

9. 8.ENUMERATION OF *SALMONELLA* SPP.

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Protocol of *Salmonella* sp.

- Aseptically weigh 25 g sample into sterile blending container.
- Add 225 ml sterile BPW (Buffer peptone Water) and blend 2 min.
- Aseptically transfer homogenized mixture to sterile wide-mouth, screw-cap jar (500 ml) or other appropriate container and incubate for 18 hr at 37°C
- Transfer **0.1** ml mixture to 10 ml Rappaport-Vassiliadis (RVS) broth (PH 5.2) and another 1 ml mixture to 10 ml tetrathionate (TT) broth.
- Incubate RVS medium 24 ± 2 h at $41.5 \pm 0.2^{\circ}\text{C}$ (circulating, thermostatically controlled, water bath). Incubate TT broth 24 ± 2 h at 37°C.
- Mix (vortex, if tube) and streak on Bismuth sulfite (BS) agar, Xylose lysine desoxycholate (XLD) agar, and Hektoen enteric (HE) agar.
- Incubate plates 24 ± 2 h at 37°C.
- Examine plates for presence of colonies that may be *Salmonella*.

TYPICAL *Salmonella* COLONY MORPHOLOGY

Pick 2 or more colonies of *Salmonella* from each selective agar after 24 ± 2 h incubation. Typical *Salmonella* colonies are as follows:

- a. **Hektoen enteric (HE) agar.** 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.
- b. **Xylose Lysine Desoxycholate (XLD) agar.** Pink 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.
- c. **Bismuth Sulfite (BS) agar.** Brown, gray, or black colonies; sometimes they have a metallic sheen. Surrounding medium is usually brown at first, but may turn black in time with increased incubation, producing the so-called halo effect.

Biochemical and serological reactions of *Salmonella*

S. No	Test or substrate	Result	
		Positive	Negative
1	Glucose (TSI)	yellow butt	red butt
2	Lysine decarboxylase broth (LDB)	purple	yellow
3	H ₂ S (TSI and LIA)	blackening	no blackening
4	Urease	rose-pink color	phenol red
5	Indole test	red color at surface	Yellow brown color at surface
6	β-galactosidase	yellow colour	-----
7	O antigen	Agglutination occurs	NO
8	Vi antigen	Agglutination occurs	NO
9	H antigen	Agglutination	

Enumeration of *Salmonella*



