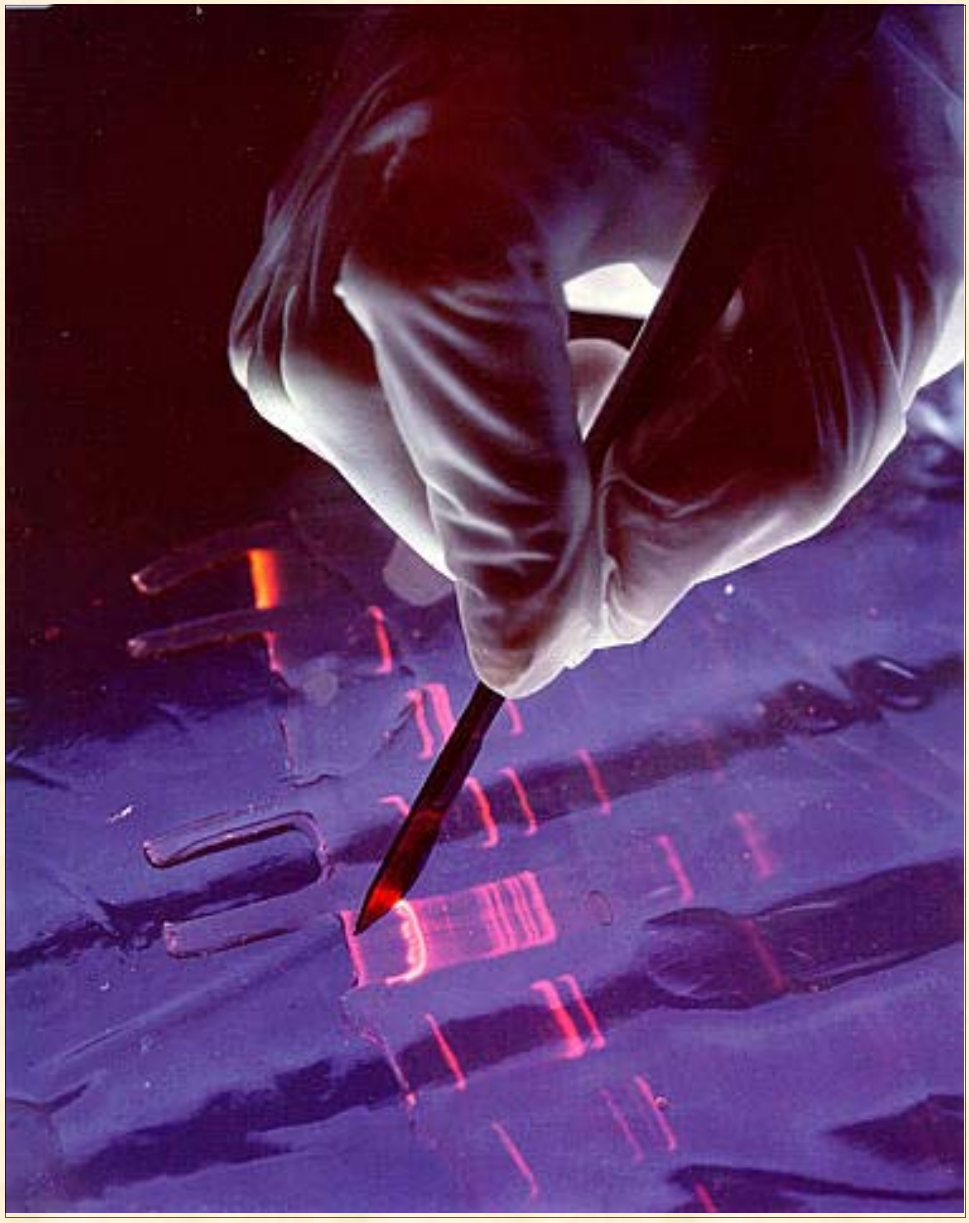
Microbiological Data Program

Progress Update and 2004 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 2006

To the Reader:

I am pleased to present the USDA Microbiological Data Program 2004 Data Summary. In 2004, MDP continued testing four commodities begun in 2002: cantaloupe, tomatoes, leaf lettuce, and romaine lettuce. These items were selected because they are high consumption fruit and vegetables in the United States. Based on consultations with the U.S. Food and Drug Administration (FDA), several commodity changes were implemented midyear in 2004. Green onions, cilantro, and parsley were introduced to the program and celery was discontinued. Leaf and romaine lettuce were combined as a single commodity with each variety being sampled at half the regular sampling rates.

