

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

SOP No.: MDP-LABOP-02		Page 1 of 16
Title: Sample Receipt, Elution, Preenrichment, and DNA Extraction		
Revision: 09	Replaces: Rv08 dated 05/01/07	Effective: 02/11/08

1. Purpose

To provide standard procedures for the USDA/AMS Microbiological Data Program (MDP) on the receipt and washing of fruit and vegetable samples, the preenrichment of the wash eluate, and the DNA extraction of the preenriched cultures. Universal Preenrichment Broth (UPB) is used as the wash eluate and as the preenrichment broth for all target organisms.

2. Scope

This 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. Outline of Procedures

Equipment and Materials	5.1
Media and Reagents	5.2
Receipt of Samples and Chain of Custody Requirements	5.3
Elution Method	5.4
Preenrichment	5.5
DNA Extraction	5.6
Flowcharts	5.7

Attachment 2, Sample Receipt Form (SRF) – downloadable version available on website at <http://www.ams.usda.gov/science/MPO/Mdp.htm>

4. References

- 4.1 SOP MDP-SAMP-PROC-2, MDP Sampling Procedures on Site
 - 4.2 SOP MDP-SAMP-PROC-3, Packing and Shipment of MDP Samples.
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- 4.3 SOP MDP-LABOP-01, Infrared (IR) Thermometer Use
- 4.4 SOP MDP-MTH-01A, Enumeration of *Escherichia coli* in Produce Samples by TEMPO[®] EC (*E. coli*) System
- 4.5 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.
- 4.6 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.
- 4.7 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.
- 4.8 Food and Drug Administration. 2000. Safer Processing of Sprouts. A Food Safety Training Program Developed by the California Department of Health Services, Food and Drug Branch and the U.S. Food and Drug Administration.
- 4.9 Hammack, T. S., Johnson, M. L., Jacobson, A. P. and W. H. Andrews. 2006. Effect of sample preparation and preenrichment media on the recovery of *Salmonella* from cantaloupes, mangoes, and tomatoes. *J. AOAC International*. 89: 180-184.

5. Specific Procedures:

- 5.1 Equipment and Materials
 - 5.1.1 Balance, capable of weighing up to 3000 g (± 1 g)
 - 5.1.2 Stomacher, Seward or equivalent
 - 5.1.3 Sterile TEMPO filter bags
 - 5.1.4 Plastic bags, sterile, suitable size and durability to hold sample and eluent (e.g., sterile 3500 stomacher bags)
 - 5.1.5 Stomacher bags with or without filter inlay (WhirlPak or equivalent)
 - 5.1.6 Forceps, tongs, slotted spoons, sterile
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- 5.1.7 Serological pipets, sterile
- 5.1.8 Thermometer, Raytek Portable IR Sensor, P/N Rayst20CRUS
- 5.1.9 Gloves, sterile
- 5.1.10 Incubator, 35 ± 2°C

5.2 Media and Reagents

- 5.2.1 Positive culture control strains grown overnight in appropriate broth. Refer to SOP MDP-QA-03 for list of specific strains used in the analyses.
- 5.2.2 Universal Preenrichment Broth (UPB), Difco Catalog # 223510 or equivalent supplemented with 0.1% Tween 80 (UPBt).
- 5.2.3 IT 1-2-3 R.A.P.I.D.[™] DNA Purification Kit, Biochem (IT Technologies Inc.)

5.3 Receipt of Samples and Chain of the Custody Requirements

- 5.3.1 The laboratory will receive three samples for each site sample of the same produce in each shipping container.
 - 5.3.1.1 Upon receipt, inspect samples for acceptability (e.g. not spoiled, rotten, or crushed) before proceeding to analysis. If at least two of the three samples are acceptable, no re-sampling is necessary. However, if only one of the three samples is acceptable, re-sampling is required. Re-sampling should occur within the same month as the initial collection, if possible. Occasionally, samples may be “made-up” the following month; however, approval from the MPO Sampling Manager or designee and the receiving laboratory is first required.
 - 5.3.1.2 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 staff shall



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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.

