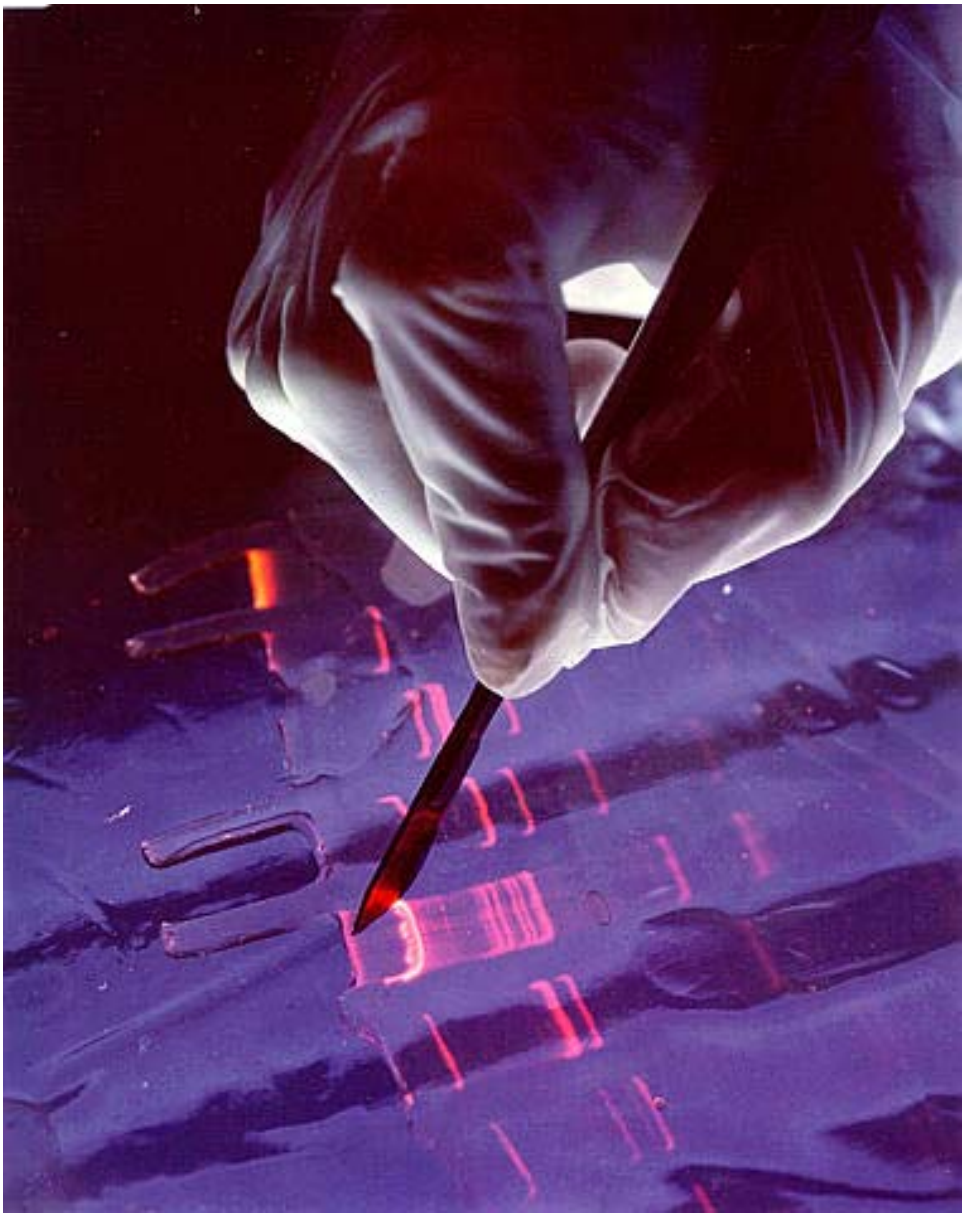
Microbiological Data Program
Progress Update and 2003 Data Summary

United States Department of Agriculture

Agricultural Marketing Service

Science & Technology Programs



United States Department of Agriculture

Marketing and Regulatory Programs

Agricultural Marketing Service

1400 Independence Ave.
Washington, DC
20250

January 2005

To the Reader:

I am pleased to present the USDA Microbiological Data Program 2003 Data Summary. In 2003, MDP continued testing the five commodities begun in 2002. Cantaloupe, tomatoes, celery, leaf lettuce, and romaine lettuce were selected because they are high consumption fruit and vegetables in the United States. Sample collection was performed using a statistical framework. The laboratory methods used in the program were primarily traditional cultural techniques. However, in 2004 MDP initiated the use of new and innovative technologies for the identification of microorganisms. A description of these technologies is provided at the end of this publication.

