Assurance® GDS *Salmonella* Tq

There are two ISO validated methods that can be followed:

NF Validation Certificate N° TRA 02/12-01/09

MicroVal Certificate No. 2015LR50

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

General Description

Assurance® GDS for *Salmonella* Tq is an automated nucleic acid amplification system for the detection of *Salmonella* in all human and animal food products and environmental samples. Assurance® GDS assays are designed for use by qualified lab personnel who follow appropriate microbiology laboratory practices.

Kit Components

Each Assurance® GDS for *Salmonella* Tq kit (**100** and **576** tests) contains the following:

- Amplification Tubes Tq
- Concentration Reagent
- Resuspension Buffer Tq
- Wash Solution

Each Assurance® GDS for *Salmonella* Tq **576ATM** kit contains the following:

- Amplification Tubes Tq
- Concentration Reagent

The following are also necessary for **576ATM** kit but sold separately:

- 61031-100 Wash Solution Kit
- 34724-100C Resuspension Buffer Tq

Equipment / Materials Required

Other necessary materials not provided include:

- Media per Appendix A
- Asssurance® GDS Rotor-Gene® thermocycler
- Rotor and locking ring
- Laptop computer and software v2.3.103
- PickPen™ device and PickPen™ tips
- Vortex mixer (IKA® MS3 or equivalent)
- Adhesive film strips
- Sample wells and sample wells base
- Resuspension plate
- Gel cooling block

Stomacher® paddle homogenizer or equivalent

Stomacher®-type bags with filter or equivalent

8-channel micropipette capable of dispensing 30 µL

Adjustable micropipette capable of accurately dispensing 1000 µL

Repeat pipette

Repeat pipette tips (0.5 mL and 10 mL)

Filter barrier micropipette tips (50 µL and 1.0 mL)

Incubator capable of maintaining 37 ± 1 °C or 41.5 ± 1 °C, depending on method

Freezer capable of maintaining -20 ± 5 °C

Refrigerator capable of maintaining 5 ± 3 °C

Additional materials for the **576**-kit include:

Variable spacing Amplification Tubes holder, 72-well

Variable spacing Amplification Tubes holder lid, 72-well

Amplification Tubes capping tool

Amplification Tubes cap rack, 72-well

Aluminum Cooling Block, 72-well

72-well rotor and locking ring

NF Validation Certificate N° TRA 02/12-01/09

Approved categories include: Food products (except sprouts), infant formula and cereals, pet foods, and environmental samples (excluding samples of primary production).

Sample Preparation

See Appendix B for Enrichment Method Tables

Test Portion Preparation & Enrichment

A. Sample Preparation & Enrichment – All foods except <u>infant formula, infant cereals, non-fat dried milk (NFDM)</u>

If alternate test portion sizes are analyzed, proportionately adjust the volume of media to maintain 1:10 ratio.

For preparations of initial suspensions, follow instructions of EN ISO 6579 and of EN ISO 6887 standards.

1. Samples without subculture in BHI

a) For **processed food** samples, add 25 g of sample to 225 mL of Buffered Peptone Water (BPW, Appendix A).

b) Homogenize or mix samples and incubate for 18 – 24 h at 37 ± 1 °C.

PROCEED TO D. SAMPLE PROCESSING PROTOCOL

2. Samples with subculture in BHI

a) For **raw** and **unprocessed** samples, add 25 g of sample to 225 mL of BPW (Appendix A).

b) Homogenize or mix samples and incubate for 18 – 24 h at 37 ± 1 °C.

c) Transfer enriched samples to BHI for 2 – 4 h at 37 ± 1 °C as described in step SAMPLE

PROCESSING PROTOCOL **(D10)**.

PROCEED TO D. SAMPLE PROCESSING PROTOCOL

B. Sample Preparation & Enrichment – <u>Environmental samples (excluding samples of primary production)</u>

1. Samples without subculture in BHI

a) **Environmental samples: food (and non-food) product contact surfaces, work surfaces and adjacent areas (i.e. blenders, work tables, drip shields, housing)**, and **process water**: Pre-moisten sterile dehydrated sponges with 10 mL BPW. Hydrate sterile swab by soaking in BPW. If neutralization is needed, substitute D/E (Dey/Engley) Broth or Letheen Broth for BPW. After collecting sample from **surface**, add sponge or swab to 100 mL or 10 mL of BPW, respectively. For **process water**, aseptically aliquot 25 mL process water to 225 mL of BPW.

b) Homogenize or mix samples and incubate for 18 – 24 h at 37 ± 1 °C.

