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Protocol for Modified Standard Method 9260.B2 for the Isolation of Salmonella from Surface Water V.2

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Protocol status: Working
This protocol was used to recovery low levels of inoculated Salmonella Typhimurium (ca. 0.30 CFU/mL) from surface water at four different USDA ARS research locations.

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Keywords: National Program 108; Environmental Microbial and Food Safety Laboratory; modified standard method 9260.B2, Isolation, Salmonella, Surface Water;

Abstract

This protocol details the Modified Standard Method 9260.B2 for the recovery and isolation of *Salmonella* from 1 L of surface water.

Attachments

413-894.docx

31KB

Materials

Supplies Needed

- Sterilized graduated cylinders (1 L, 500 mL, 250 mL)
- Whirl-Pak® bags (Nasco #CE107372386)
- Sterilized forceps
- Pall magnetic filter funnel (Pall, 47mm, 300 mL, Filter Funnel Assembly **VWR Avantor Catalog #**28143-550)
- 1 L Buchner flask
- Set up for vacuum filtration
- 70% Ethanol (for Pall funnel decontamination)
- 47-mm glass fiber filters
  Binder-Free Glass Fiber Filters Pall Laboratory **VWR Avantor Catalog #**28150-995
- Aqua Dew™ cellulose pool-filter fiber – 2 × 0.5 g (sterile)
- Sterile deionized water
- 9 L Carboy for liquid waste collection
- 2 × 1 L Beakers for decontaminating Pall funnels (to contain 70% ethanol)
- 1 X Buffered peptone water (BPW, Accumedia #7417)
- Control strain, *Salmonellatyphimurium* BIOBALL® Luminate (BioMerieux, #422190)

Protocol

2m

1 Prepare for filtration by assembling vacuum set up, including a Buchner flask (collection) that fits the Pall magnetic filter funnel and can hold 1 L of water.

Note

Note: Set up should include a rubber stopper that fits Pall magnetic filter funnel.

2 Prepare a decontamination station for magnetic filter funnel by filling 2 1 L beakers with 900 mL 70% EtOH.

Note

Note: Ensure beakers are large enough to contain filter funnel and EtOH to cover funnel pieces.

3 Place one 47-mm glass fiber filter into the bottom magnetic filter funnel (Forceps work well to gently pull one filter from stack and place into filter funnel) attached to Buchner funnel.

3.1 If needed use sterile forceps to gently 47-mm glass fiber filter into place to fit into bottom of the funnel.

Forceps are being used to press the 47 mm glass fiber filter to the bottom of the magnetic funnel. The magnetic funnel is placed in a rubber stopper, which fits into a Buchner (sidearm) flask which is connected to a trap flask (if needed) and a vacuum.

4 If needed, add Control strain to surface water sample by gently tipping vial over the opening. Close bottle tightly and mix sample thoroughly before proceeding.

