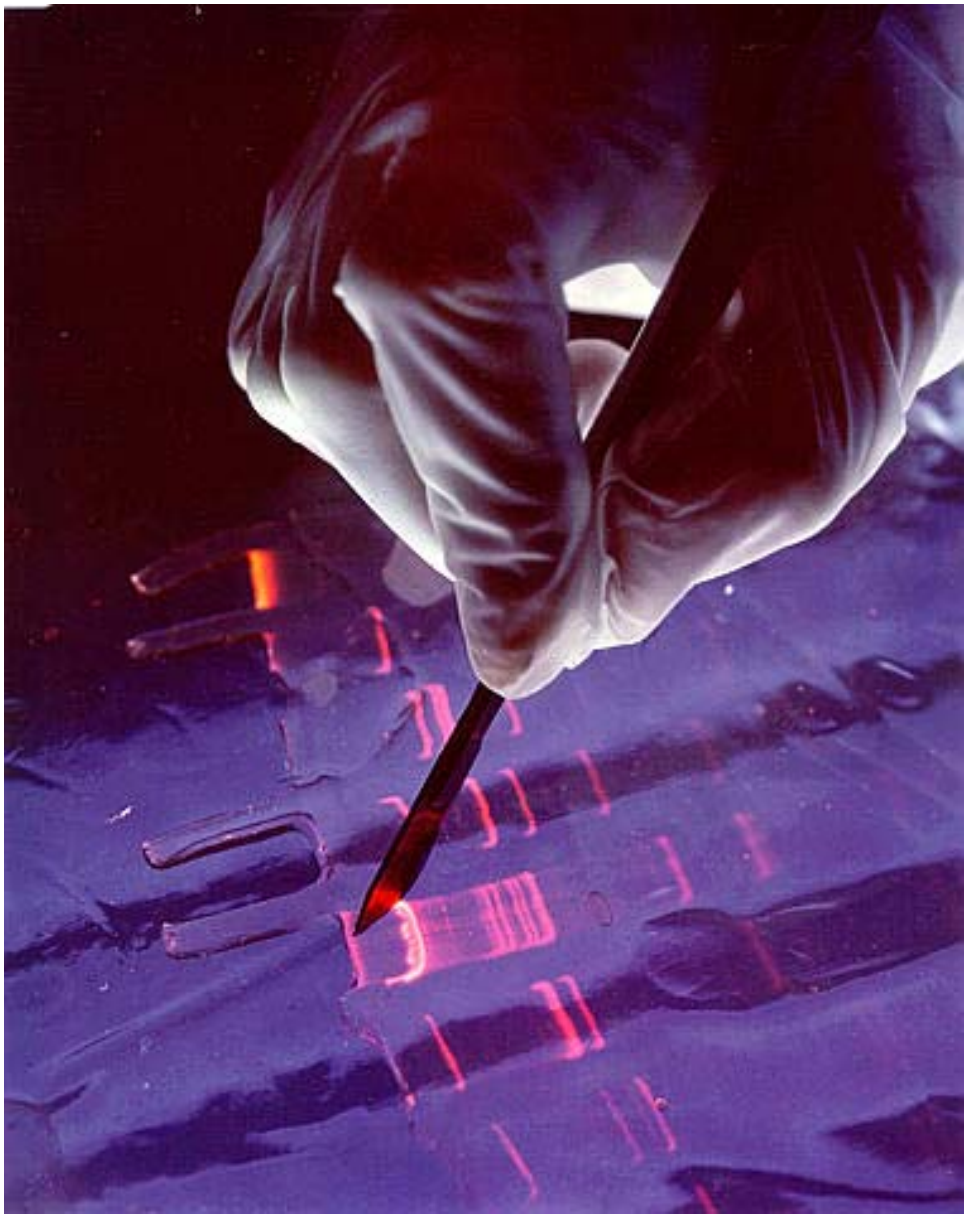
Microbiological Data Program
Progress Update and 2002 Data Summary

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
20090-6456

March 2004

To the Reader:

I am pleased to present the Microbiological Data Program Progress Update and 2002 Data Summary. In 2002, MDP tested five commodities: celery, leaf lettuce, romaine lettuce, tomatoes, and cantaloupes. Sample collection was performed using a statistical framework. These crops were selected for inclusion because they are high consumption commodities in the United States. The laboratory methods used in the program have been largely traditional cultural techniques. MDP is exploring the use of new and innovative technologies for the identification of microorganisms, which are also discussed in this publication.

The MDP is a partnership with cooperating State agencies that are responsible for sample collection and analyses. Ten States participated in 2002: California, Colorado, Florida, Maryland, Michigan, New York, Ohio, Texas, Washington, and Wisconsin. Because these States together represent all regions of the country and over half the Nation's population, these data can be used to make inferences about the national food supply.

