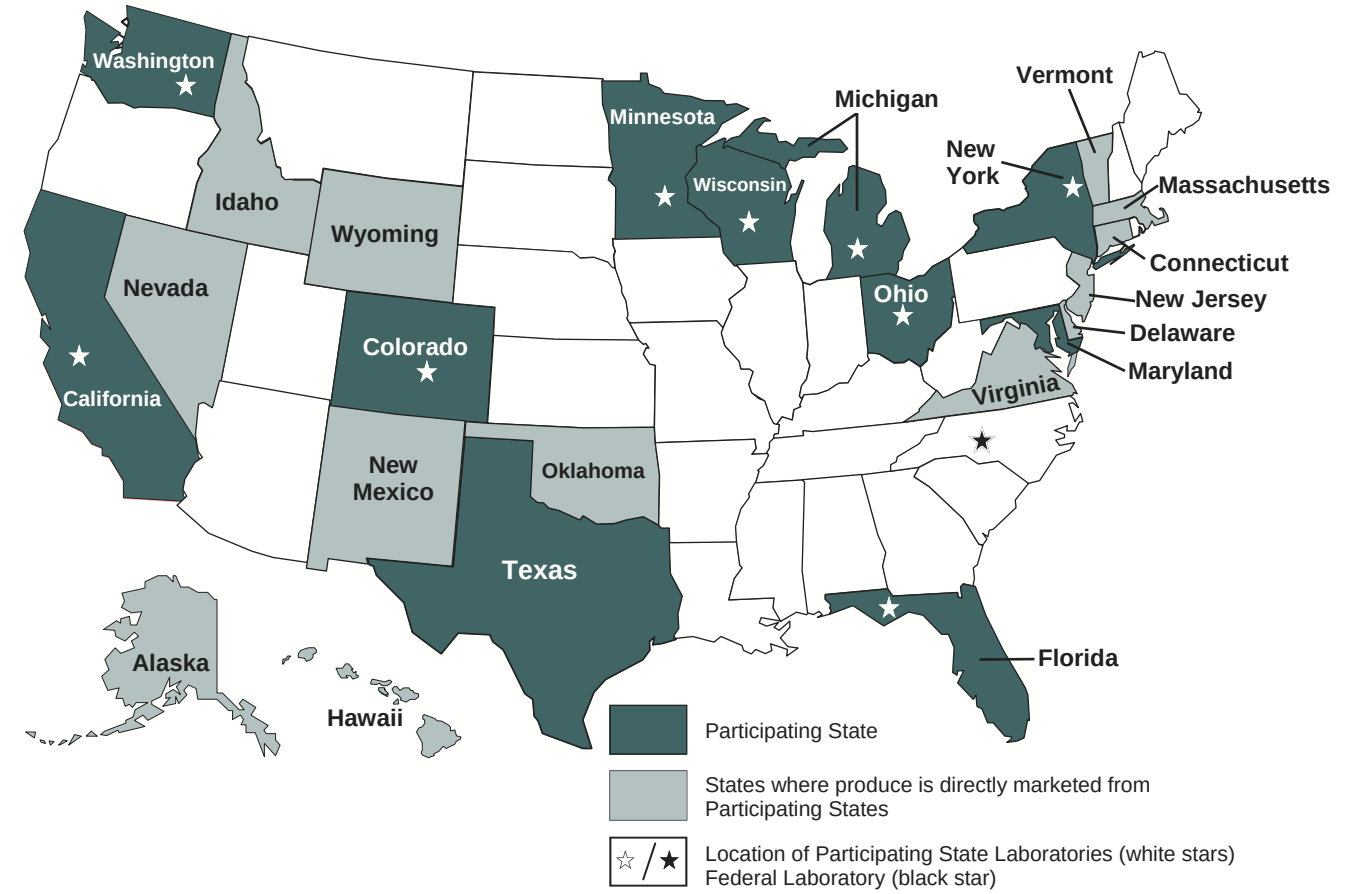


Figure 2. Program Participants. *During 2006, AMS established cooperative agreements with 11 States to sample and/or test MDP commodities. Samples collected by Maryland are analyzed by the Ohio Laboratory. Samples collected by Texas are analyzed by the National Science Laboratory in Gastonia, North Carolina. These two laboratories also analyzed California samples beginning March 2006. States that do not participate in MDP's sampling program but are in the direct distribution networks of the participating States are also shown.*

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.

