

GMIA STANDARD METHODS FOR THE TESTING OF EDIBLE GELATIN



3.2 *E. coli*

PRINCIPLE

The USP Method is a conventional cultural method that is applicable to the detection of viable *E. coli* in raw materials, finished products and environmental swabs. The entire detection testing protocol may take up to 3 days to complete. The FDA-BAM method is also applicable.

REFERENCES

Current USP Microbiological Tests.
(Also applicable is the current BAM method of Enumeration of *E. coli*)

REAGENTS AND SOLUTIONS

1. Lactose Broth
2. MacConkey Agar Medium
3. Levine Eosin-Methylene Blue Agar Medium
4. 1 N NaOH

APPARATUS

1. 500 mL Erlenmeyer flask
2. Sterile 1 mL pipette
3. Sterile Inoculating Loop
4. Water bath at 42°C
5. Refrigerator at 4°C
6. Incubator at 35°C
7. Balance
8. Bunsen Burner

PROCEDURE

Sample Preparation

1. Aseptically transfer 10 grams of sample into a sterile 500 ml Erlenmeyer flask containing 90 mL of sterile Lactose Broth Medium.
2. Swirl for 5 seconds to evenly distribute the enrichment broth through the sample.
3. For a flask of raw gelatin, swell at 25°C for 1 hour.

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4. Transfer the flask to a water bath at 42°C. Warm until completely dissolved. Swirl occasionally for 1 hour to ensure even distribution of the microorganisms. (A shaking water bath may be used).
5. Adjust pH using sterile 1 N NaOH or HCl, if necessary, to 6.8 ± 0.2 . Verify the final pH after addition of chemical (NaOH or HCl), using pH paper.
6. Incubate the broth solution for 24 ± 2 hour at 35°C.
7. Warm the broth solution in a 42°C in order to melt the gelatin.

Isolation

1. Warm the broth solution in a 42°C in order to melt the gelatin.
2. Examine the medium for growth, and if growth is present, mix by gently shaking and proceed with procedure.
3. Transfer a sterile loopful to the surface of MacConkey Agar Medium. Cover, invert, and incubate at 35°C for 24 ± 2 hours.
4. Upon examination, if none of the colonies conforms to the description given below, then the sample meets the requirements for absence of Escherichia coli.

Table – Morphologic Characteristics of Escherichia coli on MacConkey Agar Medium

Gram Stain	Characteristic Colonial Morphology
Negative Rods	Brick Red; may have surrounding zone of precipitated bile.

5. If colonies match the description given above, then proceed by transferring the suspect colonies, individually, by means of an inoculating loop, to the surface of Levine Eosin-Methylene Blue Agar Medium, plated on petri plates.
6. Cover, invert, and incubate the plates at 35°C for 24 ± 2 hours.
7. Upon examination, if none of the colonies exhibits both a characteristic metallic sheen under reflected light and blue-black appearance under transmitted light, the sample meets the requirements for the absence of E. coli.
8. The presence of E. coli can be confirmed by the API 20E, Enterotube method or other approved identification kit.