This document is intended to provide the reader with an update on the methods, modifications, and refinements made during program development, as well as provide an overview of the data obtained during the initial stages of the program. MDP data are important in developing baseline levels of targeted pathogens in the domestic food supply. MDP is a continuous data gathering program to identify microbial trends and to develop risk models. If you have comments or suggestions on how we can further improve this summary, please send electronic-mail to us at amsmpo.data@usda.gov or to our Web site 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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Acronyms

AMS	Agricultural Marketing Service
AOAC	Association of Analytical Communities
ARS	Agricultural Research Service
BAM	Bacteriological Analytical Manual
CDC	Centers for Disease Control and Prevention
CNF2	Cytotoxic Necrotizing Factor 2
DNA	Deoxyribonucleic acid
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	Environmental Protection Agency
ETEC	Enterotoxigenic *E. coli*
FDA	Food and Drug Administration
FSIS	Food Safety and Inspection Service
GFP	Green fluorescent plasmid
GLPs	Good Laboratory Practices
MDP	Microbiological Data Program
MPO	Monitoring Programs Office
NARMS	National Antimicrobial Resistance Monitoring System
NASS	National Agricultural Statistics Service
NSL	National Science Laboratory
NTEC	Necrotizing *E. coli*
PCR	Polymerase Chain Reaction
PSU	Pennsylvania State University
QA/QC	Quality Assurance/Quality Control
QAU	Quality Assurance Unit
RDE	Remote Data Entry
SOP	Standard Operating Procedure
SRC	Salmonella Reference Center
SSL	Secure Socket Layer
STa	Heat Stable Toxin a
TAC	Technical Advisory Committee
UPenn	University of Pennsylvania
USDA	United States Department of Agriculture

Preface

In 2001, AMS received Congressional funding to implement a Microbiological Data Program (MDP). The purpose of the program is to provide statistically valid information about microbial organisms on fresh fruit and vegetables. It is a voluntary data gathering program, not a regulatory enforcement effort. This MDP Progress Update and 2002 Data Summary briefly describes program activities and results.

MDP collects random produce samples from wholesale distribution centers on a year-round basis. The sampling protocol utilized provides a statistically-reliable method for collecting and analyzing samples close to consumers before other variables such as handling and treatment are introduced by distributors and retailers.

Laboratories participating in MDP screen samples of fresh produce for the presence of *Escherichia coli* (*E. coli*) with pathogenic potential and *Salmonella* spp. Scheduled for introduction in 2004 are specific screening methods for targeting enterohemorrhagic, enterotoxigenic, and Shiga-toxin producing *E. coli* of importance to human health and screening methods for *E. coli* 0157:H7 and *Shigella* species.

A number of important benefits are expected from the MDP program. Microbiological benchmark data obtained from this fresh produce screening effort will contribute significantly to a national produce microbiological baseline. Such baseline data, including the further characterization of bacterial pathogens as to virulence attributes, serotype, genomic fingerprints, and antimicrobial resistance, will provide rigorous benchmarks against which change can be measured. The data will enhance the understanding of the microbial ecology of fresh fruit and vegetables in the food supply and permit the identification of trends. MDP data also will allow better focus in the design of production and handling systems aimed at reducing potential microbiological pathogens on fresh fruit and vegetables.

The MDP program was designed and is regularly reviewed in collaboration with the Centers for Disease Control and Prevention, the Food and Drug Administration, USDA's Agricultural Research Service and National Agricultural Statistics Service, and the participating States.

Executive Summary

In fiscal year 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. AMS coordinated its MDP planning and policy requirements with the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration (FDA), the USDA Agricultural Research Service (ARS), and the USDA National Agricultural Statistics Service (NASS). Sampling and/or microbiology laboratory activities were conducted through cooperative agreements with 10 States (California, Colorado, Florida, Maryland, Michigan, New York, Ohio, Texas, Washington, and Wisconsin) and 1 Federal microbiology laboratory (AMS National Science Laboratory, Gastonia, NC). These State and Federal agencies continue to collaborate as the program advances.

The purpose of MDP is to collect information regarding the incidence and identification of targeted foodborne pathogens and indicator organisms on fresh fruit and vegetables. MDP sampling is designed to obtain a statistical representation of the United States 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. In 2002, the program tested five commodities (cantaloupe, celery, leaf lettuce, romaine lettuce, and tomatoes) for *Escherichia coli (E. coli)* and *Salmonella* spp.

For calendar year 2002, MDP analyzed a total of 10,317 samples. Eighty-six percent of the samples came from domestic sources; 11 percent of the samples were imported; and no country of origin information was obtained on 3 percent of the samples. MDP identified *E. coli* isolates with virulence factors in 0.62 percent of the samples. The presence of virulence factors does not necessarily mean that these strains are pathogenic to humans, but may have pathogenic potential. MDP screening also resulted in three *Salmonella* spp. isolates from domestic leaf lettuce samples.

MDP is pursuing the use of new technologies to streamline laboratory procedures. DNA-based technologies were implemented for *Salmonella* spp. screening in October 2003. Methods are being evaluated to enhance recovery of bacteria and to specifically capture targeted organisms such as *E. coli* O157:H7. AMS is seeking to improve data collection systems and to use improved microbial detection methods that are quicker, more reliable, and more sensitive. As the program expands, procedures and methods are being modified and refined to provide information necessary for making science-based food safety decisions.

