

**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  $300 \pm 6$  mL (or  $500 \pm 10$  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**

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- a. See SPC items 15 and 16

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**17. Reporting**

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- 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

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