PROCEED TO D. SAMPLE PROCESSING PROTOCOL

2. Samples with subculture in BHI

a) **Environmental samples: non-food contact surfaces not close to food product work surfaces (i.e. drains, floors, walls, cart wheels)** and **sweepings**: Pre-moisten sterile dehydrated sponges with 10 mL BPW. Hydrate sterile swab by soaking in BPW. If neutralization is needed, substitute D/E (Dey/Engley) Broth or Letheen Broth for BPW. After collecting sample from **surface**, add sponge or swab to 100 mL or 10 mL of BPW, respectively. For **sweepings**, aseptically add 25 g sweepings to 225 mL of BPW.

b) Homogenize or mix samples and incubate for 18 – 24 h at 37 ± 1 °C.

c) Transfer enriched samples to BHI for 2 – 4 h at 37 ± 1 °C as described in step SAMPLE PROCESSING PROTOCOL **(D10)**.

PROCEED TO D. SAMPLE PROCESSING PROTOCOL

C. Sample Preparation & Enrichment – <u>Infant formula, infant cereals, and NFDM</u>

For powdered or dry products, do not shake; instead, moisten sample with media. Allow to stand undisturbed at 18 – 27 °C (laboratory temperature) for 30 – 60 min. If after this time the powder is not homogenized, mix the contents gently till dissolved.

Note: Pre-warm the media to 37 ± 1 °C before addition to sample.

1. Samples without subculture in BHI

a) **Infant formula without probiotics**: For 10 – 375 g sample size, add to 1:10 sample-to-media ratio of BPW.

Infant cereals (without probiotics): For 10 – 375 g sample size, add to 1:10 sample-to-media ratio of BPW with amylase (BPW+a, Appendix A).

b) Homogenize or mix sample. Incubate 10 – 25 g samples for 18 – 26 h at 37 ± 1 °C. Incubate 50 – 375 g samples with pre-warmed media (37 ± 1 °C) for 18 – 29 h at 37 ± 1 °C.

PROCEED TO D. SAMPLE PROCESSING PROTOCOL

2. Samples with subculture in BHI

a) **Non-fat dry milk (NFDM)** samples: For 10 –375 g sample size, add to 1:10 sample-to-media ratio of Brilliant Green Water (BGW, Appendix A).

Infant formula with probiotics: For 10 –375 g sample size, add to 1:10 sample-to-media ratio of BPW with vancomycin (BPW+v, Appendix A).

Infant cereals (with probiotics): For 10 –375 g sample size, add to 1:10 sample-to-media ratio of BPW with vancomycin and amylase (BPW+v+a, Appendix A).

b) **All infant foods, except NFDM**: Homogenize or mix sample. Incubate 10 – 25 g samples for 18 – 26 h at 37 ± 1 °C. Incubate 50 –375 g samples with pre-warmed media (37 °C) for 18 – 29 h at 37 ± 1 °C.

NFDM samples: Incubate 10 – 25 g NFDM samples 20 – 28 h at 37 ± 1 °C. Incubate 50 – 375 g NFDM samples with pre-warmed media (37 ± 1 °C) for 20 – 29 h at 37 ± 1 °C.

c) Transfer enriched samples to BHI for 2 – 4 h at 37 ± 1 °C as described in SAMPLE PROCESSING PROTOCOL **(D10)**.

PROCEED TO D. SAMPLE PROCESSING PROTOCOL

D. Sample Processing Protocol

Change gloves prior to handling reagents

1. 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 a 0.5 mL pipette tip. Cover sample wells with adhesive film strips.
2. For **processed foods, nearby food contact surface environmental** samples, **process water**, and **infant formula and cereal (without probiotics)**, transfer 1.0 mL of **Wash Solution** to additional sample wells (1 well/sample) held in a sample well base using a repeat pipette and a 10 mL pipette tip. Cover sample wells with adhesive film strips.
3. For **raw** and **unprocessed foods**, **distant nonfood contact surface environmental** samples, **sweepings**, **NFDM**, **infant formula and cereal (with probiotics)** samples, dispense 0.5 mL of sterile Brain Heart Infusion (BHI) broth to sample wells (1 well /sample) in place of Wash Solution using a repeat pipette and a 10 mL pipette tip. Cover sample wells with adhesive film strips.
4. 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.
5. 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 prior to adding samples to a new strip. **Immediately return samples to incubator until GDS results have been obtained (maximum 24 h incubation) for confirmation, if necessary.**
6. Place sealed sample wells containing the *Salmonella* Concentration Reagent and sample 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.
7. Carefully remove and discard the adhesive film from a strip of samples. Remove the corresponding adhesive film from a strip of sample wells containing Wash Solution or BHI.
8. Load tips onto the PickPen™ device, ensuring that the tips are firmly in place on the PickPen™ tool. Extend the PickPen™ magnets and insert tips into the first strip of sample wells. Stir tips 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.
9. For **processed foods, nearby food contact surface environmental** samples, **process water**, and **infant formula and cereal (without probiotics)**, transfer PickPen™ tips to corresponding sample wells