- 5.3.1.3 Upon receipt of samples for each scheduled commodity group, the laboratory shall complete a Sample Receipt Form (SRF) and e-mail a copy of the form to the USDA/AMS Sampling Manager and/or designee. Alternatively, forms may be faxed. The SRF shall also document any uncollected samples and any problems encountered. See Attachment 2 for Sample Receipt Form.
 - 5.3.1.4 The receiving laboratory shall notify the Sampling Manager of the collection State and MPO if any samples or e-SIFs are missing.
 - 5.3.2 Upon arrival at the laboratory, take the temperature of all three samples according to SOP MDP-LABOP-01 and record the date and time of sample receipt. Do not take the temperature through the plastic bag. The bags should be sealed in such a way that they can be opened and re-sealed easily. If this is not the case, contact your sampling manager to arrange for appropriate modifications in bag closure procedures. Do not touch the produce with bare hands. Wear sterile gloves while handling the produce.
 - 5.3.3 Test each of the samples individually.
 - 5.3.4 Test all samples regardless of the temperature unless they are spoiled, rotten or, in the case of tomatoes, severely crushed.
 - 5.3.5 Refrigerate the samples until analysis begins. Perform the analysis as soon as realistically possible but no more than 48 hours after receipt in the laboratory.
- 5.4 Elution Method
- 5.4.1 Perform all manipulations using sterile technique.
 - 5.4.2 Tare a sterile bag and add sample to bag. Wear sterile gloves to remove the produce from the sample bag for transfer to other sterile bags for weighing. The laboratory may elect to add the eluent directly to the sample bag, rather than transferring the commodity to a new bag. Do not composite samples. Each sample
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shall be washed and tested individually. Remove ties or rubber bands used to bunch the produce. Remove any obvious clumps of dirt or any extraneous material clinging to the produce without damaging the sample.

- 5.4.2.1 Tomatoes: Test only whole tomatoes. Do not remove labels, stems, or leaves. Each tomato sample shall weigh at least 100 g (a roma tomato sample may include up to three tomatoes). Test all samples including those with small cuts and bruises. Only discard samples that are spoiled, rotten, or severely crushed.
 - 5.4.2.2 Cantaloupe: Test only whole cantaloupes. Do not reject cantaloupes with minor surface damage. Do not test spoiled/rotten cantaloupes.
 - 5.4.2.3 Lettuce, including the pre-cut bagged lettuce: Test minimum 100 g. Aseptically, pull the lettuce pieces out of the bag. Discard leaves that exhibit obvious wilt or excessive decay. If more than one bag is required to reach the desired amount, combine the leaves from a second bag that has an identical lot number (or product code) as the first bag. If large amount of produce is available testing 200 g of sample is optional. Use appropriate wash volume and enter the correct amount of sample tested in RDE.
 - 5.4.2.4 Green onions: Test approximately 200 g of produce. Remove and discard wilted or decayed leaves or stems. Do not cut the roots or break the stems.
 - 5.4.2.5 Parsley and cilantro: Test minimum 100 g. Aseptically, remove the samples along with leaves and stems. Discard samples that exhibit obvious wilt or excessive decay. Shake off excess dirt from the produce.
 - 5.4.2.6 Alfalfa sprouts: Test approximately 25 g of sample. Attempt to separate the sample so that it is spread out evenly in the bag.
 - 5.4.2.7 Spinach, including the pre-cut bagged spinach: Test a minimum of 100 g. Aseptically, pull the spinach pieces out of the bag. Discard leaves that exhibit obvious wilt or excessive decay. If more than one bag is required to reach the desired amount, combine the leaves from a second bag that has an identical lot number (or product code) as the first bag. If a large amount of produce is available, testing 200 g of sample is optional. Use appropriate wash volume and enter the correct weight of the sample tested in the RDE.
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5.4.3 Adding UPB + Tween (UPBt) as Eluent

- 5.4.3.1 For green onions and tomatoes add a weight of eluent (± 5 g) equal to the weight of produce to the bag.
- 5.4.3.2 For spinach, lettuce, parsley and cilantro add a weight of eluent (± 5 g) equal to two and half times (2.5 X) the weight of the produce.
- 5.4.3.3 Alfalfa sprouts: Add a weight of eluent (± 1 g) equal to nine times (9 X) the weight of the produce.
- 5.4.3.4 Cantaloupe: Add a weight of eluent equal to $\frac{1}{4}$ the weight of the cantaloupe (± 5 g).

5.4.4 Wash: In order to maximize recovery of organisms, the following elution method is employed to ensure that the wash buffer floods all surfaces of the sample.

