

**PASTEURIZED MILK CONTAINERS, CLOSURES, AND PACKAGING**  
**[Unless otherwise stated all tolerances are ±5%]**

**1. Laboratory Requirements** \_\_\_\_\_

- a. Record time and date when samples received \_\_\_\_\_
- b. Record time and date when samples examined \_\_\_\_\_

**RINSE METHOD APPARATUS**

**2. See Cultural Procedures (CP) items 1-23** \_\_\_\_\_

**3. To Add Rinse Solution to Containers** \_\_\_\_\_

- a. Sterile hypodermic syringes (capacity 20 or 100 mL) and needles \_\_\_\_\_
- b. Or, sterile pipets \_\_\_\_\_
- c. Or, sterile automatic syringe \_\_\_\_\_
- d. Or, sterile graduated cylinder \_\_\_\_\_
- e. Or, pre-dispensed dilution bottles or tubes with rinse solution (see CP item 29.f); volumes checked \_\_\_\_\_

**MATERIALS**

**4. See CP items 24-32** \_\_\_\_\_

**5. Rinse Solutions** \_\_\_\_\_

- a. Buffered Rinse Solution or Nutrient broth (see CP items 27.i-j) for Standard Plate Count (SPC) and Coliform Plate Count (CPC) agar based media \_\_\_\_\_
- b. Nutrient broth (see CP item 27.j) for 3M™ Petrifilm™ Aerobic Count (PAC), Coliform Count (PCC) and High Sensitivity Coliform Count (HSCC) plates \_\_\_\_\_

**6. Ethyl Alcohol, 70%** \_\_\_\_\_

**7. Plastic Tape** \_\_\_\_\_

**PROCEDURE**

**8. Identify Plates (SPC item 5 or Petrifilm item 6)** \_\_\_\_\_

**9. Controls (See SPC item 6 or Petrifilm item 7), in addition;** \_\_\_\_\_

- a. Transfer 1 mL of rinse solution to SPC or PAC plate for sterility control \_\_\_\_\_

**10. Rinse Solution Volumes for Collection of Surface Rinse Samples**

- a. 100 mL (+/- 2mL) for gallons (3784 mL) or larger
- b. 50 mL (+/- 1mL) for ½ gallons (1892 mL)
- c. 20 mL (+/- 0.4mL) for 100 mL to ½ pints (236 mL), pints (473 mL), and quarts (946 mL)
- d. For containers <100 mL and closures use swab method, see items 18-32
- e. Irregular shaped containers of <100 mL, use rinse method in item 10.c. Equally distribute the 20 mL among multiple units with the amount per unit no more than 20% of the volume

**11. Collection of Surface Rinse Samples**

- a. Firm walled paper containers, sealed on line
  - 1. Swab top of containers with 70% alcohol at the site of injection
  - 2. Add required amount of rinse solution to each container by injection and seal puncture with plastic tape
  - 3. Vigorously shake container length-wise on flat sides (or quadrants of round containers) 10 times, holding container horizontally
  - 4. Each shake a complete back and forth movement of approximately 20 cm
  - 5. Turn container 90° and repeat horizontal shaking treatment
  - 6. Turn container 90° twice more and repeat horizontal shaking
  - 7. Grasp container and swirl 20 times in a small flat circle while upright (top up)
  - 8. Invert (top down) and repeat swirling of container 20 times
  - 9. Stand upright and allow to drain for 1-3 min
- b. Plastic capped containers (submitted with caps)
  - 1. Swab top of container with 70% alcohol when appropriate
  - 2. Add required amount of rinse solution by aseptically removing cap, pouring in solution without touching the top and replace cap
  - 3. Complete rinse procedure as described in 11.a.3-9 above

- c. Flexible-walled containers/bags \_\_\_\_\_
  - 1. Add 100 mL aseptically by swabbing an area of tube adjacent to liner with 70% alcohol; introduce rinse by syringe and seal puncture with plastic tape \_\_\_\_\_
  - 2. Place container/bag on smooth, clean, firm horizontal surface as flat as its construction permits \_\_\_\_\_
  - 3. With hands or roller, move rinse solution back and forth 10 times, contacting all surfaces completely \_\_\_\_\_
  - 4. Lift liner and hang with "fill tube" down to permit rinse solution to collect for 1-3 min \_\_\_\_\_
  - 5. Transfer rinse solution to sterile container by cutting "fill tube" with sterile scissors \_\_\_\_\_
  