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

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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
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Texas Department of Agriculture
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Microbiological Data Program (MDP) Annual Summary, Calendar Year 2002

This summary consists of the following sections: (I.) Introduction, (II.) Sampling Operations, (III.) Laboratory Operations, (IV.) Database Management, (V.) 2002 Data Summary

I. Introduction

In 2001, Congress authorized funding for a microbiological monitoring program to establish a microbial baseline in the domestic food supply. This publication summarizes progress made toward implementation of MDP and provides an overview of data collected in 2002. The AMS Monitoring Programs Office (MPO) manages the 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.

The purpose of MDP is to collect information regarding the incidence and identification of targeted foodborne pathogens and indicator organisms on fresh fruits and vegetables. MDP is a federal-state partnership between AMS and 10 States (California, Colorado, Florida, Maryland, Michigan, New York, Ohio, Texas, Washington, and Wisconsin). MDP is a voluntary data gathering service, not a regulatory effort, and is part of the broader 1997 Presidential Food Safety Initiative.

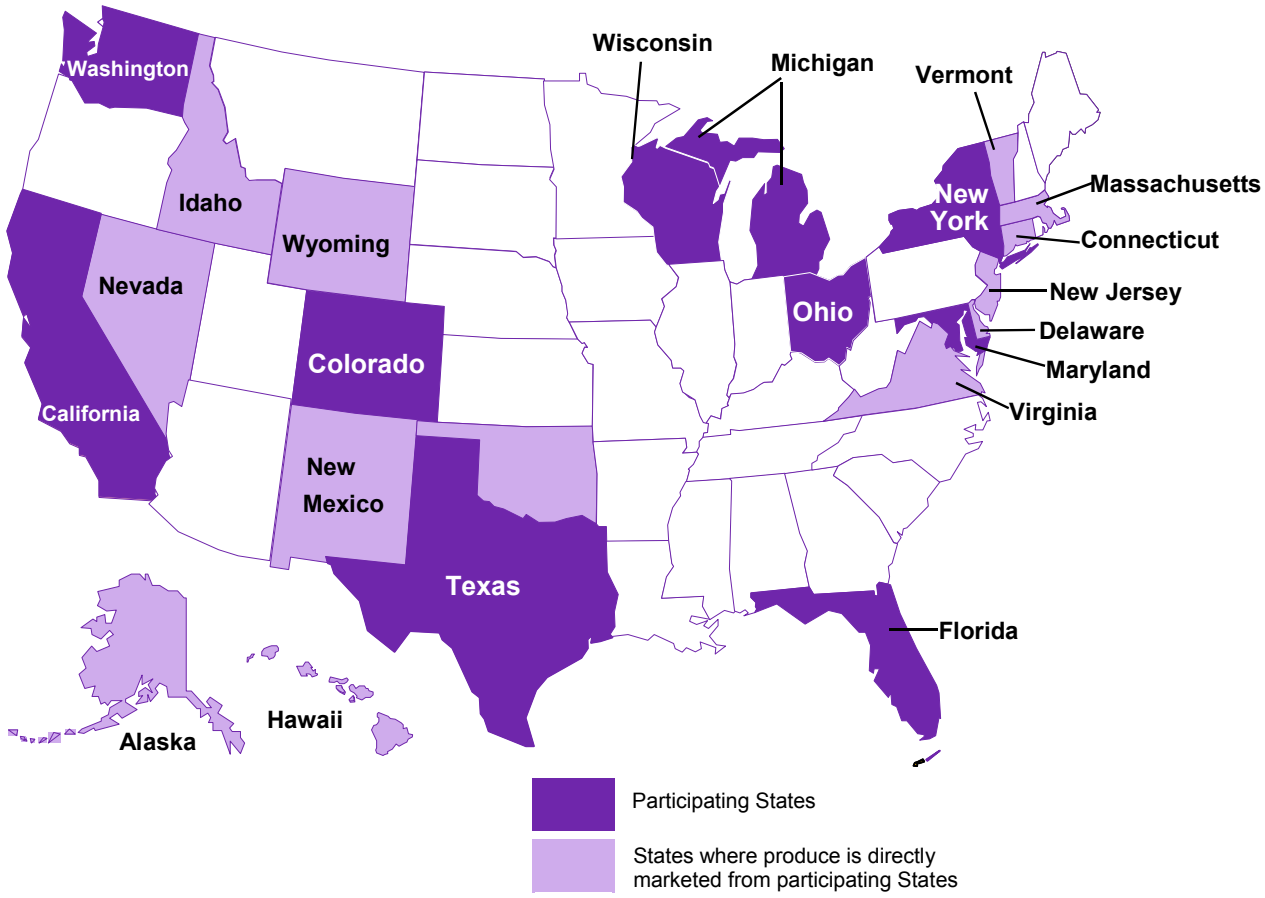
AMS coordinates its planning and policy requirements with the Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration (FDA). The USDA Agricultural Research Service (ARS) provides consultation as an independent research authority on laboratory methods. AMS and the USDA National 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 *E. coli* strains with pathogenic potential and *Salmonella* spp. Isolates of these organisms were sent to specialized laboratories for further characterization including serotyping, antibiotic resistance, and virulence attributes.

