GENERAL DESCRIPTION
Assurance GDS®, genetic detection system, for Salmonella Tq is an automated nucleic acid amplification system for the detection of Salmonella in meats, poultry, poultry rinse, seafood, dairy products, eggs, pasta, chocolate and bakery products, peanut butter, fruits and vegetables, and environmental surfaces.

Part No: 71008-100 (100 tests)
71008-576 (576 tests)

There are three validated methods that can be followed:
- AOAC® Official Method of Analysis 2009.03
- AOAC® Performance Tested Method 050602
- Health Canada Method MFLP-36

KIT COMPONENTS
Each Assurance GDS for Salmonella Tq kit contains the following:
- Amplification Tubes Tq
- Concentration Reagent
- Resuspension Buffer Tq
- Wash Solution

EQUIPMENT / MATERIALS REQUIRED
Other necessary materials not provided include:
- Media per Appendix A
- Assurance GDS Rotor-Gene®
- PickPen® and PickPen tips
- Vortex mixer
- Adhesive film
- Sample wells and sample wells base
- Resuspension Plate
- Gel Cooling Block
- Stomacher / Masticator or equivalent
- B-channel micropipette capable of dispensing 30 µL
- Repeat pipette
- Adjustable micropipettes
- Repeat pipette tips (0.5 mL and 10 mL)
- Filter barrier micropipette tips (50 µL and 1.0 mL)
- Incubator capable of maintaining 35 – 37 °C
- Additional materials for the 576 kit include:
  - Variable Spacing Multi-Channel Pipette
  - Aluminium Cooling Block, 72 well
  - 72-well rotor and locking ring

AOAC® OFFICIAL METHOD OF ANALYSIS 2009.03
Approved matrices include: Raw Beef, Raw Pork, Ground Poultry, Poultry Rinse, Raw Fish, Raw Shrimp, Orange Juice, Raw Spinach, Raw Tomato, Cantaloupe, Peanut Butter, Liquid Milk, Nonfat Dry Milk, Ice Cream, Pasta, Eggs and Environmental Surfaces

SAMPLE PREPARATION
A. Test Portion Preparation & Enrichment
(a) Add 25 g of sample to 225 mL of Buffered Peptone Water (BPW).
For raw seafood, add 25 g of sample to 225 mL BPW with novobiocin (Appendix A).
For non-fat dry milk, add 25 g of sample to 225 mL Brilliant Green Water (Appendix A).
For environmental monitoring, collect environmental surface samples with a sponge or swab hydrated with D/E (Dey/Engley) Broth or Letheen Broth. After collecting sample, add sponge or swab to 100 mL or 10 mL of BPW, respectively. If alternate test portion sizes are analyzed, proportionately adjust the volume of media to maintain 1:9 ratio.

(b) Homogenize or mix sample and incubate samples for 18 – 24 h at 35 – 37 °C. Incubate non-fat dry milk samples for 20 – 24 h at 35 – 37 °C.
(c) For high microbial load and non-fat dry milk samples, transfer enriched samples to BHI for 2 – 4 h at 35 – 37 °C as described in step B (i).

Note: Contact BioControl Systems, Inc. for recommended procedures for testing alternate sample sizes.