MDP is a partnership with cooperating State agencies that are responsible for sample collection and analyses. Eleven States participated in 2004: 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 update on the methods, modifications, and refinements made during program development, as well as an overview of the data obtained during 2004. 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
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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 2004, 11 States collected fruit and vegetable samples (California, Colorado, Florida, Maryland, Michigan, Minnesota, New York, Ohio, Texas, Washington, and Wisconsin).

The program tested eight commodities (cantaloupe, celery, leaf and romaine lettuce, tomatoes, green onions, cilantro, and parsley) for *Escherichia coli (E. coli)* with pathogenic potential and *Salmonella*. MDP analyzed a total of 11,214 samples. Seventy-six percent of the samples were from domestic sources, 20 percent were imported, and approximately 4 percent were of unspecified origin. MDP identified 43 samples carrying pathogenic *E. coli*; however, pathogenic *E. coli* strains were isolated from only 9 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 five *Salmonella* isolates: one each from cantaloupe, cilantro, green onion, lettuce (romaine), and parsley.

A number of important benefits are expected from MDP. Microbiological data obtained from this fresh produce screening effort will contribute significantly to a national produce microbiological baseline. The data will enhance the understanding of the microbial ecology of fresh fruit and vegetables in the food supply and permit the identification of long-term trends. 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 2004

This summary consists of the following sections: (I.) Introduction, (II.) Sampling, (III.) Laboratory Operations, (IV.) Database Management, (V.) Summary of 2004 Data

I. Introduction

Many eminent national scientific organizations strongly advocate microbiological monitoring (1, 2). In 2001, Congress authorized funding for a microbiological monitoring program to establish a microbial baseline for the domestic food supply. The Microbiological Data Program (MDP) was established as part of the broader 1997 Presidential Food Safety Initiative.

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 2004 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.

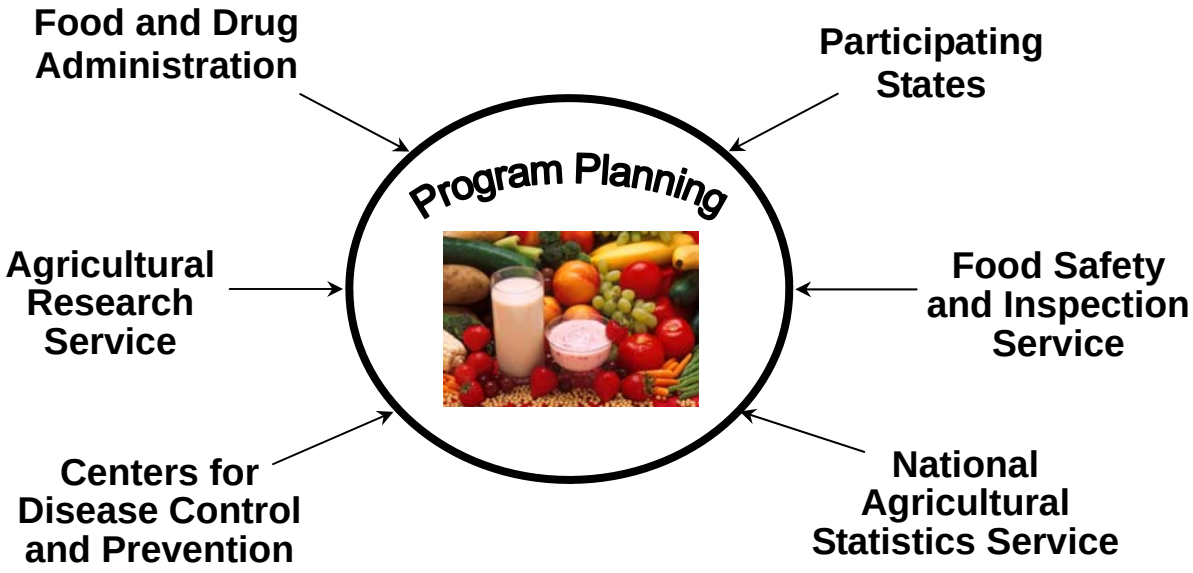
Figure 1 (a) illustrates MDP program planning activities. AMS coordinates its planning and program requirements with the Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration (FDA). The USDA Agricultural Research Service (ARS) and Food Safety and Inspection Service (FSIS) provide consultation as independent research authorities on laboratory methods. 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) also depicts MDP program testing operations. The participating State laboratories and the AMS National Science Laboratory (NSL) analyze the MDP samples collected by State samplers. FDA's Center for Veterinary Medicine (CVM) and Pennsylvania State University provide additional testing services for isolate characterization. Information on MDP data and isolates is shared with USDA's ARS and FSIS, CDC, and FDA.