The three major MDP components are illustrated in Figure 2: sample collection, laboratory analysis, and database management. Samples are collected in the 10 participating States through cooperative agreements with their respective agencies (Figure 1). 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 approximately 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 are provided by eight States (California, Colorado, Florida, Michigan, New York, Ohio, Washington, and Wisconsin) and one Federal laboratory [AMS National Science Laboratory (NSL), Gastonia, NC]. Laboratory operations are designed to minimize variability of results across laboratories. 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 will be used to establish benchmarks for the incidence of target organisms at the wholesale level, understand trends, and improve risk communication. The information from MDP could help identify technology development priorities and risk modeling needs for fresh produce in the food chain. The MDP data also could supplement the FDA/USDA "Guidance for Industry—Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables."

Figure 1. Participating States and their Geographical Distribution Areas



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.

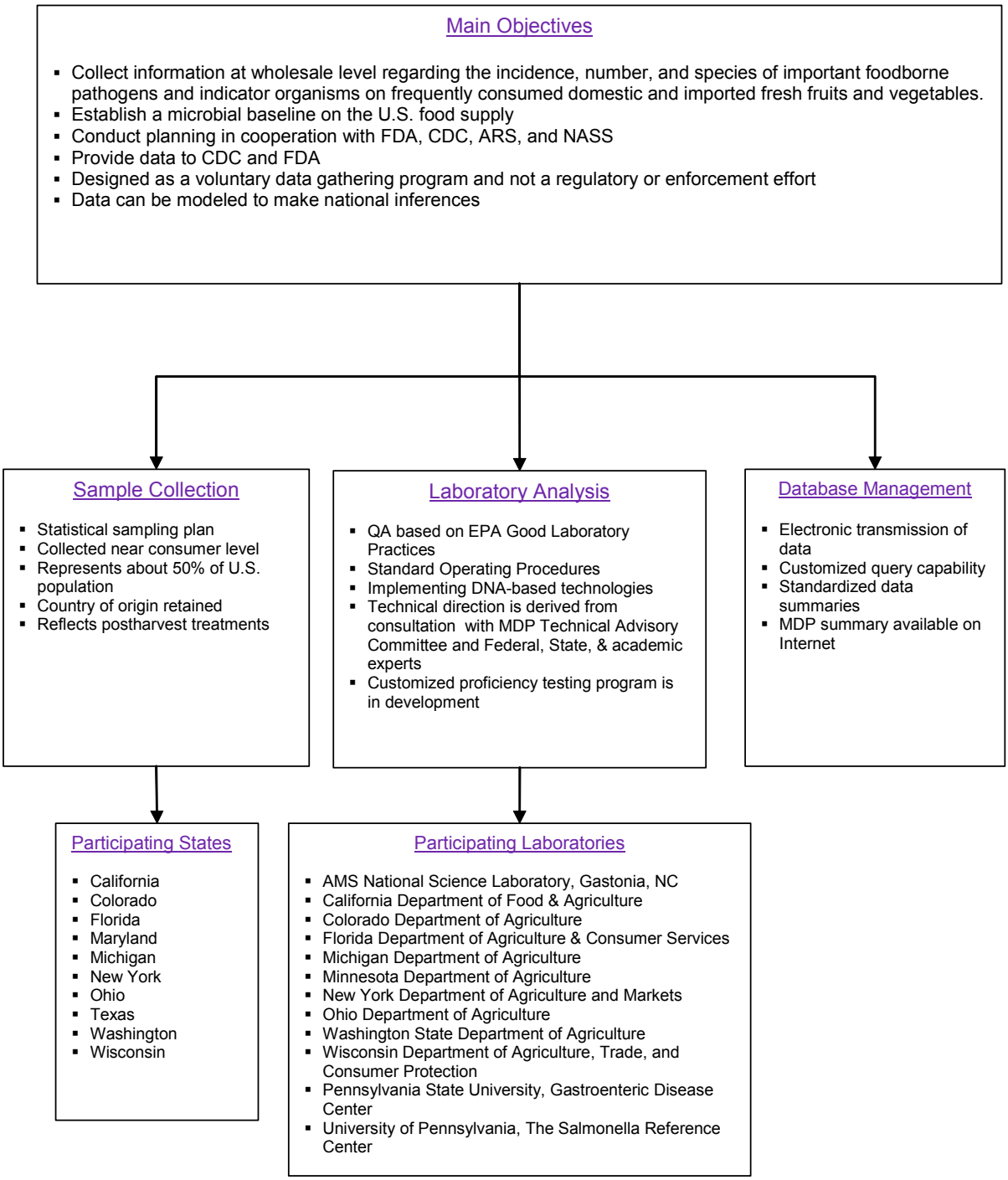
USDA is a member of the interagency Task Force on Antimicrobial Resistance established in 1999 to address antimicrobial resistance, which has been identified as a priority food safety and public health issue. As such, isolates from positive MDP samples are also being sent to ARS for testing and these data will be added to the National Antimicrobial Resistance Monitoring System (NARMS) 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 is seeking to improve data collection systems and to use improved microbial detection methods that are quicker, more reliable, and more sensitive. AMS is pursuing the use of deoxyribonucleic acid (DNA)-based detection technologies for the testing of fresh produce and is conducting validation studies at MDP laboratories.

