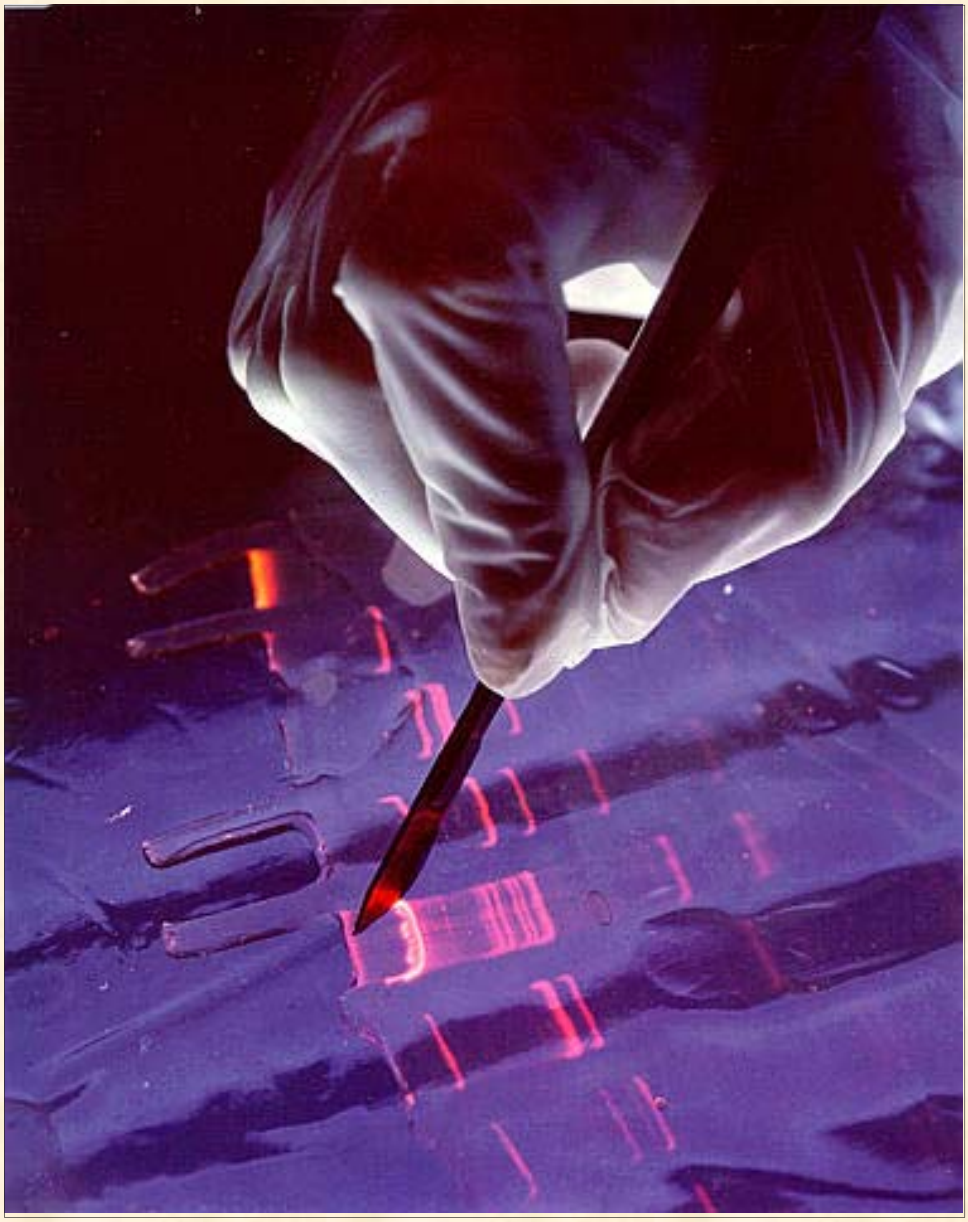
Microbiological Data Program
Progress Update and 2007 Data Summary

United States Department of Agriculture

Agricultural Marketing Service

Science & Technology Programs



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United States Department of Agriculture

Marketing and Regulatory Programs

Agricultural Marketing Service

1400 Independence Ave.
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February 2008

To the Reader:

I am pleased to present the USDA Microbiological Data Program 2007 Data Summary. In 2007, MDP tested five commodities (alfalfa sprouts, cantaloupe, bagged lettuce, green onions, and tomatoes). Bagged lettuce replaced leaf and romaine lettuces.

MDP is a partnership with cooperating State agencies that are responsible for sample collection and analyses. In 2007, 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 2007 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 www.ams.usda.gov/mdp.