containing Wash Solution and gently swirl for 10 s (do not release particles into solution). Transfer PickPen™ tips to the corresponding column of the prepared resuspension plate. With tips submerged, retract the PickPen™ magnets and tap tips gently to release particles into the Resuspension Buffer. Cover resuspension plate with adhesive film strips and continue to step (D12).

10. For **raw samples, unprocessed foods**, **distant non-food contact surface environmental** samples**, sweepings**, **NFDM**, **infant formula and cereal (with probiotics)** samples, transfer PickPen™ tips 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 of sample wells containing BHI with a new adhesive film prior to adding next set of particles to sample wells containing BHI. Incubate sample wells containing BHI and particles for 2 – 4 h at 37 ± 1°C.
11. Following incubation, transfer the particles from the BHI sample wells (see step D8) to the corresponding column of the prepared resuspension plate using the PickPen™ tips. With tips submerged, retract the PickPen™ magnets and tap tips gently to release particles into the Resuspension Buffer. Cover the resuspension plate with adhesive film and continue with step (D12).
12. Repeat steps (D7) through (D11) for all samples using new tips for each strip of samples.

PROCEED TO TEST PROCEDURE SECTION

MicroVal Certificate No. 2015LR50

Approved categories include: Raw Beef Meat, Delicatessen and Heat-Treated Meat Products, Raw Beef Meats, Raw Poultry Meats, Delicatessen and Heat-Treated Poultry Products, Multi-component and Meal Component Foods, Dairy Products, Fruits & Vegetables, and Environmental Samples.

Sample Preparation

Test Portion Preparation & Enrichment

A. Enrichment Media Preparation

1. For 25 g (mL) sample, pre-warm 225 mL sterile deionized water at 41.5 ± 1 °C overnight. On day of use, aseptically transfer 7.1 g of mEHEC® media into the pre-warmed sterile water. Gently mix to dissolve the powder. Use prepared medium within 6 h.
2. For 375 g sample, pre-warm 1500 mL sterile deionized water at 41.5 ± 1 °C overnight. On day of use, aseptically transfer 47.3 g of mEHEC® media into the pre-warmed sterile water. Gently mix to dissolve the powder. Use prepared medium within 6 h.
3. 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, dispense into desired volume and autoclave at 121 °C for 15 min. Media must be pre-warmed to 41.5 ± 1 °C overnight prior to sample addition.

B. Test Portion Preparation & Enrichment

1. **Meat Products, Delicatessen and Heat-Treated Meat Products** – Aseptically weigh 375 g test portion into 1500 mL pre-warmed (41.5 ± 1 °C) mEHEC® media. For 25 g of samples, use 225 mL of pre-warmed (41.5 ± 1 °C) mEHEC media. Homogenize or mix sample. Incubate for 10 – 18 h at 41.5 ± 1 °C.
2. **Raw Poultry, Delicatessen and Heat-Treated Poultry Products** – Aseptically weigh 25 g test portion into 225 mL pre-warmed (41.5 ± 1 °C) mEHEC® media. Homogenize or mix sample. Incubate for 10 – 18 h at 41.5 ± 1 °C.
3. **Fruits and Vegetables** – Aseptically weigh 25 g (mL) test portion into 225 mL pre-warmed (41.5 ± 1 °C) mEHEC® media. Homogenize or mix sample. Incubate for 10 – 14 h at 41.5 ± 1 °C.
4. **Multi-Component and Meal Component Foods** - Aseptically weigh 25 g (mL) test portion into 225 mL pre-warmed (41.5 ± 1 °C) mEHEC® media. Homogenize or mix sample. Incubate for 18 – 24 h at 41.5 ± 1 °C.

