Thermo Scientific SureTect Salmonella species PCR Assay shows superior performance to the bioMérieux VIDAS™ UP Salmonella (SPT) Assay

Application Note

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Key Words
Thermo Scientific™ SureTect™ Salmonella species PCR Assay, Thermo Scientific SureTect System, Thermo Scientific™ PikoReal™ Real-Time PCR Instrument, PCR, *Salmonella*, bioMerieux VIDAS™™ UP Salmonella (SPT) Assay, VIDAS™ system

Abstract
Purpose: The aim of this study was to compare the performance of the Thermo Scientific SureTect Salmonella species PCR Assay (Thermo Fisher Scientific) to the VIDAS™ UP Salmonella (SPT) Assay (bioMérieux) for detection of *Salmonella* from food samples artificially contaminated with *Salmonella* species.

Methods: Tests were carried out on raw chicken breast, raw minced beef, fresh bagged lettuce, non-fat dried milk powder and pasteurized liquid whole egg. Each food sample was combined with enrichment broth in a 1:10 ratio and artificially contaminated with stressed *Salmonella* at 3 spike levels. Following incubation, enriched food samples were tested using the SureTect Salmonella species PCR Assay and VIDAS™ UP Salmonella (SPT) Assay. All positive and negative results were confirmed according to manufacturer's instructions.

Results: The SureTect Salmonella species PCR Assay showed high sensitivity, specificity, accuracy, inclusivity and exclusivity. VIDAS™ UP Salmonella (SPT) Assay was unable to detect 48% of all *Salmonella* isolates tested. The SureTect Salmonella species PCR Assay has a more user friendly enrichment method and a quicker time to result compared to the VIDAS™ UP Salmonella (SPT) Assay when run on the mini VIDAS™. In addition, the PikoReal Real-Time PCR instrument has a small footprint and an internal amplification control is included for test reaction.

Introduction
Salmonella is one of the most frequent causes of food poisoning and a major public health problem worldwide. Foods often contaminated with *Salmonella* include meat, poultry, milk and dairy products, eggs, seafood, and some fruits and vegetables. The detection of *Salmonella* in foods before they are consumed is vital for safeguarding public health, and essential for preserving the financial health and reputation of food businesses[1].

The SureTect Salmonella species PCR Assay is a real-time PCR test which is optimized for use in conjunction with the PikoReal Real-Time PCR Instrument and the Thermo Scientific™ SureTect™ Software for the detection of *Salmonella* spp. in food and associated samples.

The VIDAS™ UP Salmonella (SPT) test is an enzyme-linked fluorescent assay (ELFA) using a recombinant phage protein based technology for use in conjunction with the automated VIDAS™ or miniVIDAS™ instruments for the specific detection of *Salmonella* spp. in food samples.

Methodology
Sample preparation
Raw chicken breast, raw minced beef, fresh bagged lettuce, non-fat dried milk powder and pasteurized liquid whole egg were tested. For each matrix tested, a total of ten bags of 25g food sample were weighed out. Twenty-five g food samples were combined with 225mL of non-selective enrichment broth, homogenized thoroughly by stomaching at 230 rpm for 30 seconds and incubated under appropriate conditions. Several AOAC-RI validated enrichment methods for the different food matrices were used during the study (see Table 1 on p. 2 for details).

Artificial contamination method
The *Salmonella* serotypes used to spike food matrices were either heat or cold stressed depending on the food matrices being tested. Isolates used to spike raw chicken breast, raw minced beef and fresh bagged lettuce were cold stressed by culturing the organism in Thermo Scientific™ Oxoid™ Tryptone Soya Broth (TSB) at 37°C for 18-24 hrs then chilling at 4°C for 12 weeks. Isolates used to spike non-fat dried milk powder and pasteurized liquid whole egg were cultured in TSB at 37°C for 18-24 hrs then heat stressed in a water-bath set at 55°C for 10 mins. The difference in log obtained between enumeration on Tryptic Soy Agar with Yeast Extract Agar (TSAYE) and enumeration on XLD Agar was calculated for each serotype. Prior to incubation, each of the food samples were individually spiked with low (1-5 CFU/25 g) and high (10-20 CFU/25 g) concentrations of *Salmonella*. For each food matrix tested, five samples were analyzed at a low spike level, three at a high spike level and two unspiked samples were analyzed as negative controls.