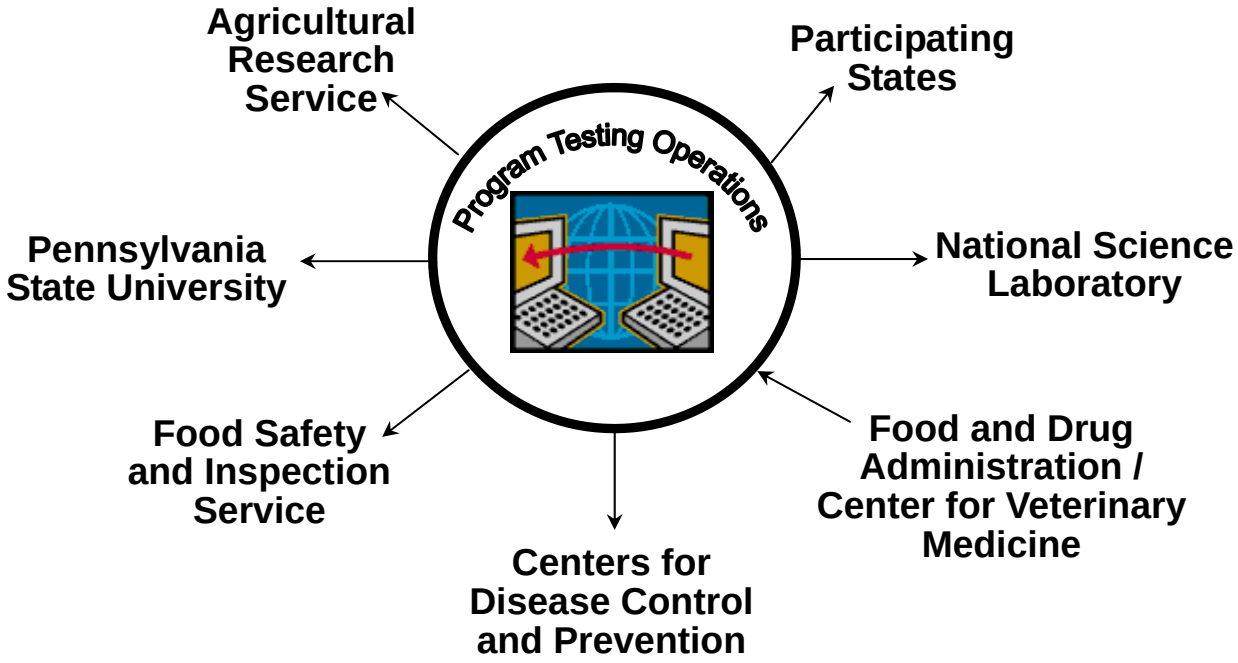
AMS used USDA consumption surveys to select commodities that are highly consumed in the United States and can be eaten raw: cantaloupe, celery, leaf lettuce and romaine lettuce, tomatoes, green onions, cilantro, and parsley. Commodities were tested for *Escherichia coli* (*E. coli)* strains with human pathogenic potential including *E. coli* O157:H7 and *Salmonella*. Isolates of these organisms were sent to specialized laboratories for further characterization including multiplex polymerase chain reaction (mPCR) screening for pathogenic *E. coli*, serotyping, testing for antimicrobial resistance and virulence attributes, and genomic fingerprinting.

Samples were collected in the 11 participating States through cooperative agreements with their respective agencies (Figure 2). 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. Also shown in Figure 2 are the 13 neighboring States that are in the direct distribution networks for the MDP collection States: Alaska, Connecticut, Delaware, Hawaii, Idaho, Massachusetts, Nevada, New Jersey, New Mexico, Oklahoma, Vermont, Virginia, and Wyoming.

Microbiology laboratory services were provided by nine States (California, Colorado, Florida, Michigan, Minnesota, New York, Ohio, Washington, and Wisconsin) and the AMS NSL.