5. **Dairy Products** – Aseptically weigh 25 g (mL) test portion into 225 mL pre-warmed (41.5 ± 1 °C) mEHEC® media. Homogenize or mix sample. Incubate for 10 – 18 h at 41.5 ± 1 °C. Transfer to 0.5 mL Brain Heart Infusion (BHI) for 2 – 4 h at 37 ± 1 °C as indicated in step C8 below.
6. **Environmental Samples** – Aseptically weigh 25 g sweepings or 25 mL process water into 225 mL pre-warmed (41.5 ± 1 °C) mEHEC® media. For environmental monitoring, pre-moisten sterile dehydrated sponges with 10 mL D/E (Dey/Engley) Broth or Letheen Broth. Hydrate sterile swab by soaking in D/E or Letheen broth. After collecting sample, add sponge or swab to 100 mL or 10 mL of mEHEC media, respectively. Incubate for 10 – 18 h at 41.5 ± 1 °C.

Note: Sponges and swabs hydrated with Neutralizing Buffer should not be used with Assurance® GDS as they may interfere with the PCR reaction.

C. Sample Processing Protocol

Change gloves prior to handling reagents.

Note: Enriched samples can be stored at 2 – 8 °C (refrigeration) for up to 72 h prior to testing with Assurance® GDS for *Salmonella* Tq.

1. 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 a 0.5 mL pipette tip. Cover sample wells with adhesive film strips.
2. Transfer 1.0 mL of **Wash Solution** to additional sample wells (1 well/sample) held in a sample well base using a repeat pipette and a 10 mL pipette tip. Cover sample wells with adhesive film strips.

   For **dairy products**, dispense 0.5 mL of sterile BHI broth to sample wells (1 well /sample) in place of Wash Solution. Cover sample wells with adhesive film strips.
3. 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.
4. 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 prior to adding samples to a new strip. **Immediately return samples to incubator until 18 h incubation time for confirmation, if necessary.**
5. 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.
6. Carefully remove and discard the adhesive film from a strip of samples. Remove the corresponding adhesive film from a strip of sample wells containing Wash Solution or BHI.
7. Load tips onto the PickPen™ device, ensuring that the tips are firmly in place on the PickPen™ tool. Extend the PickPen™ magnets and insert tips into the first strip of sample wells. Stir tips 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.
8. Transfer PickPen™ tips to corresponding sample wells containing Wash Solution and gently swirl for 5 – 10 s (do not release particles into solution). Transfer PickPen™ tips to the corresponding column of the prepared resuspension plate. With tips submerged, retract the PickPen™ magnets and tap tips gently to release particles into the Resuspension Buffer Tq. Cover the resuspension plate with adhesive film and continue with step (C10).

   For **dairy products**, transfer PickPen™ tips 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 of sample wells containing BHI with a new adhesive film prior to adding next set of particles to sample wells containing BHI. Incubate sample wells containing BHI and particles for 2 – 4 h at 37 ± 1 °C.

9. Following incubation, transfer the particles from the BHI sample wells (see step C7) to the corresponding column of the prepared resuspension plate using the PickPen™ tips. With tips submerged, retract the PickPen™ magnets and tap tips gently to release particles into the Resuspension Buffer. Cover the resuspension plate with adhesive film and continue with step (C10).
10. Repeat steps (C6) through (C9) 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

1. Prior to initial use, the gel cooling block must be stored in the freezer (-20 ± 5 °C) for minimum 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 -20 ± 5 °C.
2. 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.
3. The 72-well aluminum cooling block is for use with the **576** test kit and should be stored in the refrigerator (5 ± 3 °C).

B. Preparation of Amplification Tubes Tq

1. The Assurance® GDS Rotor-Gene® set-up and data should be completed prior to transferring samples from the resuspension plate into the Amplification Tubes Tq.
2. Remove **Amplification Tubes Tq** from foil pouch and place them in the frozen gel cooling block. Reseal pouch.
3. For the **576** test kit, use the variable spacing amplification tubes holder to slice and roll apart the Amplification Tubes. Place the lid on top of the holder.
4. Open Amplification Tubes. Transfer 30 µL of sample from resuspension plate well into each Amplification Tube using a multi-channel pipette and filter barrier tips. Firmly press down on each Amplification Tube lid to close.
5. For the **576** test kit, remove the holder lid and using the holder, roll the Amplification Tubes back together. Cap Amplification Tubes Tq using the Amplification Tubes capping tool.
6. Transfer Amplification Tubes to the aluminum block. Visually inspect each tube to ensure that the cap is securely sealed. **Store the sealed resuspension plate in the refrigerator (5 ± 3 °C)**.
7. Place Amplification Tubes into Assurance® Rotor-Gene® in sequential order, beginning with position #1. For the **100** test kit and the **576ATM** test kit, use the 36-well rotor and locking ring; for the **576** test kit, use the 72-well rotor and locking ring.