II. Sampling

The goal of the MDP sampling program is to obtain a statistical representation of the United States 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

Figure 2. Overview of MDP Management and Operations



rationale was developed by AMS Science and Technology Programs 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. The data will therefore reflect differences in microbial concentrations on produce samples across varying conditions of production, transport, and storage. Sampling is apportioned according to population of the participating State, i.e., 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). In this manner, sites located near large populations have higher volumes and therefore are more likely to be selected than sites with smaller volume.

Collecting data over time from a range of sources permits statistical statements to be made about the distribution of microorganisms within the target population. The target population is all units of a commodity available 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 requires an assumption to be made about the relationship between the participating States and the United States as a whole. The inferential population is the entire amount of the commodity actually consumed by the U.S. public during the same time frame. Because the States that participate in MDP fully represent the inferential population, MDP is a comprehensive 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 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 the (1) State of sample collection, (2) collection date, (3) commodity code, and (4) testing laboratory code. Information about the country of origin of the sample is collected, as well as any production claims, such as organic and any postharvest treatments.

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. However, sampling at a retail grocery store 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., sterilized latex gloves and sample bags). To characterize conditions during shipping, samples are measured for surface temperature at the time of collection and on receipt at the laboratory.

MDP is collecting data on cantaloupes, celery, leaf lettuce, romaine lettuce, and tomatoes. These commodities are primarily harvested 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 cantaloupes 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 atmospheres (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 will reflect any handling practice or post-harvest treatments up to that point in the food distribution chain.

III. Laboratory Operations

Nine microbiology laboratories (eight State and one Federal) perform analyses for MDP. Specialized laboratories are used to perform additional identification and antimicrobial resistance testing: The Salmonella Reference Center (SRC), University of Pennsylvania (UPenn), and The Gastroenteric Disease Center, Pennsylvania State University (PSU).

The initial laboratory methods adopted were predominantly traditional microbiological techniques; these initial methods, used in obtaining the 2002 data, are described in Section V. As the data have been obtained and reviewed, modifications are being introduced to refine and improve the techniques used to produce these data.

MDP has been evaluating the use of DNA-based polymerase chain reaction (PCR) and instruments associated with this technology. This technology has been shown to adequately address concerns such as sample matrix interferences, low cell counts, and reaction inhibition due to enrichment media. In addition, other Federal agencies such as FDA and the USDA Food Safety and Inspection Service (FSIS) have approved and are currently using these instruments and methodologies for microbial screening programs. MDP has successfully completed validation studies and began using DNA-based automated instruments for the detection of *Salmonella* spp. in October 2003. Validation studies will also be conducted to determine the use of the instrument for screening of *E. coli* O157:H7 and other targeted pathogens.

Currently, samples are washed in a buffed saline solution and all analyses are conducted from this surface wash diluent. MDP is also investigating the use of newly developed wash buffers and growth media. Trial studies are being conducted by the Minnesota Department of Agriculture to evaluate the various options and the potential for introduction into MDP methodologies.

MDP is conducting method comparison studies to validate and implement a more rapid and effective screening method for *E. coli*. This new method, which has been tested by FDA, will be evaluated by MDP laboratories for recovery of *E. coli* strains of interest to MDP.

MDP methods will be reviewed and modified as necessary to enhance productivity and to provide data that will be useful for risk model development. As new microbial technologies are developed and become commercially available, they will be evaluated for use in the program. As with all methods modifications, all programmatic quality assurance/quality control (QA/QC) criteria must be met prior to implementation by MDP laboratories.

The main objectives of the QA/QC program are to ensure the reliability of MDP data and to ensure performance equivalency of participating

laboratories. Direction for the MDP QA program is provided through written SOPs based on FDA's Bacteriological Analytical Manual (BAM) methods, Association of Analytical Communities (AOAC)-certified methods, and the Environmental Protection Agency's (EPA) Good Laboratory Practices (GLPs). SOPs provide uniform administrative, sampling, and laboratory procedures.

Positive and negative controls and a sterile media blank are required for each sample set. MDP laboratories use positive control strains of *E. coli* and *Salmonella* spp. that carry a gene for Green Fluorescent Protein (GFP) encoded in a stable plasmid. 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 are taken through the entire analytical procedure. MDP laboratories use automated instrumentation for confirmation of isolates.

A Technical Advisory Committee (TAC), comprised of microbiologists from each participating laboratory, is responsible for revising program SOPs and addressing technical and QA issues. For day-to-day quality assurance over-sight, each participating facility is required to have a Quality Assurance Unit (QAU) that operates independently from the laboratory staff. Preliminary QA/QC review procedures are performed on-site by each laboratory's QAU. Final review procedures are performed by MDP staff who are responsible for collating and reviewing data for conformance with SOPs.

