BAX® System Real-Time PCR Assay
Highly Pathogenic *Salmonella*

Product Number KIT2048

KIT CONTENTS

- 96 PCR tubes with lyophilized ready-to-use PCR mix. (1 bag of 12 x 8 strips in 96 well tray)
- 96 flat optical caps (12 x 8 strips)
- 1 bottle of protease (400 µL)
- 2 bottles of lysis buffer (12 mL)

INTENDED USE

Overview

The BAX® System Real-Time PCR Assay provides food processors and associated laboratories with a rapid, reliable method for detecting highly pathogenic *Salmonella* in various food products, including poultry, pork, and beef. This system is engineered to specifically target three *Salmonella* markers commonly found in most highly pathogenic *Salmonella* serotypes. Additionally, it has the capability to detect general *Salmonella* spp., thereby exceeding the capabilities of traditional kits that only identify general *Salmonella* spp.

This advanced kit comes in the form of ready-to-use lyophilized pellets, each individually housed in tubes, which greatly simplifies the testing process. Users can either hydrate pellets from previously prepared Salmonella-positive lysates or create colony confirmation lysates by following a simple BAX lysis protocol and initiating PCR, resulting in a seamless, efficient workflow for precise, rapid pathogen detection. Furthermore, the inclusion of an internal positive control facilitates easy verification of PCR runs.

In summary, the BAX® System Real-Time Highly Pathogenic *Salmonella* kit provides a comprehensive and reliable solution to enhance food safety protocols, offering users confidence in their ability to identify and mitigate potential risks associated with Highly Pathogenic *Salmonella* contamination.

BAX® Systems are designed for use by qualified lab personnel who follow standard microbiology laboratory practice, including the safe handling and disposal of potentially pathogenic materials. The laboratory must comply with good laboratory practice (see ISO 7218 standard).

Field of use

IMPORTANT USE AND INTERPRETATION NOTICE

INTENDED USE – RESEARCH USE ONLY

For Research Use Only. Not for use in diagnostic procedures or for making food safety-release decisions.

The BAX® System Highly Pathogenic *Salmonella* (HPS) Assay is intended for use only after an initial positive BAX System *Salmonella* spp. screening result has been obtained using the same lysate prepared from the enrichment sample or a lysate prepared from isolated colonies. The assay is designed to provide additional information on the presence of genetic biomarkers associated with *Salmonella* spp., thereby supporting research into *Salmonella* spp. population diversity.

Results generated by this assay are intended exclusively for research and ecology surveillance. They shall NOT be used for food safety, regulatory, or product release decisions.

Limitations

The assay is performed directly on BAX System *Salmonella*-positive lysates from the enrichment samples and does not differentiate individual *Salmonella* strains present within mixed populations.

If multiple *Salmonella* strains are present in the same enrichment, the detected biomarker profile represents the combined genetic content of all strains present. Therefore, pathogenicity biomarkers detected by the assay cannot be assigned to a specific strain, and the overall biomarker profile may not represent any single *Salmonella* isolate.

Detection of biomarkers associated with highly pathogenic *Salmonella* does not confirm that these biomarkers originate from a single organism or that an individual isolate possesses the complete combination of detected markers.

Characterization of individual *Salmonella* strains requires the isolation of pure colonies followed by appropriate confirmatory and molecular characterization.

Based on the USDA-ARS HPS concept

The BAX® Highly Pathogenic *Salmonella* (HPS) assay is based on the Highly Pathogenic *Salmonella* (HPS) concept developed by the U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS)[1]. The assay incorporates a selected subset of the original biomarkers to provide insights into the potential pathogenic profile of *Salmonella*. The HPS concept has primarily been evaluated using *Salmonella* genomes, isolates, and epidemiological data from the United States, with a particular focus on meat and poultry. Therefore, the distribution and predictive value of the targeted biomarkers may differ in other geographical regions or food sectors**.** The pathogenicity of *Salmonella* is determined by numerous genetic factors. The HPS concept evaluates a selected subset of biomarkers and therefore does not assess all determinants of virulence. Consequently, some strains or serovars, including attenuated vaccine strains (e.g., those of *S.* Typhimurium or *S.* Enteritidis), may produce biomarker profiles similar to those of their parental strains. HPS results should be interpreted as providing additional epidemiological insight and not as a definitive assessment of pathogenicity.