(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.*

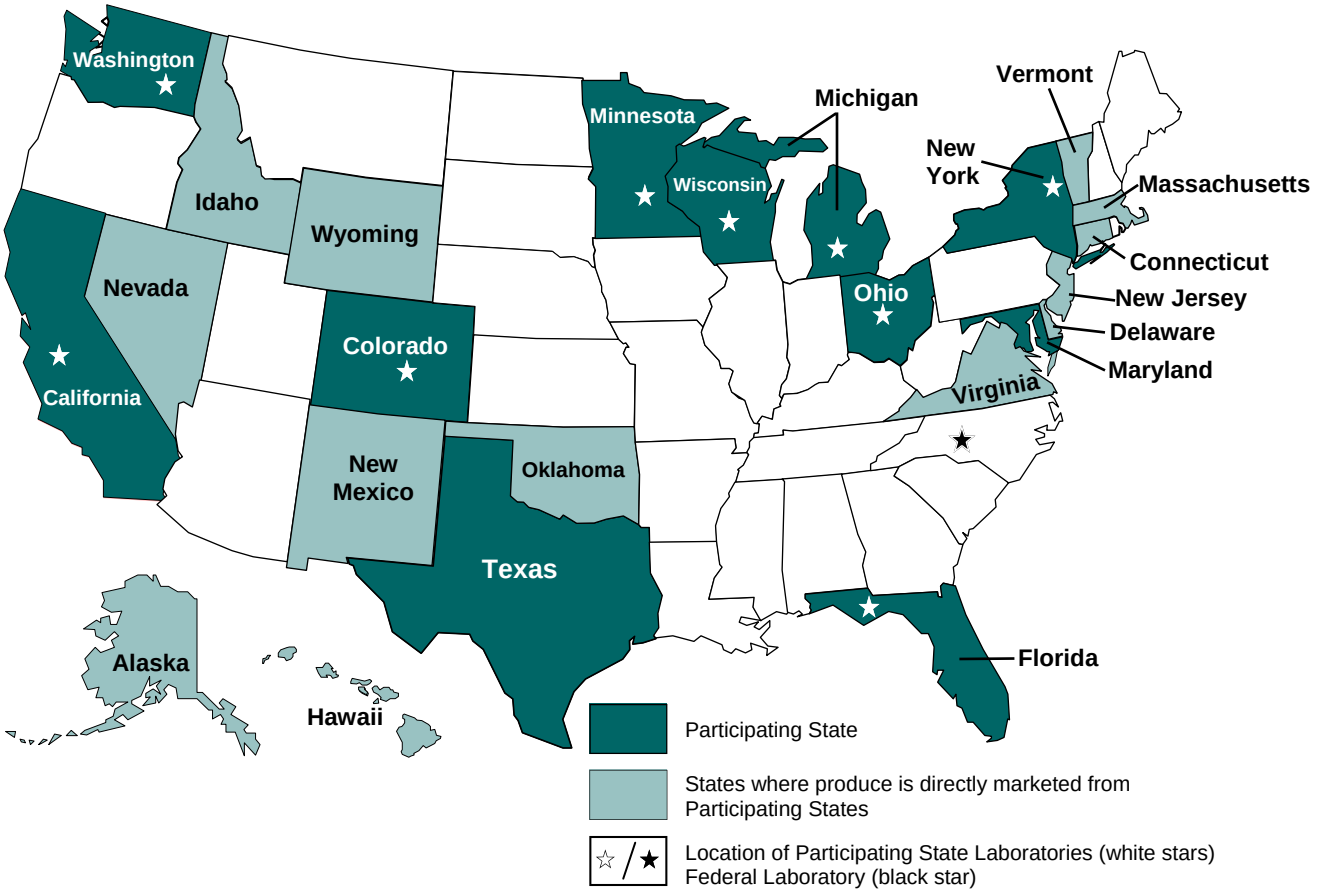


Figure 2. Program Participants. *During 2004, AMS established cooperative agreements with 11 States to sample and/or test MDP commodities. Samples collected by Maryland are analyzed by the Ohio Laboratory. Samples collected by Texas are analyzed by the National Science Laboratory in Gastonia, North Carolina. States that do not participate in MDP's sampling program but are in the direct distribution networks of the participating States are also shown.*

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.

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 and to use improved microbial detection methods that are quicker, more reliable, and more sensitive. AMS implemented DNA-based testing of samples in October 2003 following program-wide validation studies and introduced DNA-based screening for *E. coli* O157:H7 in April 2004. In 2004, all *E. coli* isolates were screened for potential human pathogenicity using mPCR technology.

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 in consultation with the 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 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.

Based on consultations with FDA, several commodity changes were implemented in 2004. Green onions, cilantro, and parsley were introduced to the program and celery was discontinued. Leaf and romaine lettuce were combined as a single commodity with each variety being sampled at half the regular sampling rates. Cantaloupe and tomatoes remained in the program at 2003 levels. 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 produce 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 MPO.

The sampling of commodities in commerce is conducted at distribution centers and terminal (wholesale) markets from which food commodities are 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 weekly 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. These 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) and 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 2004, 11,214 samples were collected and analyzed from over 700 sites across the country. Table 2

provides a detailed breakdown of sample numbers collected by commodity. As a note, cilantro and parsley were treated as a single commodity in that each product was collected at a half sampling rate (to equal the total collected for one commodity).

All samples are selected, bagged, and packed 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 re-sampled. All samples are shipped on the same day as sample collection by overnight delivery so that laboratory analysis can begin the following day.

