

# GMIA STANDARD METHODS FOR THE TESTING OF EDIBLE GELATIN



## 3. Microbiological Testing

### 3.1 Total Aerobic Count

#### PRINCIPLE

Use of plate-count agar media to enumerate in 48 hours at 35°C the level of microorganisms, including mesophilic bacteria, yeasts and moulds, by counting the colony forming units (CFU) per 1 g of gelatin.

#### REFERENCES

U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (USDA CFSAN) Bacteriological Analytical Manual (BAM) Aerobic Plate Count.

#### APPARATUS

1. Petri dishes at least 15 x 90 mm.
2. Sterile pipettes with pipetting aids.
3. Butterfield's phosphate buffer,  $90 \pm 1$  mL, sterile dilution bottles.
4. Plate Count agar or tryptic soy agar.
5. Circulating Water Bath thermostatically controlled to  $45 \pm 1^\circ\text{C}$ .
6. Incubator  $\pm 1^\circ\text{C}$ .
7. Colony counter, dark-field, Quebec, or equivalent, with suitable light source and grid plate.
8. BALANCE: with 0.1 g sensitivity.

#### PROCEDURE

##### 1/10 Solution preparation

1. Under aseptic conditions, weigh out  $10 \pm 0.1$  g of gelatin into a sterile Butterfield's phosphate buffer dilution bottle.
2. Leave the gelatin at room temperature to absorb the buffer for approximately 1 hour.
3. Transfer the bottle to the 45°C water bath.
4. Shake gently to ensure proper gelatin dissolution over a period of, maximum, one hour.

##### Inoculation

1. Aseptically transfer 1 mL of the 1/10 gelatin solution into each of two sterile Petri dishes.

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2. Immediately pour approximately 15 mL of tempered plate count agar ( $45 \pm 1^\circ\text{C}$ ) onto each dish.
3. Homogenize each plated sample and leave the agar solidify at room temperature.

## **Incubation**

1. Invert the solidified agar plates and incubate at  $35^\circ\text{C}$  for  $48 \pm 2$  hrs.

## **Aerobic plate count (apc) determination**

1. Following incubation count the number of colonies on each plate. For accurate enumeration the number of colonies counted per plate should be between 30 and 300.
2. Calculate the average of the counts and multiply by 10. Report as colony forming units per gram of gelatin (CFU/g). Report the absence of any colonies as  $<10$  CFU/g.