PRINCIPLE OF THE METHOD

See the BAX System User Guide for an overview of how the BAX System method uses automated, real-time Polymerase Chain Reaction (PCR) technology.

[1] Harhay et al., 2024. Front. Microbiol., Sec. Food Microbiology, Volume 15 https://doi.org/10.3389/fmicb.2024.1504621

MATERIALS

- BAX System Real-Time PCR Assay Highly Pathogenic *Salmonella* Part KIT2048
- BAX System start-up package (equipment and supplies for up to 192 tests)
    - BAX System Q7 cycler/detector and computer workstation
    - Heating blocks with inserts* capable of maintaining 37 ± 2 °C and 95 ± 3 °C
    - Cooling blocks with inserts*
    - PCR tube holder
    - Capping/decapping tools
    - Adjustable mechanical pipettes (5-50 µL; 20-200 µL; 100-1000 µL)
    - Repeating pipette
    - Multi-channel pipette (8 channels – 5-50 µL)
    - Cluster tubes with caps and racks
    - Pipette tips with barriers
    - Powder-free nitrile gloves
    - Stomacher with bags
    - Incubator capable of maintaining directed enrichment temperatures within ± 2 °C

Optional: PCR strip or plate centrifuges

- Without vortex: Mini microcentrifuge for 4 x 8-strips
- With vortex: Multispin MSC-6000 for 4 x 8-strips
- With vortex: CVP-2 for 12 x 8-strips and plates

*The Automated Thermal Block (Catalog No. MCH2023) may be used instead of heating and cooling blocks.

STORAGE AND SHELF LIFE

- Reagents and PCR tubes with tablets should be kept refrigerated at 2 to 8°C. Do not freeze.
- Reagents should be used by the expiration date stamped on the individual labels.
- After protease has been added to the lysis buffer, shelf life of the solution is 2 weeks when stored at 2 to 8 °C.
- If storing PCR tubes with tablets in an open kit for more than 3 weeks, seal the aluminum-lined resealable bag of PCR tubes into a larger bag with desiccant or store at 4 °C in a desiccation unit, if possible.

SAFETY PRECAUTIONS

The BAX System method includes sample enrichment procedures that nourish the growth of potential pathogens to detectable levels. Because pathogens can cause human illness, appropriate safety precautions must be taken and personal protective equipment must be worn when handling samples, media, reagents, glassware and other supplies and equipment that could be contaminated with potentially pathogenic bacteria.

Reagents used with the BAX System assays should pose no hazards when used as directed. Before using this assay, please review the Safety Data Sheet (SDS) included with your BAX System purchase; it is also available at www.hygiena.com/documents. Refer to your site practices for safe handling of materials at extreme temperatures.

SOFTWARE REQUIREMENTS

Before using this assay for the first time, install the most current version of the BAX System software, then run a calibration report to check that “Real-Time Highly Pathogenic *Salmonella*” appears in the list of calibration files. See “Troubleshooting Calibration” in the BAX System User Guide for details.

If the report list does not contain 'Real-Time Highly Pathogenic *Salmonella*', you must recalibrate the Q7 instrument to load the required dyes. Be sure to allow enough time to complete the calibration (about 1.5 to 2 hours) before starting the assay. For instructions and tips on calibrating the instrument, see the BAX System User Guide.

TEST PROTOCOLS

OPTION 1: From *Salmonella*-Positive Lysate Samples

1. Prepare Equipment as in standard PCR assays

    1.1 Make sure cooling blocks are chilled to 2 to 8 °C*.

    *If using the Automated Thermal Block, follow the instructions in the Automated Thermal Block User Guide for running the Gram-Negative program.

2. Hydrate the lyophilized PCR reagents

    2.1 Initialize the instrument by selecting RUN FULL PROCESS from the OPERATION menu.

    2.2 Place a PCR tube rack onto the chilled (2 to 8 °C) PCR cooling block.

    2.3 Arrange strips of PCR tubes according to your rack file.

    2.4 Remove the caps from the first strip of tubes.

    2.5 Transfer 30 µL Salmonella-positive lysate into PCR tubes, then seal with flat optical caps.

    2.6 Repeat with remaining strips of PCR tubes until all PCR tablets have been hydrated.

    2.7 Seal the tubes tightly with the provided 8-cap strip.

    2.8 Mix thoroughly using a vortex centrifuge. Alternatively, carefully resuspend pellet by pipetting up and down (i.e., manual mixing) multiple times in step 2.5.