Sincerely,

Lloyd C. Day
Administrator

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Acknowledgements

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State Agencies

California Department of Pesticide Regulation
Colorado Department of Agriculture
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Maryland Department of Agriculture
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Texas Department of Agriculture
Washington State 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 2007, 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 (alfalfa sprouts, cantaloupe, fresh pre-cut bagged lettuce, green onions, and tomatoes) for generic *Escherichia coli (E. coli)*, non-O157:H7 *E. coli* carrying shiga toxins and enterotoxins, *E. coli* O157:H7, and *Salmonella*. The laboratories also tested fresh pre-cut bagged lettuce samples for coliform bacteria.

MDP began sampling operations in April 2007 due to delays in the release of funds. Sampling operations ceased in September 2007 due to budget uncertainty for FY 2008; however, projects essential for efficient program and laboratory operations were continued using remaining FY 2007 funds. Results of the monitoring effort presented in this summary cover program operations from April through September 30, 2007. Projects were conducted during the period of October 1 through December 31, 2007.

MDP analyzed a total of 5,279 samples in 5 months of sampling and analytical operations. Sixty-nine percent of the samples were from domestic sources, 26 percent were imported, and 5 percent were of unspecified origin. MDP identified four samples with *E. coli* carrying shiga toxin; however, pathogenic *E. coli* strains were isolated from only two 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 for *Salmonella* spp. resulted in 40 positive samples and from these, three *Salmonella* isolates were reported from two alfalfa sprouts samples and one cantaloupe sample.

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 contribute significantly to a

national produce microbiological baseline. MDP baseline data, which in part reflect the changes in cultivation, harvesting practices, post-harvest handling and packaging of fresh produce to meet changing consumer life styles, preferences and demands, will help fine-tune Good Agricultural Practices. Furthermore, 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 2007

This summary consists of the following sections: (I.) Introduction, (II.) Sampling, (III.) Laboratory Operations, (IV.) Database Management, (V.) Summary of 2007 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 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.

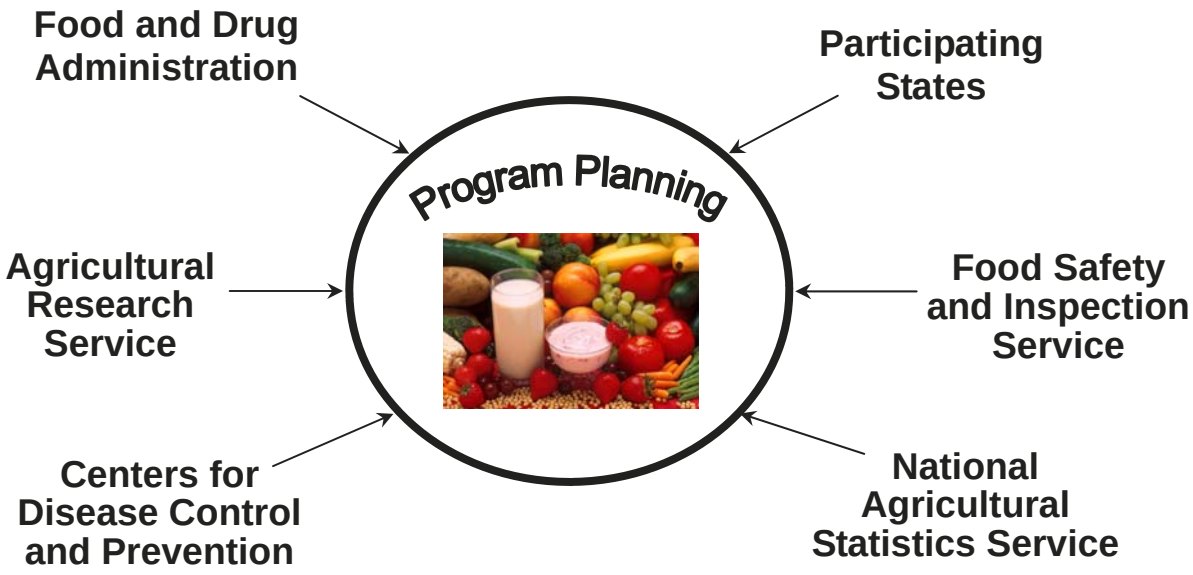
MDP's mission is to collect information regarding the incidence and identification of targeted foodborne pathogens and indicator organisms on fresh fruit and vegetables. This publication provides an overview of data collected in 2007 and summarizes program refinements made during that year. The Agricultural 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 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, 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.

