

# GMIA STANDARD METHODS FOR THE TESTING OF EDIBLE GELATIN



## 3.3 Salmonella

### PRINCIPLE

The AOAC/BAM Method is a conventional cultural method that is applicable to the detection of viable *Salmonella* in raw materials, finished products and environmental swabs. The entire detection testing protocol may take up to 5 days to complete.

### REFERENCES:

[FDA Bacteriological Analytical Manual \(BAM\), online. Updated June 2006 Chapter 5.](#)

Note: There are rapid methods for the detection of Salmonella in food and the environment, please select the link below for more information regarding rapid methods.

[Rapid Methods for Detecting Food borne Pathogens - BAM Manual Appendix 2](#)

### REAGENTS AND SOLUTIONS

- |   |                     |
|---|---------------------|
| 1. Lactose Broth                            | (BD/Difco 266520)   |
| 2. Tetrathionate Broth (TT)                 | (BD/Difco 249120)   |
| 3. Rappaport-Vassiliadis (RV)               | (BD/Difco 218581)   |
| 4. Xylose Lysine Desoxycholate (XLD) Medium | (BD/Difco 278830)   |
| 5. Hektoen Enteric (HE) Agar                | (BD/Difco 285340)   |
| 6. Bismuth Sulfite (BS) Agar                | (BD/Difco 273300)   |
| 7. Triple Sugar Iron (TSI) Agar             | (BD/Difco 226540)   |
| 8. Lysine Iron Agar (LIA)                   | (BD/Difco 284920)   |
| 9. 1 N NaOH                                 | (Aldrich 319511)    |
| 10. 1 N HCl                                 | (Aldrich 318949)    |
| 11. Papain Solution, 5%                     | (ABL-Coralase 7089) |

### APPARATUS

1. Incubator at 35°C
2. Incubator at 42°C
3. 500 mL wide-mouth, screw-cap jars, sterile (autoclavable)
4. 4 L volume autoclavable nalgene screw cap jars
5. Sterile Petri dishes

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6. 16x150mm culture tubes with caps
7. pH Paper Test Strips
8. 3 mm transfer loops (10 ul)

## Sample Preparation for Pre-enrichment of Gelatin:

**Gelatin.** Aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 mL) or other appropriate container. Add 225 mL sterile [lactose broth](#) and 5 mL 5% aqueous [papain solution](#) and mix well. For the U.S., a composite of up to 375g (15 x 25g samples) may be tested. Weigh the composited samples into a sterile, wide-mouth 4 L screw cap container and add approximately 3375 mL of Lactose broth and 75 mL of the 5% aqueous papain solution. For Europe, a composite of up to 250g (10 x 25g samples) may be tested. Weigh the composited samples into a sterile, wide-mouth 4L screw cap container and add approximately 2225 mL of Lactose broth and 50 mL of the 5% aqueous papain solution. Cap jar securely and incubate at 35°C for 60 ± 5 min. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2 using the 1N NaOH or 1N HCl. Loosen jar cap about 1/4 turn and incubate 24 ± 2 h at 35°C.

## Selective Enrichment

1. Tighten lid and gently swirl incubated sample mixture; transfer 1 mL mixture to 10 mL Rappaport-Vassiliadis (RV) medium and another 1 mL of mixture to 10 mL Tetrathionate (TT) broth.
2. Incubate the RV medium for 24 ± 2 hours at 42 °C and the TT broths 24± 2 h at 35°C.

## Selective Plating / isolation

1. Mix (vortex, if tube) and streak a 3 mm loopful of incubated TT broth on Bismuth Sulfite (BS) agar, Xylose Lysine Desoxycholate (XLD) agar, and Hektoen Enteric (HE) agar.
2. Prepare BS plates the day before streaking and store in dark at room temperature until streaked.
3. Repeat with a 3 mm loopful of SC broth.
4. Incubate plates 24 ± 2 h at 35°C.
5. Examine plates for presence of colonies suspected to be *Salmonella*.

