

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

MDP-LABOP-02 SOP Appendix D

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Lettuce (LT)

Sample Preparation - Test ~375g. If more than one bag of produce is used to constitute a sub-sample, those bags must have the same lot number or same identification code. Time stamps associated with lot numbers or codes are not applicable when time differences are less than 4 hours; if greater than 4 hours, these shall be treated as different lots. Perform all manipulations using sterile technique. Wear sterile gloves to remove the produce from the sample bag for transfer to other sterile bags for weighing. First, remove and discard any leaves that exhibit obvious wilt, cold damage or decay. Also remove any obvious clumps of dirt or any extraneous material clinging to the produce without damaging the sample. Tare a sterile bag and add the sample to bag. The laboratory may elect to add the eluent directly to the sample bag, rather than transferring the commodity to a new bag. Each sample shall be washed and incubated individually.

Eluent and Sample Wash - add sterile UPB eluent approximately equal to two and one half times **(2.5X)** the sample weight of the lettuce. Shake the sample bag vigorously for at least 20 complete vertical strokes. Shake vigorously for at least 20 horizontal strokes. Leave produce in the wash eluent in the bag. Securely close the bag and incubate at $42 \pm 2^{\circ}\text{C}$ for 22–26 hours for pre-enrichment.

NOTE: At end of the sample set-up day, massage, shake and rotate bag contents approximately 180 degrees (if the produce surface is not totally covered by UPB).

Positive Produce Control - Refer to SOP MDP-QA-03 and specific method SOPs for control set-up. Weigh one additional (~375g) sample from the leftover samples received. If necessary, excess material may be combined from the 3 sub-samples of that commodity in order to obtain the additional ~375g. Add eluent and wash. For SOPs MDP-MTH-04 and MDP-MTH-11, add 1 mL of the positive control cultures from each method to the remainder of the sample wash. Do not pool cultural (media) controls or the positive produce control. These shall be analyzed individually.

Post-enrichment for all analyses - After 22-26 hour incubation, remove UPB pre-enriched samples from the incubator. Thoroughly mix. Test samples may be pooled or tested individually. Prepare a pooled sample by aseptically removing a 10ml aliquot from each of the (same site number) individual samples and pool them together into a new suitable sterile container. The final pooled sample volume will be 30mL. Do not pool cultural (media) controls or the positive produce control. These shall be analyzed individually.

NOTE: Appropriately label the pooled sample so it can be traced back to the three individual samples. If a pooled sample tests positive during analyses, then the individual samples shall be tested.

Subsequent Analyses - Extract DNA according to section 6.6 of SOP MDP LABOP-02. Proceed with subsequent analyses according to the SOPs MDP-MTH-04 and MDP-MTH-11 for pooled and/or individual UPB-enriched samples.

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