Microbiology laboratory services were provided by nine States (California, Colorado, Florida, Michigan, Minnesota, New York, Ohio, Washington, and Wisconsin) and AMS NSL. Due to internal State funding issues, the California laboratory voluntarily discontinued program participation in February 2006. Beginning in March 2006, samples collected by California were shipped to the Ohio and AMS NSL laboratories.

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 will be added to the National

Antimicrobial Resistance Monitoring System (NARMS) database. Additionally, CVM performs genomic fingerprinting on MDP isolates for inclusion in the PulseNet system.

AMS implemented DNA-based screening for pathogenic *E. coli*, including *E. coli* O157:H7 and *Salmonella*. MDP laboratories have been using an enzyme-based assay for detection of generic *E. coli*. Beginning in 2005, mPCR technology was used to screen all *E. coli* positive samples for the presence of pathogenic *E. coli* that harbor shiga toxins (STEC) and enterotoxins (ETEC), two groups of *E. coli* that cause enteric diseases and are important to human health. As the program evolves, procedures and methods will be modified and refined to provide information necessary for making science-based food safety decisions. AMS continues to improve data collection systems for better database management and to use quicker, more reliable, and more sensitive technologies for improved microbial detection.

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 (3), 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 is a useful and defensible baseline survey.

Cantaloupe, leaf and romaine lettuce, tomatoes, and green onions remained in the program at 2005 levels. Based on consultations with FDA, alfalfa sprouts were introduced in 2006 replacing cilantro and parsley, which were treated as a single commodity in 2005. These crops 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. All samples in a State are collected on the same day or within a 2-day interval. Samples from a site consist of three individual units of produce generally collected from the same 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.

The sampling of commodities is conducted at distribution centers and terminal (wholesale) markets from which food commodities are

Commodity	Country	Number of Samples
Cantaloupe	Costa Rica	267
	Guatemala	318
	Honduras	246
	Mexico	30
	Nicaragua	3
	Peru	3
	TOTAL	981
Green Onions	Canada	18
	Chile	3
	Guatemala	24
	Mexico	1,065
	TOTAL	1,110
Lettuce	Canada	3
Tomatoes	Canada	87
	Israel	3
	Mexico	567
		657

Table 1. Distribution of Imported Samples. *This table details the number of imported samples by country of origin and by commodity.*

released to supermarkets and grocery stores, including domestic and imported commodities (refer to Table 1 and Figure 3 for sample origin information). 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 samples per commodity. Each site sample consists of three sub-samples taken from the same lot in each facility (each sub-sample is treated as a separate laboratory sample). The total number of sub-samples collected every month for each commodity is 192.

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. In 2006, 7,646 samples were collected from over 700 sites across the country and analyzed by the MDP participating State laboratories. Table 2 provides a detailed breakdown of sample numbers collected by commodity. For lettuce, either leaf or romaine varieties were eligible for sampling. In May 2006, plum tomatoes, including roma tomatoes, were added to the list of tomato varieties for collection.

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 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.