    2.9 If manual pipetting is done in 2.8, briefly spin PCR tube strips for 5 sec at 150 - 500 x g in suitable centrifuge. Otherwise, move to step 3.

3. Amplify and Detect

    3.1 At the 'Ready for Rack Load' prompt, click the NEXT button and open the instrument drawer.

    3.2 Place the rack of PCR tubes over the wells in the drawer, and check that the tubes are seated correctly.

    3.3 Close the drawer and click the NEXT button to begin automated processing.

OPTION 2: From Suspect *Salmonella* Colonies (from Selective Agar Plates)

1. Prepare Equipment

    1.1 Turn on the heating blocks to 37 °C and 95 °C*.

    1.2 Make sure cooling blocks are chilled to 2-8 °C*.

    *If using the Automated Thermal Block, follow the instructions in the Automated Thermal Block User Guide for running the Gram-Negative program.

    1.3 Power on the Q7 instrument and launch the BAX® System application.

    1.4 Create a rack file (see User Guide for details).

2. Colony Identification/Lysate Preparation

   2.1 Examine selective agar plates for suspect colonies and identify those that require confirmation.

   2.2 Add 1 mL of sterile diluent (BPW) to sterile microcentrifuge tubes.

   2.3 Pick a suspect colony using a sterile disposable needle.

   2.4 Dip the needle into the corresponding dilution tube and swirl the needle to release the colony. Remove the needle, then cap the tube and mix.

   2.5 Prepare cluster tubes and arrange in a rack according to the rack file.

   2.6 Prepare lysis reagent by adding 150 µL protease to one 12 mL bottle of lysis buffer.

   2.7 Transfer 200 µL lysis reagent to each cluster tube.

   2.8 Transfer 5 µL cell suspension from the dilution tube to the corresponding cluster tube.

   2.9 Heat at 37 °C for 20 minutes.

   2.10 Heat at 95 °C for 10 minutes.

   2.11 Cool at 2 to 8 °C for at least 5 minutes.

3. Hydrate the lyophilized PCR reagents

   3.1 Initialize the instrument by selecting RUN FULL PROCESS from the OPERATION menu.

   3.2 Place a PCR tube rack onto a chilled (2 to 8 °C) PCR cooling block.

   3.3 Arrange strips of PCR tubes according to your rack file.

   3.4 Remove the caps from the first strip of tubes.

   3.5 Transfer 30 µL lysate (from Step 2.11) into PCR tubes, then seal with flat optical caps.

   3.6 Repeat with remaining strips of PCR tubes until all PCR tablets have been hydrated.

   3.7 Seal the tubes tightly with the provided 8-cap strip.

   3.8 Mix thoroughly using a vortex centrifuge. Alternatively, carefully resuspend pellet by pipetting up and down (i.e., manual mixing) multiple times in step 3.5.

   3.9 If manual pipetting is done in 3.8, briefly spin PCR tube strips for 5 sec at 150 - 500 x g in suitable centrifuge. Otherwise, move to step 4.

4. Amplify and Detect

   4.1 At the ‘Ready for Rack Load’ prompt, click the NEXT button and open the instrument drawer.

   4.2 Place the rack of PCR tubes over the wells in the drawer, and check that the tubes are seated correctly.

   4.3 Close the drawer and click the NEXT button to begin automated processing.

RESULTS REVIEW

1. Review Results

   Qualitative results are displayed as a grid of color-cued icons in the top half of the screen.

