

**FLAT LID METHODS  
IMS #27**

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

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

a. Record time and date when samples received \_\_\_\_\_

b. Record time and date when samples examined \_\_\_\_\_

**POUR CONTACT METHOD APPARATUS**

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

**3. Forceps, Sterile** \_\_\_\_\_

a. 140 mm hemostatic type preferred \_\_\_\_\_

**4. Tweezers, Sterile** \_\_\_\_\_

**5. Petri Dishes, Sterile** \_\_\_\_\_

**MATERIALS**

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

**7. Plate Count Agar (see CP item 27.b)** \_\_\_\_\_

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

a. In covered container large enough to hold forceps and tweezers \_\_\_\_\_

**PROCEDURE**

**9. Number of lids examined is the square root of the number of items in the package to a maximum of 21** \_\_\_\_\_

**10. Identify Petri Dishes (see SPC, 2400a item 5)** \_\_\_\_\_

**11. Controls (see SPC item 6)** \_\_\_\_\_

**12. Food Contact Surface extends beyond Lip** \_\_\_\_\_

a. Pour PCA (SPC item 13) into Petri dishes to a depth of 3 mm and allow to harden \_\_\_\_\_

b. Using sterile forceps, remove and discard end unit from package \_\_\_\_\_

c. Periodically remove lids by sliding stack from package with sterile forceps \_\_\_\_\_

- d. Place lid on agar with food with food contact surface in contact with agar, pressing lid against agar to ensure contact \_\_\_\_\_
- e. Repeat until required number of lids have been selected \_\_\_\_\_
- f. Incubate plate for 24 hours at  $32 \pm 1^\circ\text{C}$  \_\_\_\_\_
- g. Remove lid from each plate, incubate plate for another 24 hours \_\_\_\_\_

**13. Food Contact Surface Recessed** \_\_\_\_\_

- a. Select lids as in 12.b & c \_\_\_\_\_
- b. Place lid in dish with food contact surface up using sterile forceps \_\_\_\_\_
- c. Pour agar into the lid to a depth of 3 mm if possible \_\_\_\_\_
- d. Incubate for 24 hours at  $32 \pm 1^\circ\text{C}$  \_\_\_\_\_
- e. With sterile forceps, remove lid from dish and slip agar out of lid into dish with the lid contact side of agar up. (Sterile tweezers may be used to loosen agar from lid) \_\_\_\_\_
- f. Incubate dishes for another 24 hours at  $32 \pm 1^\circ\text{C}$  \_\_\_\_\_

**14. If lid diameter is >13 cm or constructed so that full agar contact is not possible, use swab test (1 lid per swab)** \_\_\_\_\_

**15. Coliform Test for Flat Lids (all sizes)** \_\_\_\_\_

- a. Use swab method (items 20-35) \_\_\_\_\_

**16. Controls – For Each Group of Samples (see SPC, 2400a item 6)** \_\_\_\_\_

- a. Check sterility of agar, Petri dishes and forceps \_\_\_\_\_
- b. Air exposure plate \_\_\_\_\_

**COUNTING, RECORDING AND REPORTING**

**17. Counting Colonies** \_\_\_\_\_

- a. See SPC, 2400a item 15 & 16 \_\_\_\_\_
- b. Count after  $48 \pm 3$  hours incubation \_\_\_\_\_
- c. Record counts \_\_\_\_\_

**18. Calculations** \_\_\_\_\_

- a. Determine food contact area in sq. cm \_\_\_\_\_

b. Divide number of colonies/lid by area \_\_\_\_\_

**19. Report** \_\_\_\_\_

a. Report the number of colonies/sq. cm for each lid \_\_\_\_\_

**SWAB METHOD**

**APPARATUS AND MATERIALS**

**20. See items 2-8** \_\_\_\_\_

**21. Buffered Rinse Solution (see CP item 27.i)** \_\_\_\_\_

**22. Sodium Hexa-metaphosphate Solution or Na Citrate, 7% Solution** \_\_\_\_\_

**23. Screw-capped Vials** \_\_\_\_\_

a. 7 to 10 cm long to contain 7 mL solution \_\_\_\_\_

b. Contain 6 mL rinse solution \_\_\_\_\_

c. Sterile \_\_\_\_\_

**24. Swabs** \_\_\_\_\_

a. Calcium alginate fibers on wood stick applicator \_\_\_\_\_

b. Non-toxic; tested using *Geobacillus stearothermophilus* type assay \_\_\_\_\_

