ASSAY PRINCIPLES
Veriflow® Salmonella Species (SS) is a molecular based assay for the presumptive and qualitative detection of Salmonella species. The assay utilizes a PCR detection method coupled with a rapid, visual, flow-based assay that develops in 3 minutes post PCR amplification and requires only 18 hours of incubation for maximum sensitivity. The Veriflow® SS system eliminates the need for gel electrophoresis or fluorophore based detection of target amplifications, and does not require complex data analysis. Veriflow® SS provides the specificity and sensitivity of PCR based amplification in a cost-efficient and easy-to-use format.

INTENDED USER
The Veriflow® SS system is intended for use by personnel familiar with basic sample collection and preparation techniques associated with foodborne pathogen detection. Veriflow® SS is specifically designed to be easy-to-use and eliminates the need for advanced training in molecular biology.

MATERIALS PROVIDED
1. IS SS PCR Tube - Cat. No. IS05010200
2. 1.5 mL Sampling Tubes - Cat. No. IS0903
3. IS Buffer B - Cat. No. IS0702
4. IS VeriFlow® SS Assay Cassette - Cat. No. IS0101

MATERIALS PURCHASED SEPARATELY
1. IS BPW Broth - Cat. No. IS0305
2. IS Lactose Broth- Cat. No.IS0306
3. IS Chicken Rinse Enrichment Broth – Cat. No. IS0308

MATERIALS NEEDED FOR ENVIRONMENTAL SAMPLES
1. Dry pre-moistened sampling sponges OR swabs:
   a. Should not exceed 10 mL, and Swab should not exceed 2 mL of hydration with Dey-Engley broth.
   b. Dey-Engley broth (if necessary)
   c. 18 oz (532 mL) incubation bag for sponge samples
   d. 3 oz (58 mL) incubation bag for swab samples

MATERIALS NEEDED FOR FOOD SAMPLES
1. 24 oz (710 mL) Incubation Bags (for Chicken Rinse, Hot Dog and Milk Samples)
2. 24 oz (710 mL) or 92 oz (2721 mL) incubation bags (for Ground Beef samples)
3. 2 mg/mL Solution Novobiocin

MATERIALS NEEDED FOR ALL ENRICHMENT TYPES
1. Invisible Sentinel SimpliAmp PCR Thermocycler – Cat. No. ISTC002
2. Incubator that provides consistent and stable temperatures of 35°C ± 2°C
3. Water bath for boiling or heating block (95°C ± 2°C)
4. Heat tolerant beaker
5. Pipettes and tips capable of 5 μL, 200 μL, and 1000 μL volume transfers
6. Glassware and autoclave for media prep
7. Racks and culture bags
8. dH₂O
9. Scale for weighing of sample and media

STORAGE OF MATERIALS
The VeriFlow® SS kit components, including cassettes, growth media and buffers should be stored at room temperature (20-25°C). The IS SS PCR tubes should be stored at -20°C ± 1°C.

PRECAUTIONS
1. Salmonella species bacteria are human pathogens. All samples collected for use with the VeriFlow® SS Assay should be handled with care.
2. Assay users should observe standard BSL-2 microbiological practices and safety precautions when performing this assay. Because of high levels of enriched bacteria can result from use, immunocompromised individuals should avoid using the VeriFlow® SS system.
3. Do not use VeriFlow® SS kit past indicated expiration date.
4. Do not use media past indicated expiration date.
5. Use rehydrated media within 30 days of preparation.
6. Deviations from the assay protocol may impact overall test performance.

MEDIA PREP
BPW Broth - For environmental surfaces, chicken carcass rinses, and select food matrices
1. Add 20 grams BPW broth per 1 Liter dH₂O.
2. Autoclave at 121°C for 15 minutes.
3. Media can be stored at room temperature for 30 days.
4. NOTE: Pre-warm media to 35°C ± 2°C before use.

Lactose Broth - For milk matrices
1. Add 13 grams Lactose broth per 1 Liter dH₂O.
2. Autoclave at 121°C for 15 minutes.
3. Media can be stored at room temperature for 30 days.
4. NOTE: Pre-warm media to 35°C ± 2°C before use.

IS Carcass Rinse Enrichment Broth – for use with BPW carcass rinse
1. Add 20.5 grams of IS Carcass Rinse Enrichment broth per 1 Liter dH₂O.
2. Autoclave at 121°C for 15 minutes and allow media to cool to room temperature.
3. Pipette 4.15 mL of Novobiocin supplement per 1 liter media.
4. Media can be stored at 4°C for 30 days.
5. NOTE: Pre-warm media to 35°C ± 2°C before use.

NOTE: See Appendix 1 for matrices tested
For environmental samples, go to section I and II.
For ready-to-eat and meat samples, go to sections III and IV.
For dairy samples, go to section V.
For chicken carcass rinses, go to section VI.

