United States Department of Agriculture
Agricultural Marketing Service, Science & Technology
Microbiological Data Program

SOP No.: MDP-LABOP-02		Page 1 of 15
Title: Sample Receipt, Elution, Pre-enrichment, and DNA Extraction		
Revision: 18	Replaces: 07/01/2010	Effective: 9/1/2011

1. Purpose

To provide standard procedures for the USDA, AMS, Microbiological Data Program (MDP) on the receipt, sample preparation, pre-enrichment, and post-enrichment extraction of commodities currently analyzed for the program.

2. Scope

This Standard Operating Procedure (SOP) shall be followed by all laboratories conducting microbiological studies for MDP, including support laboratories conducting non-routine activities that may impact the program. This SOP represents minimum MDP requirements and is presented as a general guideline. Each laboratory shall have written procedures that provide specific details concerning how the procedure has been implemented in that laboratory.

3. Safety

Laboratory personnel should utilize Biosafety Level II (BSL-2) practices for MDP manipulations of known and potential pathogens. A BSL-2 laminar flow biosafety cabinet is recommended for activities with potential for producing aerosols of pathogens. Material Safety Data Sheets (MSDS) should be obtained from manufacturers for media, chemicals and reagents used in the analysis and personnel who will handle the materials should know the location of and have ready access to the MSDS sheets for reference.

4. Outline of Procedures

5. References

- Alfalfa Sprouts Sample Processing Evaluation and Investigation of Hypothesized Sprout Interference on the Modified *E. coli* MPN Method, Final study report, DCLS, Department of General Services, Commonwealth of Virginia. October 2005.

- Bacteriological Analytical Manual (BAM) Online, 8th edition. 2001. US FDA/CFSAN, http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm (last accessed 3/11)

- Evaluation of Enrichment Ability of UPB for *Salmonella* ser. Typhimurium and *E. coli* O157:H7 from Produce Commodities, Final study report, DCLS, Department of General Services, Commonwealth of Virginia. October 2005.

- Evaluation of UPB as a Wash Buffer for Produce Commodities, Final study report, Division of Consolidated Laboratory Services (DCLS), Department of General Services, Commonwealth of Virginia. October 2005.

- Hammack, T. S., Johnson, M. L., Jacobson, A. P. and W. H. Andrews. 2006. Effect of sample preparation and pre-enrichment media on the recovery of *Salmonella* from cantaloupes, mangoes, and tomatoes. J. AOAC International. 89: 180-184.

- U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health: Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition. http://www.cdc.gov/biosafety/ (last accessed 3/11)

- USDA Microbiological Data Program (MDP) 2010 Multi-Laboratory Method Verification Study: Realtime PCR assays for detecting shiga toxin DNA sequences of *E. coli* (STEC) O157:H7 and/or non-O157 serotypes. May 2010.

- SOP MDP-MTH-04, Detection of *Salmonella* in Fresh Produce by BAX® PCR

- SOP MDP-MTH-12, Detection of *Escherichia coli* O157:H7 in Fresh Produce by Real Time BAX® O157 PCR

- SOP MDP-MTH-09, Detection of *Salmonella* using VIDAS® Method

- SOP MDP-SAMP-PROC-2, MDP Sampling Procedures on Site

- SOP MDP-SAMP-PROC-3, Packing and Shipment of MDP Samples
- SOP MDP-QA-03, Quality Assurance (QA) Controls
- US Food and Drug Administration (FDA), Safer Processing of Sprouts. A Food Safety Training Program Developed by the California Department of Health Services, Food and Drug Branch and the US FDA, 2003.

6. Specific Procedures:

6.1 Equipment and Materials

- Balance, capable of weighing up to 3000g (± 1g)
- Stomacher, Seward or equivalent
- Plastic bags, sterile, suitable size and durability to hold sample and eluent (e.g., sterile 3500 stomacher bags)
- Stomacher bags with filter inlay (WhirlPak or equivalent), sterile
- Stomacher bags without filter, sterile
- Forceps, tongs, slotted spoons, tongue depressors, sterile
- Gloves, sterile
- Incubators, 35 ± 2ºC and 42 ± 2^{o}C
- Sterile blender jars with blender, optional
- Sterile containers suitable for containing 30mL of liquid
- Sterile serological pipettes
- Promega Maxwell® 16 DNA Purification System