1. Test each lot by swirling several swabs in 5 mL of sterile dilution buffer \_\_\_\_\_

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

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

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

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

**25. M-endo Broth Agar (see CP item 27.m)** \_\_\_\_\_

a. Dispense in membrane filter Petri dishes; 4-5 mL/dish \_\_\_\_\_

**26. Membrane Filters** \_\_\_\_\_

a. 0.45 µm pore size, 47 mm diameter \_\_\_\_\_

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

**27. Incubator, 35±1°C**

## PROCEDURE

### 28. Sample Size, 35 Lids/unit package

- a. See item 12.b-c for selection procedure

### 29. Identify Petri Dishes (see SPC, 2400a item 5)

### 30. Collection of Swab Samples

- a. Aseptically remove sterile swab from container
- b. Open vial of solution, wet swab and press out excess solution
- c. Holding swab at 30° angle to surface, rub over entire food surface contact area
- d. Position swab head in vial and break stick, leaving swab head in vial
- e. Repeat a-d for remainder of lids (34 vials)
- f. Repeat a-e with a second set of 35 lids for coliform determination (5 lids/vial)

### 31. Sample Measurement – SPC

- a. As described in SPC item 9, except;
  - 1. Add 1 mL sterile Na Hexa-metaphosphate or Na Citrate solution to each vial
  - 2. Shake vigorously until swabs dissolve
  - 3. Transfer vial contents to 2 plates

### 32. Sample Measurement – Coliforms

- a. Add 1 mL sterile Na Hexa-metaphosphate or Na Citrate solution to each vial
  - 1. Shake vigorously until swabs dissolve
  - 2. Add additional 1 mL phosphate or citrate solution and filter through membrane filter (item 26)
  - 3. Rinse filter and holder with sterile buffer (see CP item 25)
  - 4. Transfer filter to M-endo broth agar plate

### 33. Plating (See SPC item 13)

### 34. Controls – For Each Group of Samples (See SPC item 6)

- a. Check sterility of agars, Petri dishes, rinse solution and swabs

- b. Air exposure plate \_\_\_\_\_

**35. Incubation** \_\_\_\_\_

- a. See SPC item 14 \_\_\_\_\_
- b. Coliforms; 35±1°C for 18-24 hours \_\_\_\_\_

**COUNTING, RECORDING AND REPORTING**

**36. Counting Colonies** \_\_\_\_\_

- a. See SPC items 15 and 16 \_\_\_\_\_
- b. Count typical coliforms; dark red colonies with green metallic sheen \_\_\_\_\_

**37. Calculations** \_\_\_\_\_

- a. Determine food contact area in sq. cm \_\_\_\_\_
- b. Add colonies for 2 plates and divide by area for SPC \_\_\_\_\_

**38. Reporting** \_\_\_\_\_

- a. Report SPC as number of colonies/sq. cm \_\_\_\_\_
- b. Report coliforms as number colonies/lid \_\_\_\_\_

**ALTERNATE SWAB METHOD**

**APPARATUS AND MATERIALS**

**39. See items 2-8, 20-21, 22 and 24** \_\_\_\_\_

**40. Screw-capped Vials** \_\_\_\_\_

- a. 7 to 10 cm long to contain 10 mL of solution \_\_\_\_\_
- b. Contain 9 mL of rinse solution \_\_\_\_\_
- c. Sterile \_\_\_\_\_

**41. Plate Count Agar (see CP item 27.b)** \_\_\_\_\_

**42. Violet Red Bile Agar (see CP item 27.d)** \_\_\_\_\_

**PROCEDURE**

**43. See items 28-29** \_\_\_\_\_

**44. Collection of Swab Samples**

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- a. Aseptically remove sterile swab from container
- b. Open vial of solution, wet swab and press out excess solution
- c. Holding swab at 30° angle to surface, rub over entire food contact area
- d. Repeat b-c for remainder of lids (34)
- e. Position swab head in vial and break stick leaving swab head in vial

**45. Sample Measurement – SPC and Coliform**

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- a. As described in SPC item 9, except:
  - 1. Add 1 mL of sterile Na citrate solution to vial (see item 40)
  - 2. Shake vigorously until swab dissolves
  - 3. Transfer 2 mL of vial contents to each of 2 Petri dishes

**46. Plating (see SPC item 13)**

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- a. Add SPC to one plate
- b. Add VRBA to other plate

**47. Controls (see SPC item 6)**

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- a. Check sterility of agars, Petri dishes, rinse solution and swabs
- b. Air exposure plate

**48. Incubation (see SPC item 14)**

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**COUNTING, RECORDING AND REPORTING**

**49. Counting Colonies (see SPC items 16-18)**

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**50. Calculations**

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- a. Determine food contact area in sq. cm
- b. Multiply number of colonies on each plate by dilution factor of 5, divide by area of lid determined in item a

**51. Reporting**

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- a. Report SPC and coliform as number of colonies/sq. cm