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Selective Enrichment Protocol for *Salmonella* Isolation from Surface Water

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This protocol describes the selective enrichment of *Salmonella* from surface water samples AFTER non-selective enrichment from several different recovery methods (Modified Standard Method 9260.B2, Vertical Modified Moore Swab, or Dead-end Ultra-filtration (DEUF).

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Salmonella isolation, surface water, Selective Enrichment

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Supplies Needed

- Tetrathionate broth (TT, Acumedia #7740), Prepare this as close to day of use as possible
- Rappaport-Vassiliadis *Salmonella* enrichment broth (RV, Acumedia #7730)
- Gram-negative Hajna broth (GN, Acumedia #7218)
- Brilliant Green Sulfa agar (BGS, Acumedia #7299)
- XLT4 agar (Acumedia #7517)
- XLT4 supplement (Accumedia # 7990) - Sodium tetradecyl sulfate (Tergitol 4)
- Sterile culture tubes

Selective Enrichment – Day 1

1 Dispense **9 mL** TT (Tetrathionate) broth into sterile test tubes.

2 - Dispense **9 mL** GN (Gram-negative Hajna broth) into sterile test tubes.

3 Hand massage UPB(Universal Pre-enrichment Broth)-enriched modified Moore swab (MMS), BPW (Buffered Peptone Water)-enriched filter cakes (47 mm glass filters), or shake BPW-enriched bulk water or 2X BPW-enriched backflush (from DEUF filtration) to homogenize before transfers to *Salmonella*-selective media.

4 

Aseptically transfer **1 mL** of either UPB or BPW enrichments into selective broths and then incubate at specified temperature listed in Table 1.

A	B	C	D
Broth	**Volume (mL)**	**Enriched UPB/BPW to add (mL)**	**Incubation Time and Temperature**
TT Broth	9	1	48 h @ 37°C
GN Broth	9	1	24 h @ 37°C

Table 1. Selective broth volumes and incubation parameters for *Salmonella* isolation.

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Day 2 – Selective enrichment transfers

5

- Transfer **100 µL** of 24 h GN broth enrichment into **9.9 mL** RV (Rappaport-Vassiliadis) broth.

6 1d

Incubate the GN/RV enrichment at **37 °C** for **24:00:00** .

Day 3 – Selective enrichment transfers continued

7

Transfer **100 µL** of 48 h TT broth enrichment into **9.9 mL** RV broth.

8 1d

Incubate the TT/RV enrichment at **37 °C** for **24:00:00** .

9

- For the GN/RV enrichment, vortex tubes and use a sterile 10 µL loop to streak for isolation onto both XLT4 (Xylose Lysine Tergitol 4) (prepared with Sodium tetradecyl sulfate, XLT4 supplement) and BGS (Brilliant Green Sulfa) agar plates.

10 1d

Incubate XLT4 and BGS plates at **37 °C** for **24:00:00** .

Day 4 – Selective enrichment transfers continued

11 For the TT/RV enrichment, vortex tubes and use a sterile 10 µL loop to streak for isolation onto both XLT4 and BGS plates

12 1d

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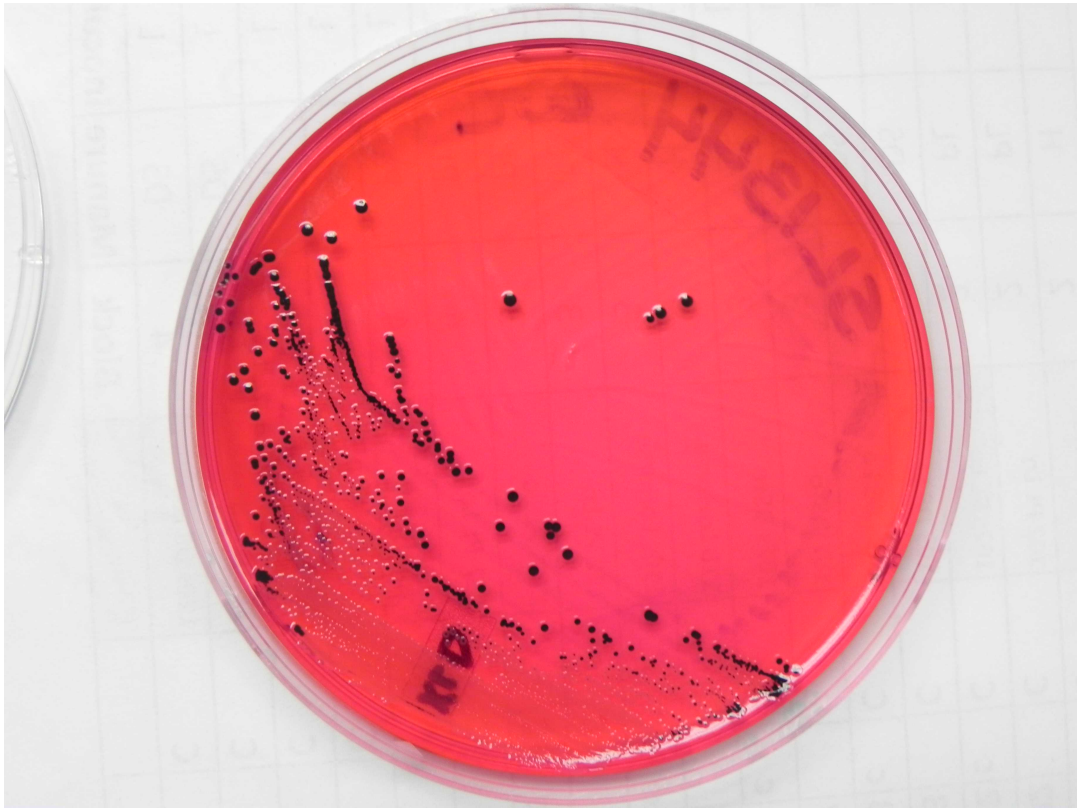
Incubate XLT4 and BGS plates at 37 °C for 24:00:00 .

