**DISINTEGRATION METHOD FOR PAPER, PAPERBOARD OR MOLDED PULP MATERIALS**
**IMS #23a**

**[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 _____

## 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 _____