

**TEMPO® AEROBIC Count and Coliform Count METHODS**  
**IMS #7b (TAC), IMS #19a (TCC)**

[Unless otherwise stated all tolerances are  $\pm 5\%$ ]

**SAMPLES**

1. **Laboratory Sample Requirements (see CP items 33 & 34)** \_\_\_\_\_  
**[For inhibitor testing requirements, refer to Section 6 of the PMO]**

**MATERIALS AND APPARATUS**

2. **TEMPO system, TEMPO Aerobic Count (TAC) assay kit and TEMPO Coliform Count (TCC) assay kit** \_\_\_\_\_

a. TEMPO System \_\_\_\_\_

1. TEMPO Reader, software version SP6 or higher \_\_\_\_\_

2. Filler Racks (6 slots) \_\_\_\_\_

3. TEMPO Filler \_\_\_\_\_

4. Incubation Racks (20 slots) \_\_\_\_\_

b. Incubator ( $32\pm 1^\circ\text{C}$ ), see CP item 15 \_\_\_\_\_

c. Pipettor (fixed volume or electronic) and/or pipets, see CP item 6 \_\_\_\_\_

d. Dispenser, 3 mL, see CP item 6 \_\_\_\_\_

e. TAC kit (red), vials and cards store at  $2-25^\circ\text{C}$  \_\_\_\_\_

1. Lot No. \_\_\_\_\_ Exp. Date: \_\_\_\_\_

2. Vials (all with same bar code) and cards (all with different bar codes) from same lot. Do not mix vials and cards from different lots \_\_\_\_\_

3. Vials and cards removed from kit box, stored at  $16-25^\circ\text{C}$ , use within two weeks, any removed vials and cards not used within two weeks discarded \_\_\_\_\_

f. TCC kit (blue), vials and cards store at  $2-25^\circ\text{C}$  \_\_\_\_\_

1. Lot No. \_\_\_\_\_ Exp. Date: \_\_\_\_\_

2. Vials (all with same bar code) and cards (all with different bar codes) from same kit lot. Do not mix vials and cards from different lots \_\_\_\_\_

- 3. Vials and cards removed from kit box, stored at 16-25°C, use within two weeks, any removed vials and cards not used within two weeks discarded

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g. TEMPO QC Kit

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- 1. Lot No. \_\_\_\_\_ Exp. Date: \_\_\_\_\_
- 2. Prior to use, allow kit to come to room temperature before removing components from box
- 3. Run once per month, maintain records
- 4. Results acceptable, if not do not test samples, contact manufacturer

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h. Sterile deionized (DI) or equivalent purified water

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**PROCEDURE**

**3. Work Area**

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- a. Bench not in direct sunlight
- b. Sanitize immediately before start of setup

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**4. Selecting Dilutions**

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a. Aerobic Count, TAC

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- 1. Recommended dilution that yield count within range
  - a. For raw milk, 1 mL of a 1:100 dilution (100 to 400,000 CFU range) is added to TAC vial which has been rehydrated with 3 mL of sterile water
  - b. For finished products, 1 mL of a 1:10 dilution (10 to 49,000 CFU range) is added to TAC vial which has been rehydrated with 3 mL of sterile water

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b. Coliform, TCC

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- 1. Recommended dilution that yield count within range
  - a. For finished products, 1 mL or 1 g of an undiluted sample is added to TCC vial which has been rehydrated with 3 mL of sterile water
  - b. If a 1/10 dilution must be prepared, associate 3 cards to the same sample ID; add 3.3 mL of the 1/10 dilution to each of 3 TCC vials rehydrated with 0.7 mL of sterile water. Enter the TEMPO 1/12 dilution into the Prep Software, continue with item 12

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## CONTROLS

### 5. Controls (TAC and TCC) \_\_\_\_\_

- a. The TEMPO certificate of quality control for each reagent kit maintained \_\_\_\_\_
  - 1. Certificate number: \_\_\_\_\_
- b. TEMPO QC Kit run monthly, see item 2.g above \_\_\_\_\_
- c. With each run prepare vial and card control for pipets or pipettor tips used by adding 1 mL of sterile water (this is a combined control) \_\_\_\_\_