MDP is a partnership with cooperating State agencies that are responsible for sample collection and analyses. Ten States participated in 2003: California, Colorado, Florida, Maryland, Michigan, New York, Ohio, Texas, Washington, and Wisconsin. Because these States together 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 2003. 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,

A. J. Yates
Administrator

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Figures and Tables

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Participating Organizations

Data presented in this report were collected and processed through the efforts of the following organizations:

State Agencies

California Department of Food and Agriculture
California Department of Pesticide Regulation
Colorado Department of Agriculture
Florida Department of Agriculture and Consumer Services
Maryland Department of Agriculture
Michigan Department of Agriculture
New York Department of Agriculture and Markets
Ohio Department of Agriculture
Texas Department of Agriculture
Washington State Department of Agriculture
Wisconsin Department of Agriculture, Trade, and Consumer Protection

Laboratories

California Department of Food and Agriculture
Division of Inspection Services
CDFA Agricultural Microbiology Laboratory
McClellan Park (McClellan AFB), Bldg. 929
5431 Arnold Ave.
Sacramento, CA 95652

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

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

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

Minnesota Department of Agriculture
Laboratory Services Division
90 West Plato Blvd.
St. Paul, MN 55107-2004

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

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

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

Washington State Department of Agriculture
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Olympia, WA 98501

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

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Web site: http://www.ams.usda.gov/science/MPO/MDP.htm

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.

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 2003, 10 States collected fruit and vegetable samples (California, Colorado, Florida, Maryland, Michigan, New York, Ohio, Texas, Washington, and Wisconsin, with Minnesota joining the program in 2004).

The program tested five commodities (cantaloupe, celery, leaf lettuce, romaine lettuce, and tomatoes) for *Escherichia coli (E. coli)* with pathogenic potential and *Salmonella*. MDP analyzed a total of 10,972 samples. Eighty-one percent of the samples were from domestic sources, 15 percent were imported, and 4 percent were of unspecified origin. MDP identified 44 *E. coli* isolates with virulence attributes. The presence of virulence attributes does not necessarily mean that these strains are pathogenic to humans, only that they may have pathogenic potential. MDP screening also resulted in three *Salmonella* isolates: one each from leaf lettuce, romaine lettuce, and tomato.

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 2003

This summary consists of the following sections: (I.) Introduction, (II.) Sampling, (III.) Laboratory Operations, (IV.) Database Management, (V.) Summary of 2003 Data

I. Introduction

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 data regarding the incidence and identification of targeted food-borne pathogens and indicator organisms on fresh fruit and vegetables. This publication summarizes progress made toward implementation of the program and provides an overview of data collected in 2003. 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.

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) provides consultation as an independent research authority 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. 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, romaine lettuce, and tomatoes. Commodities were tested for *Escherichia coli* (*E. coli)* strains with pathogenic potential and *Salmonella*. Isolates of these organisms were sent to specialized laboratories for further characterization including sero-typing, antibiotic resistance, and virulence attributes.

Figure 1 provides an overview of MDP management and operations. Figure 2 highlights participating States and their geographical distribution areas. Samples were collected in the 10 participating States through cooperative agreements with their respective agencies. Also shown are the 12 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, Vermont, Virginia, and Wyoming. Together these States represent over 50 percent of the Nation's population and all 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 (California, Colorado, Florida, Michigan, New York, Ohio, Washington, and Wisconsin) and the AMS National Science Laboratory. Laboratory operations are designed to minimize variability of results across laboratories through the use of uniform methodologies and a comprehensive quality assurance/quality control (QA/QC) program. The data are submitted electronically via a Web-based Remote Data Entry (RDE) system and entered into a central database managed by MPO in Manassas, VA.

MDP data can be used to establish baselines for the incidence of target organisms at the wholesale level, to understand trends, and to improve risk communication.