Note: For **576** test kit, after loading Amplification Tubes in the rotor and securing with locking ring, contents should be thoroughly mixed by shaking with a snapping motion. See Application Note **FRMMK.2060** for details.

8. Start Rotor-Gene® cycle. Refer to Assurance® GDS User Manual for detailed instructions on operating the Rotor-Gene®.

Note: The Assurance® Rotor-Gene® must be started within 20 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 analysis from C. Sample Processing Protocol. If No Amp repeats, contact Technical Services (BioMTS@milliporesigma.com).

No.	Color	Name	Result	Description	Kit Lot Number
1		Sample 1	Positive	Salmonella	1234567
2		Sample 2	Negative	Salmonella	1234567
3		Sample 3	No Amp	Salmonella	1234567

Note: Enriched samples can be stored at 5 ± 3 °C for up to 72 h prior to testing with Assurance® GDS for *Salmonella* Tq.

Confirmation

Method certified by MicroVal: Samples enriched in the mEHEC® enrichment media should be incubated until GDS results are obtained (maximum 24 h enrichment) at 41.5 ± 1 °C.

Note: Enriched samples can be held at 5 ± 3 °C for up to 72 h prior to confirmation. For dairy products, store mEHEC® broth (and not BHI subculture) enrichment at 5 ± 3 °C. Confirm samples by transfer to selective enrichment broth (A) or alternative confirmation (B, below).

A. If a confirmation of the positive GDS result is needed following enrichment in mEHEC® for a full 18 h,
   1. Transfer 0.1 mL of enriched sample to 10 mL Rappaport-Vassiliadis Soy (RVS) Broth.
   2. Incubate RVS broth in a water bath or incubator at 41.5 ± 1 °C for 18 – 24 h.
   3. Upon completion of incubation, streak 10 µL of RVS broth for isolation onto XLD and/or a chromogenic agar and incubate plates for 24 ± 2 h at 37 ± 1 °C.
   4. Confirm typical colonies by commercially available latex test without purification step.

B. Confirm by directly streaking enrichment to chromogenic plate of choice:
   CHROMID® *Salmonella* (bioMérieux Cat# 43621), RAPID'*Salmonella* Agar (Bio-Rad Cat# 3563961), Sigma *Salmonella* ChromoSelect Agar (Sigma Cat# 05538). Streak plate for isolation. Incubate plates for 20–24 h at 35–37°C (per manufacturer's instructions). Confirm typical colonies by latex agglutination test for *Salmonella* (Oxoid™ *Salmonella* test kit Cat# DR1108A, Microgen® *Salmonella* latex test Cat# M42CE, or equivalent).

Note: In the event of discordant results (positive presumptive results with the alternative method, non-confirmed by one the means above), the laboratory must follow the necessary steps to ensure validity of the result obtained.

Method certified NF Validation by AFNOR certification: Samples enriched in the specified Assurance® GDS enrichment media should be incubated until GDS results are obtained (maximum 24 h enrichment) at 37 ± 1 °C.

A. Enriched samples can be held at 5 ± 3 °C for up to 72 h prior to transfer to selective enrichment broth.
   In the context of NF VALIDATION, all samples identified as positive by the alternative method may be confirmed using the enrichment broth,

1. Transfer 0.1 mL of enriched sample to 10 mL Rappaport-Vassiliadis Soy (RVS) broth or Mueller Kauffman Tetrathionate broth with Novobiocin (MKTTn).
2. Incubate RVS broth in a water bath or incubator at 41.5 ± 1 °C for 18 – 24 h. Incubate MKTTn broth at 37 ± 1 °C for 18 – 24 h.
3. Upon completion of incubation, streak broth tube for isolation on appropriate selective agar (i.e. XLD) and proceed with confirmation according to the standard techniques described in the reference method of the CEN or ISO.

B. *Salmonella* may be isolated from *Salmonella* positive samples by plating the *Salmonella* Concentration Reagent which remains in the resuspension plate (step C, Test Procedure). Plate IMS beads to chromogenic agar plates. Spread plates for isolation. Incubate plates for 20–24 h at 36 ± 1 °C.

