**Selective Enrichment Protocol for *Salmonella* Isolation from Surface Water**

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 broth into sterile test tubes.
2. Dispense 9 mL GN into sterile test tubes.
3. Hand massage UPB-enriched modified Moore swab (MMS), BPW-enriched filter cakes (g47 mm glass filters), or shake BPW-enriched bulk water or 2X BPW-enrich 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.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **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 |

**Day 2 – Selective enrichment transfers**

1. Transfer 100 µl of 24 h GN broth enrichment into 9.9 ml RV broth.
2. Incubate the GN/RV enrichment at 37oC for 24 h.

**Day 3 – Selective enrichment transfers continued**

1. Transfer 100 µl of 48 h TT broth enrichment into 9.9 ml RV broth.
2. Incubate the TT/RV enrichment at 37oC for 24 h.
3. For the GN/RV enrichment, vortex tubes and use a sterile 10 µL loop to streak for isolation onto both XLT4 (prepared with XLT4 supplement) and BGS agar plates.
4. Incubate XLT4 and BGS plates at 37oC for 24 h.

**Day 4 – Selective enrichment transfers continued**

1. For the TT/RV enrichment, vortex tubes and use a sterile 10 µL loop to streak for isolation onto both XLT4 and BGS plates
2. Incubate XLT4 and BGS plates at 37oC for 24 h.
3. Inspect XLT4/BGS plates from Day 3 (GN/TT) for presumptive *Salmonella* colonies
   1. From the XLT4 plates, select presumptive environmental *Salmonella* colonies (black raised center, clear halo) and streak onto fresh XLT4 plates for isolation. \*You can divide the XLT4 plates into ½ and streak multiple colonies on one plate to save agar / plates\*.
   2. ***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**

1. Inspect BGS and XLT4 plates from TT/RV enrichment and identify presumptive *Salmonella* colonies (see above).
2. 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).
3. If non-fluorescent presumptive *Salmonella* isolates are observed, these isolates can be isolated and preserved (frozen stocks) for further characterization.