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Department of
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Science &
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TO: See Distribution List

FROM: Martha Lamont, Director
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SUBJECT: Microbiological Data Program Plan, February through June 2008

This Program Plan serves as the current Statement of Work for the period February 1, 2008 through June 30, 2008, for each State participating in the Microbiological Data Program (MDP). This document also stipulates work assignments for the Federal facility participating in MDP.

I. ADMINISTRATIVE UPDATES

- A. Program Status:** The 2008 funding for the program has been released. MDP sampling and testing activities resumed in February 2008.
- B. Personnel:** Program participants are reminded to keep MDP management informed of any critical equipment purchases, staffing issues, or expected increases in rent or sample turn-around-time (e.g., due to laboratory or office renovation/relocation). This information is required under the terms of MDP Cooperative Agreements (Section II, Responsibilities) between USDA and participating States.
- C. Sample Origin Information:** MDP began collecting sample origin information, effective February 1, 2008. Information collected includes grower, packer, distributor, country of origin, collection facility name, and lot number/product code.
- D. Data Reporting:** State and Federal program participants are reminded that routine sample results, including any in-house confirmations, must be reported within 60 days of receipt of the last sample of each batch. This requirement is reflected in Standard Operating Procedure (SOP) MDP-DATA-01, subsection 5.5.2. In response to requirements of the Office of Management and Budget (OMB) Program Assessment Rating Tool (PART), MPO will issue letters of warning to laboratories with any backlog exceeding 60 days, unless the laboratory has provided the required notification to MPO.
- E. Summary Status:** The 2007 MDP Progress Update and Data Summary will be released via the Website in March 2008. The 2004, 2005 and 2006 MDP Progress Update and Data Summaries are also available on the MDP website at <http://www.ams.usda.gov/science/MPO/Download.htm>.
- F. Financial/Cooperative Agreements:** MDP Cooperative Agreements for FY 2008 were issued February 6, 2008.

G. MDP Program Meetings: A Technical Meeting was held January 29-30, 2008 in New Orleans, Louisiana. Attendees included MDP Technical Program Managers (TPMs) and Quality Assurance Officers (QAOs) from all MDP participating States and staff from the Monitoring Programs Office. Program planning, technical, and quality assurance (QA) issues were addressed and are reflected in the Program Plan.

H. Electronic Transfer of Data:

RDE Version Upgrades: A version upgrade to the Web-based RDE system was installed in May 2007 to add some required functionality, to increase the maximum size of the user password, and to automatically prompt for a password change when the system default password is entered. With the next version upgrade, tentatively scheduled for April 2008, MPO plans to make password management more secure, increase the size of some text fields, and fix problems reported by laboratory users. A version upgrade to the RDE e-SIF system for laptops/palmtops was distributed in December 2007 that included a new feature that allows users to dynamically activate/add commodities and sites on their look-up tables. This revision will alleviate the problem where sample collectors did not have an updated commodity look-up table loaded and had to submit paper SIFs for the new commodity. MPO maintains a Change Request Database to capture all problems identified and suggestions made regarding the RDE system.

II. PROGRAM SAMPLING AND TESTING UPDATES

A. Sampling: Shipping Charts are distributed quarterly to Sampling Managers by MPO. The shipping chart covering the period February 1 through March 31, 2008 and the draft shipping chart covering the period April 1 through June 30, 2008 are attached to this document. Green onions are replaced with spinach (loose, bunched, or bagged). Alfalfa sprouts, cantaloupe, bagged lettuce, and tomatoes will continue. Samples collected in Maryland will be sent to the Ohio laboratory (OH4) and those collected in Texas will be shipped to NSL (US4). Samples collected in California will be sent to the following MDP laboratories: alfalfa sprouts and bagged lettuce to Ohio (OH4), spinach to Colorado (CO4), and cantaloupes and tomatoes to NSL (US4). Samples from all other States will be sent to the laboratory for that collection State.