6.2 Media and Reagents

- Universal Preenrichment Broth, Difco Catalog #223510 or equivalent. Follow manufacturer's instructions for preparation.
- Promega's Maxwell 16® DNA Purification System Promega's Maxwell 16® Cell DNA Purification Kit

6.3 Receipt of Samples and Chain of Custody Requirements (also refer to Attachment 1, In-Laboratory Sampling Guidance)

6.3.1 The laboratory may receive up to two (2) sets of three samples per site, resulting in a possible delivery of six samples per site to the laboratory. Alternatively, the laboratory may, on occasion, receive institutional size sample bag(s) instead of three sample bags per site of certain produce (CL, CT, LT, SP and SR). Upon receipt of an institutional size sample bag(s) (for example: ≥1 lb for SR and ≥1.5 lbs for CL and ≥ 2.5 lbs for LT and SP and large clamshells >3 dry US pints), the laboratory can split it into (3) testing samples. Alternatively, the laboratory may receive the entire case (box/flat/carton) of produce (please refer to Attachment 1, this SOP). Upon receipt of an entire case, the laboratory shall aseptically take three (3) samples per sample site for testing per applicable commodity appendix.

6.3.2 Upon receipt, inspect samples for acceptability (e.g., not spoiled, rotten, cold-damaged or crushed) before proceeding to analysis. If possible, re-sampling should occur within the same month as the initial collection. Occasionally, samples may be "made-up" the following month; however, approval from the MP Sampling Manager or designee and the receiving laboratory is first required.

6.3.3 The Sampling Manager or designee shall be notified if adequate sample sizes were not collected. If less than required sample weight is received, a re-sample may be scheduled or the laboratory has the option of analyzing the smaller sample, provided the volume of eluant added is adjusted to maintain the appropriate weight/volume ratio.

6.3.4 Each laboratory shall maintain a log of samples received which contains, at a minimum, the time and date of receipt, receiver's initials, MDP sample identification number, a unique laboratory identification number, whether the sample was analyzed, and reason if not analyzed. In lieu of a log, a compilation of printed SIFs may be maintained containing the specified information. If SIFs are not available at the time of analysis the laboratory shall ensure that the written log is complete and shall update the sample receipt information in the RDE system when the electronic SIF becomes available. For sample labeling requirements refer to SOP MDP-SAMP-PROC-02.

6.3.5 The receiving laboratory shall notify the Collection State Sampling Manager and MP if any samples or e-SIFs are missing.

6.3.6 Refrigerate all produce samples until analysis begins. Perform the analysis as soon as realistically possible but no more than 48 hours after receipt in the laboratory and

not to exceed 72 hours from time of collection. Samples must be in good condition for analysis to proceed.

6.3.7 Non-perishable samples do not need to be refrigerated prior to analysis. Samples, including any left-over samples reserved for follow-up tests, should be stored in such a manner as to maintain sample integrity (i.e. avoid exposure to extreme heat or cold). Perform the analysis as soon as realistically possible after receipt in the laboratory.

6.3.8 Until the completion of tests required for reporting positive results, retain samples' empty containers, bags, boxes, cartons, tags, twist ties, bands, etc.

6.4 **Controls -** Refer to SOP MDP-QA-03 for list of control strains of specific strains used in the analyses and specific method SOPs for control set-up. Incubate and analyze all controls listed below alongside samples. Control strains shall be maintained and passaged separately and inoculated according to internal laboratory procedures. The laboratory should determine the level of inoculum required for setting up a control culture that will give a positive result for the type of the assay to be performed. Growth yield of bacterial cultures depends on the age and type of control strains, media (rich vs. minimal media) and growth conditions (shaking vs. standing, temperature of incubation, etc.) used.

6.4.1. Positive and negative cultural control strains grown overnight in appropriate media.

6.4.2. Positive and negative cultural (media) controls: Inoculate UPB broth with control strains (dilute the control strains to appropriate levels and then inoculate the UPB broth).

6.4.3. Uninoculated media control

6.4.4. Positive produce controls – see applicable commodity Appendix

6.5 **Appendices List –** To proceed with additional sample processing steps**,** utilize applicable commodity-specific appendices listed below:

Appendix A – Cantaloupe (CN)
Appendix B – Cilantro (CL)
Appendix C – Hot Pepper (HP)
Appendix D – Lettuce (LT)
Appendix E – Spinach (SP)
Appendix F – Sprouts (SR)
Appendix G – Tomatoes (CT or TR)

Note: Because commodities are periodically deleted from/added to the Program, additional appendices may be published between revisions of this SOP. See Index of Active SOPs for all applicable appendices currently in effect.