SECTION I: SPONGE SURFACE SAMPLING AND ENRICHMENT
(Swabs see Section II)
1. If sponges are not pre-moistened, pipette 10 mL of Dey-Engley Neutralization broth to each sponge placed in an 18 oz (532 mL) incubation bag.
2. Squeeze excess moisture from sponge and remove from incubation bag.
3. Sample surface area of interest using sponge for 30 seconds.
4. Transfer sponge back to incubation bag.
5. Transfer 100 mL IS BPW Broth into bag containing sponge used for sampling, seal, and briefly (5-10 seconds) massage sponge.
6. Place bag into 35°C ± 2°C incubator, in rack, for 18-24 hours.

SECTION II: SWABS SURFACE SAMPLING AND ENRICHMENT
(Sponges see Section I)
1. If swabs are not pre-moistened, pipette 2 mL of Dey-Engley Neutralization broth to each swab placed in a 3 oz (89 mL) incubation bag.
2. Squeeze excess moisture from swab and remove from incubation bag.
3. Sample surface area of interest using swab for 30 seconds.
4. Transfer swab back to incubation bag.
5. Transfer 20 mL IS BPW Broth into bag containing swab used for sampling, seal, and briefly (5-10 seconds) massage swab.
6. Place bag into 35°C ± 2°C incubator, in rack, for 18-24 hours.

SECTION III: SAMPLING AND ENRICHMENT for 25 GRAM RTE and MEAT SAMPLE
1. Weigh out 25 gram sample.
2. Transfer 25 gram sample to 24 oz (710 mL) incubation bag.
3. Transfer 226 mL IS BPW to incubation bag containing sample.
4. Stomach 1-2 minutes to break down sample.
5. Place bag into 35°C ± 2°C incubator, in rack, for 18-24 hours.

SECTION IV: SAMPLING AND ENRICHMENT for 325 GRAM DELI MEAT SAMPLES
1. Weigh out 325 gram Meat sample.
2. Transfer 325 gram sample of Deli Meat to 92 oz (2721 mL) incubation bag.
3. Transfer 1625 mL IS BPW media to incubation bag containing sample.
4. Stomach 1-2 minutes to break down sample.
5. Place bag into 35°C ± 2°C incubator, in rack, for 18-24 hours.

SECTION V: SAMPLING AND ENRICHMENT for DAILY SAMPLES
1. Transfer 226 mL IS Lactose broth to 24 oz (710 mL) incubation bag.
2. Transfer 25 mL or 25 g sample directly to bag from step 1.
3. Agitate bag to evenly distribute sample.
4. Place bag into 35°C ± 2°C incubator, in rack, for 18-24 hours.

SECTION VI: SAMPLING AND ENRICHMENT for CHICKEN CARCASS SAMPLES
NOTE: The following chicken rinse procedure will require two separate media preparations, for rinsing (BPW) and for enrichment (IS Chicken Carcass Rinse enrichment broth media).
1. Rinse drained chicken carcass with 400 mL of IS BPW in suitable sealed bag and agitate thoroughly for 1-2 minutes.
2. Transfer 30 mL of chicken rinsate from step 2 to an 18 oz. incubation bag.
3. Pipette 36 mL of chicken carcass enrichment broth to enrichment bag
4. Place bag into 35°C ± 2°C incubator, in rack, for 18-24 hours.

SAMPLE PREP and PCR
1. Place provided 1.5 mL sampling tubes in rack (1 for each sample to be tested).
2. Remove incubation bag from incubator and agitate to suspend any settled contents.
3. Pipette 500 μL of enriched culture 1.5 mL sampling tube, seal, and invert to mix contents.
4. Boil 1.5 mL tube with sample in water bath or heating block for 10 ± 1 minute and allow to cool to room temperature (20-25°C).
5. N. Samples can be stored sealed at -20°C ± 2°C, pre or post boil, for 1 week prior to step below.
6. Transfer 5 mL of broth from cooled sample from step 4 above to thawed VeriFlow® SS PCR tube for each sample (have VeriFlow® SS PCR tube for 10 ± 1 minutes at room temperature [20-25°C] and use immediately).
7. NOTE: Open VeriFlow® SS PCR tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
8. Place VeriFlow® SS PCR Tube into IS PCR Thermocycler, select “VFLOWSS” program and press “START RUN” as directed by the Thermocycler User Guide.

CASSETTE SAMPLE ANALYSIS
1. Remove tubes from IS PCR Thermocycler and add 4 drops of BUFFER B directly to each VeriFlow® SS PCR tube.
2. Transfer entire contents (200 μL) of VeriFlow® SS PCR tube directly to VeriFlow® SS cassette sample window with pipette. A separate VeriFlow® SS cassette must be used for each PCR tube.
3. Allow VeriFlow® SS cassette to develop for 2 minutes ± 15 seconds.
4. Add 4 drops of BUFFER B directly to each VeriFlow® SS Assay cassette sample window.
5. Allow VeriFlow® SS cassette to develop for 1 minute ± 15 seconds.
6. NOTE: VeriFlow® SS cassette can be developed for up to 120 min before proceeding to step 6.
7. Retrack VeriFlow® SS cassette switch and record results.
   a. The appearance of one red line (control) in the VeriFlow® SS cassette sample window indicates a negative result.
   b. The appearance of two red lines (control and test) in the VeriFlow® SS cassette sample window indicates a positive result.