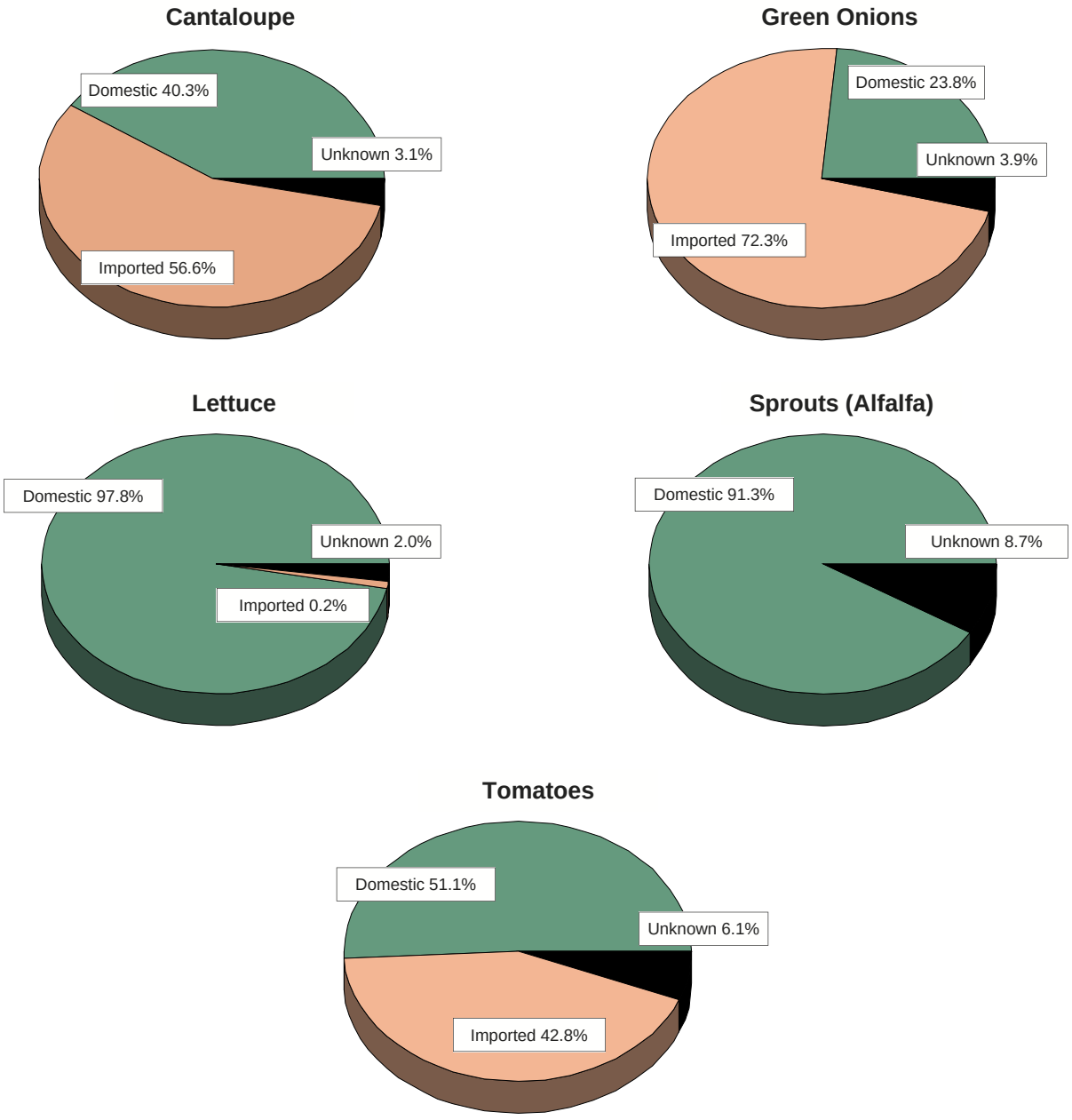


Figure 3. Commodity Origin. *The proportion of domestic, imported or unknown origin for each commodity is depicted for samples tested in 2006.*

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 Ohio laboratory and AMS NSL, Gastonia, NC, for analysis.

Cantaloupe, leaf and romaine lettuce, tomatoes, green onions, and alfalfa sprouts were collected and tested as commodities for 2006. These commodities are harvested primarily by hand although some mechanical harvesting does occur. Alfalfa sprouts are most often grown in drums and packaged in controlled environments. The produce may be packaged in the field or taken to a packinghouse (e.g., tomatoes require classification for color and/or size). At the packinghouse, the produce is cleaned, trimmed, sized, sorted, 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). To minimize spoilage and bruising, the produce is often harvested before reaching full ripeness.

State	Cantaloupe	Green Onions	Lettuce (Leaf or Romaine)	Tomatoes	Sprouts (Alfalfa)	Total	*E. coli*	*E. coli* O157:H7	*Salmonella*
California	336	336	336	336	330	1,674	1,674	1,674	1,674
Colorado	48	48	48	48	48	240	240	240	240
Florida	168	168	168	168	168	840	840	840	840
Maryland	96	96	96	96	87	471	471	471	471
Michigan	144	144	144	144	144	720	720	720	720
Minnesota	48	48	48	48	48	240	240	240	240
New York	216	216	216	216	216	1,080	1,080	1,080	1,080
Ohio	144	144	141	143	138	710	710	710	710
Texas	189	192	189	192	189	951	951	951	951
Washington	96	96	96	96	96	480	480	480	480
Wisconsin	48	48	48	48	48	240	240	240	240
Totals	1,533	1,536	1,530	1,535	1,512	7,646	7,646	7,646	7,646

Table 2. Samples Collected and Analyzed by State. *This table shows the number of samples collected by each State by commodity and the total number of collected samples tested for each organism.*

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), 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.