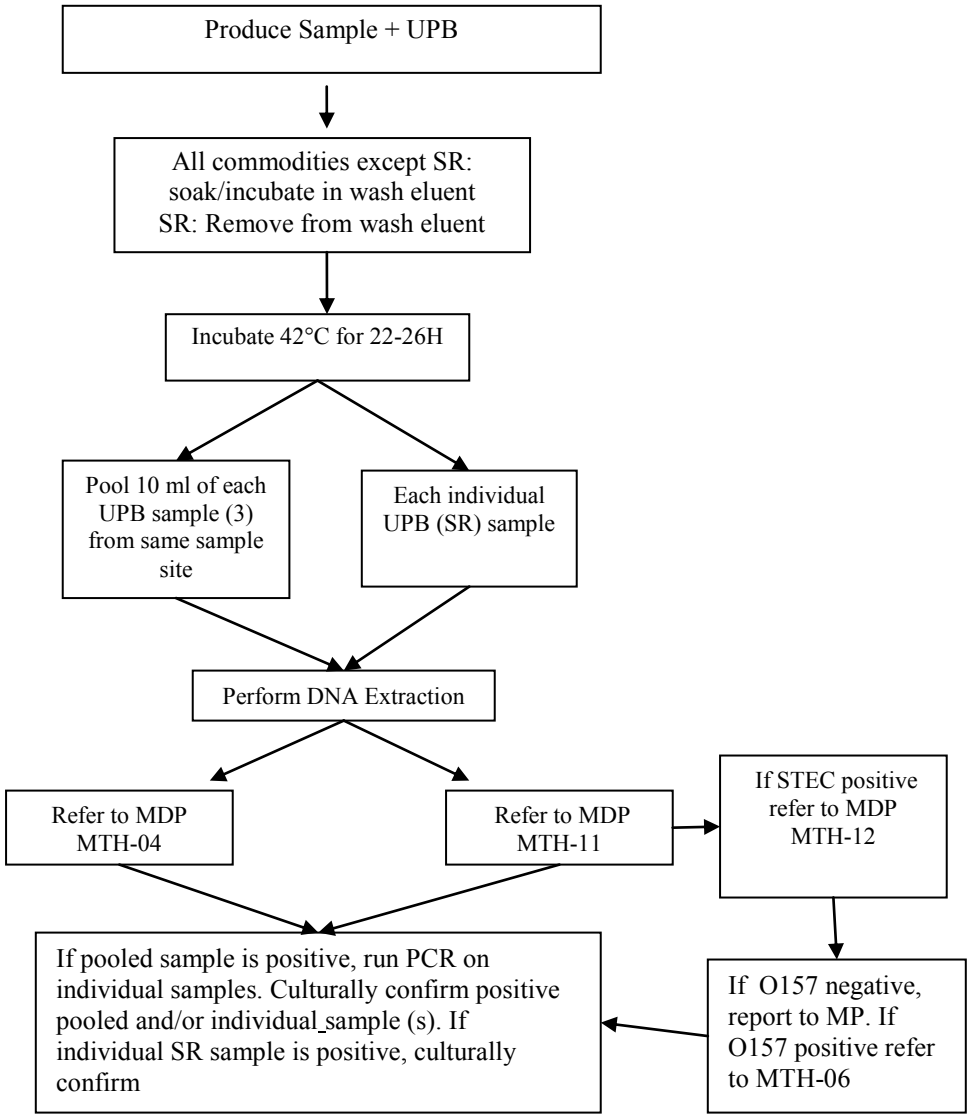
6.6 **DNA Extraction** - Extract and purify DNA from the UPB enriched pooled and/or individual samples according to the manufacturer's instructions for the Maxwell® 16 Cell DNA Purification Kit with the following exception: Use 400µL sample volume. Use 300uL elution buffer.

In case of Maxwell® 16 equipment failure, contact MPD for alternative DNA extraction instructions.