Figure 1. Overview of MDP Management and Operations

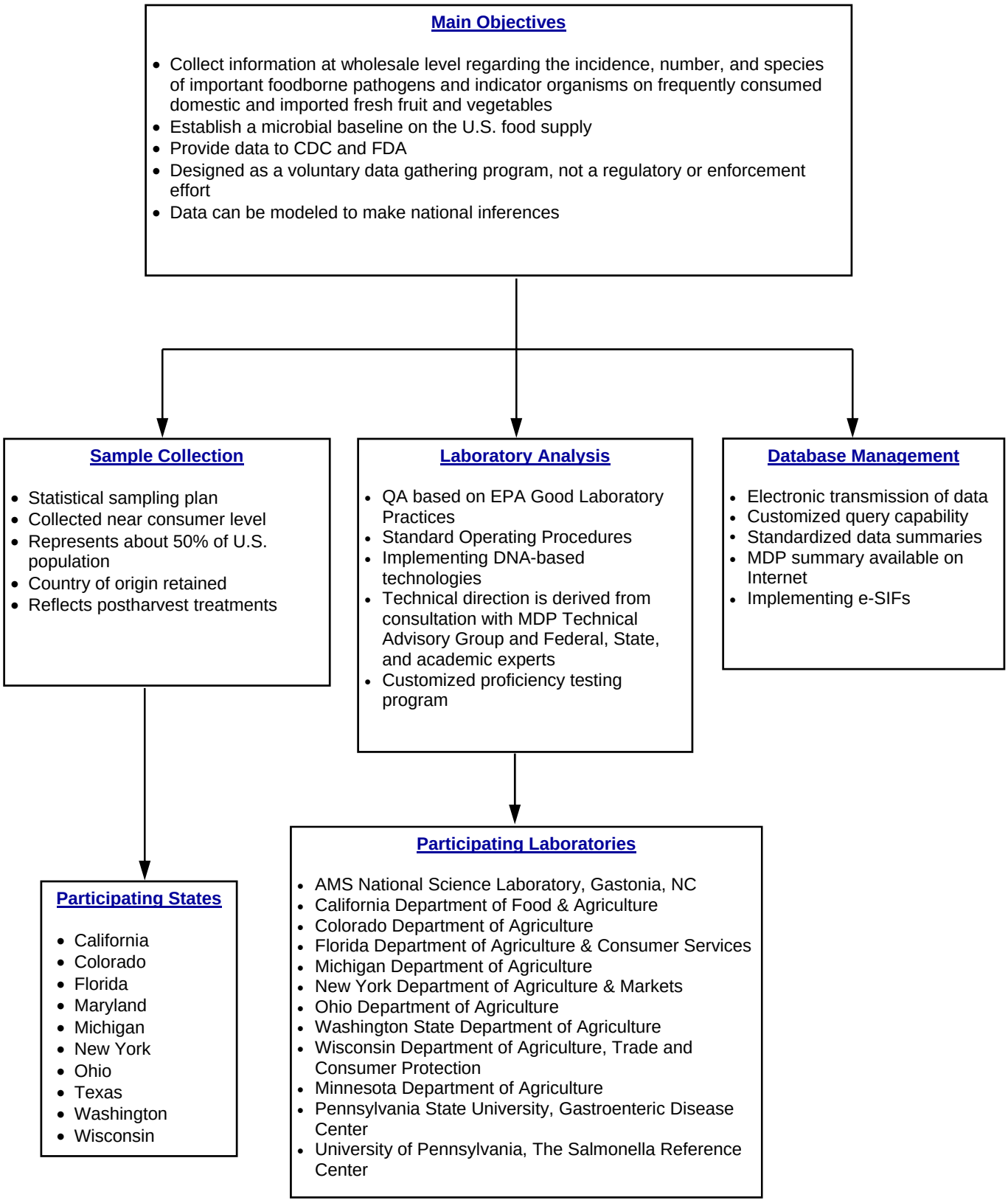
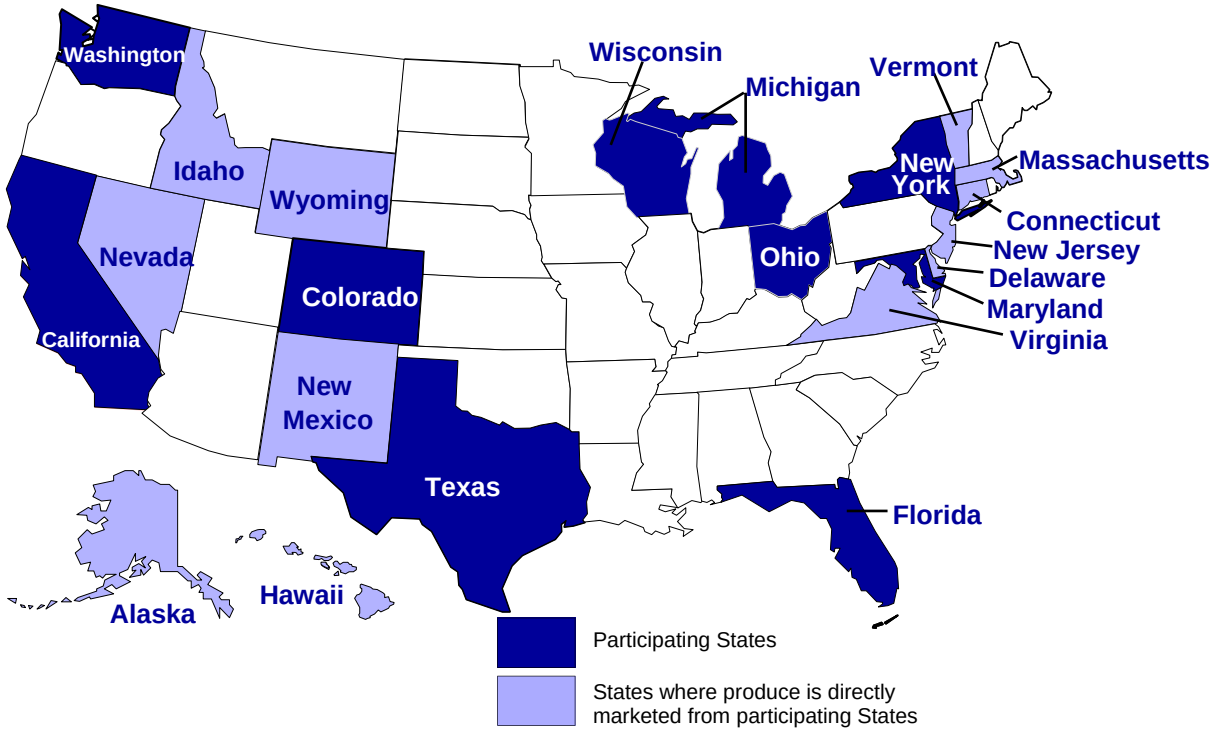