- 5.4.4.1 Tomatoes: Cup the produce in both hands from the outside of the sample bag. Rub the produce, turning as needed. Shake vigorously for 20 complete up and down strokes. Shake vigorously for 20 side to side strokes.
- 5.4.4.2 Cantaloupe: Cup the produce in both hands from the outside of the sample bag. Rub the produce, turning as needed. Shake vigorously for 20 complete up and down strokes. Shake vigorously for 20 side to side strokes.
- 5.4.4.3 Lettuce, spinach, green onions, parsley and cilantro: Shake vigorously for 20 complete up and down strokes. Shake vigorously for 20 side to side strokes.
- 5.4.4.4 Alfalfa sprouts: Remove as much air as possible and close the bag. Rock the bag, alternating from side to side, back and forth, and up and down for approximately 10 seconds, at a rate of about one full side to side motion per second. Place bag in stomacher for 2 minutes at normal speed (medium setting).

5.4.5 Distribution of Wash Eluate

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- 5.4.5.1 For SOP MDP-MTH-01A, Enumeration of *Escherichia coli* in Produce Samples by TEMPO[®] EC (*E. coli*) System, SOP MDP-MTH-01B, Enumeration of Coliform Bacteria in Produce Samples by TEMPO[®] CC (Coliform Count) System and SOP MDP-MTH-01C, Enumeration of Total Viable Count (TVC) in Produce Samples by TEMPO[®] TVC System. Pipet 10 to 25 mL of sample wash into a sterile TEMPO filter bag.
- 5.4.5.2 For ColiComplete MPN refer to SOP MDP-MTH-01, *Escherichia coli* MPN method.
- 5.4.5.3 Set up positive produce controls (refer to flowcharts in Section 5.7) according to the type of produce. Gently mix the controls.

5.4.5.3.1 For Alfalfa Sprouts: Weigh one additional 25 g sample from the leftover samples received. If necessary, samples may be combined from the 3 sub-samples of that commodity in order to obtain the 25 g. Add eluent and wash according to sections 5.4.3 and 5.4.4. Separate the produce from the wash. Remove a 25 mL aliquot and add 1 mL of the positive culture control from SOPs MDP-MTH-01 *Escherichia coli* MPN method or MDP-MTH-01A Enumeration of *Escherichia coli* in Produce Samples by TEMPO[®] EC (*E. coli*) System. Refer to SOP MDP-MTH-01 *Escherichia coli* MPN method or MDP-MTH-01A Enumeration of *Escherichia coli* in Produce Samples by TEMPO[®] EC (*E. coli*) System for set up of controls. For SOPs MDP-MTH-04, MDP-MTH-05, and MDP-MTH-07 add 1 mL of the positive control cultures from each method to the remainder of the sample wash.

5.4.5.3.2 For Green Onions: Weigh one additional sample; approximately 200 g of green onions from the leftover samples received. If necessary, samples may be combined from the 3 sub-samples of that commodity in order to obtain the 200 g. Add eluent and wash according to sections 5.4.3 and 5.4.4. Separate the produce from the wash. Remove a 25 mL aliquot and add 1 mL of the positive culture control from SOP MDP-MTH-01 *Escherichia coli* MPN method or MDP-MTH-01A Enumeration of *Escherichia coli* in Produce Samples by TEMPO[®] EC (*E. coli*) System. Refer to SOP

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MDP-MTH-01 *Escherichia coli* MPN method or MDP-MTH-01A Enumeration of *Escherichia coli* in Produce Samples by TEMPO[®] EC (*E. coli*) System for set up of controls. Remove the washed produce. For SOPs MDP-MTH-04, MDP-MTH-05, and MDP-MTH-07 add 1 mL of the positive control cultures from each method to the remainder of the sample wash.

5.4.5.3.3. For Cantaloupe and Tomatoes: Use one additional whole cantaloupe and tomato. Add eluent and wash according to sections 5.4.3 and 5.4.4. Remove a 25 mL aliquot and add 1 mL of the positive culture control from SOP MDP-MTH-01 *Escherichia coli* MPN method or MDP-MTH-01A Enumeration of *Escherichia coli* in Produce Samples by TEMPO[®] EC (*E. coli*) System. Refer to SOP MDP-MTH-01 *Escherichia coli* MPN method or MDP-MTH-01A Enumeration of *Escherichia coli* in Produce Samples by TEMPO[®] EC (*E. coli*) System for set up of controls. For SOPs MDP-MTH-04, MDP-MTH-05, and MDP-MTH-07 add 1 mL of the positive control cultures from each method to the remainder of the sample wash.