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Table 1. Enrichment methods for SureTect Salmonella species PCR Assay and VIDAS™ UP Salmonella (SPT) Assay

Food matrix	Conditions	SureTect Salmonella species PCR Assay	VIDAS™ UP Salmonella (SPT) Assay
Raw chicken breast	Primary enrichment	BPW (ISO)	BPW
	Supplement	None	Salmonella supplement
	Incubation time	20-24 hrs	18-24 hrs
	Incubation temperature	37±1°C	42±1°C
Raw minced beef	Primary enrichment	BPW (ISO)	BPW
	Supplement	None	Salmonella supplement
	Incubation time	18-24 hrs	18-24 hrs
	Incubation temperature	37±1°C	42±1°C
Fresh bagged lettuce	Primary enrichment	BPW (ISO)	BPW
	Supplement	None	Vancomycin supplement
	Incubation time	20-24hrs	18-24 hrs
	Incubation temperature	37±1°C	42±1°C
Non-fat dried milk powder	Primary enrichment	BPW (ISO)	BPW
	Supplement	None	Salmonella supplement
	Incubation time	18-24 hrs	18-24 hrs
	Incubation temperature	37±1°C	42±1°C
Pasteurized liquid whole egg	Primary enrichment	BPW (ISO)	pre-warmed BPW
	Supplement	None	None
	Incubation time	18-24 hrs	16-24 hrs
	Incubation temperature	37±1°C	42±1°C

SureTect Salmonella species PCR Assay procedure

Following appropriate enrichment of food samples, the SureTect Salmonella species PCR Assay was performed according to the manufacturer's Instructions for Use (IFU). In summary, 10µL of each enriched food sample was added to the prefilled SureTect Lysis Tubes (prepared by additionally adding Proteinase K Reagent) and the sample lysed according to the SureTect lysis protocol (37°C for 10 mins followed by 95°C for 5 mins). Once lysed, 20µL of the lysate was added to the SureTect PCR Tubes (contain lyophilised PCR reagents). Then the SureTect PCR Tubes were loaded into the PikoReal Real-Time PCR instrument and the PCR run was started. After approximately 1 hr and 20 mins, the SureTect Salmonella species PCR Assay results were automatically interpreted as 'positive' or 'negative' by the SureTect Software.

All results from the SureTect System were confirmed culturally using the SureTect confirmation method as stated in the IFU. Enriched samples were streaked onto Thermo Scientific™ Oxoid™ *Brilliance*™ Salmonella Agar and incubated at 36±1°C for 24 hrs. Presumptive positive purple colonies observed on *Brilliance* Salmonella Agar were confirmed using Thermo Scientific™ Oxoid™ Salmonella Latex Kit and Thermo Scientific™ Remel™ Salmonella O Polyvalent agglutinating sera.

VIDAS™ UP Salmonella (SPT) Assay procedure

Following appropriate enrichment of food samples, the VIDAS™ UP Salmonella (SPT) Assay was performed according to the manufacturer's Instructions for Use (IFU). In summary, 500µL of each enriched sample was added to the first tube of the single-dose reagent strip. Before running on the miniVIDAS™ instrument, each strip was heated in the VIDAS™ Heat & Go dry heating system at 131°C for 5±1 mins then cooled to room temperature for 10 mins. Heat-treated strips and the Solid Phase Receptacle (SPR™) were inserted into the appropriate position on the miniVIDAS™ instrument and the run was initiated. In approximately 48 mins, VIDAS™ UP Salmonella (SPT) Assay results were automatically calculated and interpreted by the miniVIDAS™ instrument as 'positive' or 'negative' and then printed out.

All VIDAS™ UP Salmonella (SPT) Assay results were confirmed following the USDA-FSIS method[2] for raw minced beef and raw chicken, and the FDA-BAM method[3] for all other food matrices. After incubation, 100µL of enriched sample was subcultured into 10mL of Rappaport-Vassiliadis Soya (RVS) Broth and 1mL of enriched sample was subcultured into 10mL of Muller Kaufmann Tetrathionate Novobiocin (MKTTn) Broth. The RVS and MKTTn broths were incubated at 42°C and 37°C, respectively for 24 hrs. A 10µL loop of each broth was streaked onto *Brilliance* Salmonella Agar; plates were incubated at 36±1°C for 24 hrs. Presumptive positive purple colonies observed on *Brilliance* Salmonella Agar were confirmed using Salmonella Latex Kit and Salmonella O Polyvalent agglutinating sera.