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 http://www.ams.usda.gov/science/MPO/SOPs.htm.

III. Laboratory Operations

Participating microbiology laboratories tested samples of MDP commodities 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 samples that tested positive for the presence of *E. coli*. Isolates of these organisms were sent to the Gastroenteric Disease Center at Pennsylvania State University (PSU) and FDA/CVM for further characterization. Tests performed by PSU and FDA CVM included serotyping, testing for antimicrobial resistance and virulence attributes, and genomic fingerprinting. In addition, the Commonwealth of Virginia Division of Consolidated Laboratory Services (DCLS) developed a realtime PCR based screening method for *Shigella* for MDP.

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 24 hours to allow for different sample arrival times from the various collection sites. Only excess soil was removed prior to testing.

Samples were washed in Universal Preenrichment Broth (UPB) and all analyses were conducted from this surface wash eluent. For *E. coli* assays, an AOAC®-approved enzyme-based method specific for detecting *E. coli* was used. Enumeration was accomplished using the standard Most Probable Number (MPN) method. The presumptive *E. coli* positive cultures were screened by each laboratory via multiplex DNA-based PCR procedures for shiga toxin-producing *E. coli* (STEC) and enterotoxigenic *E. coli* (ETEC). MDP used DNA-based PCR assays and automated instruments for the detection of *Salmonella* and enterohemorrhagic *E. coli* O157:H7 in produce samples. Cultural and Immunomagnetic Separation (IMS) technology

were employed for isolation of target bacteria. Automated biochemical tests and cultural methods were used in the verification of any preliminary findings.

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 2001 Bacterial Analytical Methods (BAM), AOAC® methods, the FSIS Microbiological Laboratory Guide, and the Environmental Protection Agency's Good Laboratory Practices. MDP analytical methods are published at http://www.ams.usda.gov/science/MPO/SOPs.htm. SOPs provide uniform administrative, sampling, and laboratory procedures.

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 *Salmonella 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 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, 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 on-site by each laboratory's QAU. Final review procedures are performed by MDP staff responsible for collating and reviewing data for conformance with SOPs.

Laboratory performance was monitored through on-site reviews by MDP 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/password accounts and user access rights for the various system functions based on position requirements. The RDE system utilizes Secure Socket 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