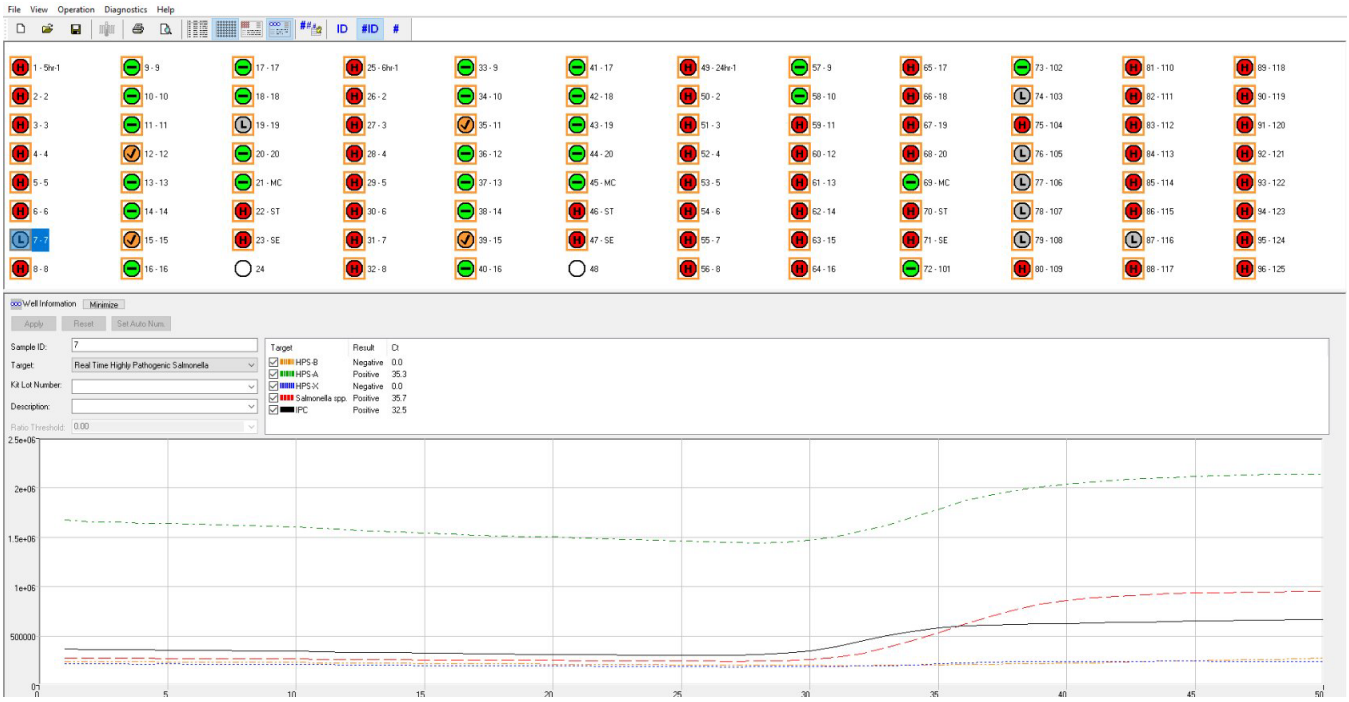

Figure 1: Screenshot from BAX Q7 Software showing Highly Pathogenic *Salmonella* assay menu options. The top panel is the rack-view grid of color-coded icons with HPS-positive wells shown as Red (M/H), *Salmonella* spp. positive wells or *Salmonella* + A target positive wells shown as Grey (L), and negative wells shown as Green (-) icons. The bottom panel shows the amplification PCR curves for the selected sample.

- Green (-) = Negative for *Salmonella* spp. and HPS targets
- Grey (L) = *Salmonella* spp. **and/or** 'A' target detected
- Red (M) = 'B' target **or** 'X' target detected
- Red (H) = 2 or 3 HPS targets detected
- Orange (✓) = Secondary enrichment recommended
- Yellow (?) = Indeterminate
- Yellow (? with slash) = Signal error

Note: For lysates with low concentrations of *Salmonella* DNA near the limit of detection, it is possible that not all targets (HPS markers or *Salmonella* spp.) that exist in the sample will be detected.

If the Ct value for Salmonella spp. is very high and no 2 or 3 HPS targets are positive, the software displays the orange icon "Secondary enrichment recommended." By performing a secondary enrichment as outlined in Appendix A, the concentration of *Salmonella* present will increase and a potential HPS misclassification due to insufficient DNA concentration will be avoided. On the other hand, a sample can be positive for HPS even if the result for *Salmonella* spp. is negative.

DISPOSAL

Decontaminate materials and dispose of biohazardous waste per your site practices and as required by federal, state and local regulations.