B. Testing: Green onions are replaced with spinach (loose, bunched, or bagged). Alfalfa sprouts, cantaloupes, bagged lettuce, and tomatoes will continue. The Ohio laboratory (OH4) will analyze all samples collected by Ohio and Maryland as well as alfalfa sprouts and bagged lettuce collected by California. NSL (US4) will analyze all samples collected by Texas and cantaloupe and tomato samples collected by California. Colorado will analyze all samples collected by Colorado and spinach collected by California. All other States will analyze samples collected in that State.

Testing Changes: Some testing changes will be introduced based on special studies/validations performed during 2007 and discussions during the January 2008 Technical Meeting. These changes include:

- (1) All laboratories will perform a method tryout for the addition of spinach to the program. The method tryout will be performed prior to sample analysis and will be based on the protocol provided.

- (2) All laboratories will capture total viable count (TVC) for bagged lettuce and bagged spinach. A new Organism/Test code of “TVC” has been activated in RDE so that a TVC result can be reported for bagged lettuce and bagged spinach samples only. Information detailing this change has been sent to all laboratories. Laboratories will use the TEMPO[®] system to determine TVC. This method is specified in SOP MDP-MTH-01C [Enumeration of Total Viable Count (TVC) in Produce Samples by the TEMPO[®] TVC System, Original Version, 03/01/08]. The laboratories will perform method validation prior to sample analysis based on the protocol provided.
- (3) All laboratories will begin testing *Shigella* following method validation according to the protocol provided. Extracted DNA and an aliquot of each overnight-enriched culture sample will be held frozen at -20°C and -70° C, respectively, until method validation has been completed and testing is ready to begin. Testing will be performed according to SOP MDP-MTH-08 [Detection of *Shigella* spp. in Fresh Produce by Realtime Polymerase Chain Reaction (rtPCR) and Cultural Isolation and Identification, Original Version, 03/01/08].

Target Microorganisms:

- (1) *Escherichia coli* (*E. coli*): MDP laboratories will continue to test all samples for *E. coli* using the TEMPO[®] system. Method procedures are detailed in SOP MDP-MTH-01A [Enumeration of *Escherichia coli* in produce samples by TEMPO[®] EC (*E. coli*) Method, Original Version, 05/01/07].
- (2) Coliform Count: All laboratories will capture total coliform bacteria for bagged lettuce and bagged spinach using the TEMPO[®] system. This method is specified in SOP MDP-MTH-01B [Enumeration of Coliform Bacteria in Produce Samples by TEMPO[®] CC (Coliform Count) System, Original Version, 05/01/07].
- (3) TVC Count: All laboratories will capture TVC data for bagged lettuce and bagged spinach using the TEMPO[®] system. This method is specified in SOP MDP-MTH-01C [Enumeration of Total Viable Count (TVC) in Produce Samples by the TEMPO[®] TVC System, Original Version, 03/01/08].
- (4) Pathogenic *E. coli*: All laboratories will continue to screen all samples for pathogenic *E. coli* according to SOP MDP-MTH-07 [Detection of Pathogenic *E. coli* in Fresh Produce by Multiplex PCR (mPCR) and Cultural Isolation and Identification, Revision 3, 05/01/07]. The mPCR assay tests for two types of pathogenic *E. coli*: (a) shiga toxin-producing *E. coli* (STEC) that carry genes coding for shiga toxins (Stx) 1 and 2 and (b) enterotoxigenic *E. coli* (ETEC) that carry genes coding for enterotoxins, heat labile (LT), and heat stable (ST) toxins.
- (5) *Salmonella*: MDP laboratories will continue to screen all samples for *Salmonella* (presence or absence) by BAX[®]. Method procedures are detailed in SOP MDP-MTH-04 (Detection of *Salmonella* in Fresh Produce by BAX[®] PCR, Revision 2, 01/01/06). Presumptive positive samples are subjected to enrichment and isolation as described in SOP MDP-MTH-03A (Isolation and Identification of *Salmonella* from Fresh Produce using Cultural Methods, Revision 1, 01/01/06).
- (6) *E. coli* O157:H7: MDP laboratories will continue to screen all samples for *E. coli* O157:H7 (presence or absence) by BAX[®]. Method procedures are detailed in