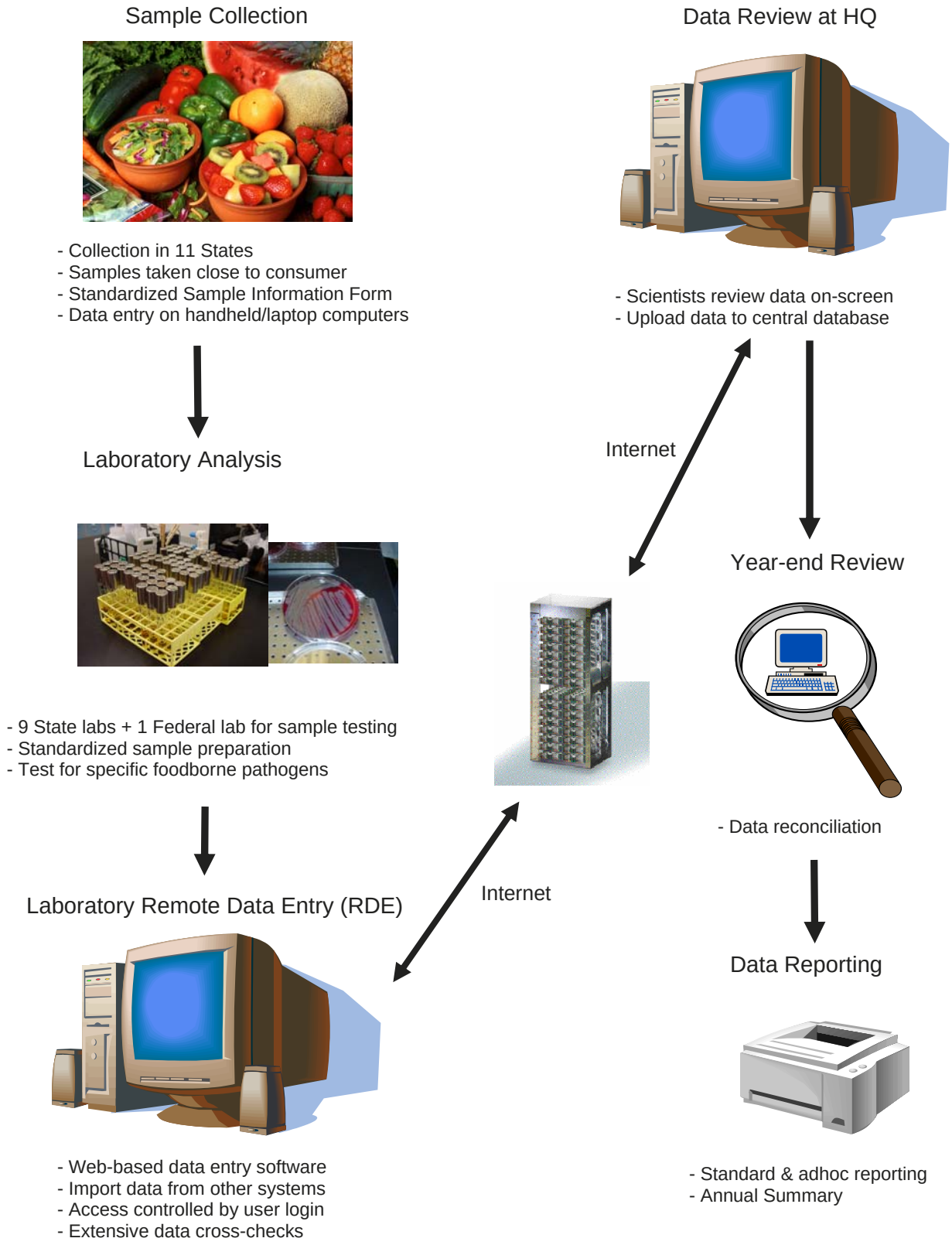


Figure 4. MDP Data Pathway. *An illustration of MDP data path from sample collection, through laboratory analysis and reporting.*

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 in a Windows® operating environment. Access to the central MDP database is limited to MDP headquarters personnel and is controlled through password protection and user access rights. The system is backed up each night and back-up tapes are sent to off-site storage once a week.

V. Summary of 2006 Data

MDP discontinued sampling operations on August 31, 2006 due to budget uncertainty for FY 2007. Consequently, results presented in this summary cover only eight months of program operations. MDP collected a total of 7,646 samples of cantaloupe (1,533), green onions (1,536), lettuce (1,539), tomatoes (1,535) and alfalfa sprouts (1,512).

Table 1 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. Sixty-one percent of the samples were from domestic sources, 34 percent were imported, and 5 percent were of unspecified origin. Table 2 shows the distribution of samples among each commodity and collection State.

In 2006, produce washes were preenriched in a single broth, Universal Preenrichment Broth (UPB), in an effort to streamline the screening process for all target bacteria. The BAX® instrument, an automated PCR system, was used for screening samples for the presence of *Salmonella* and enterohemorrhagic *E. coli* O157:H7. Unlike in previous years, 2006 samples were not pooled in order to test each sample individually. Genomic DNA was extracted from each of the 7,646 preenriched cultures. Appropriate aliquots of the genomic DNA samples were used for all BAX PCR testing of *Salmonella* and *E. coli* O157:H7. Similarly, appropriate aliquots of extracted DNA samples were used for screening for the

Commodity	Number of Samples Tested	Number of Samples Screened by mPCR	Number of Pathogenic *E. coli*-Positive Samples
Cantaloupe	1,533	225	3
Green Onions	1,536	366	8
Lettuce	1,530	294	8
Sprouts (Alfalfa)	1,512	612	9
Tomatoes	1,535	94	1
Total	7,646	1,591	29

Table 3. 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.*

presence of pathogenic *E. coli* by mPCR (refer to Table 3).

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 MDP-approved commercial biochemical kit or system. 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, antimicrobial resistance testing, and genomic fingerprinting.