Figure 2. Participating States and their Geographical Distribution Areas



The information gathered from MDP can help identify technology development priorities and risk modeling needs for fresh produce in the food chain. The MDP data can also supplement the FDA/USDA "Guidance for Industry—Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables." The "Guide," developed in concert with industry, has fostered proactive leadership and broad adherence to good agricultural practices as well as a commitment to continually seeking practices based on the best available science that will minimize microbial contamination.

USDA is a member of the interagency Task Force on Antimicrobial Resistance established in 1999 to seek better surveillance and education regarding optimal use of antimicrobials. Isolates from MDP samples testing positive for *Salmonella* or *E. coli* were sent to Pennsylvania State University (Penn State) for antimicrobial resistance testing. These data will be added to the National Antimicrobial Resistance Monitoring System database.

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.

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 FDA, CDC, and NASS (1).

The sampling of commodities in commerce is conducted at wholesale markets and/or distribution centers on a year-round basis and 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 in the State, the greater the number of samples taken. 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.

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 time frame (e.g., one 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 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.

MDP sampling is 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.

MDP uses Sample Information Forms (SIFs) to document information required for chain-of-custody, which is captured in the MDP database files. Sample collectors use the forms to record information such as (1) the State of sample collection, (2) the collection date, (3) the commodity code, and (4) the testing laboratory code. Other information collected includes the country of origin of the sample, any production claims (such as organic), and any postharvest treatments. A customized software application allows States to capture SIFs electronically using laptop or hand-held computers. MDP samples are collected aseptically by trained collectors who safeguard sample integrity. Food samples are collected at terminal markets and large chain store distribution centers from which food commodities are released to supermarkets and grocery stores. If samples are not available at the designated site, an alternate site can be used. Sample collection at retail grocery stores, however, is not permitted because commodity handling practices at this level in the distribution chain may vary.

State population figures are used to assign the number of samples scheduled for collection each month. These population-based numbers are as follows: California, 14; Colorado, 2; Florida, 7; Maryland, 4; Michigan, 6; New York, 9; Ohio, 6; Texas, 8; Washington, 4; and Wisconsin, 2. This schedule results in a monthly target of 62 samples per commodity. Each site sample collected consists of three individual units, which are treated as three distinct laboratory samples. Samples are collected and transported using aseptic techniques (i.e., sterile latex gloves and sample bags). Samples are measured for surface temperature at the time of collection and on receipt at the laboratory to characterize conditions during shipping.