5.4.5.3.4 For spinach, lettuce, including the pre-cut bagged spinach or lettuce, parsley and cilantro: Weigh one additional 100 g sample. If necessary, samples may be combined from the 3 sub-samples of that commodity in order to obtain the 100 g. Add eluent and wash according to sections 5.4.3 and 5.4.4. Remove a 25 mL aliquot and add 1 mL of the positive culture control from SOP MDP-MTH-01 *Escherichia coli* MPN method or MDP-MTH-01A Enumeration of *Escherichia coli* in Produce Samples by TEMPO[®] EC (*E. coli*) System. Refer to SOP MDP-MTH-01 *Escherichia coli* MPN method or MDP-MTH-01A Enumeration of *Escherichia coli* in Produce Samples by TEMPO[®] EC (*E. coli*) System for set up of controls. For SOPs MDP-MTH-04, MDP-MTH-05, and MDP-MTH-07 add 1 mL of the positive control cultures from each method to the remainder of the sample wash.

Note: MDP-MTH-05 positive control can be used as the positive control for MDP-MTH-07.

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5.5 Preenrichment (Refer to Flowchart in 5.7)

- 5.5.1 For Cantaloupes: Leave the produce in the wash eluent in the bag. Remove excess air from the bag, close the bag and incubate at $35 \pm 2^{\circ}\text{C}$ for 18–24 hours for preenrichment. NOTE: At the end of the sample set-up day, rotate produce approximately 180 degrees if the produce surface is not immersed in UPBt.
- 5.5.2 For Tomatoes: Leave the produce in the wash eluent in the bag. Remove excess air from the bag, close the bag and incubate at $35 \pm 2^{\circ}\text{C}$ for 18–24 hours for preenrichment. NOTE: At the end of the sample set-up day, rotate produce approximately 180 degrees if the produce surface is not immersed in UPBt.
- 5.5.3 For Spinach and Lettuce: Leave the produce in the wash eluent in the bag. Close the bag and incubate at $35 \pm 2^{\circ}\text{C}$ for 18–24 hours for preenrichment. NOTE: At the end of the sample set-up day, rotate the lettuce approximately 180 degrees if the produce surface is not immersed in UPBt.
- 5.5.4 For Alfalfa Sprouts: Separate the disintegrated strings of sprouts from the wash and incubate the remaining wash at $35 \pm 2^{\circ}\text{C}$ for 18–24 hours for preenrichment.
- 5.5.5 For Green Onions, parsley and cilantro: Separate the washed produce from the wash and incubate the remaining wash at $35 \pm 2^{\circ}\text{C}$ for 18–24 hours for preenrichment.
- 5.5.6 Controls: Refer to specific method SOPs for control strains and set-up.
 - 5.5.6.1 Positive and negative culture controls: Inoculate UPBt broths with control strains (see flowcharts in 5.7).
 - 5.5.6.2 Positive produce controls: See flowchart in 5.7.
 - 5.5.6.3 Incubate UPB cultures at $35 \pm 2^{\circ}\text{C}$ for 18–24 hours for preenrichment.

5.6 DNA Extraction

- 5.6.1 For all samples and controls, extract DNA from the UPBt preenrichment cultures according to the manufacturer's instructions for the IT 1-2-3 DNA purification kits using the following modifications:
 - 5.6.1.1 Use 100 μL of UPBt culture in place of swab.



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- 5.6.1.2 In “Part 2: Wash Filter – DNA Cleanup”, after second centrifugation step, open vials and set on countertop for 5 minutes with lids off to facilitate evaporation of ethanol. Perform these steps in a clean environment to minimize contamination.
- 5.6.1.3 In “Part 3: DNA Elution”, add 100 µL of buffer 3 instead of 400 µL for a more concentrated sample.
- 5.6.2 Store the extracted DNA at 2-8°C for short-term storage or -20°C for long-term storage. Refrigerate the UPBt preenrichment cultures until the PCR assays are completed. Refrigerate positive UPBt preenrichment cultures until isolation steps are completed.
- 5.6.3 Proceed with subsequent analyses according to the SOPs for those analytes.

Disclaimer: Reference to brand names (kits, equipment, media, reagents, etc.) does not constitute endorsement by this agency.

