

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

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Title: Sample Receipt and Elution Procedure		
Revision: 04	Replaces: 08/15/03	Effective: 08/01/04

1. Purpose:

To provide standard procedures for receipt and washing of fruit and vegetable samples for the USDA/AMS Microbiological Data Program (MDP).

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.

3. Outline of Procedure:

Equipment and Materials	Section 5.1
Media and Reagents	Section 5.2
Receipt of Samples	Section 5.3
Elution Method	Section 5.4

4. References:

- 4.1 MDP-LABOP-02, Sample Receipt and Elution Procedure
 - 4.2 MDP-LABOP-02 Attachment 1, Fabrication of the California Cantaloupe Shaker Adapter
 - 4.3 SAMP-PROC-2, MDP Sampling Procedures on Site.
 - 4.4 SAMP-PROC-3, Packing and Shipment of MDP Samples.
 - 4.5 USDA/FSIS: SOP No. MLG Appendix 1.02; Rev.02, effective 7/3/03
 - 4.6 Baylis CL, MacPhee S, and Betts RP. 2000. Comparison of two commercial preparations of buffered peptone water for the recovery and growth of Salmonella bacteria from foods. *Journal of Applied Microbiology*. 89: 501-510.
 - 4.7 Andrews WH and Hammack ST. 1998. Food sampling and preparation of sample homogenate. FDA. BAM Chapter 1, edition 8, revision A. <http://www.cfsan.fda.gov/~ebam/bam-1.html> (last accessed 07-12-04)
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5. Specific Procedures:

5.1 Equipment and Materials

- 5.1.1** Balance, minimum 1000 g and capable of weighing up to 3000 g
- 5.1.2** Rotary or orbital shaker, set at 2.54 cm (1 inch) stroke
- 5.1.3** In-house adaptation for orbital shaker bed to shake cantaloupe samples (e.g. California Shaker Adaptor, Attachment 1)
- 5.1.4** Plastic bags, sterile, suitable size to hold sample and eluent (e.g., sterile 3500 stomacher bags)
- 5.1.5** Forceps, tongs, slotted spoons, sterile
- 5.1.6** Thermometer, Raytek Portable IR Sensor, P/N Rayst20CRUS
- 5.1.7** Gloves, sterile

5.2 Media and Reagents

- 5.2.1** Buffered Peptone Water (BPW) plus 0.1% Tween 80
- 5.2.2** Sodium hydroxide (NaOH) solution for pH adjustment
- 5.2.3** Hydrochloric acid (HCl) for pH adjustment

Prepare buffered peptone water plus 0.1% Tween 80 (v/v) for use as eluent

Peptone	10.0 g
Sodium chloride	5.0 g
Sodium phosphate, dibasic	3.5 g
Potassium phosphate, monobasic	1.5 g
Tween 80	1.0 mL
Distilled water	1.0 L



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Dissolve dry ingredients in distilled water, add 1.0 mL of Tween 80 and dissolve again. Adjust to a final pH of 7.2 ± 0.2 at room temperature (approximately between 22-25°C) using NaOH or HCl. Commercially prepared dehydrated media may also be used. The media must be prepared according to manufacturer's instructions with a final concentration of 0.1% Tween 80 in BPW. Dispense into appropriate containers and autoclave at $120 \pm 1^\circ\text{C}$ for 15 minutes. Commercially prepared liquid media may also be used.

5.3 Receipt of Samples

- 5.3.1** The laboratory will receive 3 samples (or multiples thereof) of the same produce in each shipping container. The laboratory manager is required to notify the State Sampling Manager if samples do not arrive on schedule or in acceptable condition. Notification by e-mail shall be made on the day of sample receipt if unacceptable conditions occur. Description of the sample arrival condition shall also be entered into the RDE system under "Comments" if the condition is other than acceptable. The MPO Sampling Manager shall be notified if/when persistent or serious problems occur.
- 5.3.2** Upon arrival at the laboratory, take the temperature of all 3 samples and record the date and time of sample receipt. Carefully open a sample bag to obtain direct access to the surface of the sample. Determine the temperature of the produce by pointing and activating the IR thermometer at the surface of each sample. Record the surface temperature of the sample. **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 all laboratory samples regardless of the temperature unless they are spoiled, rotten, or , in the case of tomatoes, severely crushed.
- 5.3.4** Refrigerate the samples until analysis begins. Perform the analysis as soon as realistically possible but no more than 24 hours after receipt in the laboratory.
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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. If the commodity arrives in a sterile bag, the laboratory may elect to add the diluent directly to that bag, rather than transferring the commodity to a new bag. Do not composite samples. Each sample is 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 them without damaging the sample.

5.4.2.1 Tomatoes: Test only whole tomatoes. Do not remove labels, stems or leaves. Each tomato sample should weigh at least 100 g. 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 each of the 3 cantaloupe samples individually. Test only whole cantaloupes. Do not reject cantaloupes with minor surface damage. Do not test spoiled/rotten cantaloupes.

5.4.2.3 Lettuce: Test approximately 200 g of outer lettuce leaves. Remove and discard any outer leaves that exhibit obvious wilt or decay. Aseptically pull whole leaves of lettuce from the base of the loose-leaf lettuce. Test only whole leaves of lettuce, but do not reject lettuce leaves that are torn or have breaks in their surface.

5.4.2.4 Green onion, parsley and cilantro: Test approximately 200 g of produce. Remove and discard wilted or decayed leaves or stems. Do not cut the roots or break the stems.