During 2003, MDP collected data on cantaloupe, tomatoes, celery, leaf lettuce, and romaine lettuce. These commodities are harvested primarily by hand although some mechanical harvesting does occur. The produce may be packaged in the field (except tomatoes, which require classification for color and size) or taken to a packinghouse. 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. Tomatoes and cantaloupe often 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, tomatoes are often artificially ripened using techniques such as ethylene oxide gas. Therefore, the data reflect handling practices and postharvest treatments.

III. Laboratory Operations

Nine microbiology laboratories performed analyses for MDP. Specialized laboratories, including the Salmonella Reference Center (SRC), University of Pennsylvania (UPenn), and the Gastroenteric Disease Center, Penn State, performed serotyping, antimicrobial resistance, and virulence attribute testing. In addition, the Minnesota Department of Agriculture laboratory performed method development studies 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 a buffered solution and all analyses were conducted from this surface wash eluent. MDP also tested the use of newly developed wash buffers and growth media. An Enzyme-linked immunofluorescent assay (ELFA) and cultural microbiological techniques, described in Section V, were used to obtain data through September 2003. As data were obtained and reviewed, modifications were introduced to refine and improve the techniques used to produce these data.

MDP evaluated the use of DNA-based polymerase chain reaction (PCR) and instruments associated with this technology to compare performance with the ELFA method. PCR technology has been shown to adequately address concerns such as sample matrix interferences, low cell counts, and reaction inhibition due to enrichment media. MDP successfully completed validation studies and began using DNA-based automated instruments for the detection of *Salmonella* isolates in October 2003. AOAC®, an internationally recognized organization that validates analytical methods for testing food and agricultural products, approved the use of these instruments and methodologies for microbial screening programs; the USDA Food Safety and Inspection Service (FSIS) also uses this technology for microbiological screening programs.

MDP methods were routinely reviewed and modified as necessary to enhance productivity and to provide data that will be useful for risk model development. As new microbial technologies became commercially available, they were evaluated for use in the program. As with

all methods modifications, all programmatic QA/QC criteria must be met prior to implementation by MDP laboratories.

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 Bacteriological Analytical Manual (BAM) (2) methods, AOAC®-certified methods, 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 used positive control strains of *E. coli* and *Salmonella* 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 revised program SOPs 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 operates independently from the laboratory staff. Preliminary QA/QC review procedures were performed on-site by each laboratory's QAU. Final review procedures were performed by MDP staff that is 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. MDP laboratories participated in a check sample program administered through AOAC®.

IV. Database Management

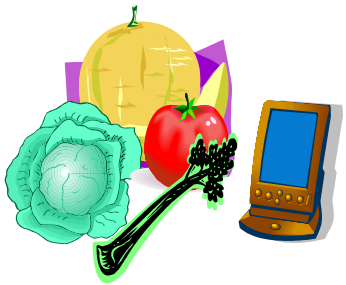
MDP maintains an electronic database that serves as a central data repository. The central database resides at the Monitoring Programs Office in Manassas, VA. The data captured and stored in the MDP database include product information and analytical findings for each sample collected along with QA/QC results for each set of samples. The MDP data life-cycle is depicted in Figure 3.

MDP utilizes a Web-based 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 technology to encrypt all data passed between users' computers and the central Web server. At MDP headquarters, the RDE system allows scientists to review and approve the data for inclusion in the central database. A separate Windows-based system allows sample collectors to electronically capture the standardized Sample Information Form (e-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

Figure 3. Data Life Cycle

Sample Collection

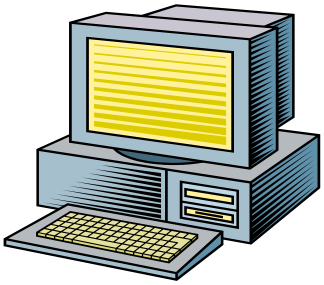


- Collection in 10 States
- Samples taken close to consumer
- Standardized Sample Information Form
- Data entry on handheld/laptop computers

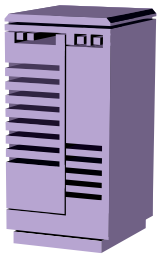
Laboratory Analysis

- 8 State laboratories + 1 Federal laboratory for sample testing
- Standardized sample preparation
- Test for specific foodborne pathogens

Lab Remote Data Entry (RDE)



Internet

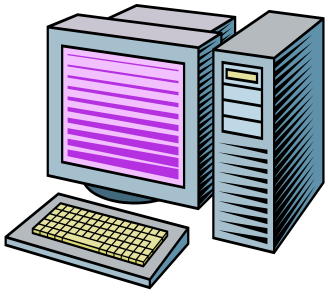


- Web-based data entry software
- Import data from other systems
- Access controlled by user login
- Extensive data cross-checks