B. Sample Preparation Protocol
Change gloves prior to handling reagents.
(a) Vortex Concentration Reagent. Immediately transfer 20 µL to each of the required number of Assurance GDS sample wells (1 well/sample) using a repeat pipette and 0.5 mL pipette tip. Cover sample wells with adhesive film strips.
(b) Transfer 1.0 mL of Wash Solution to additional sample wells (1 well/sample) using a repeat pipette and 10 mL pipette tip.
For high microbial load and non-fat dry milk samples, dispense 0.5 mL of sterile Brain Heart Infusion (BHI) broth to sample wells (1 well/sample) in place of Wash Solution.
For raw ground poultry, add 0.7 mL of Wash Solution to the sample wells containing Concentration Reagent and dispense 0.5 mL of sterile BHI to a separate set of sample wells. Cover all sample wells with adhesive film strips.
(c) Transfer 45 µL of Resuspension Buffer Tq to the wells in the resuspension plate using a repeat pipette and a 0.5 mL pipette tip. Cover resuspension plate with adhesive film strips.
(d) Carefully remove adhesive film from 1 strip of sample wells. Add 1.0 mL of incubated sample to each sample well containing Concentration Reagent.
For raw ground poultry, transfer 0.3 mL of incubated sample to wells containing Concentration Reagent and Wash Solution. Avoid transferring food particles. A new pipette tip must be used for each sample. Cover each strip of sample wells with a new adhesive film strip prior to adding samples to a new strip. Immediately return samples to incubator.
(e) Place sealed sample wells on the vortex mixer and vortex at approximately 900 rpm for 10-20 min. If necessary, adjust rpm to be certain that liquid does not contact adhesive film.
(f) Carefully remove and discard adhesive film strip from a strip of samples. Remove corresponding film strip from a strip of sample wells containing Wash Solution or BHI.
(g) When analyzing peanut butter samples, load PickPen tips onto PickPen tool. Insert tips into the sample wells without extending the magnets. Stir 5 – 10 s to remove excess fat accumulation. Discard tips and continue with step (h).
(h) For all samples load tips onto the PickPen, ensuring that the tips are firmly in place on the PickPen tool. Extend the PickPen magnets and insert into the first strip of sample wells. Stir gently for 30 s while continually moving up and down from the surface to the bottom of the well. Gently tap the PickPen tips against the side of the sample wells to remove excess media droplets.
(i) Transfer PickPen to corresponding sample wells containing Wash Solution and gently swir for 5 s (do not release particles into solution). Transfer PickPen to the corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen magnets and tap gently to release particles into the Resuspension Buffer Tq. Cover resuspension plate with adhesive film strips and continue with step (j).

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For high microbial load and non-fat dry milk samples transfer PickPen to corresponding sample wells containing BHI. With tips submerged, retract the PickPen magnets and tap gently to release particles into the BHI. Cover each strip with a new adhesive film strip prior to adding samples to a new strip. Incubate sample wells containing BHI and particles for 2 – 4 h at 35 – 37 °C. For raw ground poultry, incubate sample wells for a minimum of 3 h.

Following incubation, transfer the particles from the BHI sample wells to the corresponding row of the prepared resuspension plate using the PickPen. With tips submerged, retract the PickPen magnets and tap gently to release particles into the Resuspension Buffer Tq. Cover the resuspension plate with adhesive film and continue with step (j).

(j) Repeat steps (f) through (i) for all samples using new tips for each strip of samples.

PROCEED TO TEST PROCEDURE SECTION

AOAC® PERFORMANCE TESTED METHOD 050602
Approved matrices include: Raw Ground Beef, Raw Beef Trim, RTE Meat, Raw Spinach, Leaf Lettuce, Mixed Greens, Strawberries, Almonds and Environmental Surfaces

SAMPLE PREPARATION
A. Enrichment Media Preparation
(a) For 25 g sample, prewarm 225 mL sterile deionized water at 41 – 43 °C overnight. On day of use, aseptically transfer 7.1 g of BioControl mEHEC media into the prewarmed sterile water. Gently mix to dissolve the powder. Use prepared medium within 6 h.

(b) For 375 g sample, prewarm 1500 mL sterile deionized water at 41 – 43 °C overnight. On day of use, aseptically transfer 47.3 g of BioControl mEHEC media into the prewarmed sterile water. Gently mix to dissolve the powder. Use prepared medium within 6 h.

(c) Alternatively, mEHEC media can be prepared in advance and autoclaved. Add 31.6 g media per liter of deionized water. Stir to dissolve the powder. Place sealed sample wells on the vortex mixer and vortex at approximately 900 rpm for 10 – 20 min. If necessary, adjust rpm to be certain that liquid does not contact adhesive film.

(d) Carefully remove and discard adhesive film strip from a strip of samples. Remove corresponding film strip from a strip of sample wells containing Wash Solution or BHI.

(e) Load tips onto the PickPen, ensuring that the tips are firmly in place on the PickPen tool. Extend the PickPen magnets and insert into the first strip of sample wells. Stir gently for 30 s while continually moving up and down from the surface to the bottom of the well. Gently tap the PickPen tips against the side of the sample wells to remove excess media droplets.

(f) Transfer PickPen to corresponding sample wells containing Wash Solution and gently swirl for 5 s (do not release particles into solution). Transfer PickPen to the corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen magnets and gently tap to release particles into the Resuspension Buffer Tq.

(i) Repeat steps (f) through (h) for all samples using new tips for each strip of samples.

(j) Cover resuspension plate with adhesive film strips.

HEALTH CANADA METHOD MFLP-36
Approved matrices include: Raw Beef, Raw Ground Poultry, Frozen Ground Poultry, Raw Shrimp, Egg, Liquid Milk, Milk Chocolate, Chocolate Cake and Environmental Surfaces

SAMPLE PREPARATION
A. Test Portion Preparation & Enrichment
(a) Add 25 g of sample to 225 mL of Buffered Peptone Water (BPW).