Laboratory performance is also monitored through on-site reviews by MDP staff to determine compliance with MDP SOPs. Corrective actions, if necessary, are performed as a result of on-site reviews. Performance equivalency of the participating laboratories is monitored by a program-wide proficiency testing program. MDP laboratories have participated in a check sample program administered through AOAC. MPO is working to develop a customized proficiency testing program.

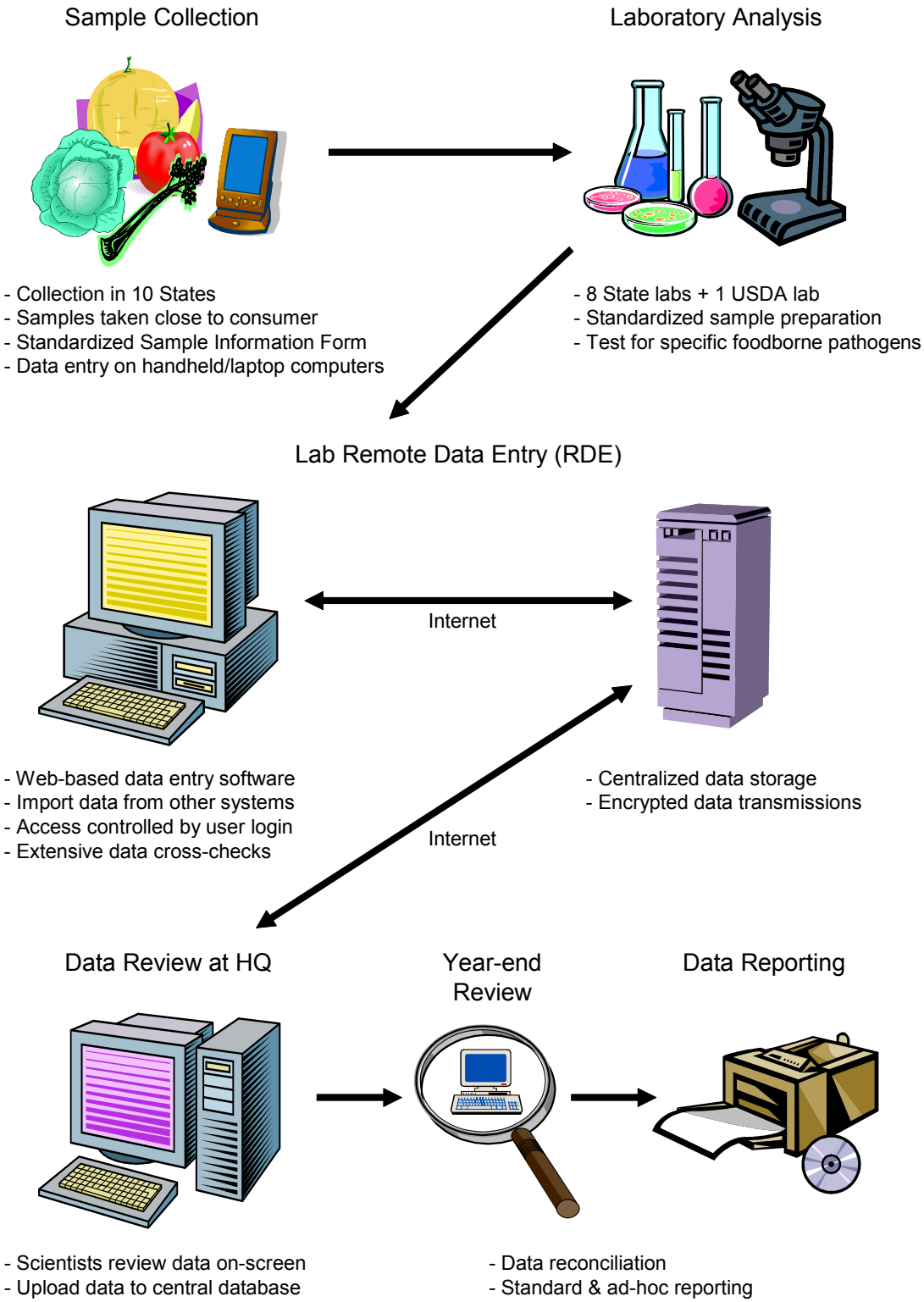
IV. Database Management

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 (SSL) technology to encrypt all data passed between users' computers and the central Web server. At MDP headquarters, the RDE system allows the staff scientists to review and approve the data for inclusion in the central database.

The RDE data entry screens have extensive edits and cross-checks built in to ensure that valid 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 ad-hoc queries (data searches) 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. MDP headquarters plans to migrate to the Microsoft SQL Server 2000 environment for more secure and robust database management.

Figure 3. Data Life Cycle



Access to the central MDP database is limited to MDP headquarters personnel and is controlled through password protection and user access rights. System back-ups are done each night and back-up tapes are sent to off-site storage once a week.