TECHNICAL ASSISTANCE

For questions, comments or assistance, please contact your local distributor or contact us at www.hygiena.com/support.

You can also call 800-863-6842 in the US, 1- 302-695-5300 outside the US, or email us at diagnostics.support@hygiena.com.

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4. The accuracy of the BAX® System can be affected by factors over which Hygiena has no control, including, without limitation, the use of the Equipment, assays and/or media in a manner that is contrary to the conditions of use, the procedures or the instructions specified by Hygiena. Because of the large number of factors over which Hygiena has no control, Hygiena makes no promise or guarantee of the accuracy of, or results obtained from the use of the BAX® System. In particular, Hygiena disclaims any warranty or liability and assumes no responsibility whatever for the failure of the BAX® System due, in whole or in part, to user's failure to: (a) properly maintain Equipment, (b) maintain specified operating or storage conditions, (c) follow the specified instructions, or (d) use the proper microbiological techniques consistent with the standard of care accepted in the industry for the proper collection, storage, handling and preparation of the sample.
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6. The BAX® System is designed to test only for the presence of the target organisms specified in the particular assay. The BAX® System has been tested against many, but not all, strains of the target within the sample types specified in the user documentation. Hygiena, therefore, cannot and does not make any representation or warranty that the BAX® System is capable of detecting every organism in the target genus, serotype, or species in any sample source. Accordingly, the BAX® System should not be used as the sole test for the release of user's products, nor should it be used as the sole basis for determining the safety of user's products.
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8. THE SOLE AND EXCLUSIVE REMEDY OF CUSTOMER/USER, AND THE SOLE AND EXCLUSIVE LIABILITY OF HYGIENA, ITS AFFILIATES, DISTRIBUTORS, LICENSORS OR REPRESENTATIVES FOR ANY AND ALL CLAIMS, INCLUDING BREACH OF WARRANTY, TORT, CONTRACT, STRICT LIABILITY, NEGLIGENCE OR OTHERWISE, SHALL BE LIMITED TO THE FOLLOWING:

   a) Should Equipment fail to conform with the Paragraph 2 warranty, Hygiena shall, at its option: repair or replace the non-conforming Equipment with new or refurbished (repaired or rebuilt) functionally equivalent Equipment or refund the purchase price; (b) Should BAX® Software fail to conform with the Paragraph 3 warranty, Hygiena will replace it free of charge; (c) For all other claims, Hygiena may, at its option, refund the purchase price or replace the Equipment, assays or media; (d) In all cases, user is responsible for the repackaging and return of non-conforming Equipment, along with the reinstallation of new or refurbished Equipment; and (e) Equipment, assays or media shall not be returned without prior written permission from Hygiena, and then only in the manner prescribed by Hygiena. The maximum liability of Hygiena, its affiliates, distributors and licensors, and whether or not based on negligence, shall not exceed in the aggregate the amount equal to: (a) the purchase price of the BAX® System, assay or media for which damages are claimed, or (b) in the case of BAX® Software, the amount paid for the software (if licensed separately) or twenty-five thousand dollars ($25,000.00USD). Customer/user shall

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Appendix A: Secondary Enrichment Protocols

For instances when the BAX® System Real-Time PCR Assay for *Salmonella* identifies sample wells that display an "Orange" result, which may indicate the presence of HPS pathogens, it is recommended to perform a secondary enrichment followed by PCR analysis to determine the precise HPS positive results (accurate for any of the indicated markers).

There are three (3) secondary enrichment options, each using the primary enrichment as a starting point and utilizing a reference method based on the matrix type. The three reference method options are:

1. The FDA-BAM Method for *Salmonella* Isolation-Selective Enrichment – ideal for anything except poultry, meat and fish (matrices such as produce or whole eggs).
2. The MLG-4 (USDA) Method for Isolation and Identification of *Salmonella* – ideal for meat, poultry, fish and processed eggs.
3. The ISO 6579-1:2017/Amd.1:2020(E) (Broad guidance for *Salmonella*).

The details for secondary enrichment follow.