For raw seafood, add 25 g of sample to 225 mL BPW with novobiocin (Appendix A).

For chocolate products, add 25 g of sample to 225 mL reconstituted nonfat dry milk. Homogenize sample allow to sit at room temperature for 1 h and adjust pH to 6.6-7.0. Add 0.45 mL of 1% Brilliant Green Dye Solution (1 g Brilliant Green Dye in 100 mL sterile deionized water) and mix.

For environmental monitoring, collect environmental surface samples with a sponge or swab hydrated with D/E (Dey/Engley) Broth or Letheen Broth. After collecting sample, add sponge or swab to 100 mL or 10 mL of prewarmed (41 – 43 °C) mEHEC media respectively. Masticate sponge and media to mix well. Incubate for 8 – 24 h at 41 – 43 °C.

C. Sample Preparation Protocol
Change gloves prior to handling reagents.

(a) Vortex Concentration Reagent. Immediately transfer 20 µL to each of the required number of Assurance GDS sample wells (1 well/sample) using a repeat pipette and 0.5 mL pipette tip. Cover sample wells with adhesive film strips.

(b) Transfer 1.0 mL of Wash Solution to additional sample wells (1 well/sample) using a repeat pipette and 10 mL pipette tip.

(c) Transfer 45 µL of Resuspension Buffer Tq to the wells in the resuspension plate using a repeat pipette and a 0.5 mL pipette tip. Cover resuspension plate with adhesive film strips.

(d) Carefully remove adhesive film from 1 strip of sample wells. Add 1.0 mL of incubated sample to each sample well containing Concentration Reagent. Avoid transferring food particles. A new pipette tip must be used for each sample. Cover each strip of sample wells with a new adhesive film strip prior to adding samples to a new strip. Immediately return samples to 42 °C incubator.

(e) Place sealed sample wells on the vortex mixer and vortex at approximately 900 rpm for 10 – 20 min. If necessary, adjust rpm to be certain that liquid does not contact adhesive film.

(f) Carefully remove and discard adhesive film strip from a strip of samples. Remove corresponding film strip from a strip of sample wells containing Wash Solution or BHI.

(g) Load tips onto the PickPen, ensuring that the tips are firmly in place on the PickPen tool. Extend the PickPen magnets and insert into the first strip of sample wells. Stir gently for 30 s while continually moving up and down from the surface to the bottom of the well. Gently tap the PickPen tips against the side of the sample wells to remove excess media droplets.

(h) Transfer PickPen to corresponding sample wells containing Wash Solution and gently swirl for 5 s (do not release particles into solution). Transfer PickPen to the corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen magnets and gently tap to release particles into the Resuspension Buffer Tq.

(i) Repeat steps (f) through (h) for all samples using new tips for each strip of samples.

(j) Cover resuspension plate with adhesive film strips.

Note: Contact BioControl Systems, Inc. for recommended procedures for testing alternate sample sizes.
B. Sample Preparation Protocol

Change gloves prior to handling reagents.

(a) Vortex Concentration Reagent. Immediately transfer 20 µL to each of the required number of Assurance GDS sample wells (1 well/sample) using a repeat pipette and 0.5 mL pipette tip. Cover sample wells with adhesive film strips.

(b) Transfer 1.0 mL of Wash Solution to additional sample wells (1 well/sample) using a repeat pipette and 10 mL pipette tip. For high microbial load and chocolate samples, dispense 0.5 mL of sterile Brain Heart Infusion (BHI) broth to sample wells (1 well/sample) in place of Wash Solution.

For raw ground poultry, add 0.7 mL of Wash Solution to the sample wells containing Concentration Reagent and dispense 0.5 mL of sterile BHI to a separate set of sample wells. Cover all sample wells with adhesive film strips.

(c) Transfer 45 µL of Resuspension Buffer Tq to the wells in the resuspension plate using a repeat pipette and a 0.5 mL pipette tip. Cover resuspension plate with adhesive film strips.

(d) Carefully remove adhesive film from 1 strip of sample wells. Add 1.0 mL of incubated sample to each sample well containing Concentration Reagent.

For raw ground poultry, transfer 0.3 mL of incubated sample to wells containing Concentration Reagent and Wash Solution. Avoid transferring food particles. A new pipette tip must be used for each sample. Cover each strip of sample wells with a new adhesive film strip prior to adding samples to a new strip. Immediately return samples to incubator.

