Hygiena™ BAX® System Weak Positive Analysis & Confirmation Techniques

Although the below verification and confirmation protocols should be effective for most sample matrices, some particularly difficult matrices may require additional steps. For more information, contact Diagnostics technical support at 1-800-863-6842 or diagnostics.support@hygiena.com.

If confirmation difficulties of typical weak or strong positive results continue to occur or if the rates of confirmation difficulties increase over time, it is good laboratory practice to:

- Include regular amplicon swab testing of the lab environment
- Test control samples of the various enrichment medias, buffers, and lysis solution to ensure no contamination with target organism DNA has occurred
- Reference Appendix B: PCR Contamination Control in the BAX Users Guide for more information and recommended areas for amplicon swabbing

Weak Positive *Listeria* Analysis and Confirmation:

1) If the typical weak positive result is located next to or near strong BAX positive results:
    a. Retest in duplicate or quadruplicate from the last enrichment to rule out any possible contamination from the pipetting transfers, decapping of cluster tube caps, or handling of strong positive sample enrichments
    b. See the Acting on Positives and Retesting Statistics Technical Bulletins (MTD-2007, MTD-2025, MTD-2055) for more information

2) A retesting option for typical weak positive result when using the BAX System PCR assay for Genus *Listeria* is:
    a. Retest at minimum in duplicate with the BAX System Real-Time PCR assay for Genus *Listeria* from the last enrichment of the weak positive sample.
        i. BAX System Real-Time PCR assay for Genus *Listeria* has a different genetic target on the primer, so it will only repeat as a positive result if viable *Listeria* DNA is present in the enrichment
        ii. A positive result due to amplicon contamination in the lab will not repeat due to the different primer in assay
        iii. The BAX System Real-Time PCR assay for Genus *Listeria* assay has greater than or equal sensitivity compared to the BAX System PCR assay for Genus *Listeria*.
    b. If the enrichment retest is positive using the Real-Time assay and you still don't get a confirmation on the plate, then it's just difficult to confirm and we suggest using our Herculean Protocol for *Listeria* confirmation outlined below.

3) Herculean Protocol for *Listeria* Confirmation (Figure 1):
    a. Plate 100 µL from final enrichment (MOPS-BLEB, LEB Complete, or 24 LEB Complete) to MOX, Ottaviani & Agosti (O&A or Aloa), and PALCAM agars
        i. Incubate at 37°C for up to 48 h
    b. From the final enrichment (MOPS-BLEB, LEB Complete, or 24 LEB Complete), transfer 100 µl to 9.9 mL Fraser Broth
        i. Incubate at 35°C for 18-24 h

ii. Alternative media option: transfer 100 µl to 9.9 mL 24 LEB Complete media , it has a gentle chemistry formulation that can allow a weak *Listeria* to grow.

c. Following 24 h incubation of Fraser Broth and/or 24 LEB Complete, plate 100 µl to MOX, Ottaviani & Agosti (O&A or Aloa), and PALCAM
   i. Incubate at 37°C for up to 48 h
d. Re-incubate the Fraser Broth or 24 LEB Complete enrichment for an additional 24 h at 35°C.
   i. Total enrichment time across multiple medias of 72 h to improve growth and isolation
   ii. NOTE: Plate on MOX, O&A, and PALCAM agars or only to the respective agars that have shown growth from the prior plating attempts

