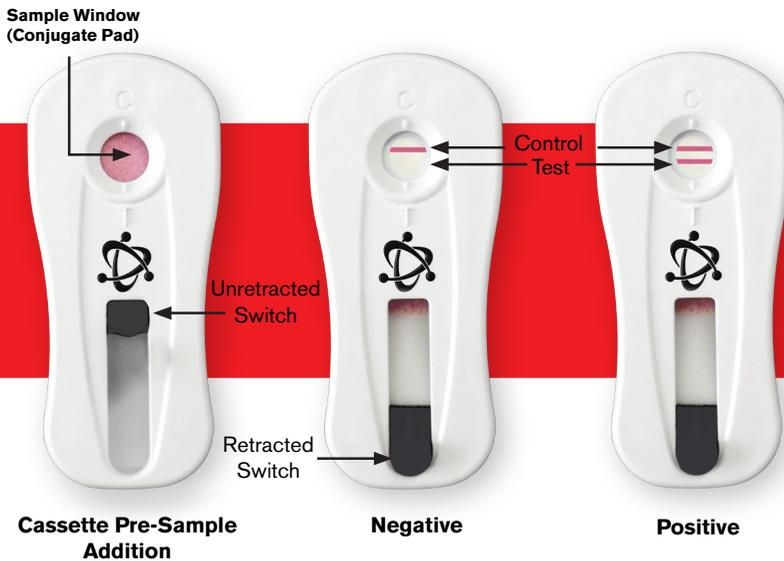


APPENDIX 1: ASSAY APPLICABILITY

Spices

APPENDIX 2: RESULTS INTERPRETATION



The control line, as indicated by the letter C on the test cassette, should always develop. The test line, as indicated by the letter T on the cassette, will only develop in the event of a positive sample for *Salmonella species*. If the control line fails to develop, the test is invalid, and will need to be repeated.

APPENDIX 3: CONFIRMATION OF RESULTS

Presumptive positive samples must be confirmed by the FDA BAM Chapter 5 method for the detection of *Salmonella species* from spice Samples.

Enriched samples (un-boiled) from the Veriflow procedure can be transferred to secondary enrichment for confirmation following the necessary steps described in the FDA BAM Chapter 5 method.

APPENDIX 4: DISPOSAL

Invisible Sentinel devices are for single use only. Decontaminate all surfaces, media and reagents and discard in accordance with local, state, and federal regulations.

CUSTOMER SERVICE

Invisible Sentinel customer service and technical assistance can be reached Monday-Friday between 9AM and 5PM Eastern Standard Time by calling 215-966-6118 and asking for an Invisible Sentinel sales or technical representative. Training on this product and all Invisible Sentinel test kits is available.

SDS INFORMATION AVAILABLE

Safety Data Sheets (SDS) are available for this test kit and all of Invisible Sentinel's test kits by calling Invisible Sentinel at 215-966-6118.



V. IS0972.2

INVISIBLE SENTINEL

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THE POWER OF
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ASSAY PRINCIPLES

Veriflow® *Salmonella species Spice* (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 18-24 hours of incubation for maximum sensitivity. The Veriflow® *SS Spice* system eliminates the need for gel electrophoresis or fluorophore based detection of target amplifications, and does not require complex data analysis. Veriflow® *SS Spice* provides the specificity and sensitivity of PCR based amplification in a cost-efficient and easy-to-use format.

INTENDED USER

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

Invisible Sentinel® and Veriflow® are trademarks of Invisible Sentinel, Inc., of Philadelphia, PA. U.S. Patent No. 8,183,059 and other patents pending. Purchase and use of this product is subject to Invisible Sentinel's Terms and Conditions of Sale located at <http://www.invisiblesentinel.com>.

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MATERIALS PROVIDED

1. IS *SS Spice* PCR tube - Cat. No. IS0523
2. IS Buffer B - Cat. No. IS0702
3. IS Veriflow® *SS Spice Assay Cassette* - Cat. No. IS0101
4. 1.5 mL *SS Spice* Sampling Tubes - Cat. No. IS0973

MATERIALS NEEDED for SPICE SAMPLES

1. Enrichment/incubation bags
2. Incubator that provides continuous and stable temperatures of 35°C ± 2°C
3. Heating block (95°C ± 2°C)
4. Invisible Sentinel SimpliAmp PCR Thermocycler Cat No. ISTC002
5. Pipettes and tips for 5, 50, and 200 µl volumes
6. Glassware and autoclave for media prep
7. Racks for culture bags and 1.5 ml tubes
8. dH₂O
9. Scale for weighing of sample and media

STORAGE OF MATERIALS

The Veriflow® *SS Spice* kit components, including cassettes, growth media and buffers should be stored at room temperature (20-25°C). The Veriflow® *SS Spice* PCR tubes should be stored at -20°C ± 2°C.