- d. Irregular shaped containers of <100 mL \_\_\_\_\_
  - 1. Swab top of container with 70% alcohol when appropriate e.g. at injection site \_\_\_\_\_
  - 2. Aseptically add required amount of rinse solution to each container, seal with cap or appropriate sterile closure \_\_\_\_\_
  - 3. Complete rinse procedure as described in 11.a.3-9 above \_\_\_\_\_
  - 4. Transfer rinse solutions of the multiple containers in sequence by aseptically removing cap or sterile closure, pouring solution into a common sterile container without touching the tops and replacing cap or sterile closure on the sterile container \_\_\_\_\_

**12. Sample Measurements** \_\_\_\_\_

- a. As described in SPC items 9 & 10 or Petrifilm items 10 & 11, except: \_\_\_\_\_
  - 1. For Residual Bacterial Count (RBC), pipet 2 mL portion in a single SPC plate or pipet two 1 mL portions on 2 PAC plates \_\_\_\_\_
  - 2. For Residual Coliform Count (RCC), pipet 10 mL of remaining rinse solution among 3 CPC plates, or pipet ten 1 mL portions of remaining rinse solution on 10 PCC plates or two 5 mL portions on 2 HSCC plates \_\_\_\_\_

**13. Pouring Agar (See SPC item 13)** \_\_\_\_\_

**14. Incubating Plates (See SPC item 14 or Petrifilm item 13)** \_\_\_\_\_

**15. Confirmation Test for CPC (See SPC item 17.c)** \_\_\_\_\_

**16. Counting and Recording Colonies**  
(See SPC items 15-17 or Petrifilm items 14-16)

- a. Count obtained from RBC plate(s) recorded as colonies counted
- b. If no colonies on RBC plate(s), record as 0
- c. Count obtained from RCC plates recorded as colonies counted
- d. If no colonies on RCC plates, record as 0
- e. Values are recorded as number of colonies per container

**REPORTS**

**17. Reporting Counts**

- a. Report computed bacterial count as RBC/container
  - 1. Containers rinsed with 20 mL
    - a. 2 mL plated for RBC, multiply colony count by 10
  - 2. Containers rinsed with 50 mL
    - a. 2 mL plated for RBC, multiply colony count by 25
  - 3. Containers rinsed with 100 mL
    - a. 2 mL plated for RBC, multiply colony count by 50
- b. Report computed coliform count as RCC/container
  - 1. Containers rinsed with 20 mL
    - a. 10 mL plated for RCC, multiply colony count by 2
  - 2. Containers rinsed with 50 mL
    - a. 10 mL plated for RCC, multiply colony count by 5
  - 3. Containers rinsed with 100 mL
    - a. 10 mL plated for RCC, multiply colony count by 10
- c. If no colonies appear on plate(s), report as less than n/container, substituting for n the number that would be reported if 1 colony had been counted from the volume of rinse solution plated and multiplied by appropriate factor

**SWAB METHOD**

**APPARATUS**

**18. See CP items 1-23** \_\_\_\_\_

**19. Screw-capped Containers** \_\_\_\_\_

a. 7 to 10 cm long to contain: \_\_\_\_\_

1. 5 mL rinse solution for non-soluble swabs (see item 5) \_\_\_\_\_

2. 4.5 mL rinse solution for alginate swabs (see item 5, SPC & CPC only) \_\_\_\_\_

b. Sterile \_\_\_\_\_

**20. Swabs** \_\_\_\_\_

a. Cotton, non-absorbent (firmly twisted to about 5 mm diameter by 2 cm long over one end of applicator stick 12-15 cm long) \_\_\_\_\_

b. Or, calcium alginate fibers (SPC & CPC only) \_\_\_\_\_

c. Or, polyester or rayon fibers \_\_\_\_\_

d. Commercial source, sterile, non-toxic in protected containers \_\_\_\_\_

1. Supporting documentation from manufacturer \_\_\_\_\_

2. Maintain records \_\_\_\_\_

**MATERIALS**

**21. See items 4 & 5** \_\_\_\_\_

**22. Sodium Hexa-metaphosphate Solution, 10% (if calcium alginate swabs used, SPC & CPC only), sterile** \_\_\_\_\_

**23. Shaking Machine, optional (See SPC item 8.c or PAC item 9.c)** \_\_\_\_\_

**PROCEDURE**

**24. Identify Plates (See SPC item 5 or Petrifilm item 6)** \_\_\_\_\_

**25. Controls (See SPC item 6 or Petrifilm item 7), in addition;** \_\_\_\_\_

a. Pipet 1 mL of rinse solution to SPC or PAC plate for sterility control \_\_\_\_\_

