Confirmation Protocol for *Salmonella*

Introduction

The following protocol has been developed by Hygiena™ to confirm the presence of *Salmonella* in food samples that were enriched according to the BAX® System method. The protocol has been modified from USDA-FSIS procedures.

Depending on the food type, samples are pre-enriched in either buffered peptone water, buffered peptone water with novobiocin, lactose broth, universal pre-enrichment broth, reconstituted nonfat dry milk, brilliant green water or trypticase soy broth. All samples (except meat and poultry, natural cheese, and raw frozen fish) then benefit from a 3-hour regrowth in BHI. These differences in enrichment, however, can make it difficult to confirm positive BAX® System results with traditional culture methods, especially when the sample contains low levels of the bacteria.

Various other factors over which the BAX® System has no control can also affect confirmation results. Low levels of *Salmonella* in a sample may be lost during refrigeration of the enrichment. Amplicon contamination in a lab might yield an initial positive result that does not confirm. Electrical noise, mislabeling and other errors can sometimes yield a non-confirmable positive result.

Although this confirmation protocol should be effective for most samples, some particularly difficult samples may require additional steps. For more information, contact technical support (800-863-6842).

Equipment and Supplies

- Incubator, static (42 ± 0.5°C)
- Micropipettors to deliver 15-1000 µl with sterile disposable filtered micropipette tips
- Mechanical pipettor with 1.0 ml, 5.0 ml 10.0 ml sterile pipettes
- Inoculating loops, “hockey sticks” or spreaders
- Dilution tubes (sterile >100 ml)

Media, Reagents and Cultures

- TT broth (Hajna)
- Rappaport Vassiliadis (RV) broth
- Brilliant green sulfa agar (BGS) plates
- Xylose lysine Tergitol™ 4 agar (XLT4) plates

- Original sample enrichments used with BAX® System
- Original regrowths used with the BAX® System
- Triple sugar iron agar (TSI) slants
- Lysine iron agar (LIA) slants

Procedure

1. Add 5 mL original sample enrichment to 100 mL TT broth.
2. Add 1 mL original sample enrichment to 100 mL RV.
3. Incubate the broths at 42 ± 0.5°C for 22-24 hours.
4. Remove two plates of each agar type (BG and XLT4) from refrigeration for a total of 4 plates per sample.
5. Spread TT suspension on BG and XLT4 plates, using 100 µL for each plate.
6. Spread RV suspension on BG and XLT4 plates, using 100 µL for each plate.
7. Examine plates at 18-24 hours for *Salmonella* and pick well-isolated typical colonies.

   • BGS - Select colonies that are pink and opaque with a smooth appearance and entire edge surrounded by a red color in the medium. On very crowded plates, look for colonies that give a tan appearance against a green background.

   • XLT4 - Select black colonies or red colonies with or without black centers. The rim of the colony may still be yellow in 24 hours; later it should turn red.
8. Pick at least three colonies from each plate, if available. (NOTE: Before any sample is reported as *Salmonella*-negative, a total of three typical colonies, if available, from each selective agar plate must be examined). Pick only from the surface and center of the colony. Avoid touching the agar because these highly selective media suppress growth of many organisms that may be viable.
9. Inoculate TSI and LIA slants in tandem with a single pick from a colony by stabbing the butts and streaking the slants in one operation. If screw cap tubes are used, the caps must be loosened. Incubate at 35 ± 2°C for 24 ± 2 hours.
10. Examine TSI and LIA slants as sets. Note the colors of butts and slants, blackening of the media and presence of gas as indicated by gas pockets or cracking of the agar. Note also the appearance of the growth on the slants along the line of streak. Discard, or re-streak for isolation, any sets that show "swarming" from the original site of inoculation. Discard sets that show a reddish slant in lysine iron agar. Isolates giving typical *Salmonella* spp. reactions and isolates which are suggestive, but not typical of *Salmonella* spp. should be confirmed by a combination of biochemical and serological procedures.

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