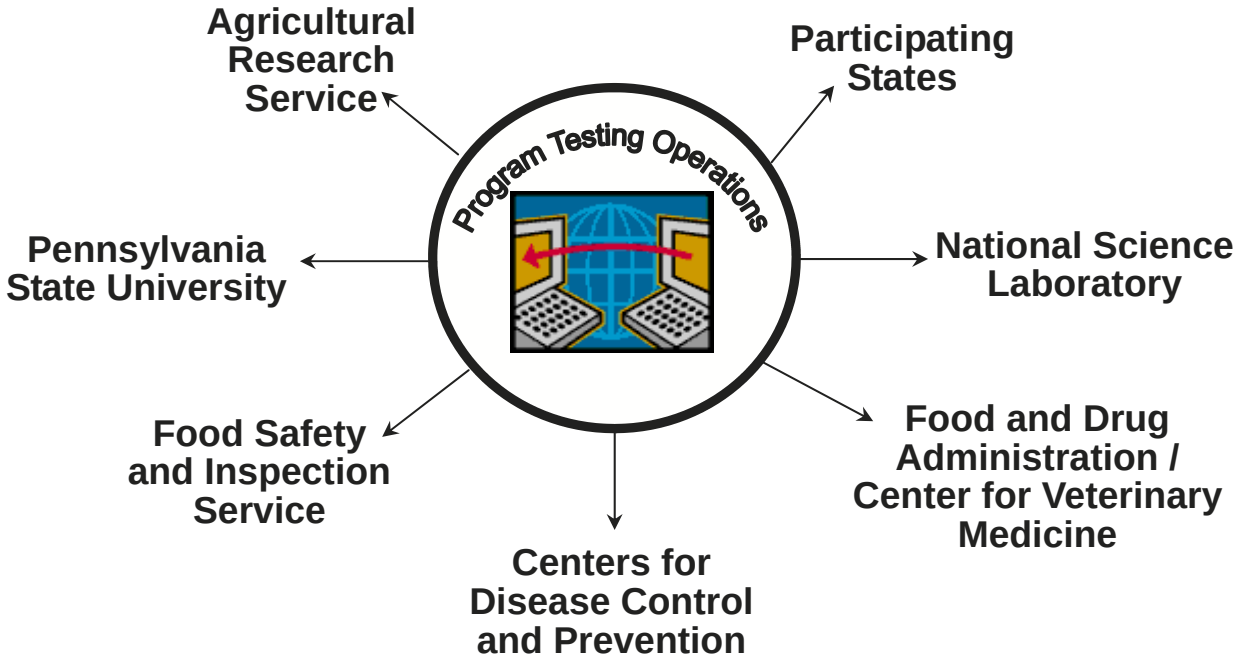
Figure 1 (b) depicts MDP program testing operations. The participating State laboratories and AMS National Science Laboratory (NSL) analyze MDP samples collected by trained State sample collectors. FDA's Center for Veterinary Medicine (CVM) and Pennsylvania State University (PSU) provide additional testing services for isolate characterization. Information on MDP data and isolates is shared with 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 2007 included: alfalfa sprouts, cantaloupe, fresh pre-cut bagged lettuce, green onions, and tomatoes. Commodities were tested for generic *Escherichia coli* (*E. coli*), *E. coli* strains with human pathogenic potential including *E. coli* O157:H7, and *Salmonella*. MDP laboratories also performed multiplex polymerase chain reaction (mPCR) screening for pathogenic *E. coli* on all samples. Isolates of these organisms were sent to specialized laboratories for further characterization including serotyping, testing for antimicrobial resistance and virulence attributes, and genomic fingerprinting. Additionally, the laboratories screened all fresh pre-cut bagged lettuce samples for coliform bacteria.