Isolation of *Salmonella* using Concentration Reagent Remainder in Resuspension Plate Equipment / Materials Required

Necessary materials in addition to those needed for the SECONDARY SCREENING PROTOCOL:

Wash Solution

Sterile disposable agar plate spreaders

Incubator capable of maintaining 36 ± 1 °C (for incubation of plates)

1. Add 100 µL Wash Solution, using a repeat pipette and a 0.5 mL pipette tip, to the required number of wells in the Resuspension plate (1 well/sample). Cover resuspension plate wells containing Wash Solution with adhesive film strips.
2. From the sample resuspension plate previously used for GDS *Salmonell*a analysis (step B4, Test Procedure), briefly pipette up and down the IMS beads in resuspension plate well to mix (approximately 15 µL volume), as IMS beads will fall to bottom of well during storage.
3. Remove 1 adhesive film strip from Wash Solution resuspension plate from step 1. Transfer 15 µL suspended IMS beads to 1 resuspension plate well containing Wash Solution.
4. Briefly pipette up and down the Wash Solution to mix beads in well. Transfer 50 µL IMS beads from the Wash Solution resuspension plate to chromogenic plate of choice: Sigma *Salmonella* ChromoSelect Agar (Sigma Cat#05538) or CHROMID *Salmonella* (bioMérieux Cat# 43621) or RAPID' *Salmonella* Agar (Bio-Rad Cat# 3563961). Spread beads for isolation. Incubate plates for 22 ± 2 h at 36 ± 1 °C (per Manufacturer's instructions).
5. Confirm typical colonies by latex agglutination test for *Salmonella* (Oxoid™ *Salmonella* test kit Cat#DR1108A, Microgen® *Salmonella* latex test Cat#M42CE, or equivalent).

Note: Original GDS *Salmonella* resuspension buffer plate can be stored at 5 ± 3 °C (refrigeration) for up to 24 h prior to confirmation.

C. *Salmonella* may be culturally isolated from *Salmonella* positive samples using the *Salmonella* Concentration Reagent (included with Assurance® GDS for *Salmonell*a kit), which contains a mixture of IMS particles containing all *Salmonella* serogroups. This method utilizes the Assurance® GDS PickPen™ device to isolate using the *Salmonella* Concentration Reagent. Plate IMS beads to chromogenic agar plates. Spread plates for isolation. Incubate plates for 20–24 h at 36 ± 1 °C.

Isolation of *Salmonella* using Concentration Reagent and PickPen™ Device Equipment / Materials Required

Necessary materials in addition to those needed for the SECONDARY SCREENING PROTOCOL:

Salmonella Concentration Reagent

Wash Solution

Sterile disposable agar plate spreaders

Incubator capable of maintaining 36 ± 1 °C (for incubation of plates)

Note: For **dairy products**, no BHI subculture should be performed for confirmation; instead, IMS-process as non-dairy samples.

1. Aliquot 20 µL of homogenized *Salmonella* Concentration Reagent, using a repeat pipette and a 0.5 mL pipette tip, into the GDS sample wells (1 well/sample). Cover sample wells with adhesive film strips.
2. Add 1 mL, using a repeat pipette and a 10 mL pipette tip, of Wash Solution into another set of GDS sample wells (1 well/sample). Cover sample wells with adhesive film strips.
3. Add 100 µL of Wash Solution, using a repeat pipette and a 0.5 mL pipette tip, to the required number of wells in the resuspension plate (2 wells/sample). Cover resuspension plate wells containing Wash Solution with adhesive film strips.
4. Carefully remove the adhesive film from 1 strip of GDS sample wells containing Concentration Reagent. Following incubation, gently mix enriched presumptive positive samples by hand to ensure homogeneity. Add 1 mL of the enriched sample to each sample well. Avoid transferring food particles. A new pipette tip must be used for each sample. Seal each row of the sample wells with new adhesive film strip.
5. Place the sealed sample wells containing the *Salmonella* Concentration Reagent and enrichments on the plate vortex mixer at approximately 900 RPM for 5-15 min.
   Note: If necessary, adjust the RPM to be certain that liquid does not contact adhesive film.
6. Carefully remove and discard the adhesive film from 1 strip of samples. Remove the corresponding adhesive film from sample wells contains Wash Solution.
7. Load tips onto the PickPen™ device, ensuring that the tips are firmly in place on the PickPen™ tool. Extend the PickPen™ magnets and insert tips into the first strip of sample wells. Stir tips gently for at least 30 s while continually moving up and down from the surface to the bottom of the wells. Gently tap the PickPen™ tips against the side of the sample wells to remove excess media droplets.
8. Transfer the PickPen™ tips to corresponding sample wells containing Wash Solution and gently swirl for at least 10 s. Do not release particles into solution. Gently tap the PickPen™ tips against the side of the sample wells to remove excess Wash Solution droplets.
9. Transfer PickPen™ tips to the corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen™ magnets and tap tips gently to release particles into the Wash Solution.
10. Make an additional 1:10 dilution of the *Salmonella* Concentration Reagent (IMS beads) in Wash Solution. Perform dilutions in the resuspension plate wells.
11. Briefly pipette up and down Wash Solution to resuspend beads. Transfer 50 µL of 1:10 diluted beads from the resuspension plate to chromogenic plate of choice: Sigma *Salmonella* ChromoSelect Agar (Sigma Cat#05538) or CHROMID *Salmonella* (bioMérieux Cat# 43621) or RAPID' *Salmonella* Agar (Bio-Rad Cat# 3563961). Spread beads for isolation. Incubate plates for 22 ± 2 h at 36 ± 1 °C (per Manufacturer's instructions).
12. Confirm typical colonies by latex agglutination test for *Salmonella* (Oxoid™ *Salmonella* test kit Cat#DR1108A, Microgen® *Salmonella* latex test Cat#M42CE, or equivalent).