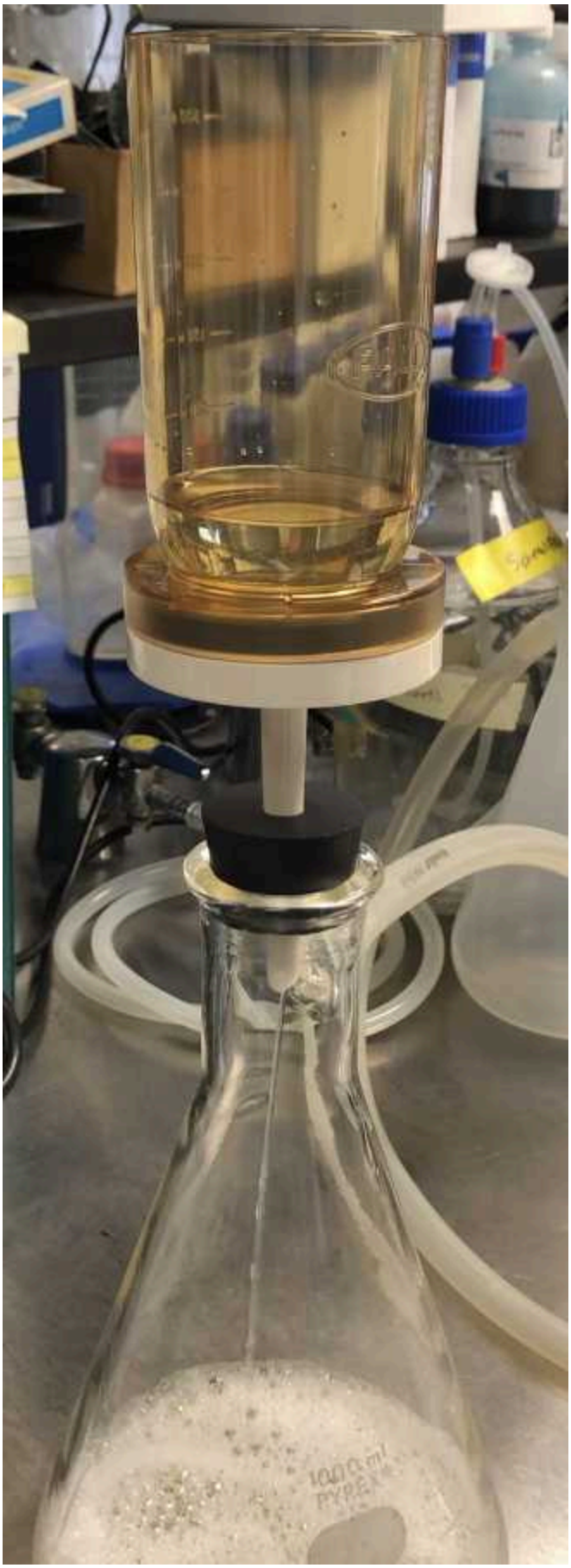
5 Measure 2 different sterile 0.5 g portions of the cellulose pool-filter fiber (Aqua Dew™), contained in sterile tubes, and suspend ONE of those portions in 15 mL of sterile deionized water.

6 Pre-load glass filter with the pool-filter fiber by applying vacuum and pouring the 15 mL water / pool-filter fiber suspension over the glass fiber filter.

7 Add the second 0.5 g portion of pool-filter fiber to the 1 L of sample water, close bottle tightly, and vigorously mix suspension.

8 Filter water sample by carefully pouring into 300 mL funnel and over pre-loaded 47 mm glass filter and allow vacuum to completely filter water sample.

8.1


1000 ml
PYREX

Water contained in 300 mL magnetic funnel and flowing over 47mm glass fiber filter with vacuum pressure applied.

9 If desired, use sterile deionized water to rinse off pool-filter fiber from sides of Pall filter funnel before turning off vacuum.

10 Carefully and aseptically, using sterilized forceps, transfer the 47 mm glass fiber filter (filter cake) from the filter funnel into a Whirl-Pak® sample bag.

Magnetic funnel containing 47 mm glass fiber filter is separated from base, inverted, and sterile forceps are used to nudge or poke glass fiber filter (filtercake) into sterile Whirl-Pak bag.

Note

NOTE: It is easier to pull magnetic portion of filter funnel off, tip on its side, and gently push filter cake from the bottom with sterile forceps, allowing it to fall into Whirl-Pak® bag.

11 Add 25 mL of 1 X BPW to Whirl-Pak bag and massage until filter cake until broken up.

12 Decontaminate filter funnel pieces (2) individually by dropping them into first beaker of 70% EtOH. Allow for a minimum of 00:02:00 contact time before transferring to second 70% EtOH bath. After a second 2-min soak, rinse filter funnel pieces with Del water and allow to dry.

2m

13 Incubate Whirl-Pak bag containing BPW-enriched filter cake for 18-24 h at 37 °C.

Note

For next steps, refer to “Selective Enrichment Protocol for *Salmonella* Isolation from Surface Water”.