13 Inspect XLT4/BGS plates from Day 3 (GN/TT) for presumptive *Salmonella* colonies.

13.1 From the XLT4 plates, select presumptive environmental *Salmonella* colonies (black raised center, clear halo) and streak onto fresh XLT4 plates. *Salmonella* colonies on BGS appear opaque and surrounded by red color on all edges.



Colonies of *Salmonella* Typhimurium isolated on XLT4 from pure culture (black raised center, clear halo).

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Colonies of *Salmonella* spp. isolated on BGS agar from pure culture (opaque colony surrounded by red).

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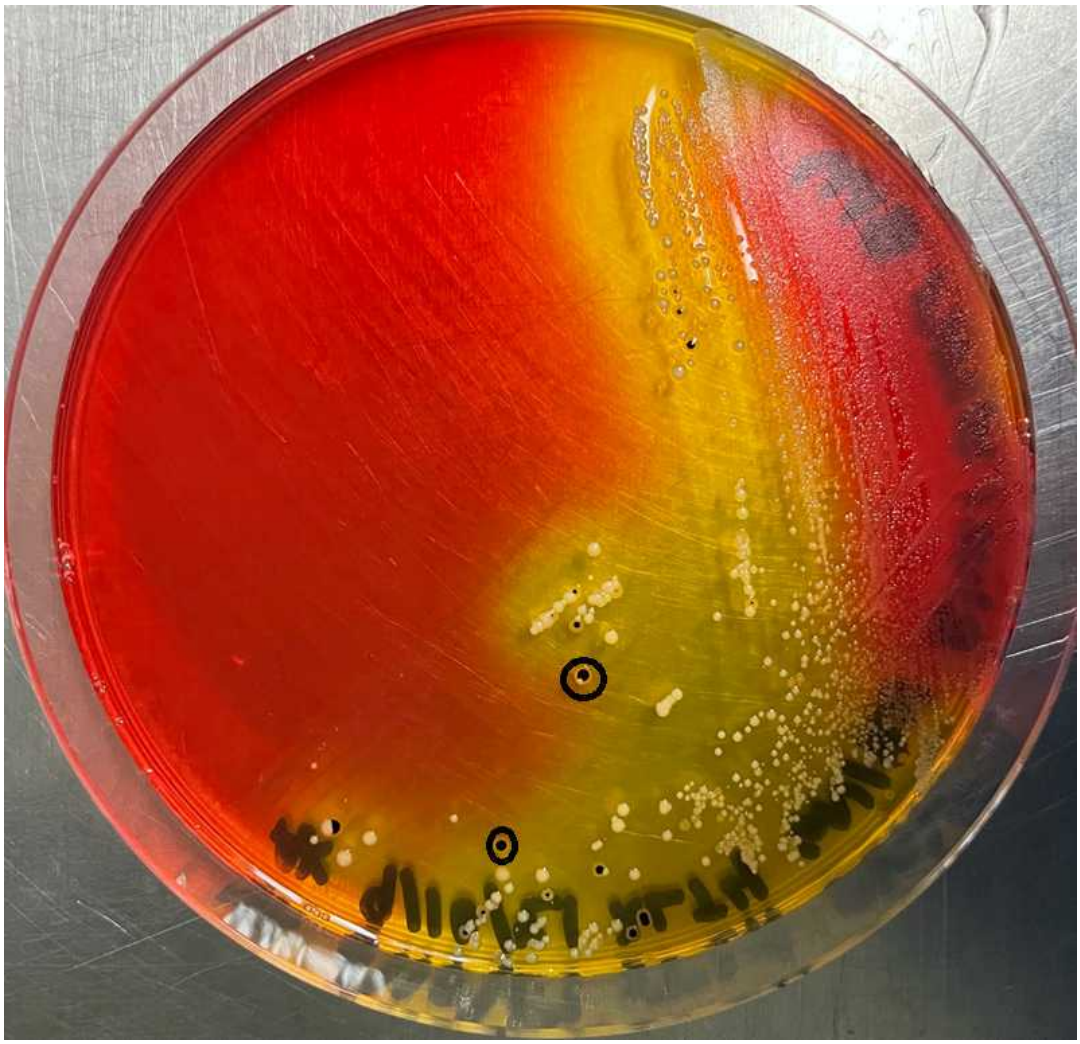

Colonies of *Salmonella* Typhimurium isolated from selective enrichment of surface water plated on XLT4: black colonies (surrounded by black circles for emphasis).

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Colonies of *Salmonella* Typhimurium isolated from selective enrichment of surface water plated on BGS; opaque colonies (surrounded by black circles for emphasis).

You can divide the XLT4 plates into ½ and streak multiple colonies on one plate to save agar / plates.

***Note**:* If control strain used**,** *for BioBall gfp-Salmonella Typhimurium, colonies should fluoresce when held under a UV light (395 nm).*

Day 5 – Colony confirmation

14 Inspect BGS and XLT4 plates from TT/RV enrichment and identify presumptive *Salmonella* colonies (see above).

15 Colonies from both Day 4 and 5 can be subjected to further confirmation using triple sugar iron (TSI) agar, lysine iron agar (LIA), and/or PCR (see following sections).

16 If non-fluorescent presumptive *Salmonella* isolates are observed, these isolates can be isolated

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and preserved (frozen stocks) for further characterization.

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