- b. For calcium alginate swab, break off swab head in container with 4.5 mL rinse solution plus 0.5 mL Na Hexa-metaphosphate solution and continue as described in 27.a.1, pipetting 1 mL rinse solution to plate for RBC sterility control of swab and bottle \_\_\_\_\_
- c. For all other fibers, break off swab head in container with 5 mL rinse solution and continue as described in item 27.a.2 & 27.b, pipetting 1 mL rinse solution to plate for RBC sterility control of swab and bottle \_\_\_\_\_

**26. Collection of Swab Samples from product contact surfaces** \_\_\_\_\_

- a. 250 sq cm of product contact surface must be swabbed or five 50 sq.cm for a total of 250sq. cm (calculate or use template – must be sterile if swab will be in contact with template) \_\_\_\_\_
- b. Aseptically remove sterile swab from container \_\_\_\_\_
- c. Open vial of solution, wet swab and press out excess solution \_\_\_\_\_
- d. Holding swab at 30° angle to surface, rub over 50 sq. cm area three times, reversing direction between successive strokes \_\_\_\_\_
  - 1. For snap or screw cap closures, calculate number of closures required for product contact surface area of 50 sq. cm \_\_\_\_\_
  - 2. For cup shaped containers, determine 50 sq. cm for the product contact surface \_\_\_\_\_
- e. Rinse swab in solution and press out excess \_\_\_\_\_
- f. Swab four additional 50 sq. cm areas \_\_\_\_\_
- g. After fifth area has been swabbed, position swab head in vial and break stick, leaving swab head in vial \_\_\_\_\_

**27. Sample Measurement** \_\_\_\_\_

- a. As described in SPC items 9 & 10; \_\_\_\_\_
  - 1. For calcium alginate, add 0.5 mL of sterile Na Hexa-metaphosphate solution (see item 22) to 4.5 mL rinse solution in vial and shake until dissolved **[Not acceptable for use with 3M Petrifilm]** \_\_\_\_\_
  - 2. For all other fibers: \_\_\_\_\_
    - a. Shake swab container 50 times \_\_\_\_\_
    - b. Each shake a complete back and forth movement of approximately 15 cm \_\_\_\_\_
    - c. Strike palm of hand at end of each cycle \_\_\_\_\_

- d. Complete shaking in approximately 10 sec \_\_\_\_\_
- b. As described in Petrifilm items 10 & 11; \_\_\_\_\_
  - 1. Shake swab container 50 times \_\_\_\_\_
  - 2. Each shake a complete back and forth movement of approximately 15 cm \_\_\_\_\_
  - 3. Strike palm of hand at end of each cycle \_\_\_\_\_
  - 4. Complete shaking in approximately 10 sec \_\_\_\_\_
- c. For RBC, pipet 1 mL portion to a single SPC or PAC plate \_\_\_\_\_
- d. For RCC, pipet 3 mL to a single CPC plate or three 1 mL portions on three PCC plates \_\_\_\_\_

**28. Pouring Agar (See SPC item 13)** \_\_\_\_\_

**29. Incubation (See SPC item 14 or Petrifilm item 13)** \_\_\_\_\_

**30. Confirmation for CPC test (See SPC item 17.c)** \_\_\_\_\_

**31. Counting and Recording Colonies (See SPC items 15-17 or Petrifilm items 14-16)** \_\_\_\_\_

- a. Count obtained from RBC plates, record as colonies counted \_\_\_\_\_
- b. If no colonies on RBC plates, record as 0 \_\_\_\_\_
- c. Count obtained from RCC plate(s) record as colonies counted \_\_\_\_\_
- d. If no colonies on RCC plate(s), record as 0 \_\_\_\_\_

**REPORTS**

**32. Reporting Counts** \_\_\_\_\_

- a. Report the count in 31.a as the RBC/50 sq. cm \_\_\_\_\_
- b. If no colonies on RBC plate, report as < 1/50 sq cm \_\_\_\_\_
- c. Report the count in 31.c as the RCC \_\_\_\_\_
- d. If no colonies on RCC plate(s), report as < 1 \_\_\_\_\_