Note: In the event of discordant results (positive presumptive results with the alternative method, non-confirmed by one the means above), the laboratory must follow the necessary steps to ensure validity of the result obtained.

Storage

Store Assurance® GDS for *Salmonella* Tq kit components at 5 ± 3 °C. Kit expiration is provided on the product box label.

Precautions

Comply with Good Laboratory Practice (refer to EN ISO 7218 standard)

Do not use test kit beyond expiration date on the product box label.

SAFETY

Assurance® GDS for *Salmonella* Tq kit — This product is not intended for human or veterinary use. Assurance® GDS for *Salmonella* Tq must be used as described in the package insert. Contents of the test may be harmful if swallowed or taken internally. The user should read, understand, and follow all safety information in the instructions for the Assurance® GDS for *Salmonella* Tq kit. Retain the safety instructions for future reference.

Do not open or autoclave used Amplification Tubes. After run is complete, place used Amplification Tubes into a sealed container with sufficient volume of a 10% bleach solution to cover tubes for a minimum of 15 min or double bag amplification tubes and dispose outside of the lab. Follow all applicable local, state/provincial, and/or national regulations on disposal of wastes. If contamination is suspected, moisten paper towel with 10% bleach solution and wipe all lab benches and equipment surfaces. Avoid spraying bleach solution directly onto surfaces. Allow bleach solution to remain on surfaces for a minimum of 15 min before wiping clean with 70% isopropyl alcohol solution.

To prepare 10% bleach solution, add 10 mL of commercially available bleach containing at least 5% sodium hypochlorite to 90 mL of deionized water. The minimum final concentration of sodium hypochlorite in the bleach solution should be 0.5%. The bleach solution is stable for 7 days from preparation. To prepare 70% isopropyl alcohol solution, add 70 mL of pure isopropyl alcohol to 30 mL of deionized water or buy commercially available 70% isopropyl alcohol.

Assurance® GDS Rotor-Gene® thermocycler — Improper use of the Assurance® GDS Rotor-Gene® may cause personal injuries or damage to the instrument. Some components may pose a risk of personal injury due to excessive heat if improperly handled. For safe use, the instrument must only be operated by qualified laboratory personnel who have been appropriately trained. Servicing of instrument must only be performed by MilliporeSigma Service Engineers.

Sample Enrichment — To reduce the risks associated with exposure to chemicals and biohazards, perform pathogen testing in a properly equipped laboratory under the control of trained personnel. Always follow standard laboratory safety practices, including wearing appropriate personal protective apparel and eye protection while handling reagents and contaminated samples. Avoid contact with the contents of the enrichment media and reagent tubes after amplification. Dispose of enriched samples according to current industry standards. Decontaminate and dispose of materials in accordance with good laboratory practices and in accordance with local, state, and federal regulations.

Salmonella Precautions — *Salmonella* are biosafety level-2 organisms. Biological samples**,** such as enrichments**,** have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations on disposal of biological wastes. Wear appropriate protective equipment which includes, but is not limited to: protective eyewear, face shield, laboratory coat, and gloves. All work should be conducted in properly equipped facilities utilizing the appropriate safety equipment (e.g., physical containment devices). Individuals should be trained in accordance with applicable regulatory and company/institution requirements before working with potentially infectious materials. All enrichment broths should be sterilized following any culture-based confirmatory steps. Clean the workstations and laboratory equipment with a disinfectant of choice before and after lab activities (sodium hypochlorite solution, phenol solution, quaternary ammonium solution, etc.).

NF Validation certificate granted by AFNOR Certification for TRANSIA® PLATE Listeria as an alternative method of analysis for all food products and industrial production environmental samples in relation to the reference method described in the ISO EN 11290-1 international standard in accordance with EN ISO 16140-2 (2016). For more information about the NF VALIDATION certification, please refer to the certificate available at http://nf-validation.afnor.org/en

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ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS

http://nf-validation.afnor.org/en

Appendix A – Enrichment Media Recipes

Buffered Peptone Water (BPW)
Follow the manufacturer's instructions for preparation of media.