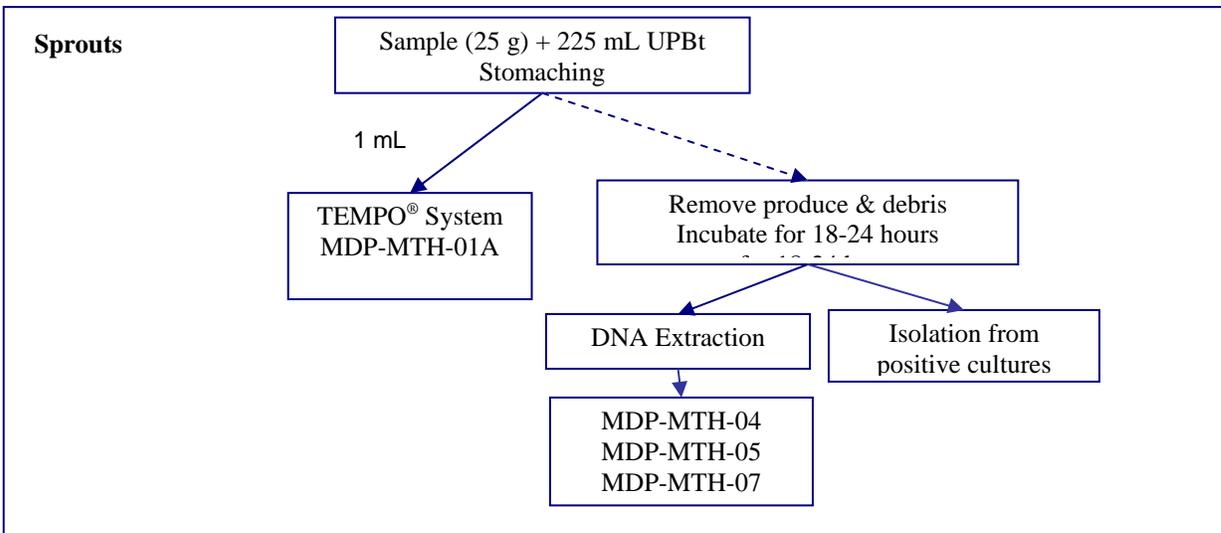
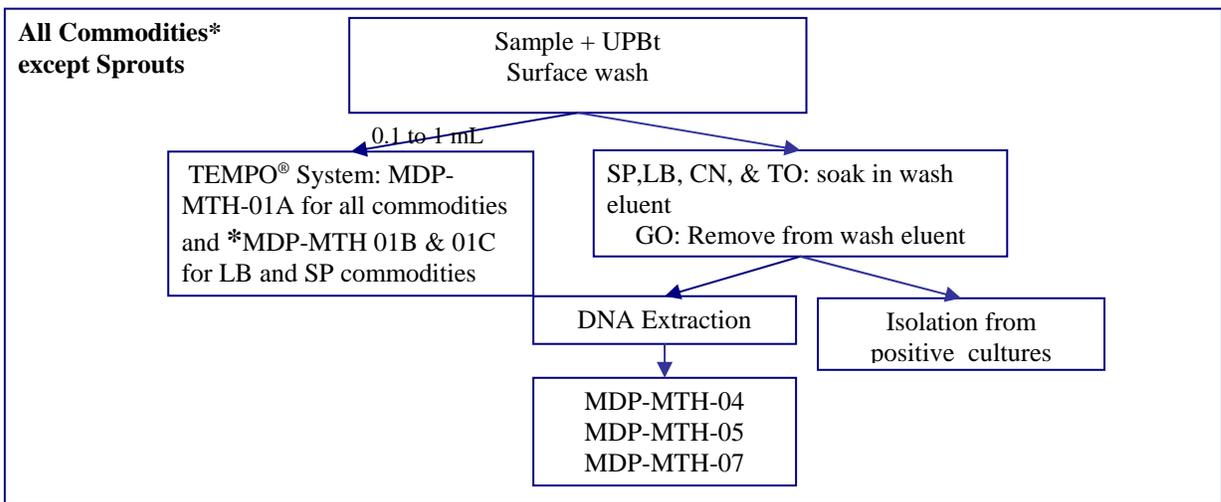