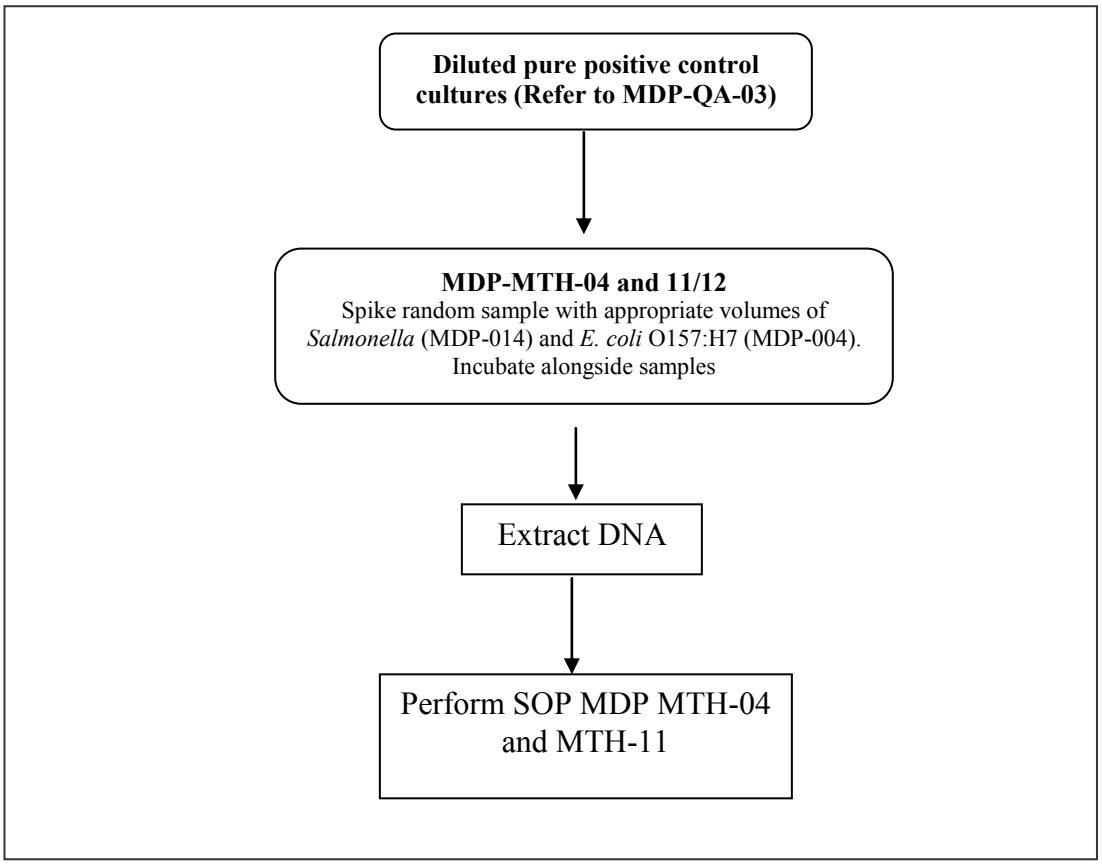
6.7 **DNA Storage** – Store the extracted DNA at 2-8^{o}C, not to exceed 48 hours, for short term storage or at -20 ± 5^{o}C for a maximum of 6 months and then at -75 ±10^{o}C for long term storage.

6.8 Refrigerate all samples and controls until all analyses are completed. If pursuing a positive isolation, maintain all sample-associated materials (washes, containers, tag, bands, ties, etc.) or clear photographs of everything, until receiving release notification from MPD.

Sample Setup (Fresh Produce Only) Flowchart 6.6.1



Positive (Produce Controls Flowchart 6.6.2 (Fresh Produce Only)



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ELECTRONICALLY REPRODUCED SIGNATURES - ORIGINAL SIGNATURE PAGE MAINTAINED BY USDA, AMS, S&T, MONITORING PROGRAMS OFFICE

Revision 18 June 2011 Monitoring Programs Division

- Added words to Title, Section 6.3, "(also refer to Attachment 1, In-Laboratory Sampling Guidance)"
- Replaced >3 lbs with >2.5 lbs, Section 6.3.1
- Added Attachment 1, In-Laboratory Sampling Guidance
- Revised Appendix D
- Revised Appendix E
- Revised Appendix G