V. 2002 Data Summary

In 2002, the first full year of testing, MDP screened commodities for the presence of *E. coli* and *Salmonella* spp. Both organisms are of public health significance. *E. coli* has historically been used as an indicator of fecal contamination in food and water. *Salmonella* spp. are most often implicated in foodborne outbreaks involving produce (2). The 2002 data set represents 1 year of data and is insufficient to draw any inferences. Baseline studies require data generated over more than one growing season and representing several years.

Table 1 shows the number of samples collected by each State and tested for *E. coli* and *Salmonella* spp. Commodities in the program for 2002 included celery, leaf lettuce, romaine lettuce, and tomatoes; in July 2002, cantaloupe was added. These crops were selected for inclusion in the program because they are high consumption items in the U.S. diet and can be consumed raw.

Figure 4 shows the proportion of domestic and imported samples for all of the 10,317 samples analyzed. This figure also shows the portion of domestic and imported samples for each of the five commodities in the program: cantaloupe, celery, leaf lettuce, romaine lettuce, and tomatoes. No other information regarding the source of the samples is collected, as congressional language required the sampling to occur in a blind fashion.

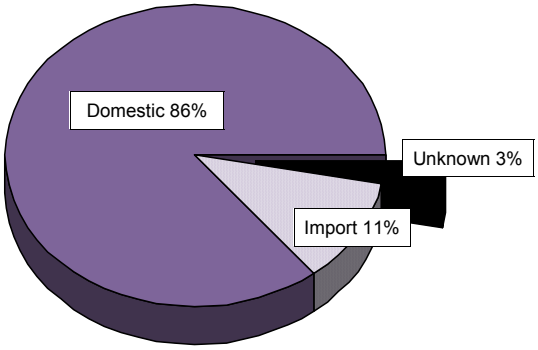
Samples from a site consist of three individual units generally collected from the same container. All samples in a State are collected on the same day or within a two-day interval. Inferences cannot reasonably be made from the sample units to the lots from which they originate, because the units do not provide

Table 1. Number of Samples Collected and Analyzed by State

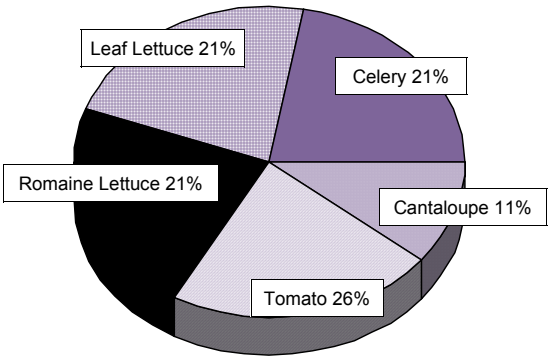
Collection State	Cantaloupe	Celery	Leaf Lettuce	Romaine Lettuce	Tomatoes	Total	Samples Analyzed for	
							E. coli	Salmonella
California	249	504	492	492	624	2,361	2,361	2,361
Colorado	36	72	72	72	90	342	342	342
Florida	126	250	249	254	291	1,170	1,134	1,170
Maryland	68	141	140	144	179	672	672	672
Michigan	107	216	213	213	268	1,017	1,015	1,017
New York	162	311	324	322	387	1,506	1,505	1,505
Ohio	96	202	212	213	252	975	975	975
Texas	129	273	273	261	349	1,285	1,285	1,285
Washington	72	138	139	141	179	669	667	668
Wisconsin	33	69	66	65	87	320	320	320
TOTAL	1,078	2,176	2,180	2,177	2,706	10,317	10,276	10,315

Figure 4. Commodity Origin and Distribution

Origin



Commodity Distribution



Commodity	Origin	No. of samples	Percent of Total
Cantaloupe	Domestic	961	89.1
	Import	72	6.7
	Unknown	45	4.2
		1,078	
Celery	Domestic	2,083	95.7
	Import	54	2.5
	Unknown	39	1.8
		2,176	
Lettuce, Leaf	Domestic	2,117	97.1
	Import	18	0.8
	Unknown	45	2.1
		2,180	
Lettuce, Romaine	Domestic	2,108	96.8
	Import	33	1.5
	Unknown	36	1.7
		2,177	
Tomatoes	Domestic	1,647	60.9
	Import	930	34.4
	Unknown	129	4.7
		2,706	
	TOTAL	**10,317**	

enough information to produce statistically reliable lot estimates. However, over time, statistical methods can be applied to make national inferences from the data.

Upon arrival at the testing facility, samples are visually examined for acceptability and discarded if determined to be damaged (decayed, extensively bruised, or spoiled). Samples are refrigerated until analysis begins. Laboratories are permitted to refrigerate commodities for up to 24 hours to allow for different sample arrival times from the various collection sites. Only excess soil is removed prior to testing.

With the inception of MDP in 2001, experts from State and Federal governments, industry, and academia were consulted in program development and implementation of methodologies. The samples were screened for *E. coli* using an FDA BAM method. If *E. coli* isolates were detected, additional screening for potentially pathogenic strains of *E. coli* was conducted. *E. coli* isolates were sent to PSU for further identification and characterization, including serotyping, antimicrobial resistance testing, and testing for virulence attributes.