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5.7 Flowcharts

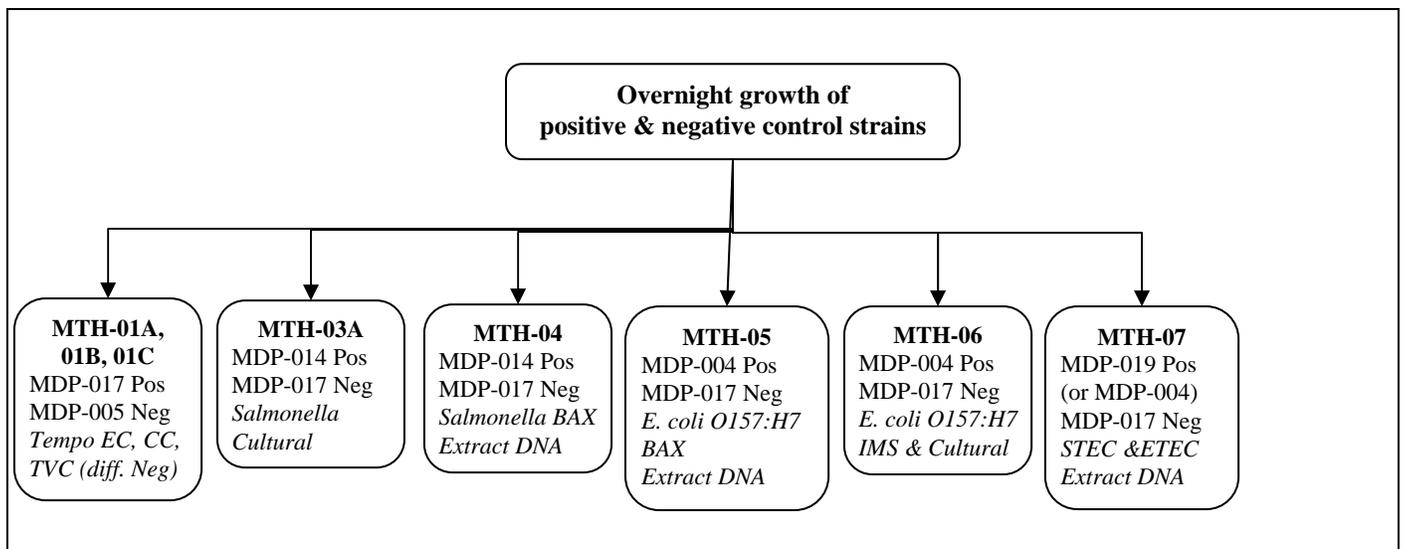
Sample Setup



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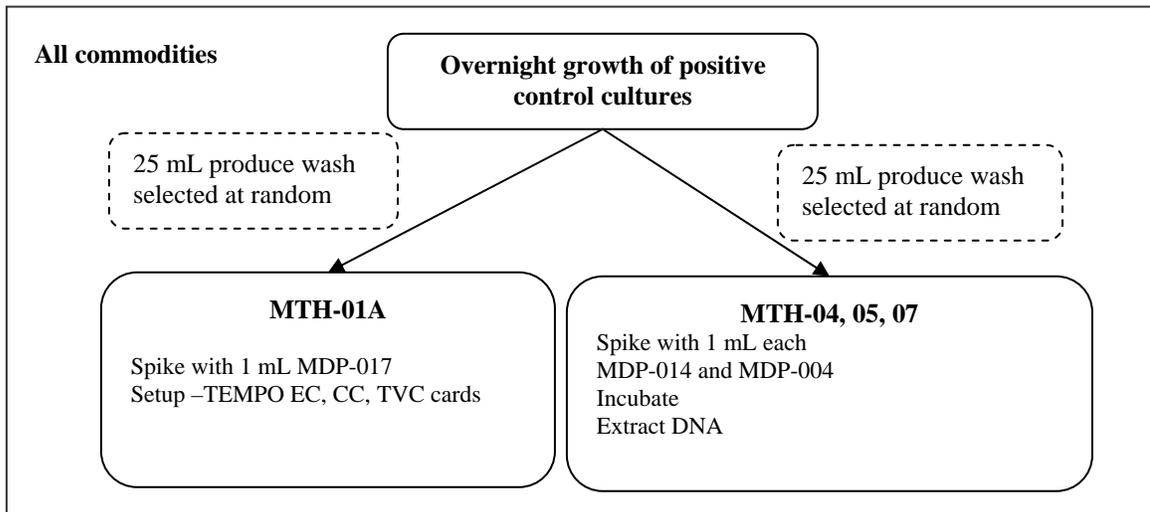
Positive and Negative Controls



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Positive Produce Controls



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Revision 05

July 2004

Monitoring Programs Office

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