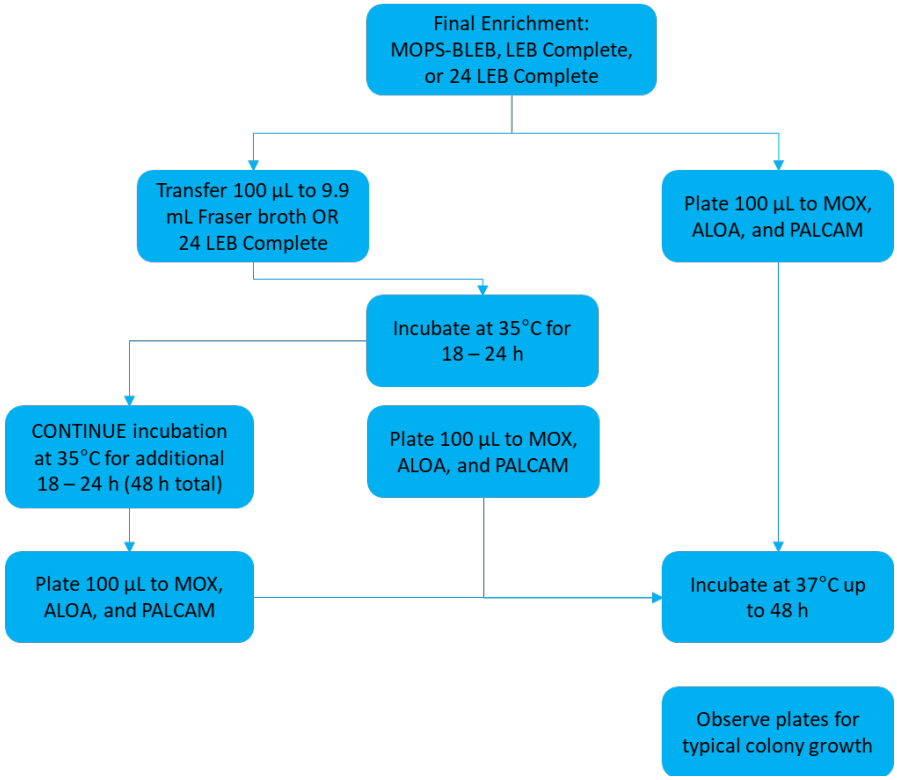

Figure 1. Herculean Protocol for *Listeria* Confirmation

4) Additional verification and confirmation method that can be attempted:
   a. Following the protocol in the User's Guide Appendix C: Confirmation Utility, utilize Sheep's Blood agar isolation and pick any isolated colonies to run on the Hygiena BAX Q7 System by
      i. If the sheep's blood agar plate has many colonies or has lawn growth
         1. Pick similar morphology or lawn streak into 300 µl of buffer (BPW, TSB, BHI) in a cluster tube, cap, and vortex
         2. Transfer 5 µl into the prepared BAX lysis buffer and follow recommended procedures
         3. Analyze using BAX Q7 system
      ii. If a positive, streak from the 300 µl cluster tube to MOX, Ottaviani & Agosti, or PALCAM for isolation.

Salmonella Weak Positive Analysis and Confirmation:

1) If the typical weak BAX positive result from the BAX System PCR Assay for *Salmonella* 2 or BAX System Real-Time PCR Assay for *Salmonella* is located next to or near strong BAX positive results of the respective assay:
    a. Retest in duplicate or quadruplicate from the last enrichment to rule out any possible contamination from the area amplicon, pipetting transfers, decapping of cluster tube caps, or handling of the strong positive sample enrichments
    b. See the Acting on Positives and Retesting Statistics Technical Bulletins (MTD-2007, MTD-2025, MTD-2055) for more information on the math behind this

2) Modified USDA/FSIS *Salmonella* confirmation method recommended by Hygiena (Figure 2):
    a. Transfer **5 mL** of original sample enrichment to **100 mL** Tetrathionate (TT) broth and **1 mL** original sample enrichment to **100 mL** Rappaport-Vassiliadis (RV) broth
        i. incubate at 42 ± 0.5°C for 22-24 hours
    b. From incubated TT solution:
        i. Spread plate 100 µl onto BGS and XLT4
        ii. Incubate at 35°C for up to 48 h
    c. From incubated RV solution:
        i. Spread plate 100 µl onto BGS and XLT4
        ii. Incubate at 35°C for up to 48 h
    d. Examine plates after 18-24 hours of incubation:
        i. Pick well-isolated typical colonies.
            1. 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
            2. 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
        ii. If no colonies are present, incubate at 35°C for remainder of 48 h
    e. Before any potential (non-typical) *Salmonella* isolate is reported as *Salmonella*-negative:
        i. Pick a total of three typical colonies, if available, from each selective agar plate
            1. 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
        ii. Transfer picked colonies to TSI and LIA slants in tandem with a single pick from a colony from the BGS or XLT4 plates by stabbing the butts and streaking the slants in one operation.
            1. If screw cap tubes are used, the caps must be loosened
            2. Incubate at 35 ± 2°C for 24 ± 2 h
        iii. Examine TSI and LIA slants as sets
            1. 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

2. Note also the appearance of the growth on the slants along the line of streak
3. Discard, or re-streak for isolation, any sets that show "swarming" from the original site of inoculation
4. Discard sets that show a reddish slant in LIA

iv. Isolates which are suggestive, but not typical of *Salmonella* spp. can be confirmed by running the isolate on the BAX System Real-Time PCR assay for *Salmonella* or BAX System PCR assay for Salmonella 2 by following the procedure in the BAX Users Guide in Appendix C: Confirmation Utility.