- Centralized data storage
- Encrypted data transmissions

Internet

Data Review at HQ

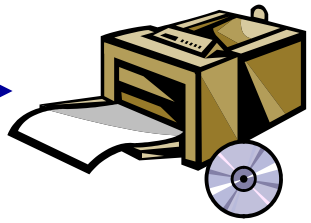


- Scientists review data on-screen
- Upload data to central database

Year-end Review

- Data reconciliation
- Standard & ad-hoc reporting

Data Reporting



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. The central MDP database is maintained using Microsoft® Access in a Windows® 2000 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 2003 Data

In 2003, the second full year of testing, MDP screened 10,972 samples for the presence of *E. coli* and *Salmonella*. *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 involving produce (3). Consequently, 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 2003 provided a second 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.

Commodities tested in 2003 remained the same as those in 2002: cantaloupe, tomatoes, celery, leaf lettuce, and romaine lettuce. These crops were selected because they are high consumption fruit and vegetables in the U.S. diet and are often consumed raw. All samples in a State are collected on the same day or within a two-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.

Table 1 shows the distribution of samples among each commodity and collection State. Figure 4 illustrates the proportion of samples that were domestic, imported, and of unknown origin for each commodity. Table 2 specifies the distribution of imported samples by commodity and country of origin.

The samples were screened for *E. coli* using an AOAC®-certified method. When *E. coli* was detected through the initial screening process, the organism was isolated and identified. To screen for potentially pathogenic strains, the isolates were sent to Penn State for further identification and characterization, including serotyping, antimicrobial resistance testing, and testing for virulence attributes.

To determine pathogenic potential, Penn State tested the isolates for the presence of 13 different virulence-specific genes associated with 7 different categories of pathogenic *E. coli*. A summary of the analysis of pathogenic *E. coli* by virulence attributes is presented in Table 3. The isolates listed in Table 3, containing Enterohemorrhagic *E. coli*, Enterotoxigenic *E. coli*, and Enteropathogenic *E. coli*, are of importance to human health. Most of the remaining isolates listed can be grouped as Necrotoxigenic *E. coli,* Enteroaggregative *E. coli,* and Enteroinvasive *E. coli* based on presence of virulence attributes. Penn State also classified the *E. coli* strains

Table 1. Number of Samples Collected and Analyzed by State

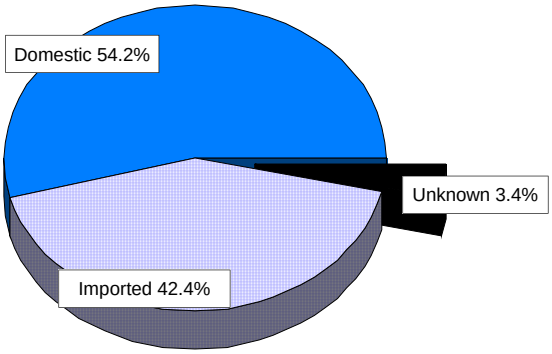
State	Cantaloupe	Tomatoes	Celery	Leaf Lettuce	Romaine Lettuce	Total	Samples Analyzed for	
							E. coli	*Salmonella*
California	504	504	504	501	504	2,517	2,517	2,517
Colorado	72	72	72	72	72	360	360	360
Florida	248	249	249	252	252	1,250	1,250	1,250
Maryland	138	144	141	126	117	666	666	666
Michigan	216	213	213	216	213	1,071	1,071	1,071
New York	324	324	324	324	324	1,620	1,620	1,620
Ohio	214	212	216	213	219	1,074	1,074	1,074
Texas	283	280	275	282	285	1,405	1,405	1,402
Washington	122	132	144	144	141	683	683	679
Wisconsin	69	64	52	72	69	326	326	326
TOTAL	2,190	2,194	2,190	2,202	2,196	10,972	10,972	10,965