Inclusivity testing

Thirty-three Salmonella isolates covering a wide variety of serotypes (including *Salmonella enterica* subspp. *enterica* serotype Dublin, *Salmonella enterica* subspp. *enterica* serotype Gallinarum, *Salmonella bongori*, *Salmonella enterica* subspp. *enterica* serotype Typhimurium and *Salmonella enterica* subspp. *enterica* serotype Enteritidis)

were cultured in Thermo Scientific™ Oxoid™ Buffered Peptone Water (BPW) (ISO) at 37°C for 18-24 hrs. A 0.5 McFarland standard of each culture was prepared and diluted in Thermo Scientific™ Oxoid™ Maximum Recovery Diluent (MRD) to achieve an inoculum level of 10^4 CFU/mL. The lysis procedures detailed in the method section above for both assays were followed and then the *Salmonella* isolates were analyzed using the SureTect Salmonella PCR Assay and VIDAS™ UP Salmonella (SPT) Assay.

Exclusivity testing

Thirty-one non-Salmonella isolates including *Citrobacter freundii*, *Citrobacter koseri*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Haemophilus influenzae* and *Staphylococcus aureus* were cultured in TSB and incubated at 37°C for 18-24 hrs. Once each culture was diluted to match a 0.5 McFarland standard (approximately 10^8 CFU/mL), the lysis procedures for both assays were performed. The non-Salmonella isolates were then analyzed using the SureTect Salmonella species PCR Assay and VIDAS™ UP Salmonella (SPT) Assay.

Workflow

The workflow of the SureTect Salmonella species PCR Assay and VIDAS™ UP Salmonella (SPT) Assay was studied by comparing criteria such as assay kits contents and equipment, enrichment protocols, handling time, PCR run time and confirmation methods required to perform the assays.

Results and discussion

High Performance of the SureTect Salmonella species PCR Assay

Sensitivity, specificity and accuracy for both the SureTect Salmonella species PCR Assay and VIDAS™ UP Salmonella (SPT) Assay for all the food matrices analyzed were calculated. Results are presented in Tables 2-6.

Table 2. Performance of SureTect Salmonella species PCR assay and VIDAS™ UP Salmonella (SPT) Assay when testing raw chicken breast with *Salmonella* Mbandaka

		Confirmatory method	
		+	-
SureTect Salmonella species PCR assay	+	8	0
	-	0	2
Sensitivity (high spike)	100% (95% CI=100%)		
Sensitivity (low spike)	100% (95% CI=100%)		
Overall sensitivity	100% (95% CI=100%)		
Specificity	100% (95% CI=100%)		
Accuracy	100% (95% CI=100%)		

		Confirmatory method	
		+	-
VIDAS™ UP Salmonella (SPT) Assay	+	8	0
	-	0	2
Sensitivity (high spike)	100% (95% CI=100%)		
Sensitivity (low spike)	100% (95% CI=100%)		
Overall sensitivity	100% (95% CI=100%)		
Specificity	100% (95% CI=100%)		
Accuracy	100% (95% CI=100%)		

Table 3. Performance of SureTect Salmonella species PCR assay and VIDAS™ UP Salmonella (SPT) Assay when testing raw minced beef with *Salmonella* Agona

		Confirmatory method	
		+	-
SureTect Salmonella species PCR assay	+	7	0
	-	0	3
Sensitivity (high spike)	100% (95% CI=100%)		
Sensitivity (low spike)	100% (95% CI=100%)		
Overall sensitivity	100% (95% CI=100%)		
Specificity	100% (95% CI=100%)		
Accuracy	100% (95% CI=100%)		

		Confirmatory method	
		+	-
VIDAS™ UP Salmonella (SPT) Assay	+	6	0
	-	0	4
Sensitivity (high spike)	100% (95% CI=100%)		
Sensitivity (low spike)	100% (95% CI=100%)		
Overall sensitivity	100% (95% CI=100%)		
Specificity	100% (95% CI=100%)		
Accuracy	100% (95% CI=100%)		

Table 4. Performance of SureTect Salmonella species PCR assay and VIDAS™ UP Salmonella (SPT) Assay when testing fresh bagged lettuce with *Salmonella* Newport

		Confirmatory method	
		+	-
SureTect Salmonella species PCR assay	+	8	0
	-	0	2
Sensitivity (high spike)	100% (95% CI=100%)		
Sensitivity (low spike)	100% (95% CI=100%)		
Overall sensitivity	100% (95% CI=100%)		
Specificity	100% (95% CI=100%)		
Accuracy	100% (95% CI=100%)		