### 6. TEMPO Vial Preparation \_\_\_\_\_

- a. Refrigerated vials and cards allowed to warm to room temperature for 15 min \_\_\_\_\_
- b. Until use card transfer tubes protected from contamination \_\_\_\_\_
- c. Prime 3 mL sterile water dispenser (item 2.d) to waste 3 times \_\_\_\_\_
- d. Label each vial with sample ID and dilution factor \_\_\_\_\_
- e. Log on to the TEMPO preparation station \_\_\_\_\_
- f. Identify samples to be tested, dilution factors and enter the vial identifier barcodes using the bar code reader (follow manufacturer's instructions for the preparation station user interface) \_\_\_\_\_
- g. Remove cap on culture medium vials and add 3 mL of sterile water \_\_\_\_\_

## DILUTING SAMPLES

### 7. Sample Agitation \_\_\_\_\_

- a. When appropriate, wipe top of unopened containers with sterile, ethyl alcohol-saturated cloth \_\_\_\_\_
- b. Before removal of any portion or sub-samples, thoroughly mix contents of each container \_\_\_\_\_
  - 1. Mix raw sample(s) by shaking 25 times in 7 sec with a 1 ft movement (containers approx.  $\frac{3}{4}$  full) \_\_\_\_\_
  - 2. Mix retail milk samples by inverting containers top to bottom, then bottom to top (a complete half circle or 180 degrees) without pausing, 25 times \_\_\_\_\_
- c. Remove test portion within 3 min of sample agitation \_\_\_\_\_

**8. Dilution Agitation**

- a. Before removal of any portion, shake each dilution bottle 25 times in 7 sec with a 1 ft. movement
- b. Remove test portion within 3 min of dilution agitation
- c. Mechanical shakers may be used only if a laboratory provides validation data on a specific unit. Data must pass validation criteria

**9. Sample and Dilution Measurement, Pipets**

- a. Use separate sterile pipets for the initial transfers from each container, adjust pipets in pipet container without touching the pipets
- b. Do not drag pipet tip over exposed exterior of pipets in pipet container
- c. Do not drag pipet across lip or neck of sample container or dilution blank
- d. Insert pipet not more than 2.5 cm (1") below sample surface or dilution surface (avoid foam and bubbles)
- e. Using pipet aid, draw test portion above pipet graduation mark and remove pipet from liquid (mouth pipetting not permitted)
- f. Adjust test volume to mark with lower side of pipet:
  - 1. In contact with inside of sample container (above the sample surface)
  - 2. Or, in contact with inside of dilution blank neck or area above buffer on straight-walled container
  - 3. Ensure excess liquid does not adhere when pipet is removed from the sample container or dilution blank
- g. For dilutions, dispense test portion (1 mL or 11 mL) to dilution blank (with lower side of pipet in contact with neck of dilution blank, or area above buffer on straight-walled containers) with column drain of 2-4 sec
- h. Dispense test sample or dilution to rehydrated vial with lower side of pipet in contact with neck of vial with a drain of 2-4 sec
- i. Discard pipets into disinfectant OR dispose into biohazard bags or containers to be sterilized (using this method of disposal does not require placing into disinfectant first)

**10. Sample and Dilution Measurement, Pipettors, [for electronic pipettors follow Manufacturer instructions]    Mechanical \_\_\_\_\_    Electronic \_\_\_\_\_**

- a. Each day before use, vigorously depress plunger 10x to redistribute lubrication and assure smooth operation (mechanical pipettors) \_\_\_\_\_
- b. Before each use examine pipettor to assure that no liquid is expelled from the pipettor nose-cone (contaminated), if fouling is detected do not use until cleaned as per manufacturer recommendation \_\_\_\_\_
- c. Use separate sterile tip for the initial transfers from each container \_\_\_\_\_
- d. Depress plunger to first stop (mechanical pipettors) \_\_\_\_\_
- e. Do not drag tip/barrel across lip or neck of sample container or dilution blank, and do not allow pipettor barrel within sample container \_\_\_\_\_
- f. Insert tip approximately 0.5-1.0 cm below sample or dilution surface (avoid foam and bubbles) \_\_\_\_\_
- g. With pipettor vertical, slowly and completely release plunger on mechanical pipettor and remove from sample or dilution \_\_\_\_\_
- h. Touch off lower side of tip:
  - 1. To inside of sample container above the sample surface, excess liquid not adhering to tip \_\_\_\_\_
  - 2. Or to the inside of dilution blank neck or area above buffer on straight-walled containers, excess liquid not adhering to tip \_\_\_\_\_
    - a. For dilutions, hold pipettor nearly vertical with lower side of tip touching neck of dilution blank (or area above buffer on straight-walled containers), dispense test portion to blank by slowly depressing plunger to stop (mechanical pipettor) and then remove tip from container \_\_\_\_\_
    - b. For two (2) stop pipettors, depress plunger to second stop with tip remaining in contact with dilution blank and then remove tip from container \_\_\_\_\_
- i. Do not lay pipettor down once sample is drawn up, use vertical rack or charging stand if necessary \_\_\_\_\_
- j. Dispense test sample or dilution to rehydrated vial with lower side of pipettor tip in contact with neck of vial (mechanical pipettor) \_\_\_\_\_
- k. Discard tips into disinfectant OR dispose into biohazard bags or containers to be sterilized, (using this method of disposal does not require placing into disinfectant first) \_\_\_\_\_