(e) Place sealed sample wells on the vortex mixer and vortex at approximately 900 rpm for 10-20 min. If necessary, adjust rpm to be certain that liquid does not contact adhesive film.

(f) Carefully remove and discard adhesive film strip from a strip of samples. Remove corresponding film strip from a strip of sample wells containing Wash Solution or BHI.

When analyzing peanut butter samples, load PickPen tips onto PickPen tool. Insert tips into the sample wells without extending the magnets. Stir 5 – 10 s to remove excess fat accumulation. Discard tips and continue with step (h).

(g) For all samples load tips onto the PickPen, ensuring that the tips are firmly in place on the PickPen tool. Extend the PickPen magnets and insert into the first strip of sample wells. Stir gently for 30 s while continually moving up and down from the surface to the bottom of the well. Gently tap the PickPen tips against the side of the sample wells to remove excess media droplets.

(h) Transfer PickPen to corresponding sample wells containing Wash Solution and gently swirl for 5 s (do not release particles into solution). Transfer PickPen to the corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen magnets and tap gently to release particles into the Resuspension Buffer Tq. Cover resuspension plate with adhesive film strips and continue with step (j).

For high microbial load samples transfer PickPen to corresponding sample wells containing BHI. With tips submerged, retract the PickPen magnets and tap gently to release particles into the BHI. Cover each strip with a new adhesive film strip prior to adding samples to a new strip. Incubate sample wells containing BHI and particles for 2-4 h at 35-37°C. For raw ground poultry, incubate sample wells containing BHI and particles for 2-4 h at 35-37°C. Following incubation, transfer the particles from the BHI sample wells to the 2nd set of wells containing wash solution and gently swirl for 5 seconds (do not release particles into solution). Transfer samples to the corresponding row of the prepared resuspension plate using the PickPen. With tips submerged, retract the PickPen magnets and tap gently to release particles into the Resuspension Buffer Tq. Cover the resuspension plate with adhesive film and continue with step (j).

For chocolate samples, transfer PickPen to the 1st set of wells containing wash solution and gently swirl for 5 seconds (do not release particles into solution). Transfer PickPen to corresponding sample wells containing BHI. With tips submerged, retract the PickPen magnets and tap gently to release particles into the BHI. Cover each strip with a new adhesive film strip prior to adding samples to a new strip. Incubate sample wells containing BHI and particles for 2-4 h at 35-37°C. Following incubation, transfer the particles from the BHI sample wells to the 2nd set of wells containing wash solution and gently swirl for 5 seconds (do not release particles into solution). Transfer samples to the corresponding row of the prepared resuspension plate using the PickPen. With tips submerged, retract the PickPen magnets and tap gently to release particles into the Resuspension Buffer Tq. Cover the resuspension plate with adhesive film and continue with step (j).

(j) Repeat steps (f) through (j) for all samples using new tips for each strip of samples.

PROCEED TO TEST PROCEDURE SECTION

TEST PROCEDURE

Change gloves prior to handling reagents.

A. Preparation of Gel Cooling Block

(a) Prior to initial use, the gel cooling block must be stored in the freezer (-25 to -15°C) for 6 h. When frozen, the gel cooling block will change color from pink to purple. When not in use the gel cooling block should continue to be stored at -25 to -15°C.

(b) Between each use the gel cooling block should be returned to the freezer until it has turned completely purple, indicating it is ready for use. This may take up to 2 h.

(c) The aluminum cooling block is for use with the 576 test kit and should be stored in the refrigerator (2 – 8°C). To use, place the aluminum cooling block on top of the gel cooling block.

B. Preparation of Amplification Tubes

(a) The Assurance GDS Rotor-Gene set up and data entry should be completed prior to transferring samples from the resuspension plate into the Amplification Tubes.

(b) Remove Amplification Tubes Tq from foil pouch and place them in the frozen gel cooling block (aluminum cooling block for 576 test kit). Reseal pouch.

(c) Transfer 30 µL of sample from the resuspension plate wells into each Amplification Tube using a multi-channel pipette and filter barrier tips. Firmly press down on each Amplification Tube lid to close. Visually inspect each tube to ensure that the cap is securely sealed.

(d) Prior to placing in rotor, invert Amplification Tubes and shake with a snapping motion to thoroughly mix contents.