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.*

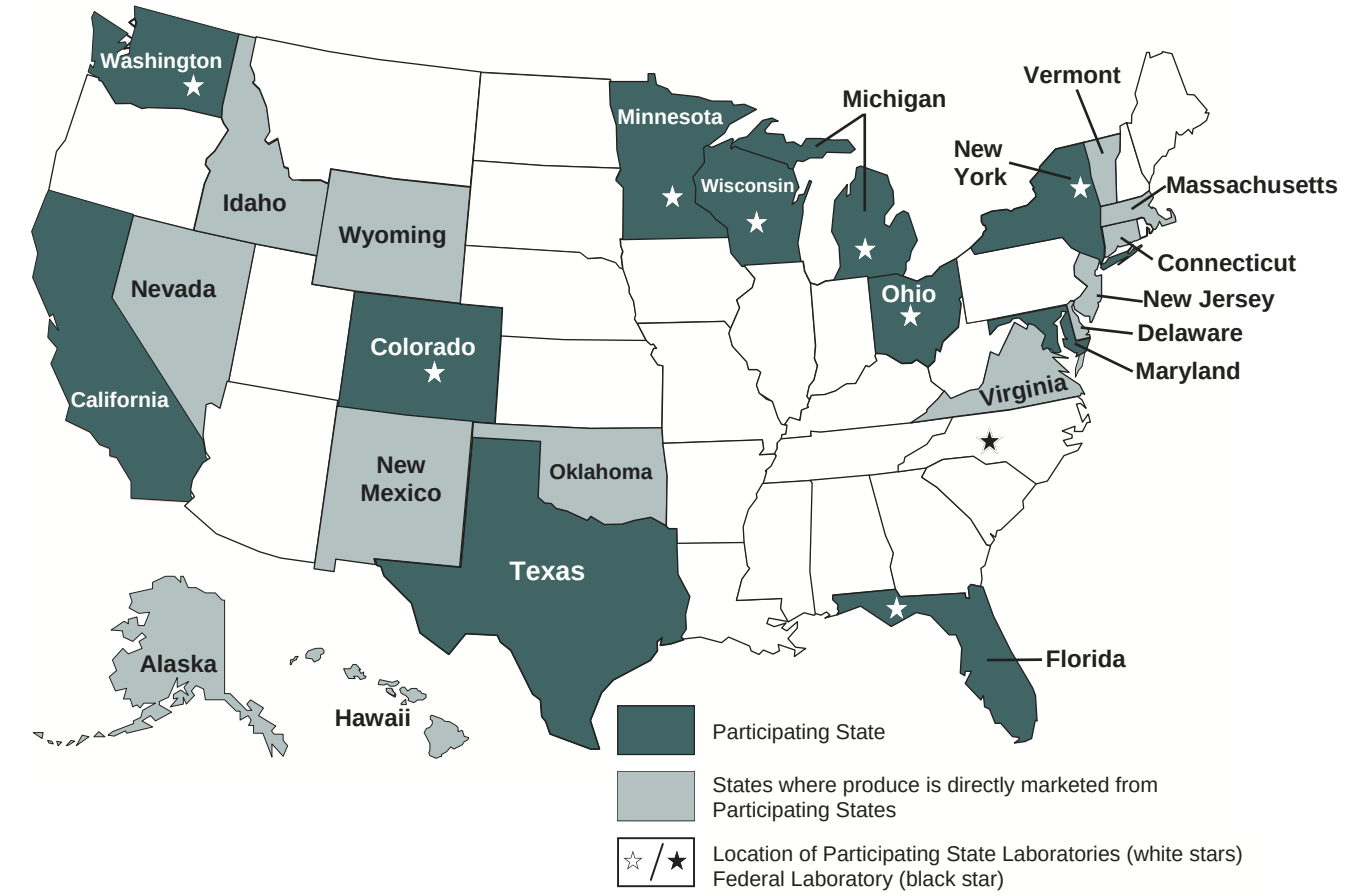


Figure 2. Program Participants. *During 2007, AMS established cooperative agreements with 11 States to sample and/or test MDP commodities. Samples collected by California, Maryland, and Texas are analyzed by the Ohio laboratory and the National Science Laboratory in Gastonia, North Carolina. In addition, 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.*

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 eight States (Colorado, Florida, Michigan, Minnesota, New York, Ohio, Washington, and Wisconsin) and one USDA AMS facility, NSL. Samples collected by California, Maryland, and Texas were analyzed by the Ohio and AMS NSL laboratories. In addition, the Colorado laboratory analyzed cantaloupes and green onions collected by Florida.

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 detection of generic *E. coli* and the Most Probable Number (MPN) analysis with an automated system for detection and enumeration of *E. coli*. This new system is based on the same unique enzyme assay; however, the experimental setup, assay and the capture and analysis of data have been automated. The automated system allowed the laboratories to reduce personnel 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 pre-cut fresh bagged lettuce for coliform bacteria; 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*.

AMS employed DNA-based screening for pathogenic *E. coli*, including *E. coli* O157:H7 and Salmonella. All samples were screened for the presence of pathogenic *E. coli* that harbor shiga toxins (STEC) and enterotoxins (ETEC), 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 (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.