Table 2. Distribution of Imported Samples

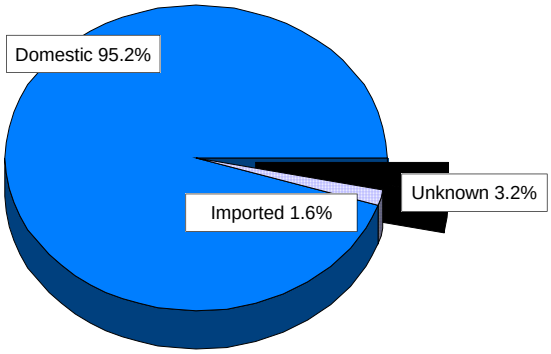
Commodity	Country Name	Number of Samples
Cantaloupe	Costa Rica	273
	Dominican Republic	58
	Guatemala	296
	Honduras	204
	Guatemala / Honduras	3
	Mexico	36
	Unknown Country	59
		929
Celery	Canada	1
	Mexico	33
		34
Lettuce, Leaf	Canada	6
Lettuce, Romaine	Canada	6
	Unknown Country	3
		9
Tomatoes	Canada	114
	Israel	3
	Mexico	527
	Netherlands	3
	Spain	5
	Unknown Country	3
		655

Figure 4. Commodity Origin

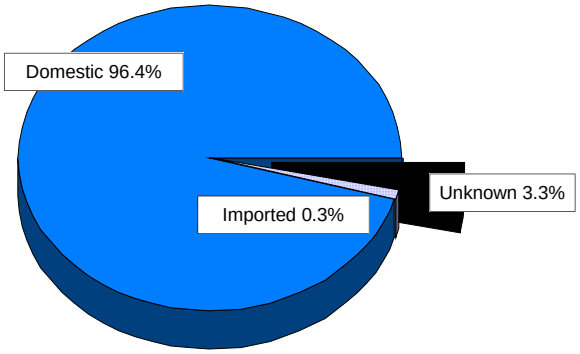
Cantaloupe



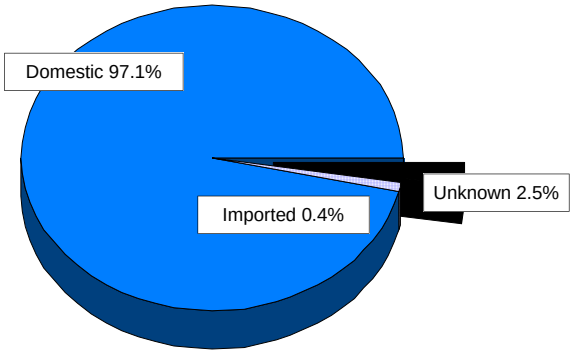
Celery



Leaf Lettuce



Romaine Lettuce



Tomatoes

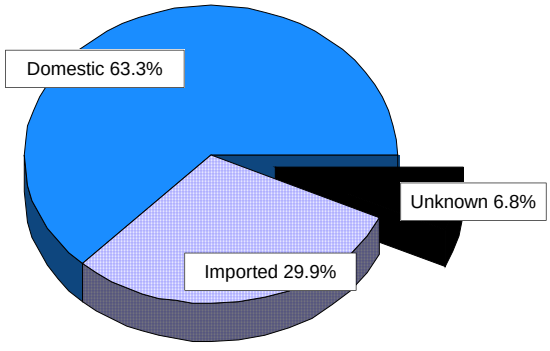


Table 3. Summary of Analysis of Pathogenic *E. coli* by Virulence Attributes

E. coli Type*	Number of Isolates	Virulence Attributes	Attribute Description
EHEC	3	Stx-1, Stx-2, HlyA	Shiga Toxins, EHEC Hemolysin
ETEC	2	Heat Stable (STa, STb), Heat Labile (LT)	Toxins
EAggEC	4	EAggEC	Plasmid-encoded Factor
EPEC	11	Eae	Virulence Factor (Intimin)
NTEC	14	CNF-1 and CNF-2	Cytotoxic Necrotizing Factor
EIEC	3	IpaH	Invasive Plasmid Antigen
Other	7	K1	Capsular Antigen

* See Definitions

based on the somatic "O" type and flagellar "H" type antigens using an enzyme-linked immunosorbent assay method, commonly known as ELISA.

The number of samples for each commodity analyzed for *E. coli* with subsequent characterization of the isolated organism is shown in Table 4. Virulence attributes were identified in 44 *E. coli* isolates—4 on imports (2 tomatoes and 2 cantaloupes), 4 on samples of unknown origin, and 36 on domestic samples. These isolates may have pathogenic potential but cannot be characterized as pathogenic to humans. An isolate must carry several genes to be positively characterized as a pathogen. MDP isolates were tested for those genes referenced in Table 3 and were shown to carry only one of them. The additional testing required in order to determine the actual pathogenicity of the organism to humans is not within the scope of MDP.