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

The commodities collected and tested in 2004 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 which 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-

Commodity	Country	Number of Samples
Cantaloupe	Costa Rica	245
	Dominican Republic	27
	Guatemala	432
	Honduras	231
	Mexico	30
	Nicaragua	9
	Unknown	18
		992
Celery	Canada	3
	Mexico	24
		27
Green Onions	Canada	12
	Guatemala	12
	Mexico	444
		468
Lettuce	Canada	6
	Mexico	15
		21
Parsley	Mexico	27
Tomatoes	Belgium	3
	Canada	93
	Mexico	537
	Netherlands	3
		636

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

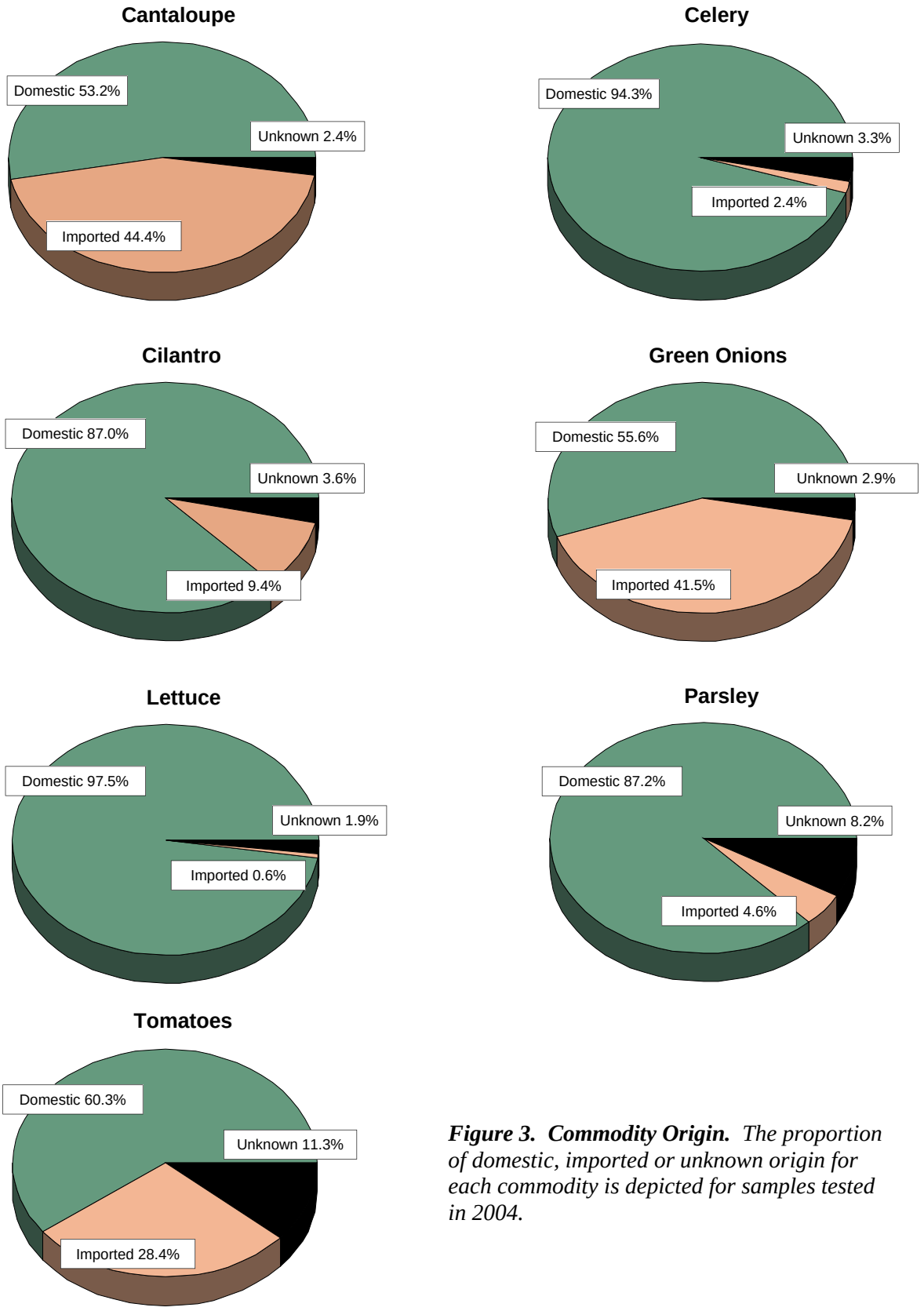


Figure 3. Commodity Origin. *The proportion of domestic, imported or unknown origin for each commodity is depicted for samples tested in 2004.*

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. 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 post-harvest 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 hand-held 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-