Alfalfa sprouts, cantaloupes, green onions, and tomatoes remained in the program at 2006 levels. Based on consultations with FDA, fresh pre-cut bagged lettuce was introduced in 2007 replacing leaf and romaine lettuce. 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. All samples in a State are collected on the same day or within a 2-day interval. The samples of cantaloupes and tomatoes collected from a site consist of three individual units of produce generally collected from the same container. Fresh pre-cut bagged lettuce and alfalfa sprouts samples generally represent individual samples. Inferences cannot reasonably be made from the sample units to the lots from which they originate because the units

Commodity	Country	Number of Samples
Cantaloupe	Costa Rica	75
	Guatemala	144
	Honduras	81
	Mexico	6
	TOTAL	306
Green Onions	Canada	3
	Chile	3
	Guatemala	18
	Mexico	669
	TOTAL	693
Lettuce	Canada	3
	Chile	9
	Mexico/USA	9
	TOTAL	21
Tomatoes	Canada	93
	Mexico	276
	TOTAL	369

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

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 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 sites sampled per commodity. At each site, 3 samples are collected from the same lot in each facility for a total of 192 samples collected every month for each commodity.

Distribution centers and terminal markets in each State are selected at random based on probability proportional to the site's distribution volume (i.e., the amount of produce that moves through the site). Therefore, the larger the site, the greater the chance it will be sampled. If the commodity of interest is not available at the designated primary site, an alternate site may be chosen. MDP does not allow samples to be taken from public markets or retail stores because of the potential for contamination by the consumer and because commodity handling practices at this level in the distribution chain may vary widely. Between April and September 2007, 5,279 samples were collected from over 260 sites across the country and analyzed by MDP

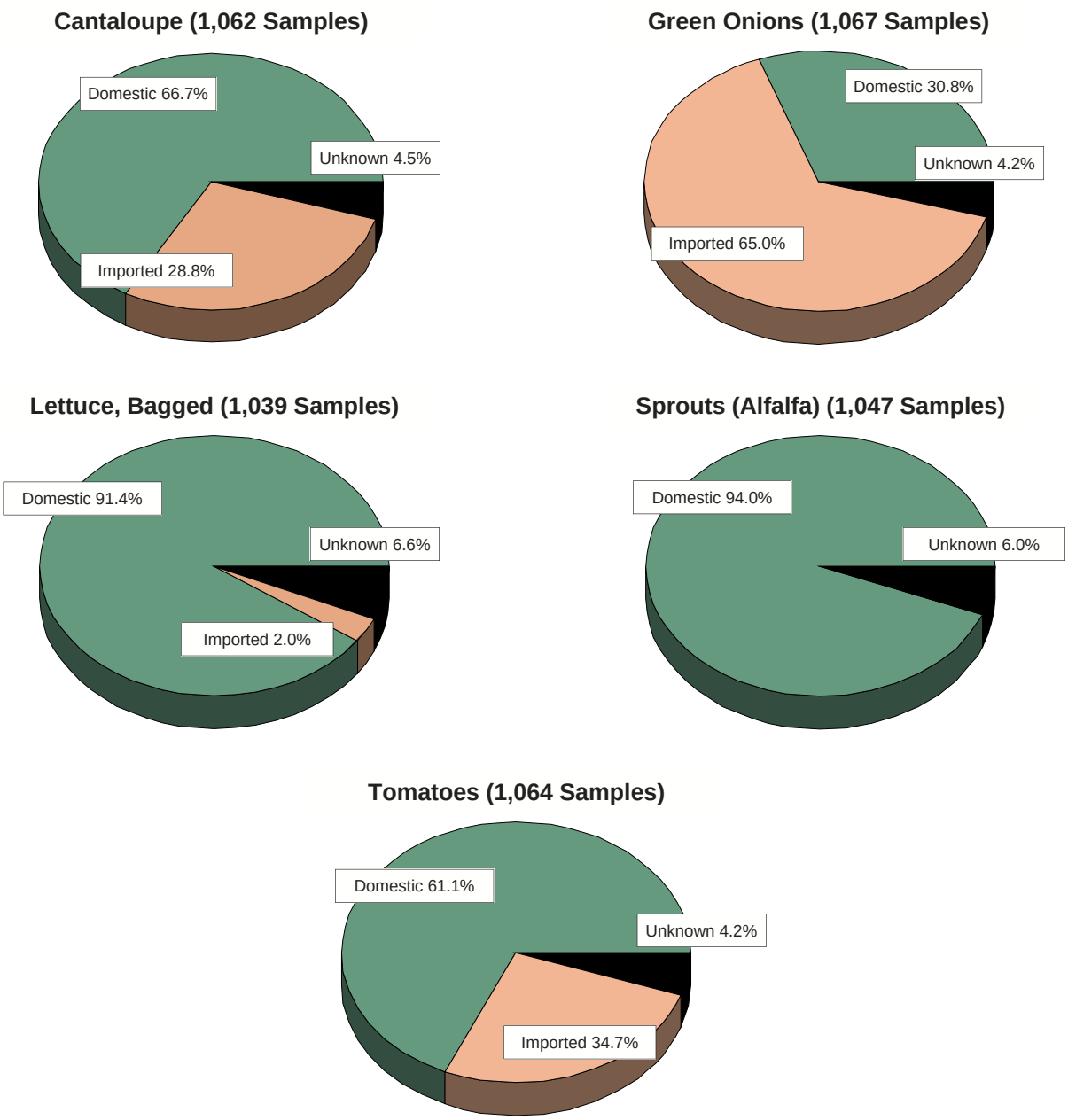


Figure 3. Commodity Origin. *The proportion of domestic, imported or unknown origin for each commodity is depicted for samples tested in 2007.*