Salmonella spp. isolates were identified using a fully automated enzyme-linked immunosorbent assay (ELISA) system. Isolates were then sent to the SRC at UPenn for further identification (serotyping) and to PSU for antimicrobial resistance testing. In addition, selected isolates of *E. coli* and *Salmonella* spp. were sent to ARS for antimicrobial resistance testing and inclusion into the NARMS database.

Identification of isolates was confirmed using either a conventional biochemical testing system or an AOAC-certified or MDP-accepted commercial biochemical kit or system. MDP analytical methods are published at http://www.ams.usda.gov/science/MPO/SOPs.htm

MDP utilized the legacy RDE system, which was a customized software application developed at MDP headquarters, to capture and report MDP data. The legacy RDE system provided interactive data entry screens to participating laboratories in a distributed, Windows-based application. The MDP results were electronically transmitted to MDP headquarters through an e-mail system.

In 2002, three domestic leaf lettuce samples were found to be positive for *Salmonella* spp. The numbers of each commodity analyzed for *Salmonella* spp. with subsequent characterization is shown in Table 2. Confirmed cultures were sent to the SRC at UPenn for serotyping; all three isolates were further identified as *Salmonella typhimurium* group B strains.

The data show increased *E. coli* detections in the spring and the fall. To determine pathogenic potential, PSU tested the isolates for the presence of 13 different virulence-related genes associated with 7 different categories of virulent *E. coli* (Table 3). The predominant virulence factors were Cytotoxic Necrotizing Factor 2 (CNF2) and Heat Stable Toxin a (STa), which are associated with cytotoxic necrotizing *E. coli* (NTEC) and enterotoxigenic *E. coli* (ETEC), respectively.

The numbers of each commodity analyzed for *E. coli* with subsequent characterization are shown in Table 4. The virulence factors identified by commodity are shown in Table 5. Virulence attributes were identified in 64 *E. coli* isolates. These isolates may have pathogenic potential but cannot be characterized as pathogenic to humans. The ability of a bacterial pathogen to cause a disease in humans requires a complex interplay of proteins encoded by numerous genes including a few genes from the host. PSU also classified the *E. coli* strains based on the somatic "O" type antigens using the ELISA method designed specifically for their identification.

The traditional microbiological methods used by MDP laboratories in 2002 were resource-intensive and less sensitive. DNA-based technologies offer rapid detection with greater sensitivity and specificity. Therefore, MDP is pursuing the use of new technologies to improve sensitivities and refine laboratory procedures.

Table 2. Summary of Sample Analysis for Salmonella

Commodity	Number of Samples Tested for *Salmonella*	Number of Positive Isolates	Percent of Samples Testing Positive
Cantaloupe	1,077	0	0.00
Celery	2,175	0	0.00
Leaf Lettuce	2,180	3	0.14
Romaine Lettuce	2,177	0	0.00
Tomato	2,706	0	0.00
TOTAL	10,315	3	0.03

Table 3. Virulence Attributes

	E. coli	**Attributes**
EHEC	Enterohemorrhagic	Shiga-toxins SLT-1 and SLT-2
	--	Hemolysin HlyA
	--	Invasive trait (Intimin-*eae*)
ETEC	Enterotoxigenic	Toxins (Heat stable –STa, STb; Heat Labile-LT
EPEC	Enteropathogenic	Invasive character (Intimin *eae*-α)
EAEC	Enteroaggregative	Gene associated with the virulent plasmid
NTEC	Necrotizing cytotoxic	Cytotoxic Necrotizing Factor (CNF-1 and 2)
EIEC	Enteroinvasive	*IpaH* gene known to be associated with EIEC
Others		K1 capsular antigen

Table 4. Summary of Sample Analysis for E. coli

Commodity	Number of Samples Tested for *E. coli*	Number of Isolates Testing Positive for Virulence Attributes*	Percent of Isolates Testing Positive for Virulence Attributes
Cantaloupe	1,077	2	0.19
Celery	2,174	3	0.14
Leaf Lettuce	2,161	27	1.25
Romaine Lettuce	2,158	29	1.34
Tomato	2,706	3	0.11
TOTAL	10,276	64	0.62

* The presence of virulence factors does not necessarily mean that these strains are pathogenic to humans, but may have pathogenic potential.

Table 5. Virulence Factors by Commodity

Virotype	Celery	Cantaloupe	Leaf Lettuce	Romaine Lettuce	Tomato	Virulent Attributes	Attribute Description
EHEC	-	1	8	7	1	SLT-1, SLT-2, HlyA, Intimin eae-gamma	Shiga-toxins
ETEC	-	-	6	2	1	Heat stable (Sta, STb), Heat Labile (LT)	Toxins
EPEC	1	-	2	3	-	EAggEC	Invasive Character
NTEC	1	1	9	12	-	CNF-1, CNF-2	Cytotoxic Necrotizing Factor
EIEC	-	-	1	-	-	IpaH	
Other	1	-	2	5	1	K1	Capsular antigen
	3	2	28	29	3		

Note: One isolate from leaf lettuce had more than 1 virulence attribute (EHEC and NTEC), resulting in 65 virulence factors from 64 isolates.

References

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