State	Cantaloupe	Celery	Cilantro	Green Onions	Lettuce (Leaf or Romaine)	Parsley	Tomatoes	Total	*E. coli*	*E. coli* O157:H7	*Salmonella*
California	497	252	135	252	753	141	504	2,534	2,531	1,905	2,534
Colorado	72	36	18	36	108	18	72	360	360	252	360
Florida	252	126	69	126	378	66	250	1,267	1,267	88	1,264
Maryland	144	69	33	72	210	33	144	705	705	492	705
Michigan	216	108	54	108	324	54	213	1,077	1,077	753	1,077
Minnesota	12	0	12	12	12	12	12	72	72	72	72
New York	324	162	81	162	486	81	323	1,619	1,619	1,133	1,619
Ohio	215	108	51	108	321	54	215	1,072	1,072	749	1,072
Texas	285	144	71	144	429	72	288	1,433	1,433	997	1,433
Washington	144	72	33	72	213	39	144	717	717	525	717
Wisconsin	72	36	18	36	106	18	72	358	358	252	358
Totals	2,233	1,113	575	1,128	3,340	588	2,237	11,214	11,211	8,018	11,211

Note: There were three samples that were analyzed for *E. coli*, but not *Salmonella*. There were three other samples that were analyzed for *Salmonella*, but not *E. coli* (explains 11,214 vs. 11,211 total).

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.*

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

Ten microbiology laboratories performed analyses for MDP in 2004. The Minnesota Department of Agriculture laboratory began analyzing routine samples in September 2004 after previously performing method development studies for MDP. Further testing on positive culture samples and isolates was performed by the Florida Department of Agriculture and Consumer Services (FDACS), the Gastroenteric Disease Center at Pennsylvania State University, and FDA/CVM. These additional tests included multiplex polymerase chain reaction (mPCR) screening for pathogenic *E. coli*, serotyping, testing for antimicrobial resistance and virulence attributes, and genomic fingerprinting.

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 buffered peptone water and all analyses were conducted from this surface wash eluent. Refinements to methods were introduced in 2004 to enhance sensitivity and selectivity for target pathogens in MDP commodities. As with all method modifications, all program quality assurance (QA)/quality control (QC) criteria must be met prior to implementation by MDP laboratories.

Prior to February 2004, MDP used the FDA 2001 Bacteriological Analytical Manual (BAM)-specified (4) traditional gas-production method for detecting thermotolerant fecal *E. coli* and the Most Probable Number (MPN) method for enumeration. In February 2004, MDP switched to an AOAC®-approved method based on a more sensitive enzyme-based assay specific for detecting *E. coli*, with enumeration accomplished using the standard MPN method. The presumptive *E. coli* positive cultures were sent to the Florida laboratory (FDACS) for mPCR screening for shiga-toxin producing and enterotoxigenic *E. coli*.

MDP used DNA-based polymerase chain reaction (PCR) assays and automated instruments for the detection of *Salmonella* and enterohemorrhagic *E. coli* O157:H7 (introduced in April 2004) 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 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 BAM methods, 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 having to perform lengthy biochemical tests. All controls and blanks were taken through the entire analytical procedure. MDP laboratories used automated instrumentation for confirmation of isolates.

A Technical Advisory Group, comprised of microbiologists from each participating laboratory, provided technical feedback on program SOP revisions and addressed technical and QA 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 that are 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. Performance equivalency of the participating laboratories was monitored by a program-wide proficiency testing program.

IV. Database Management

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

MDP utilizes 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 on handheld or laptop computers. The e-SIF system generates formatted text files containing sample information that are e-mailed to MDP headquarters and then imported into the Web-based RDE system.

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

At MDP headquarters, the RDE system allows scientists to review and approve the data for inclusion in the central database. The central MDP database is maintained using Microsoft® 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 2004 Data

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. Seventy-six percent of the samples were from domestic sources, 20 percent were imported, and approximately 4 percent were of unspecified origin. Table 2 shows the distribution of samples among each commodity and collection State.

In 2004, the third full year of testing, MDP collected 11,214 samples. Of these, 11,211 samples were screened for the presence of *E. coli* and *Salmonella*; and 8,018 samples were screened for enterohemorrhagic *E. coli* O157:H7. Table 2 shows the number of samples collected and analyzed by each State. *E. coli* has been used as an indicator of fecal contamination in food and water; pathogenic *E. coli* and *Salmonella* are frequently implicated in foodborne outbreaks where produce was involved (1). Consequently,