5.4.3 Add sterile buffered peptone water containing 0.1% Tween 80 as eluent to bag with sample.

5.4.3.1 For all produce, except cantaloupes, add a weight of eluent equal to the weight of produce (± 5 g) to the bag

5.4.3.2 Cantaloupes: Add a weight of eluent equal to one-quarter the weight of the cantaloupe (± 5 g).

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- 5.4.4** In order to maximize recovery of organisms, the following wash method is employed to ensure that the wash buffer floods all surfaces of the sample. After removing as much air possible, close and seal the bag so that no fluid can leak during the wash.
- 5.4.4.1 Tomatoes:** 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. Gently shake the bag to dislodge any produce adhering to itself and/or the bag. Vigorous shaking, resulting in rupture or breakage of produce is not required. Place bag on rotating shaker and shake the samples at 160 rpm, stroke size 2.54 cm (1 inch) for 3.5 minutes \pm 0.5 minutes. Repeat the manual shaking described above for approximately 10 seconds. Repeat the mechanical shaking for 3.5 minutes \pm 0.5 minutes. Give the bag a final manual shaking for approximately 5 seconds.
- 5.4.4.2 Cantaloupes:** 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 bagged cantaloupes in round containers on shaker adaptation and shake at 160 rpm, stroke size 2.54 cm (1 inch) for 3.5 minutes \pm 0.5 minutes. Repeat the manual shaking described above for approximately 10 seconds. Repeat the mechanical shaking for 3.5 minutes \pm 0.5 minutes. Give the bag a final manual shaking for approximately 5 seconds.
- 5.4.4.3 Lettuce, green onions, parsley, and cilantro:** attempt to separate the sample so that it is spread out evenly in the bag. Follow instructions in 5.4.4.2.
- 5.4.5** Use the eluate as the sample. Proceed with subsequent analyses according to the SOPs for those analytes.
- 5.4.6** Excess wash must be refrigerated until all tests have been successfully completed. For ease of storage, the produce may be aseptically removed from the bag and discarded or the eluate may be transferred to a sterile container.
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Fabrication of the California Cantaloupe Shaker Adapter For mechanical elution of surface bacteria from cantaloupes.

Overview: Several 5-quart plastic pails are glued to a plywood sheet which is screwed to the top metal bed plate of the orbital shaker. Cantaloupes in double plastic bags with Butterfield's-Tween are shaken according to the draft Cantaloupe Sample-Wash MDP SOP. (See CN Photo #1) *First suggested on 2/6/02 by Dwight Harder, Director of the Arizona Agriculture Laboratory.*

1. Purchase or collect a few empty, clean 5-quart plastic pails, with relatively vertical sides. These are commonly used for ice cream (vanilla is good with cantaloupe). They can also be purchased from a hardware store, Target, Wal-Mart, etc. Determine the arrangement of several pails to be lined up contiguously on a sheet of 3/8", 1/2" or 5/8" thick plywood approximately the dimensions or larger of the bed of your orbital shaker. Circumscribe the circumference of the pails in the position where they will be later glued (i.e, draw a circle around each pail). See CN Photo #2. Do not use "chip board", "particle board" or "wafer board". It is good to remove the metal or plastic bails (handles) from the pails as they will chatter during shaking.
2. Cut plywood to accommodate the number of pails you will glue to the plywood. California has 12 pails (4 rows of 3 ea) on a plywood board measuring 23" by 32" for our shaker which has an 18" x 30" bed.
3. The plywood must be screwed to the metal bed of the shaker, and able to be removed on the days commodities other than CNs are washed. If possible, remove the metal top bed plate from the shaker. California's is attached to the shaker with 4 large slotted screws. Use a large enough screwdriver so as not to strip the screw slots. Position the removed shaker plate upside down on the upside-down plywood and mark at least 4 predrilled and threaded holes in the shaker plate to align and drill holes through the plywood. Be sure screw holes will not be obstructed by plastic pails later, to ensure convenient removal of the CN Shaker adapter from the shaker bed plate. Reassemble bed plate back onto the shaker. This is a good time to perform inspection preventive maintenance and belt-tightening of the shaker, if needed.
4. Obtain machine screws of proper length, diameter, and thread pitch to screw the plywood sheet to the metal shaker bed plate. Use washers, and be sure the screws are tight and do not protrude through the shaker bed plate to obstruct the shaking function. (See CN photo #3)
5. Glue plastic pails to the plywood in the locations previously marked using construction adhesive, subfloor adhesive, or "Liquid Nails". This is sold by hardware stores and building-supply outlets (Home Depot, Lowes) in 10.5 Fl.Oz. "caulking" tubes (< \$3.00). You will also need a "caulking" gun (<< \$5.00). Tight adhesion is guaranteed by using plenty of goo, and scooting the pails side-to side prior to their final location and setting of the adhesive. Tight adhesion is facilitated by weighting each pail down with 2-4 quarts of water (in plastic bags) during the 1-2 day curing of the adhesive. Work in a well

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ventilated area and do not use so much goo that large quantities ooze from the sides of the bases of the pails. Although this is usually not a problem, it is wise to initially try your adhesive with one of your specific pails to determine if the volatile organic solvent in the adhesive softens the plastic of the pail.

6. Test your Cantaloupe adapter and shaker in advance of receiving melons by placing a volume of water in plastic bags in each pail equivalent to a melon plus an equal volume by weight of water. Large cantaloupes weigh about 1.2 Kg, so 2.4 liters of water would be a good volume for the test. Such weights, more than other commodities, will cause the shaker to shimmy. Be sure it is secure. California's is on wheels, which must be stabilized.

NOTE: The California MDP Lab., in collaboration with the Food Safety & Health unit of USDA's ARS-WRRC in Albany, CA is microbiologically validating the elution efficiency of this method for the MDP. Results are very promising, and will be forthcoming soon to MDP, in advance of publication. This is the most promising and feasible method for MDP of the 4 we have evaluated.



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