

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

SOP No.: MDP-MTH-01C		Page 1 of 6
Title: Enumeration of Total Viable Count (TVC) in Produce Samples by the TEMPO® TVC System		
Revision: Original	Replaces: None	Effective: 03/01/08

1. Purpose & Principle

The TEMPO® is an automated system designed to provide a standard procedure for enumeration of total viable count (TVC) of aerobic mesophilic bacteria in fresh fruit and vegetables. In the TEMPO® system, a card carrying 3 sets of 16 wells (48 wells in total) is used to represent a Most Probable Number (MPN) method. A fluorescent signal, generated upon the reduction of a compound present in the culture medium by bacterial growth, is used in the detection and enumeration.

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. For MDP, only fresh pre-cut bagged lettuce and bagged spinach are to be analyzed for TVC per this SOP.

3. Outline of Procedures

- 5.1 Equipment and Materials
- 5.2 Media and Reagents
- 5.3 List of Controls
- 5.4 TEMPO® TVC Method
- 5.5 Reporting Data

4. References

- 4.1. TEMPO® TVC REF 80 007 dated 03/2007, provided by bioMerieux® SA
 - 4.2. TEMPO® QC REF 80 000 dated 04/2004, provided by bioMerieux® SA, for the verification of TEMPO Reader measurement performance
 - 4.3. SOP MDP-LABOP-02, Sample Receipt, Elution, Preenrichment, and DNA Extraction.
 - 4.4. SOP MDP-DATA-01, Data Entry, Record Keeping, and Results Reporting
 - 4.5. SOP MDP-QA-03, Quality Assurance Controls
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4.6. Evaluation of Universal Pre-enrichment Broth (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.

5. Specific Procedures

5.1. Equipment and Materials

- 5.1.1. TEMPO® system, bioMerieux
- 5.1.2. Incubator, 35 ± 1°C
- 5.1.3. Serological pipets, sterile
- 5.1.4. Vortex mixer
- 5.1.5. TEMPO® incubation rack
- 5.1.6. Dispensers, Ceramus or equivalent
- 5.1.7. TEMPO® filler rack
- 5.1.8. TEMPO® filter bags, sterile

5.2. Media and Reagents

- 5.2.1. TEMPO® TVC cards
- 5.2.2. TEMPO® TVC culture medium vials
- 5.2.3. TEMPO® QC kit
- 5.2.4. Sterile deionized water
- 5.2.5. Eluent: Universal Preenrichment Broth (UPB) from Difco or equivalent, supplemented with 0.1% Tween 80 (UPBt)
- 5.2.6. Control culture broth: UPBt or any sterile non-specific rich media
- 5.2.7. Sterile Butterfield's Phosphate Buffer (BPB) for diluting control cultures and/or samples

5.3. List of controls (specific strains are listed in SOP MDP-QA-03)

- 5.3.1. Positive culture control: 1 mL overnight culture, diluted appropriately (e.g. ~100 CFU/mL) in sterile BPB or any sterile non-specific rich media



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- 5.3.2. Positive produce culture control (refer to SOP MDP-LABOP-02): 1 mL spiked culture
- 5.3.3. Media controls: Sterile UPBt.
- 5.3.4. Carry all controls through this entire procedure. If any of the controls fail to yield a satisfactory result refer to SOP MDP-QA-03. The expected results of QC control cultures after a minimum of 40-48 hours of incubation should conform to those shown below:

QC Culture	Enumeration Range
Uninoculated media	Low threshold < 250
Positive Control	Higher than low threshold
Produce Control	Higher than low threshold

5.4. TEMPO® TVC Method

Follow the manufacturer's instructions for handling, storage and use of the TEMPO® cards and reagents vials, reconstitution of culture reagents, and TEMPO® cards setup and filling.

- 5.4.1. Remove TEMPO® TVC cards and media vials and allow them to reach room temperature.
- 5.4.2. For samples, transfer 10 to 25 mL of each sample wash from SOP MDP-LABOP-02 to a sterile TEMPO® filter bag.
- 5.4.3. Refer to manufacturer's instructions for TEMPO® card setup.
- 5.4.4. Reconstitute the contents of each TEMPO® TVC media vial as follows:
 - 5.4.4.1. Dispense 3.9 mL of sterile water into a TEMPO® TVC media vial.
- 5.4.5. Aseptically transfer 1 mL of sample wash from 5.4.2 above to 9 mL of sterile BPB. Vortex to mix sample.
 - 5.4.5.1. Add 0.1 mL of the diluted sample directly to the reconstituted TVC media vial and mix by vortexing (~3 seconds).
 - 5.4.5.2. Follow the manufacturer's instructions for card filling, incubation and reading the cards.
 - 5.4.5.3. Enter a 1/1000 dilution into the TEMPO set-up screen for bagged pre-cut lettuce and bagged spinach.

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5.5. Results and Interpretation

- 5.5.1. The results are automatically read and analyzed by the computer. The enumeration number represents CFU per milliliter of the eluate. Report CFU/mL, as displayed by the computer, for each sample. Normalization of data per gram of the produce will be performed by MPO.
- 5.5.2. Report CFU/mL in the RDE according to SOP MDP-DATA-01

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



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