PRECAUTIONS

1. *Salmonella species* bacteria are human pathogens. All samples collected for use with the Veriflow® *SS Spice* assay should be handled with care.
2. Assay users should observe standard BSL-2 microbiological practices and safety precautions when performing this assay. Because high levels of enriched bacteria can result from use, immunocompromised individuals should avoid using the Veriflow® *SS Spice* system.
3. Do not use Veriflow® *SS Spice* 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.

MATRIX GUIDE

Spice Type	Recommended Media and Sample to Media Ratio	Enrichment Time at 35°C
Black/white pepper, celery seed or flakes, chili powder, cumin, paprika, parsley flakes, rosemary, sesame seed, vegetable flakes, nutmeg, turmeric, coriander	BPW or TSB 1:9 (e.g. 25 grams + 225 mL)	24-26 hours
Onion flakes, onion powder, garlic flakes	TSB 1:9 with 0.5% K ₂ SO ₃ (e.g. 25 grams + 225 mL)	24-26 hours
Basil, marjoram, cilantro, thyme	BPW or TSB 1:19 (e.g. 25 grams + 475 mL)	24-26 hours
Allspice, cinnamon, cloves, oregano	TSB 1:100 (e.g. 25 grams + 2500 mL)	24-26 hours

TSB = Tryptone Soy Broth, BPW = Buffered Peptone Water. Follow manufacturer directions for media preparation. Note: **pre-warm media to 35°C ± 2°C** before use. Compatibility of assay is not limited to spices listed above.

SAMPLING and ENRICHMENT for 25 and 375 GRAM SAMPLES

1. Weigh out 25 or 375 gram sample.
2. Transfer 25 or 375 gram sample to sufficiently sized incubation bag.
3. Transfer required volume and media type to incubation bag containing sample.
4. Agitate sample to homogenize (or stomach as necessary).
5. Place bag into 35°C ± 2°C incubator, in rack, for 24-26 hours, as detailed above.

SAMPLE PREP and PCR

1. Place provided 1.5 mL *SS Spice* sampling tubes in rack (1 for each sample to be tested).
2. Remove culture enrichment bag from incubator and agitate to suspend any settled contents.
3. Pipette 50µL of enriched culture to 1.5 mL sampling tube, seal, and invert to mix sample.
4. Boil 1.5 mL tube with sample in heating block for 10 ± 1 minute and allow to cool for at least 10 minutes at room temperature (20-25°C).
 - a. Note: Samples can be stored sealed at -20°C ± 2°C, pre or post boil, for 1 week prior to step 5 below.
5. Transfer 5µL of liquid from cooled sample from step 4 above to thawed Veriflow® *SS Spice* PCR tube for each sample (thaw PCR tube for 10 ± 1 minutes at room temperature (20-25°C) and use immediately).
 - a. Note: Open Veriflow® *SS Spice* PCR tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
6. Place Veriflow® *SS Spice* PCR tube into Invisible Sentinel PCR Thermocycler, select "SS SPICE" program and press "START RUN" as directed by the Thermocycler User Guide.
7. Upon completion of "SS SPICE" program, press "STOP RUN" and proceed to Cassette Sample Analysis section.

CASSETTE SAMPLE ANALYSIS

1. Remove tubes from Invisible Sentinel SimpliAmp PCR Thermocycler and add 4 drops of BUFFER B directly to each Veriflow® *SS Spice* PCR tube.
2. Transfer entire contents (200 µL) of Veriflow® *SS Spice* PCR tube directly to Veriflow® *SS Spice* Assay cassette sample window with pipette. A separate Veriflow® *SS Spice* Assay cassette must be used for each Veriflow® *SS Spice* PCR tube.
3. Allow test to develop for 2 minutes ± 15 seconds.
4. Add 4 drops of BUFFER B directly to each Veriflow® *SS Spice* Assay cassette sample window.
5. Allow test to develop for 1 minute ± 15 seconds.
 - a. Note: Cassette may develop for up to 120 minutes before proceeding to Step 6.
6. Retract switch and immediately record results.
 - a. The appearance of one red line (control) in the cassette window indicates a negative result.
 - b. The appearance of two red lines (control and test) in the cassette window indicates a positive result.