(e) Place Amplification Tubes into Assurance Rotor-Gene in sequential order, beginning with position #1. For the 100 test kit, use the 36-well rotor and locking ring; for the 576 test kit, use the 72-well rotor and locking ring. Start Rotor-Gene cycle. Refer to Assurance GDS user manual for detailed instructions on operating the Rotor-Gene.

NOTE: The Assurance GDS Rotor-Gene must be started within 15 min after addition of the samples to the Amplification Tubes.
RESULTS
Upon completion of the run, the Assurance GDS Rotor-Gene software will provide a results table. Each sample will be identified as Positive, Negative, or No Amp.

Positive: Samples are positive for Salmonella.
Negative: Samples are negative for Salmonella.
No Amp: Amplification did not occur. Repeat the test beginning from Step B. Sample Preparation Protocol. If the No Amp result repeats, contact BioControl Technical Service.

CONFIRMATION
Samples enriched as specified by AOAC OMA 2009.03 should be incubated for 20 - 24 h and samples enriched as specified by AOAC PTM 050602 should be incubated for 16 -24 h prior to transfer to a selective enrichment broth for confirmation.
Presumptive positive samples should be confirmed from the retained Assurance GDS enrichment media via:

Health Canada Method MFLP-36: Presumptive positive results may be confirmed from the primary enrichment broth by proceeding with the plating and confirmation steps of an appropriate reference method such as MFHPB-20. http://www.hc-sc.gc.ca/fn-an/res-rech/analy-meth/microbiovolume2/mfhpb20-01-eng.php

APPENDIX A – Enrichment Media Recipes

Buffered Peptone Water
Suspend 20 g of dehydrated Buffered Peptone Water (BPW) in 1L of purified water. Mix thoroughly and dispense into desired aliquots. Autoclave at 121 °C for 15 min.

Buffered Peptone Water w/ Novobiocin
Prepare BPW as described above. On day of use, add 1 mL of 0.4% Novobiocin solution to 225 mL BPW.

0.4% Novobiocin Solution
Dissolve 0.4 g of Novobiocin (sodium salt) in 100 mL of sterile purified water.

Brain Heart Infusion
Suspend 37 g of Brain Heart Infusion in 1L of purified water. Mix thoroughly and dispense into desired aliquots. Autoclave at 121°C for 15 min.

Brilliant Green Water
Create a 1% Brilliant Green Dye stock solution by dissolving 1 g Brilliant Green Dye in 100 mL of sterile purified water (Do not autoclave).
To prepare Brilliant Green Water, add 2 mL of the 1% Brilliant Green Dye stock solution to 1L of sterile purified water.

Reconstituted Nonfat Dry Milk
Suspend 100g dehydrated nonfat dry milk in 1 L deionized water. Autoclave for 15 minutes at 121°C.

PRODUCT WARRANTY
BioControl Systems, Inc. (BCS) warrants this product to be free from defects in materials and workmanship, when stored under labeled conditions and used as intended until the expiration date stated on the package. BCS agrees during the applicable warranty period to replace all defective products after return to BCS. BCS shall not have obligation under this Limited Warranty to make replacements which result, in whole or in part, from negligence of the Buyer, or from improper use of the products, or use of the product in a manner for which it was not indicated. Buyer shall notify BCS of any products which it believes to be defective during the warranty period. At BCS option, such products shall be returned to BCS, transportation and insurance prepaid. BCS shall replace any such product found to be defective, at no charge. Should BCS examination not disclose any defect covered by the foregoing warranty, BCS shall so advise Buyers and dispose of the product in accordance with Buyer’s instructions.

This product contains MGB Eclipse™ probes and primers which are manufactured for BioControl Systems, Inc. by Epoch Biosciences. Certain reagents are covered by patents owned by Epoch Biosciences including, but not limited to, US Patents 6,112,894; 6,127,121; 5,801,155; 6084,102; 6,426,408; 6,492,346 and 6,485,906, and there is no implied license for any other use with respect to this product. The purchase price of this product includes a limited, nontransferable license under US patent nos. 6,030,787, 5,723,591; and 5,876,930 and corresponding foreign patents to use only the amount of MGB Eclipse detection reagents provided in the product for use only in the provided kit and solely for food and environmental testing, the express purposes noted in the instructions. This product may be used in the Polymerase Chain Reaction (“PCR”) process, certain aspects of which are covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffman-La Roche Ltd (“Roche”). A license to use the PCR process accompanies the purchase of polymerase reagents from licensed suppliers, including those recommended by BioControl Systems when used in conjunction with an authorized thermal cycler, or is available from Applied BioSystems.


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