FDA BAM Method for *Salmonella* Isolation-Selective enrichment

1. Prepare two media types for inoculation with the primary enrichment.
    a. Media 1: Rappaport-Vassiliadis (RV) Broth
    Transfer 0.1 ± 0.02 mL of the primary enrichment into 10 mL of RV Broth.
    Vortex to mix thoroughly.
    Incubate at 42 ± 0.5 °C for 22-24 hours.
    b. Media 2: Tetrathionate (TT) Broth
    Transfer 1 mL of the primary enrichment into 10 mL of TT Broth.
    Vortex to mix thoroughly.
    Incubate at 42 ± 0.5 °C for 22-24 hours.
2. Combination Step (Post-Incubation)
    a. Combine the two incubated selective media (RV and TT broths) at 1:1 ratio. This mixture will be the final broth sample to be tested.
    b. Optional:
    Prepare two separate mixtures by combining each selective broth individually with the primary enrichment broth as follows:
        - *Broth Mix 1 1:1 ratio of incubated RV broth and sterile primary enrichment broth*
        - *Broth Mix 2 1:1 ratio of incubated TT broth and sterile primary enrichment broth*

    This process will provide two test samples from the same primary enrichment broth.
3. From the final broth mix(es), prepare to transfer 5 µL to 200 µL of BAX lysis reagent for Real Time Highly pathogenic *Salmonella* assay. (see Step 2.8, pg. 5)

MLG 4(USDA) Isolation and identification of *Salmonella*

1. Prepare two media types for inoculation with the primary enrichment.
    a. Media 1: Rappaport-Vassiliadis (RV) Broth
    Transfer 0.1 ± 0.02 mL of the primary enrichment into 10 mL of RV Broth.
    Vortex to mix thoroughly.
    Incubate at 42 ± 0.5 °C for 22-24 hours.
    b. Media 2: Tetrathionate (TT) Hajna Broth
    Transfer 1 mL of the primary enrichment into 10 mL of TT Hajna Broth.
    Vortex to mix thoroughly.
    Incubate at 42 ± 0.5 °C for 22-24 hours.
2. Combination Step (Post-Incubation)
    a. Combine the two incubated selective media (RV and TT Hajna broths) at 1:1 ratio. This mixture will be the final broth sample to be tested.
    b. Optional:
    Prepare two separate mixtures by combining each selective broth individually with the primary enrichment broth as follows:
        - *Broth Mix 1 1:1 ratio of incubated RV broth and sterile primary enrichment broth*
        - *Broth Mix 2 1:1 ratio of incubated TT Hajna broth and sterile primary enrichment broth*

    This process will provide two test samples from the same primary enrichment broth.
3. From the final broth mix(es), prepare to transfer 5 µL to 200 µL of BAX lysis reagent for Real Time Highly pathogenic *Salmonella* assay. (see Step 2.8, pg. 5)

ISO 6579-1:2017/Amd.1:2020(E) (Broad guidance for *Salmonella*)

1. Prepare two media types for inoculation with the primary enrichment.
    a. Media 1: Rappaport-Vassiliadis Soya Pepton (RVS) Broth
    Transfer 0.1 ± 0.02 mL of the primary enrichment into 10 mL of RVS Broth.
    Vortex to mix thoroughly.
    Incubate at 41.5 ± 1.0 °C for 24 ± 3 hours.
    b. Media 2: MKTTn broth (Muller-Kauffmann Tetrathionate-Novobiocin enrichment broth)
    Transfer 1 mL of the primary enrichment into 10 mL of MKTTn Broth.
    Vortex to mix thoroughly.
    Incubate at 34 to 38 °C for 24 ± 3 hours.
2. Combination Step (Post-Incubation)
    a. Combine the two incubated selective media (RVS and MKTTn broths) at 1:1 ratio. This mixture will be the final broth sample to be tested.
    b. Optional:
    Prepare two separate mixtures by combining each selective broth individually with the primary enrichment broth as follows:
        - *Broth Mix 1 1:1 ratio of incubated RVS broth and sterile primary enrichment broth*
        - *Broth Mix 2 1:1 ratio of incubated MKTTn broth and sterile primary enrichment broth*

    This process will provide two test samples from the same primary enrichment broth.
3. From the final broth mix(es), prepare to transfer 5 µL to 200 µL of BAX lysis reagent for Real Time Highly pathogenic *Salmonella* assay. (see Step 2.8, pg. 5)