

**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 ± 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

- 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

- 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)

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

47. Controls (see SPC item 6)

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

48. Incubation (see SPC item 14)

COUNTING, RECORDING AND REPORTING

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

50. Calculations

- 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

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