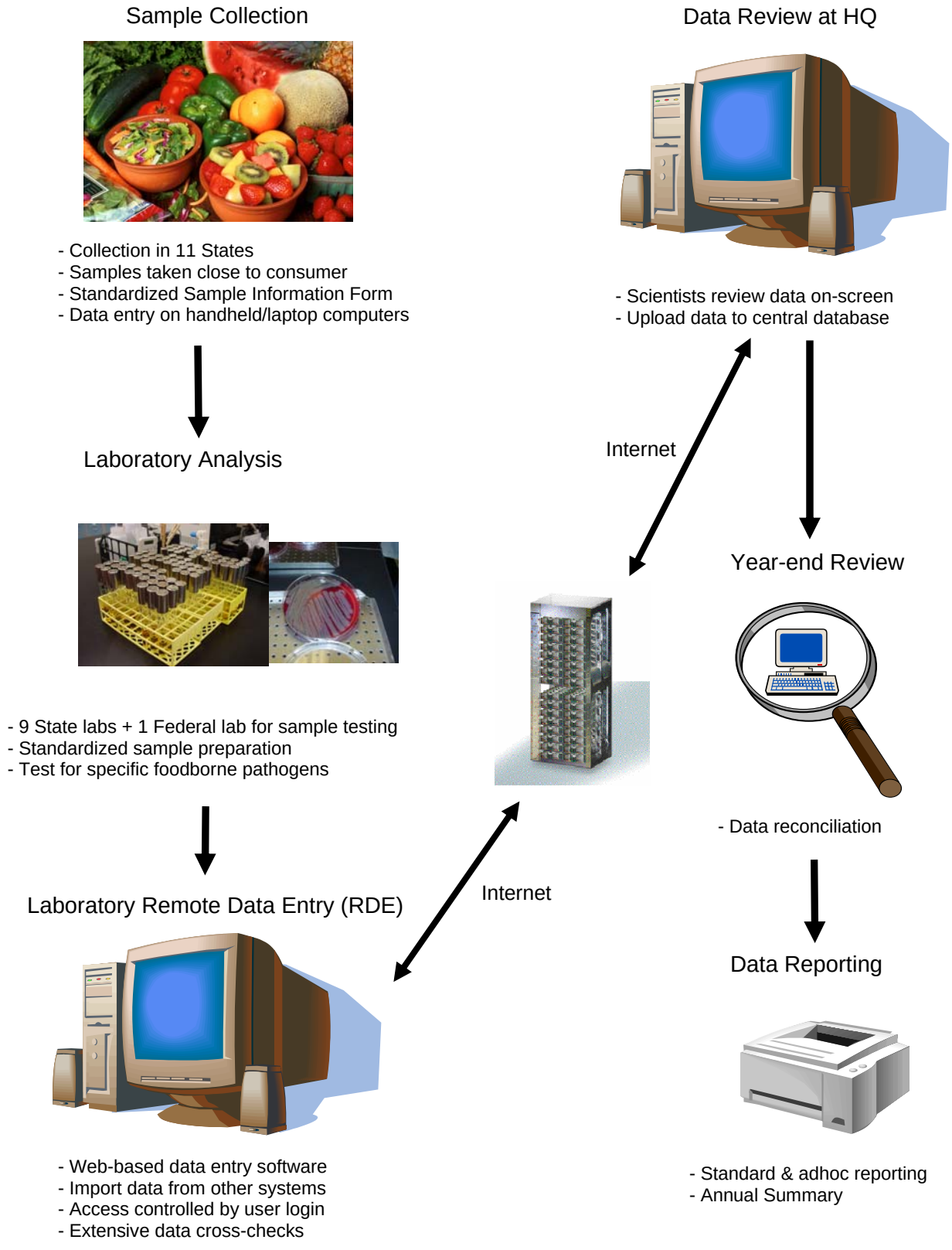


Figure 4. MDP Data Pathway. *An illustration of MDP data path from sample collection, through laboratory analysis and reporting.*

these organisms are of public health significance. Baseline data-gathering efforts designed to identify relevant trends ideally require data generated over multiple growing seasons that span several years. Although 2004 provided a third year of data for MDP, continued data collection is needed before multi-year inferences can be made. Additionally, MDP began implementing major changes in detection technology that will further affect data interpretation.

The 11,211 samples were initially screened for *E. coli* using an AOAC-official method for detection and enumeration. Presumptive *E. coli*-positive samples were further screened for pathogenic *E. coli* that harbor shiga-toxins (STEC) and entero-toxins (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 43 samples; however, pathogenic *E. coli* strains were isolated in only 9 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 micro-flora in comparison to a small number of target bacterial cells; differential growth rates of various bacteria; and additional growth requirements.

The 9 isolates were sent to Pennsylvania State University for serotyping and further characterization, including 13 virulence-specific genes associated with different categories of pathogenic *E. coli*. FDA/CVM conducted tests on antimicrobial resistance and genomic fingerprinting on these isolates.