laboratories. Table 2 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 will request that the particular commodity be sampled again. All samples are shipped on the same day as sample collection by overnight delivery so that laboratory analysis can begin the following day.

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

Alfalfa sprouts, cantaloupe, fresh pre-cut bagged lettuce, green onions, and tomatoes were collected and tested as commodities for 2007. For fresh pre-cut bagged lettuce, all lettuce varieties were acceptable, whether

State	Cantaloupe	Green Onions	Lettuce (Bagged)	Sprouts (Alfalfa)	Tomatoes	Total	*E. coli*	*E. coli* O157:H7	*Salmonella*	Total Coliform
California	237	231	234	237	234	1,173	1,173	1,173	1,173	234
Colorado	36	36	36	36	36	180	180	180	180	36
Florida	117	120	117	117	120	591	591	591	591	96
Maryland	63	66	51	66	63	309	309	309	309	51
Michigan	99	102	102	90	98	491	491	491	491	84
Minnesota	33	33	33	33	33	165	165	165	165	33
New York	150	150	150	150	150	750	750	750	750	150
Ohio	96	98	96	96	99	485	485	485	485	96
Texas	132	132	120	123	132	639	639	639	639	120
Washington	66	66	66	66	66	330	330	330	330	66
Wisconsin	33	33	34	33	33	166	166	166	166	34
Totals	1,062	1,067	1,039	1,047	1,064	5,279	5,279	5,279	5,279	1,000

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.*

single or mixed. Bags containing lettuce mixed with spinach or other greens were not acceptable. 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, 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). 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), 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.

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 samples of MDP commodities for generic *E. coli*, *E. coli* strains carrying shiga toxins and enterotoxins, including *E. coli* O157:H7, and *Salmonella*. MDP laboratories performed mPCR screening of all samples for pathogenic *E. coli*, based on the presence of gene coding for shiga toxins and enterotoxins. Isolates of these organisms were sent to the Gastroenteric Disease Center at 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. The laboratories also participated in the validation of a realtime PCR-based screening method for *Shigella* detection.

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.

All samples were washed in Universal Preenrichment Broth (UPB) with 0.1% Tween® (Polysorbate 80). Cantaloupes, bagged lettuce, green onions, and tomatoes were manually washed and alfalfa sprouts were blended using a Stomacher® blender. For *E. coli* and coliform assays, an AOAC®-certified enzyme-based automated TEMPO® system was used for detection and enumeration of *E. coli* and coliform bacteria (bagged lettuce samples only). This system uses the standard Most Probable Number (MPN) method for enumeration.

In order to improve pathogen detection, cantaloupe, bagged lettuce, and tomato samples were soaked overnight. For alfalfa sprouts and green onions, any debris/plant material was removed from the wash prior to overnight incubation. Genomic DNA was extracted from each enriched sample and purified for use in detecting pathogens via DNA-based PCR assays. All samples were screened by mPCR procedures for shiga toxin-producing *E. coli* (STEC) and entero-toxigenic *E. coli* (ETEC). MDP laboratories used PCR assays and automated instruments for the detection of *Salmonella* 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 verification of isolates for the target pathogens.

During 2007, the laboratories assessed 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. MDP also investigated a semi-automated system for repetitive sequence-based PCR (rep-PCR) DNA fingerprinting.

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 www.ams.usda.gov/mdp. SOPs provide uniform administrative, sampling, and laboratory procedures. MDP laboratories participated in the proficiency testing of generic *E. coli* in produce samples, administered by MDP. Several MDP laboratories also participated in *E. coli* O157:H7 and *Shigella* proficiency testing for foods, including produce, administered by the Food Emergency Response Network (FERN).

