

**DISINTEGRATION METHOD FOR PAPER,
PAPERBOARD OR MOLDED PULP MATERIALS
IMS #26**

[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 _____

APPARATUS AND MATERIALS

2. See Cultural Procedures (CP) items 1-32 _____

3. Pipets _____

a. Sterile _____

b. 10 mL capacity with 3 mm tip opening _____

c. Or, 20 mL with large-bore opening _____

4. Pipet Containers (see CP item 7) _____

5. Sterile Scalpel or Scissors _____

6. Sterile Forceps _____

7. Disintegration Blender _____

a. High speed, electronically operated _____

b. Sterile corrosion-resistant cup _____

c. Capacity: 500 mL, optionally 1000 mL _____

8. Ethyl Alcohol, 70% _____

a. In covered container large enough to hold scalpels, scissors and forceps _____

9. Dilution Buffer (see CP item 25.a & c) _____

a. In containers filled to contain 300 ± 6 mL (or 500 ± 10 mL) _____

10. Sterile Kraft Paper or Envelopes _____

PROCEDURE

[Not applicable when wax, plastic or metal is food contact surface]

11. Use Sterile Cutting Device

- a. Cut 100 g from butt roll
- b. Transfer to sterile wrapper or envelope
- c. Trim off 5 cm of outer edge of sample sheet
- d. With sterile forceps, cut into 0.5 cm pieces 3 g of center portion into sterile Petri dish
- e. Transfer this 3 g portion into sterile disintegrator cup containing 300 mL dilution buffer (or 5 g in 500 mL)

12. Blending (take care to avoid dust, moisture and other contaminants)

- a. Place cup on blender motor
- b. Run at high speed for 30 sec; check to ensure no particles are on side of cup or trapped beneath blade
- c. Continue high speed blending for a total of 2 min depending on paper type. Check at intervals for particles on side of cup

PLATING

13. Plating

- a. Using sterile pipet, divide 10 mL of disintegrated sample equally among 3 plates (optionally use 5 plates)
- b. Pour agar (see SPC item 13)

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

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

15. Incubation (see SPC item 14)

- a. 32±1°C for 48±3 hours

COUNTING, RECORDING AND REPORTING

16. Counting Colonies

- a. See SPC items 15 and 16

17. Reporting

- a. Multiply the sum of the colonies on 3 (5) plates by 10 and record
- b. Report as the number of colonies/g of stock