Buffered Peptone Water w/ Vancomycin (BPW+v)
Prepare BPW as described above. On day of use, add 0.675 mL of 0.2% Vancomycin solution to 225 mL BPW (10.1 mL of Vancomycin solution to 3375 mL BPW).

Buffered Peptone Water w/ Amylase (BPW+a)
Prepare BPW as described above. On day of use, add 2.25 mL of 1% Amylase solution to 225 mL BPW (33.8 mL of Amylase solution to 3375 mL BPW).

Buffered Peptone Water w/ Vancomycin and Amylase (BPW+v+a)
Prepare BPW as described above. On day of use, add 0.675 mL of 0.2% Vancomycin solution and 2.25 mL of 1% Amylase solution to 225 mL BPW (10.1 mL of Vancomycin solution and 33.8 mL of Amylase solution to 3375 mL BPW).

0.2% Vancomycin Solution
Dissolve 0.2 g of vancomycin hydrochloride in 100 mL of sterile deionized water. Store in dark at 2 – 8 °C.

1% Amylase Solution
Dissolve 1.0 g of alpha-Amylase (~50 U/mg) in 100 mL of sterile deionized water. Store in dark at 2 – 8 °C.

Note: Verify amylase source does not also contain dextrin by manufacturer. Ensure amylase is from bacterial origin. We recommend Sigma #10070 or MP Biologics #0210044725.

Brain Heart Infusion

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

1% Brilliant Green Dye Solution

Create a 1% Brilliant Green Dye stock solution by dissolving 1 g Brilliant Green Dye in 100 mL of sterile deionized water. Do not autoclave.

Brilliant Green Water (BGW)

To prepare Brilliant Green Water, add 2 mL of the 1% Brilliant Green Dye solution to 1 L of sterile deionized water.

Appendix B – Enrichment Methods

Table 1: Sample Type and Enrichment Method for *Salmonella* in All Foods (except Infant Formula, Infant Cereals and NFDM) and Environmental Surfaces– NF Validation (**TRA 02/12-01/09**)

Food Type / Environmental Sample	**Media**	**Sample size**	**Sample:Media Ratio**	**Enrichment Time**	**Enrichment Temperature**
No BHI subculture					
Processed food types (see exceptions below)	BPW	25 g	1:10	18 – 24 h	37 °C
Environmental samples (Nearby food contact surfaces*)	BPW	Swab Sponge	10 mL 100 mL	18 – 24 h	37 °C
Process water	BPW	25 mL	1:10	18 – 24 h	37 °C
With BHI subculture					
Raw and unprocessed food types	BPW	25 g	1:10	18 – 24 h	37 °C
Environmental samples (Distant non-food contact surfaces**)	BPW	Swab Sponge	10 mL 100 mL	18 – 24 h	37 °C
Dust, sweepings	BPW	25 g	1:10	18 – 24 h	37 °C

* Environmental samples: Food (and non-food) product contact surfaces, work surfaces and adjacent areas (i.e. blenders, work tables, drip shields, housing)
**Environmental samples: non-food contact surfaces not close to work surfaces (i.e. drains, floors, walls, cart wheels)

Table 2: Sample Type and Enrichment Method for *Salmonella* (Infant Formula, Infant Cereals, and NFDM)

Food Type	**Media**	**Sample size**	**Sample:Media Ratio**	**Enrichment Time**	**Enrichment Temperature**
No BHI subculture					
Infant Formula <u>without</u> Probiotics	BPW	10 – 25 g	1:10	18 – 26 h	37 °C
		50 – 375 g		18 – 29 h	37 °C
Infant Cereals <u>without</u> Probiotics	BPW+a	10 – 25 g	1:10	18 – 26 h	37 °C
		50 – 375 g		18 – 29 h	37 °C
With BHI subculture					
Infant Formula <u>with</u> Probiotics	BPW+v	10 – 25 g	1:10	18 – 26 h	37 °C
		50 – 375 g		18 – 29 h	37 °C
Infant Cereals <u>with</u> Probiotics	BPW+v+a	10 – 25 g	1:10	18 – 26 h	37 °C
		50 – 375 g		18 – 29 h	37 °C
NFDM	BGW	10 – 25 g	1:10	20 – 28 h	37 °C
		50 – 375 g		20 – 29 h	37 °C

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