## 11. Samples Other than Milk

- a. Weigh 11 g aseptically into a 99 mL dilution blank heated to 40-45°C

## 12. TEMPO Analysis

- a. Mix inoculated vials for 10 sec at maximum setting using a vortex-type mixer
- b. Check that the codes, colors and abbreviations (red=AC and blue=CC) on the cards and the vials of inoculated medium, match for lot being used
- c. Associate the identifier of the test sample with card and scan card barcodes corresponding inoculated medium vials with cards
- d. Put the vials containing the inoculated medium in the filling rack
- e. Insert the TAC or TCC card in the slot opposite the labeled vial, and aseptically place the transfer tube of the card inside the vial. The rack can hold up to 6 vials and cards and enables 1-6 TEMPO cards to be filled simultaneously
- f. Place filler rack containing a maximum of 6 samples is placed into the TEMPO Filler. After the cards have been filled (inoculated medium is completely aspirated into the card), the TEMPO Filler cuts and hermetically seals the cards all within 3 min
- g. Remove the filling rack from the TEMPO Filler and visually check that the vials are empty (inspect vial, if more than a small residual remains, discard and set up new vial and card)
- h. Prepare 1 filling rack of up to six samples at a time. Complete and place in incubator within 15 min of inoculating the vials. Multiple racks may be run if can be completed within 15 min (Scanning the card bar codes initiates a 15 min clock)
- i. Take the cards out of the rack, visually inspect for bubbles and if present tap cards to cause bubbles to rise to the top
- j. Transfer filled cards into incubation racks, insert the cards into the slots with the label on the card facing the user (towards the rack handle)
- k. Label tray with time of first and last card placed
- l. Each incubation rack can hold up to 20 cards Do not insert cards in between the slots
- m. Dispose of the used vials and transfer tubes into a biohazard receptacle
- n. Incubate TAC cards for 22-28 hours at 32 ±1°C and TCC cards for 22-27 hours at 32 ±1°C

### 13. Results and Interpretation

- a. Log on to the reading station.
- b. Place the incubation rack containing the cards to be read into the reader and assure rack properly seated in reader
- c. The reader scans the barcode of each card and interprets the results of fluorescence in the wells. It automatically associates the sample identifier with the type of test, the dilution and the enumeration results
- d. Reading of the TAC cards may be deferred at the end of incubation by storing them at 2-8°C for a maximum of 48 hours. In this case, allow the cards to come to room temperature (approximately 5-15 min) before introducing refrigerated cards into the reader (do not do as routine practice)
  - 1. If not possible to read cards at once, the activation of refrigeration Management and type must have been previously configured in TEMPO Admin. This functionality enables the warning « **card read too late** » obtained for a test result, to be replaced by a message indicating that the card has been refrigerated
- e. Editing the results: on the reading station screen, the number of colony forming units (CFU) per gram or milliliter of initial product is associated with the sample identifier, the parameter tested and the analysis date
- f. The reading station user interface enables the results to be printed out or transmitted to the laboratory information management system (LIMS). It also enables the records of the results obtained the previous days to be consulted
- g. At the end of the analysis, remove the cards from the rack and dispose of them into a biohazard receptacle

### REPORTING

#### 14. Reporting (see CP item 34.b.2.d)

**[When samples are demonstrated to contain inhibitors, no bacteria counts are reported; report as positive for inhibitors or growth inhibitors (GI)]**

- a. TEMPO AC
  - 1. Report computed count as TAC/mL or /g
- b. TEMPO CC
  - 1. Report count as TCC/mL or /g

- c. Report only first two left-hand digits \_\_\_\_\_
  - 1. If the third digit is 5 round the second number using the following rules \_\_\_\_\_
    - a. When the second digit is odd round up (odd up, 235 to 240) \_\_\_\_\_
    - b. When the second digit is even round down (even down, 225 to 220) \_\_\_\_\_
- d. If a laboratory accident renders a plate uncountable, report as laboratory accident (LA) \_\_\_\_\_