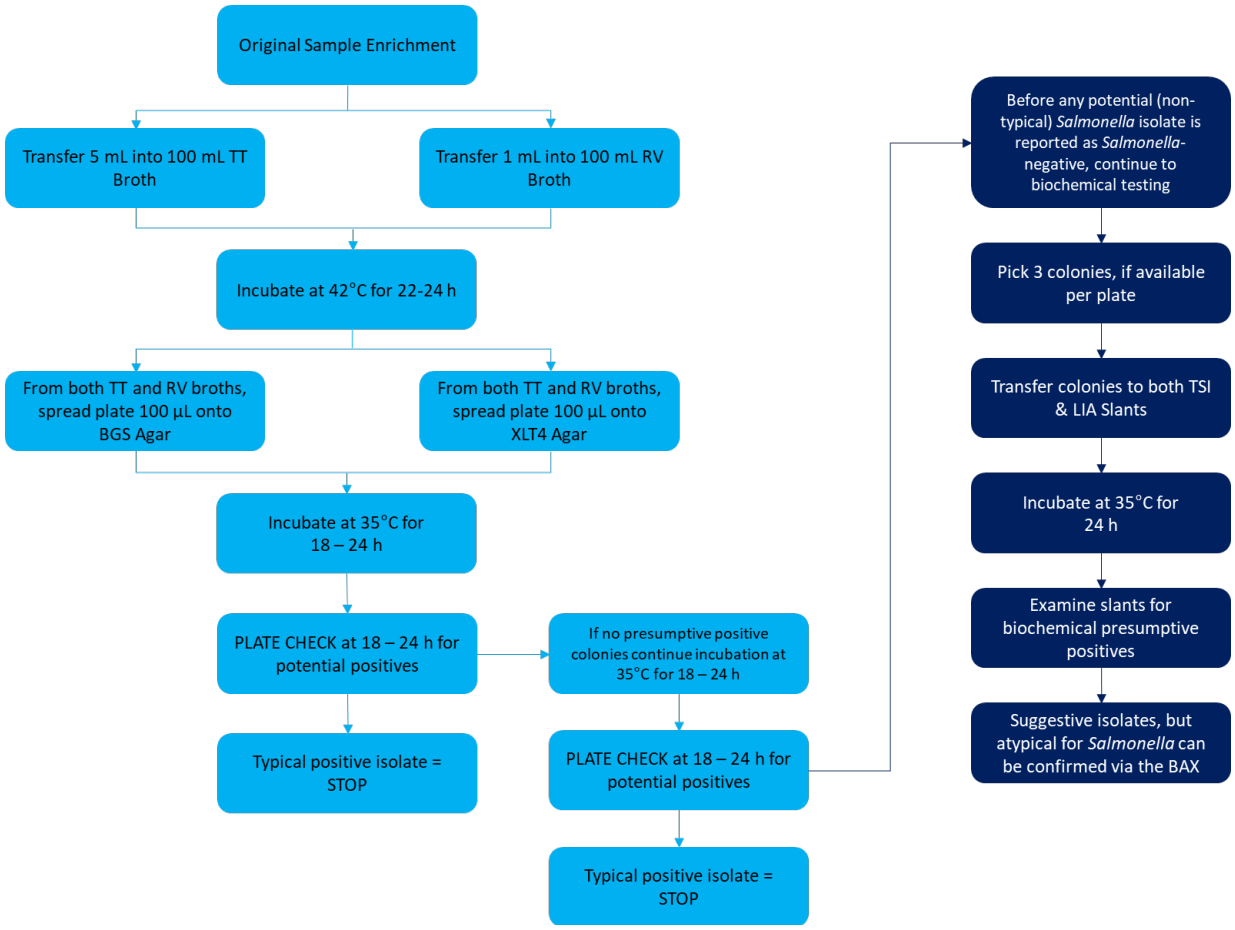

Figure 2. Modified USDA/FSIS *Salmonella* confirmation method recommended by Hygiena