Revision 17 August 2010 Monitoring Programs Office

- Section 6.4, deleted "Target concentration of 100-10000 cfu/ml should be used." **and** "Final concentration of inoculated control wash should be greater than minimum detectable limit of assay equipment being utilized." **and replaced with** "The laboratory should determine the level of inoculum required for setting up a control culture that will give a positive result for the type of assay to be performed. Growth yield of bacterial cultures depends on the age and type of control strains and media (rich vs. minimal media) and growth conditions (shaking vs. standing, temperature of incubation, etc.) used."
- Section 6.4.1 revised to read as "Positive and negative cultural control strains grown in appropriate media."
- Section 6.4.2 revised to read as "Positive and negative cultural (media) controls: Inoculated UPB broth with control strains (dilute the control strains to appropriate levels and then inoculate the UPB broth)."
- Removed "1ml each" from Flowchart 6.6.2 and replace with "appropriate volumes"

Revision 16 June 2010 Monitoring Programs Office

- Storage temperature and time changed- section 6.7
- Reformatted and renumbered
- Added Safety, new Section 3
- Updated References, Section 5
- Deleted "Controls", old Section 5.3
- Deleted "Elution Method", old Section 5.5
- Deleted "Adding Eluent", old Section 5.6
- Deleted "Wash", old Section 5.7

- Deleted "Pre-enrichment", old Section 5.8
- Deleted "Post-enrichment for SLM analysis", old Section 5.9
- Deleted "Post-enrichment for ECO157:H7 for LT, SP, CL and GO", old Section 5.10
- Deleted "Post-enrichment for ECO157:H7 for SR", old Section 5.11
- Deleted "DNA Extraction", old Section 5.12
- Added words "see applicable commodity Appendix" to new Section 6.4.4
- Added "Appendices List", new Section 6.5
- Revised "box contents" directly below "Perform DNA Extraction box" in Flowchart 6.5.1 **from** "Perform BAX PCR for *Salmonella* (all commodities). Perform *E. coli* O157:H7 (LT, SP, CL, and SR only). Perform mPCR for ETEC/STEC (all commodities)." **to read as** "Using DNA extracts of UPB enriched samples, perform BAX® *Salmonella* PCR and ETEC/STEC mPCR (all commodities). Using DNA extracts of mEC enriched samples, perform BAX® *E. coli* O157:H7 PCR (LT, SP, CL, and SR only)."
- Corrected Revision History 5
- Included Revision Histories 4 to Original

Revision 15 December 2009 Monitoring Programs Office

- Added Outline of Procedures, Section 3
- Added word "/clover" where "alfalfa" was present in SOP
- Alphabetized and removed numbering from References,Section 4
- Deleted "Lactose broth, follow manufacturer's instructions for preparation.", Section 5.2
- Moved "Positive culture control strains…" from Section 5.2 to Section 5.3.1
- Removed green onions, Section 5.3.3.1
- Removed peanut butter, Section 5.3.3.8
- Removed "GO" from Section 5.4.1
- Added words "cold damaged", Section 5.4.2
- Moved "Remove ties or rubber bands…." from Section 5.5.1 to Section 5.5
- Added sample weight "~50g" to Section 5.5.1
- Removed green onions, Section 5.5.6
- Removed "Remove ties or rubber bands…" from new Section 5.5.6
- Removed words "or leaves" from Section 5.5.7

- Moved sentences around; no content change, Section 5.5.8
- Removed peanut butter, Section 5.5.10
- Moved sentences to and removed words "green onions", Section 5.6
- Removed peanut butter, Section 5.6.1
- Condensed elution procedures, Section 5.7
- Added "Tomatoes (Roma and round): Prior to addition of UPB and incubation, thoroughly disrupt each tomato's outer skin.", Section 5.7.1
- Deleted Sections 5.8.1 through 5.8.3 and replaced with "For all produce **except sprouts**: Leave the produce in the wash eluent in the bag. Close the bag and incubate at 35 ± 2ºC for 22–26 hours for pre-enrichment ", Section 5.8
- Added words "is optional and can" to Section 5.9.1
- Removed words "green onions", Section 5.10
- Added words "…individual samples (sprouts)…", Sections 5.12.1 and 5.12.2
- Added "or disrupt tomato skins prior to adding UPB" in first block of Flowchart 5.13.1
- Corrected mEC+n incubation times to read "22-24 H", removed "SR" from far left block and removed "GO" from Flowchart 5.13.1