SOP MDP-MTH-05 (Detection of *Escherichia coli* O157:H7 in Fresh Produce by BAX[®] PCR, Revision 2, 01/01/06). Presumptive positive samples are subjected to IMS procedures and cultural confirmation, as described in SOP MDP-MTH-06 (*Escherichia coli* O157:H7 in Fresh Produce by BAX[®] System, Revision 3, 05/01/07).

- (7) *Shigella*: All laboratories will begin screening all samples for *Shigella* using realtime PCR. Method validation will be performed according to an established protocol prior to routine sample testing. Extracted DNA and an aliquot of each overnight-enriched culture sample will be held frozen at -20°C and -70° C, respectively, until method validation has been completed and testing is ready to begin. Method procedures are detailed in SOP MDP-MTH-08 [Detection of *Shigella* spp. in Fresh Produce by Realtime Polymerase Chain Reaction (rtPCR) and Cultural Isolation and Identification, Original Version, 03/01/08].

C. Quality Assurance:

Proficiency Testing Program: The next proficiency testing (PT) round will be introduced in July/August 2008. The test organism will be unknown to the laboratories. Each laboratory should perform all MDP tests on the samples provided.

SOPs: The following new and revised SOPs will be issued February/March 2008, and reflect the changes noted under Section (B), Testing, of this document:

- SOP MDP-LABOP-02: Sample Receipt, Elution, Preenrichment and DNA Extraction, Rv. 09, 02/11/08.
- SOP MDP-DATA-01: Data Entry, Record Keeping and Results Reporting, Rv. 04, 03/01/08.
- SOP MDP-MTH-01C: Enumeration of Total Viable Count (TVC) in Produce Samples by the TEMPO[®] TVC System, Original Version, 03/01/08
- SOP MDP-MTH-08: Detection of *Shigella* spp. in Fresh Produce by Realtime Polymerase Chain Reaction (rtPCR) and Cultural Isolation and Identification, Original Version, 03/01/08.
- SOP MDP-QA-02: Proficiency Test Samples, Rv. 02, 03/01/08.
- SOP MDP-QA-03: Quality Assurance (QA) Controls, Rv. 04, 03/01/08.

SOPs are posted to the MDP website at the time of distribution to program participants. Refer to: <http://www.ams.usda.gov/science/MPO/SOPs.htm>.

D. Archiving and Additional Testing:

Archival of Isolates: NSL (US4) serves as a centralized location for archival of isolates as well as a distribution center for isolates from MDP testing laboratories to the reference laboratories.

Additional Testing by Reference Laboratories: All target organisms are frozen in Microbank[™] vials and shipped to NSL (US4). Vials are shipped by NSL (US4) to the FDA/Center for Veterinary Medicine (CVM) laboratory in Laurel, Maryland for antimicrobial resistance testing for inclusion in the National Antimicrobial Resistance

Monitoring System (NARMS) and pulsed-field gel electrophoresis (PFGE) analysis for inclusion in PulseNet. *Salmonella* and *E. coli* O157 isolates are also serotyped by FDA/CVM. Pathogenic *E. coli* isolates are shipped by NSL (US4) to Pennsylvania State University (PSU) for serotyping and testing for additional virulence attributes. *Shigella* isolates will be sent to FDA/CVM for antimicrobial resistance testing and PFGE and to PSU for serotyping and virulence attributes.

- E. Transfer of Data:** AMS transfers data to the Centers for Disease Control and Prevention (CDC) and FDA on a semi-annual basis. In addition, MDP data is given to USDA's Food Safety and Inspection Service (FSIS) and Agricultural Research Service (ARS).