A fully automated ELFA system was used to screen for *Salmonella* through September 2003. In October 2003, the BAX® instrument, an automated PCR system, was introduced for *Salmonella* screening. Pooled samples are screened initially; if a positive result is obtained, the three individual samples are tested. Positive samples were cultured for isolation and identification of the organism. Identification of isolates was confirmed using either a conventional biochemical testing system, an AOAC® performance-tested kit, or a MDP-accepted commercial biochemical kit or system. Isolates were then sent to the SRC at UPenn for further characterization (serotyping) and to Penn State for antimicrobial resistance testing.

Three samples, all of domestic origin, were found to contain *Salmonella*: one each of leaf lettuce, romaine lettuce, and tomato. The numbers of each commodity analyzed for *Salmonella* with subsequent characterization are shown in Table 5. Based on serotyping, the leaf lettuce isolate was identified as *Salmonella muenchen* group C2 and the romaine lettuce isolate was identified as *Salmonella poona* group G1. The species identity for the tomato isolate was not determined; however, the isolate belonged to group C2.

MDP is now implementing an enzyme-based assay to detect *E. coli* followed by multiplex PCR techniques to detect *E. coli* with pathogenic potential. In 2004, *E. coli* O157:H7 was added to the program as a target organism with screening performed using PCR techniques. MDP plans to add *Shigella* in 2005 and is investigating DNA-based screening methods for this organism.

Table 4. Summary of Sample Analysis for *E. coli*

Commodity	Number of Samples Tested	Number of Virulent Strains Isolated	Percent of Isolates Testing Positive for Virulence Attributes	Breakdown in Virulent Attributes
Celery	2,190	3	0.14	1-Eae, 1-Stx-1+CNF2, 1-CNF1+K1
Cantaloupe	2,190	7	0.32	3-Eae (1-Eae), 2-IpaH, 2-K1 (1-K1)
Leaf Lettuce	2,202	15	0.68	1-CNF1, 5-CNF2, 1-EAggEC, 3-Eae, 2-K1
Romaine Lettuce	2,196	17	0.77	1-Sta, 1-CNF1+K1, 1-Stx-1+Stx-2+HlyA 5-CNF2, 1-IpaH, 2-K1, 3-EAggEC, 5-Eae 1-Sta+HlyA
Tomatoes	2,194	2	0.09	1-Stx-2, 1-EAggEC
	10,972	**44**		

Table 5. Summary of Sample Analysis for *Salmonella*

Commodity	Number of Samples Tested	Number of Positive Isolates	Percent of Samples Testing Positive for Isolates
Celery	2,190	0	0
Cantaloupe	2,184	0	0
Leaf Lettuce	2,202	1	0.045
Romaine Lettuce	2,195	1	0.046
Tomato	2,194	1	0.046
	10,965	**3**	

References:

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2. Bacteriological Analytical Manual (BAM) (2001). US Food and Drug Administration, http://www.cfsan.fda.gov/~ebam/bam-toc.html.
3. Scientific Criteria to Ensure Safe Food (2003). Institute of Medicine, National Research Council of the National Academies. The National Academies Press, Washington, DC.

Disclaimer:

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Definitions:

Antigen: A substance (such as a toxin or enzyme) capable of stimulating an immune response.

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.

Check 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.

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). O157:H7 is the prototypic EHEC.

Enteroinvasive *E. coli* (EIEC): strains that closely resemble *Shigella* and cause an invasive, dysenteric form of diarrhea in humans.

Enteropathogenic *E. coli* (EPEC): Strains of *E. coli* that cause a profuse watery diarrheal disease, a leading cause of infantile diarrhea in developing countries. Pathogenesis of EPEC involves intimin protein (encoded by *eae* gene) and EPEC adherence factor.

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.

Enteroaggregative *E. coli* (EAggEC): Strains of *E. coli* that resemble ETEC in causing non-bloody diarrhea in children. They attach to tissue culture cells in an aggregative manner. Their significance to human diseases is uncertain.

Enzyme-linked fluorescent assay (ELFA): Similar to ELISA; the secondary antibodies are tagged with a fluorescent substrate.

Enzyme-linked immunosorbent assay (ELISA): A technique for detecting and measuring antigens or antibodies in a solution. The presence of specific antigens which bind to the antibodies is detected by the application of secondary antibodies that have been tagged with a fluorescent or an enzymatic substrate.

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

Indicator organism: A microorganism or group of microorganisms whose presence indicates in-sanitation or fecal contamination.

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*.

Necrotoxigenic *E. coli* (NTEC): pathogenic *E. coli* strains that carry cytotoxic necrotizing toxins CNF-1 and CNF-2 and are known to cause extra-intestinal (urinary tract) infections in humans.

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.

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 probably 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.