Positive and negative controls and a sterile media blank were required for each sample set. MDP laboratories use positive control strains of *E. coli* O157:H7 and *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

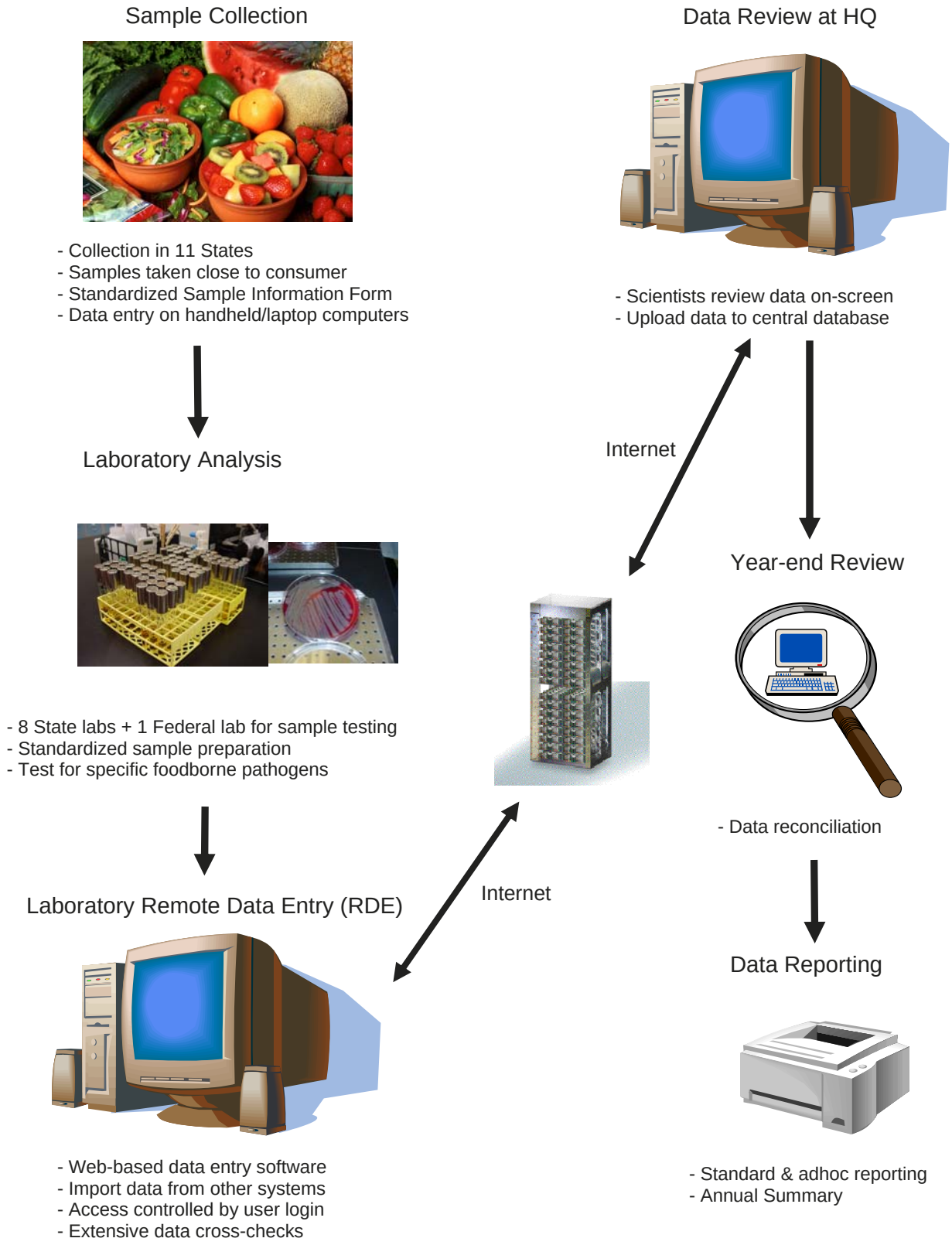


Figure 4. MDP Data Pathway. *An illustration of MDP data path, from sample collection through laboratory analysis and reporting.*

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

IV. Database Management

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

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

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

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

At MDP headquarters, the RDE system allows scientists to review and approve the data for inclusion in the central database. The central MDP database is maintained using Microsoft® 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 2007 Data

MDP began sampling operations in April 2007 due to delays in the release of funds. Sampling

operations ceased in September 2007 due to budget uncertainty for FY 2008. Consequently, results presented in this summary cover only 5 months of program operations. MDP collected a total of 5,279 samples--alfalfa sprouts (1,047), cantaloupe (1,062), pre-cut fresh bagged lettuce (1,039), green onions (1,067), and tomatoes (1,064).

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-nine percent of the samples were from domestic sources, 26 percent were imported, and 5 percent were of unspecified origin. Table 2 shows the distribution of samples among each commodity and collection State.