Revision 14 September 2009 Monitoring Programs Office

- Updated References, Section 3
- Revised Sections 4.1 through 4.13
- Revised Sample Setup Flowchart
- Revised Positive Produce Control Flowchart

Revision 13 March 2009 Monitoring Programs Office

- UPDATED REFERENCES
- UPDATED SPECIFIC PROCEDURES (5.1.3.)
- UPDATED MEDIA AND REAGENTS (5.2.4.)
- UPDATED CONTROLS (5.3.1.3.6.)
- UPDATED RECEIPT OF SAMPLES… (5.4.1.)
- UPDATED ELUTION METHOD (5.5.1.8, 5.5.2.2.2., 5.5.3.3.)
- UPDATED PRE-ENRICHMENT (5.6.4.)
- UPDATED POST-ENRICHMENT (5.7.4., 5.7.5.)

- UPDATED DNA EXTRACTION (5.8.4.)
- UPDATED FLOWCHARTS

Revision 12 February 2009 Monitoring Programs Office

- Deleted the word "produce" and replaced it with "commodity" in 5.3.1
- Added a peanut butter matrix control to this SOP
- Removed restrictive language from 5.4.2.8 and added a procedure for samples containing a top oil layer.

Revision 11 February 2009 Monitoring Programs Office

- Added 4.8 to the Reference Section
- Added peanut butter as a commodity for testing according to this SOP
- Revised 5.3.2

Revision 10 January 2009 Monitoring Programs Office

- Removed requirements for measuring sample temperatures
- Removed testing provisions for TVC, CC and *Shigella*

Revision 09 February 2008 Monitoring Programs Office

- Added spinach as a commodity for testing according to this SOP

Revision 08 April 2007 Monitoring Programs Office

- Removed requirement for checking sample origin information

Revision 07 April 2007 Monitoring Programs Office

- Introduced pre-cut bagged lettuce replacing whole leaf or romaine lettuce
- Analysts to inspect the sample origin information and report if missing
- Introduced addition of 0.1% Tween 80 to the Universal Preenrichment Broth (UPB) as wash
- Removed mechanical shaking for washing produce samples
- Introduced vigorous up and down and side by side manual shaking for all commodities
- Cantaloupe, lettuce, and tomato samples would be soaked
- Washed samples of green onions and stomached samples of alfalfa sprouts would be removed prior to overnight incubation

- All samples would be screened for STEC and ETEC by mPCR
- Introduced adding back addition volumes of sterile UPBt to washed samples after removing aliquot for *E. coli* MPN and/or TEMPO methods
- Revised the 5.7 flowcharts to reflect changes in the SOP

Revision 06 May 2006 Monitoring Programs Office

- Added chain of custody requirements to receipt of samples
- Increased allowable refrigeration time from 24 to 48 hours after receipt in laboratory

Revision 05 January 2006 Monitoring Programs Office

- Introduced sprouts as new commodity
- Replaced wash eluate (BPW + 0.1% Tween) with Universal Preenrichment Broth
- Replaced various preenrichment broths in subsequent SOPs with UPB as a single broth
- Added DNA extraction step
- For positive produce control, combined all positive strains into one preenriched UPB sample

Revision 04 July 2004 Monitoring Programs Office

- Updated references
- Introduced new commodities
- Introduced manual followed by mechanical shaking

Revision 03 September 2003 Monitoring Programs Office

- Updated references
- Changed wash buffer from 1.0% Tween 80 in Butterfield's phosphate buffer to buffered peptone water with 0.1% Tween 80

Revision 02 May 2003 Monitoring Programs Office

- Re-formatted and re-numbered
- Combined elution procedure for all commodities
- Removed section 5.3, Definition of Sample
- Added orbital shaker to equipment under Section 5.1.2
- Changed wording on shaker adaptation under section 5.1.3
- Changed wording on taking temperature of specific commodities, section 5.3.2

- Added extensive freezing or spoilage to section 5.3.3
- Added "outer leaves" to 5.4.3.2
- Changed wording on section 5.4.6.1 from 5-qt plastic pails to round containers
- Deleted sub sections under section 5.4 dealing with MPN calculations

Revision 01 February 2002 Monitoring Programs Office

- Changed maximum laboratory holding time prior to analysis from 72 hours to 24 hours in subsections 5.4.b and 5.5.a

Original July 2001 Monitoring Programs Office

- Established standard procedures for the receipt and washing of MDP samples