Eight of the nine isolates carried two or more toxins. Four carried toxin genes from both the STEC and ETEC pathogenic classes. Two of the isolates showed resistance to various antimicrobial agents. For an isolate to be characterized as a human pathogen and cause disease, there must be an interplay of several proteins including toxins, encoded by respective genes. MDP only identified toxin genes; additional testing required in order to determine the actual pathogenicity of these isolates is not within the scope of MDP. The results of testing conducted by Pennsylvania State University and FDA/CVM are shown in Table 4.

Commodity	Number of Samples Tested	Number of Pathogenic *E. coli*-Positive Samples
Cantaloupe	2,233	2
Celery	1,113	1
Cilantro	574	8
Green Onions	1,128	2
Lettuce	3,339	19
Parsley	588	10
Tomatoes	2,236	1
Total	11,211	43

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.*

In 2004 the BAX® instrument, an automated PCR system, was used for *Salmonella* screening. In April 2004, MDP introduced screening for enterohemorrhagic *E. coli* O157:H7 by BAX-based PCR. For all BAX determinations, pooled samples were initially screened. If a positive result was obtained, the three individual samples were tested. 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. Isolates were then sent to FDA's CVM for serotyping, antimicrobial resistance testing, and genomic fingerprinting.

As depicted in Table 5, a total of 11,211 samples were screened for *Salmonella* by BAX-PCR. Seventeen of these individual samples were positive and five *Salmonella* isolates were obtained: one each from cantaloupe, cilantro, green onion, lettuce (romaine), and parsley. These five isolates were sent to FDA's CVM for identification by serotyping, antimicrobial resistance, and genomic fingerprinting. Table 6 identifies each isolate and lists the associated serogroup. One isolate, *S. poona*, belonging to

Commodity	Pathogenic Class	Toxin Genes Identified	Serotyping		Pulsed-Field Gel Electrophoresis
			O Antigen	H Antigen	
Cantaloupe	STEC/ETEC	STa, Stx-1	NT	52	
Cilantro	STEC/ETEC	STa, Stx-1	NT	52	
Cilantro	ETEC	LT, STb	73w	14	
Cilantro	STEC/ETEC	STb, Stx-2, hlyA	NT	19	
Green Onion[1]	ETEC	LT, STa	19	H+	
Lettuce	STEC/ETEC	STb, Stx-2, eae, hlyA	121	19	
Lettuce	ETEC	STa	116w	28	
Parsley[2]	ETEC	STa, STb	117	21	
Parsley	ETEC	STa, STb	5w	10	

STEC Shiga-toxin producing *E. coli.*
ETEC Enterotoxigenic *E. coli.*
Stx-1/2 Shiga-toxin 1 or 2, eae intimin; hlyA-hemolysin.
NT Non typable.
[1] Resistance to antimicrobials: Ampicilin, Streptomycin, Sulfasoxazole, Tetracycline, Trimethoprim/sulfamethoxazole
[2] Resistance to antimicrobials: Streptomycin, Sulfasoxazole

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. Also shown is an image of the pulsed-field gel electrophoresis (PFGE) analysis performed for each isolate.*

serogroup G, is under further investigation. Two isolates, *S. newport* and *S. oraninenburg* belong to serogroup C while *S. enteritidis* and *S. anatum* belong to serogroups D and E, respectively. These four isolates were not resistant to any of the antimicrobial agents tested. Also shown in Table 6 is an image of the PFGE analysis performed for each isolate.

No enterohemorrhagic *E. coli* O157:H7 strain was isolated from the 8,018 samples screened, although there were 6 samples that tested positive by BAX-PCR. In this case, as with pathogenic *E. coli* analysis, a number of factors can be involved in the rate of 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	2,233	3	1
Celery	1,113	0	0
Cilantro	572	3	1
Green Onions	1,128	1	1
Lettuce	3,340	3	1
Parsley	588	0	1
Tomatoes	2,237	7	0
TOTALS	11,211	17	5

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*	*Newport*	C_2	
Cilantro	*Salmonella*	*Anatum*	E_1	
Green Onion	*Salmonella*	*Enteritidis*	D_1	
Lettuce (Romaine)	*Salmonella*	*Poona**	G	
Parsley	*Salmonella*	*Oraninenburg*	C_1	

* Under further investigation.

Table 6. Salmonella Identification, Serogroup, and Genomic Fingerprinting. *This table summarizes the genus, species, and serogroup for each of the five Salmonella isolates obtained in 2004. Also shown is an image of the pulsed-field gel electrophoresis (PFGE) analysis performed for each isolate.*

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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: Refers to 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 insanitation 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 (as 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 the 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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