### HEKTOEN ENTERIC (HE) AGAR

Typical *Salmonella* colonies may appear blue-green to blue colonies with or without black centers. Many cultures of *Salmonella* may produce colonies with large, glossy black centers or may appear as almost completely black colonies. Atypically, a few *Salmonella* species produce yellow colonies with or without black centers

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## BISMUTH SULFITE (BS) AGAR

Typical *Salmonella* colonies may appear brown, gray, or black; sometimes they have a metallic sheen. Surrounding medium is usually left brown at first, but may turn black in time with increased incubation, producing the so-called halo effect. Some strains may produce green colonies with little or no darkening of surrounding medium

## XYLOSE LYSINE DESOXYCHOLATE (XLD) AGAR

Typical *Salmonella* colonies may appear pink with or without black centers. Many cultures of *Salmonella* may have large, glossy black centers or may appear as almost completely black colonies. Atypically, a few *Salmonella* species produce yellow colonies with or without black centers.

## ATYPICAL *Salmonella* COLONY MORPHOLOGY

In the absence of typical or suspicious *Salmonella* colonies, search for atypical *Salmonella* colonies as follows:

1. **HE and XLD agars.** Atypically a few *Salmonella* cultures produce yellow colonies with or without black centers on HE and XLD agars. In the absence of typical *Salmonella* colonies on HE or XLD agars after  $24 \pm 2$  h incubation, then pick 2 or more atypical *Salmonella* colonies.
2. **BS agar.** Atypically some strains produce green colonies with little or no darkening of the surrounding medium. If typical or suspicious colonies are not present on BS agar after  $24 \pm 2$  h, then do not pick any colonies but reincubate an additional  $24 \pm 2$  h. If typical or suspicious colonies are not present after  $48 \pm 2$  h incubation, then pick 2 or more atypical colonies.

## Biochemical Media /screening and identification

1. Select 2 or more colonies typical of (or suspected to be) *Salmonella* from each selective agar. If no typical colonies are present, select representatives of atypical colony types to confirm the absence of atypical *Salmonella*. Inoculate into Triple Sugar Iron (TSI) agar and Lysine Iron Agar (LIA). If BS plates have no colonies typical of (or suspected to be) *Salmonella* or no growth whatsoever, incubate them an additional  $24 \pm 2$  h. ***If no growth is detected on any of the selective agar plates, results may be reported as negative per sample size tested.***
2. Lightly touch the very center of the colony to be picked with sterile inoculating needle and inoculate TSI agar slant by streaking agar slant and stabbing agar butt. Without flaming, inoculate LIA by stabbing agar butt twice and then streaking slant. Since lysine decarboxylation reaction is strictly anaerobic, the LIA slants must have a deep butt (4 cm). Store picked selective agar plates at 5-8°C.

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3. Incubate TSI agar and LIA slants at 35°C for 24 ± 2 h.
4. Cap tubes loosely to maintain aerobic conditions while incubating slants to prevent excessive H<sub>2</sub>S production. *Salmonella* in culture typically produces alkaline (red) slant and acid (yellow) butt, with or without production of H<sub>2</sub>S (blackening of agar) in TSI agar. In LIA, *Salmonella* typically produces alkaline (purple) reaction in butt of tube. Consider only distinct yellow in butt of tube as acidic (negative) reaction. Do not eliminate cultures that produce discoloration in butt of tube solely on this basis. Most *Salmonella* cultures produce H<sub>2</sub>S in LIA. Some non-*Salmonella* cultures produce a brick red reaction on LIA slants.
5. Suspect cultures should be streaked onto Brain Heart Infusion Agar plates and incubated overnight at 35°C. Micro Id (Rummel), API 20e (bioMerieux) or other approved identification kit may be run to get a quick identification as *Salmonella*. Always do an oxidase test before running Micro ID. Only oxidase negative cultures are run on Micro ID.

All cultures that give an alkaline butt in LIA, regardless of TSI reaction, should be retained as potential *Salmonella* isolates and submitted for biochemical and serological tests. Cultures that give an acid butt in LIA and an alkaline slant and acid butt in TSI should also be considered potential *Salmonella* isolates and should be submitted for biochemical and serological tests. Cultures that give an acid butt in LIA and an acid slant and acid butt in TSI may be discarded as not being *Salmonella*.