All samples were washed or blended (alfalfa sprouts) in Universal Preenrichment Broth (UPB) in order to streamline the screening process for all target bacteria. *E. coli* and coliform (bagged lettuce only) enumerations were performed using the automated TEMPO® system. A soaking step was implemented for cantaloupe, bagged lettuce, and tomatoes to improve pathogen detection. 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 (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 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, antimicrobial resistance testing, and genomic fingerprinting.

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

For detection and enumeration of generic *E. coli*, 5,279 samples were screened using the automated TEMPO® system and an AOAC-certified method. All 5,279 samples were also screened for pathogenic *E. coli* that harbor shiga toxins (STEC) and enterotoxins (ETEC)

Commodity	Number of Samples Tested	Number of Samples Screened by mPCR	Number of Pathogenic *E. coli*-Positive Samples
Cantaloupe	1,062	1,062	1
Green Onions	1,067	1,067	0
Lettuce, Bagged	1,039	1,039	2
Sprouts (Alfalfa)	1,047	1,047	1
Tomatoes	1,064	1,064	0
Total	5,279	5,279	4

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.*

(refer to Table 3) using a mPCR assay developed by FDA. Four samples were identified as positive for pathogenic *E. coli* carrying shiga toxin genes. Successful isolation of shiga toxin-carrying *E. coli* strains (STEC) was attained from two 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 the small number of target bacterial cells, differential growth rates of various bacteria, and additional growth requirements.

The two isolates were sent to PSU for serotyping and further characterization and to FDA/CVM for antimicrobial resistance testing. PSU conducted tests that included 12 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 antimicrobial resistance for 15 different antibiotics and genomic fingerprinting on these isolates. The results of PSU and FDA/CVM testing are shown in Table 4. One each of the STEC strain was isolated from cantaloupe and fresh pre-cut bagged lettuce. Both strains carried additional virulence-related genes and they were not resistant to any of the antimicrobial compounds tested. 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.

Coliform Bacteria

Coliform bacteria are normally found in large numbers in fresh produce samples because produce is grown in an open, natural environment. However, fresh pre-cut bagged lettuce is a ready-to-eat product that is washed before packaging. Therefore, only bagged lettuce samples were tested for the presence of coliform bacteria using the automated TEMPO® system. In this assay, bacteria that produce acid from lactose fermentation are enumerated. The data show a significant reduction of coliform bacteria when compared to fresh lettuce, demonstrating that washing and other steps taken in the preparation of ready-to-eat fresh pre-cut bagged lettuce greatly reduced the number of coliform bacteria normally expected in lettuce.

Commodity	Pathogenic Class	Toxic Genes Identified	Serotyping		Pulsed-Field Gel Electrophoresis
			O Antigen	H Antigen	
Cantaloupe	STEC	Stx-1, Cnf-2	88	38	
Lettuce, Bagged	STEC	Stx-2, HlyA, EAggEC	Neg	8	

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

HlyA - hemolysin A

Cnf-2 - cytotoxic necrotizing factor 2

EAggEC - Enteroaggregative *E. coli*

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.*

Commodity	Number of Samples Tested	Number of Positive Individual Samples	Number of Positive Isolates
Cantaloupe	1,062	6	1
Green Onions	1,067	9	0
Lettuce, Bagged	1,039	6	0
Sprouts (Alfalfa)	1,047	9	2
Tomatoes	1,064	10	0
TOTALS	5,279	40	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.*

Salmonella

As depicted in Table 5, a total of 5,279 samples were screened for *Salmonella* by BAX-PCR. Of these samples, 40 were positive and 3 *Salmonella* isolates were obtained: 1 from cantaloupe and 2 from alfalfa sprouts. These 3 isolates were sent to FDA/CVM for identification by serotyping, antimicrobial resistance for 15 antibiotics, and genomic fingerprinting. Table 6 identifies each isolate and the associated serogroup. The isolate from cantaloupe, *S. tucson*, belonging to serogroup H, was resistant to sulfisoxazole, an antimicrobial agent. The isolates found in alfalfa sprouts, *S. dessau*, belonging to serogroup E4, and *S. mbandaka*, belonging to serogroup C1, were also resistant to sulfisoxazole.

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

No enterohemorrhagic *E. coli* O157:H7 strain was isolated from the 5,279 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.

	Serotype/Identification			
Commodity	**Genus**	**Species**	**Serogroup**	**Pulsed-Field Gel Electrophoresis**
Cantaloupe	*Salmonella*	*tucson*	H	
Sprouts (Alfalfa)	*Salmonella*	*mbandaka*	C1	
Sprouts (Alfalfa)	*Salmonella*	*dessau*	E4	

Table 6. Salmonella Identification and Serogroup. *This table summarizes the genus, species, and serogroup for each of the three Salmonella isolates obtained in 2007.*

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 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.

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.