Generic *E. coli* and Pathogenic *E. coli*

The 7,646 samples were initially screened for generic *E. coli* using an AOAC-official method for detection and enumeration which resulted in 1,591 samples that tested positive for *E. coli.* These *E. coli* positive samples were further screened for pathogenic *E. coli* that harbor shiga toxins (STEC) and enterotoxins (ETEC) (refer to Table 3) using a multiplex polymerase chain reaction (mPCR) assay developed by FDA. Toxin genes associated with pathogenic *E. coli* were found in 29 samples. Successful isolation of pathogenic *E. coli* strains was attained for six of these samples. In addition to the technological differences between the detection by PCR and isolation by cultural means, several other factors influence the rate of successful isolation including: an overwhelming amount of background microflora in comparison to the small number of target bacterial cells, differential growth rates of various bacteria, and additional growth requirements.

The six isolates were sent to PSU for serotyping and further characterization and to FDA/CVM for antimicrobial resistance testing. PSU conducted tests that included 13 virulence-specific genes associated with different classes of pathogenic *E. coli*. FDA/CVM conducted tests on antimicrobial resistance and genomic fingerprinting on these isolates. The results of PSU and FDA/CVM testing are shown in Table 4. Five of the pathogenic *E. coli* isolates were from lettuce and one from alfalfa sprouts. Five out of six isolates carried more than one toxin

Commodity	Pathogenic Class	Toxic Genes Identified	Serotyping		Pulsed-Field Gel Electrophoresis
			O Antigen	H Antigen	
Alfalfa Sprouts	STEC	STa, Stx-2, HlyA	36	14	
Lettuce	STEC	Stx-2	Neg	2 or 35	
Lettuce	ETEC	LT, STb	N	14	
Lettuce	STEC	Stx-2, HlyA	8	28	
Lettuce	STEC	Stx-2, HlyA	8	28	
Lettuce	STEC	Stx-1, Stx-2, HlyA	141	38	

HlyA - hemolysin

LT - heat-labile toxin

STx - shiga toxin

ST - heat-stable toxin

pos - novel positive reaction that did not fall into any known standards

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

Table 4. 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.*

gene. One ETEC isolate, carrying both the heat-labile and heat-stable enterotoxins, was resistant to antimicrobial agent, sulfasoxazole. One of the STEC isolates, that carried a single toxin gene, was found to be resistant to antimicrobial agents, tetracycline and sulfasoxazole. 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 actual pathogenicity of these isolates, is not within the scope of MDP.

Salmonella

As depicted in Table 5, a total of 7,646 samples were screened for *Salmonella* by BAX PCR. Twenty-two of these samples were positive and three *Salmonella* isolates were obtained: one each from cantaloupe, green onion, and alfalfa sprouts. These three isolates were sent to FDA/CVM for identification by serotyping, antimicrobial resistance, and genomic fingerprinting. Table 6 identifies each isolate and the associated serogroup. The isolate from green onions, *S. agona*, belonging to serogroup B, was resistant to antimicrobial agent, tetracycline. The isolate found in alfalfa sprouts, *S. havana*, belonging to serogroup G and the isolate from cantaloupe, *S. sandiego*, belonging to serogroup B, were found sensitive to antimicrobial agents tested.

***E. coli* O157:H7**

No enterohemorrhagic *E. coli* O157:H7 strain was isolated from the 7,646 samples screened, although 3 samples tested positive by BAX PCR. 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.

Commodity	Number of Samples Tested	Number of Positive Individual Samples	Number of Positive Isolates
Cantaloupe	1,533	5	1
Green Onions	1,536	4	1
Lettuce	1,530	5	0
Sprouts (Alfalfa)	1,512	7	1
Tomatoes	1,535	1	0
TOTALS	7,646	22	3

Table 5. Summary of Analysis for Salmonella. *This table shows the number of samples screened for Salmonella, the number of positive individual samples, and the number of isolates obtained.*

	Serotype/Identification			
Commodity	**Genus**	**Species**	**Serogroup**	**Pulsed-Field Gel Electrophoresis**
Cantaloupe	*Salmonella*	*sandiego*	B	
Green Onions	*Salmonella*	*agona*	B	
Alfalfa Sprouts	*Salmonella*	*havana*	G	

Table 6. Salmonella Identification and Serogroup*. This table summarizes the genus, species, and serogroup for each of the three Salmonella isolates obtained in 2006.*

References:

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Definitions:

Antimicrobial resistance: 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.

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 encoding this protein is used as a marker in control cultures.

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.

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.

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.