		Confirmatory method	
		+	-
VIDAS™ UP Salmonella (SPT) Assay	+	8	0
	-	0	2
Sensitivity (high spike)	100% (95% CI=100%)		
Sensitivity (low spike)	100% (95% CI=100%)		
Overall sensitivity	100% (95% CI=100%)		
Specificity	100% (95% CI=100%)		
Accuracy	100% (95% CI=100%)		

Table 5. Performance of SureTect Salmonella species PCR assay and VIDAS™ UP Salmonella (SPT) Assay when testing pasteurized liquid whole egg with *Salmonella* Indiana

		Confirmatory method	
		+	-
SureTect Salmonella species PCR assay	+	8	0
	-	0	2
Sensitivity (high spike)	100% (95% CI=100%)		
Sensitivity (low spike)	100% (95% CI=100%)		
Overall sensitivity	100% (95% CI=100%)		
Specificity	100% (95% CI=100%)		
Accuracy	100% (95% CI=100%)		

		Confirmatory method	
		+	-
VIDAS™ UP Salmonella (SPT) Assay	+	8	0
	-	0	2
Sensitivity (high spike)	100% (95% CI=100%)		
Sensitivity (low spike)	100% (95% CI=100%)		
Overall sensitivity	100% (95% CI=100%)		
Specificity	100% (95% CI=100%)		
Accuracy	100% (95% CI=100%)		

Table 6. Performance of SureTect Salmonella species PCR assay and VIDAS™ UP Salmonella (SPT) Assay when testing non-fat dried milk powder with *Salmonella* Anatum

		Confirmatory method	
		+	-
SureTect Salmonella species PCR assay	+	6	1
	-	0	3
Sensitivity (high spike)	100% (95% CI=100%)		
Sensitivity (low spike)	100% (95% CI=100%)		
Overall sensitivity	100% (95% CI=100%)		
Specificity	75.0% (95% CI=48.2-100%)		
Accuracy	90.0% (95% CI=71.4-100%)		

		Confirmatory method	
		+	-
VIDAS™ UP Salmonella (SPT) Assay	+	3	0
	-	4	3
Sensitivity (high spike)	50.0% (95% CI=19.0-81.0%)		
Sensitivity (low spike)	40.0% (95% CI=35.2%-44.8%)		
Overall sensitivity	42.8% (95% CI=12.1-73.5%)		
Specificity	100% (95% CI=100%)		
Accuracy	60.0% (95% CI=29.7-90.4%)		

The SureTect Salmonella species PCR Assay was able to detect low (1-5 CFU/25 g) and high (10-20 CFU/25 g) levels of *Salmonella* spp., showing 100% sensitivity for all food matrices tested. SureTect Salmonella species PCR Assay also showed 100% specificity and accuracy for raw chicken breast, raw minced beef, fresh bagged lettuce and pasteurized liquid whole egg.

Specificity of SureTect Salmonella species PCR assay for non-fat dried milk powder was reduced due to a positive result on the assay not being confirmed via the culture method. This may be a limitation of the culture method; the limit of detection for PCR may be lower than a culture method which would imply the culture method failed to detect *Salmonella* Anatum and the SureTect Salmonella species PCR assay result was correct.

VIDAS™ UP Salmonella (SPT) Assay showed poor overall performance for non-fat dried milk powder, failing to detect Salmonella Anatum in 4 samples, thus giving a low sensitivity and accuracy result.

Inclusivity / Exclusivity

SureTect Salmonella species PCR Assay showed superior inclusivity compared to the VIDAS™ UP Salmonella (SPT) Assay.

All 33 *Salmonella* isolates tested were detected by the SureTect Salmonella species PCR Assay. Sixteen *Salmonella* isolates (*Salmonella enterica* subspp. *enterica* serotype Gallinarum (2), *Salmonella arizoniae* (6), *Salmonella diarizoniae*, *Salmonella* Urbana, *Salmonella bongori*, *Salmonella* Kedougou, *Salmonella* Schalkwijk, *Salmonella subterranea*, *Salmonella enterica* subspp. *enterica* serotype Typhimurium and *Salmonella* Waycross) were not detected by the VIDAS™ UP Salmonella (SPT) Assay.

None of the 31 non-Salmonella species tested (including *C. freundii*, *C. koseri*, *P. mirabilis*, *P. vulgaris*, *E. cloacae*, *E. aerogenes*, *E. amnigenus*, *H. influenzae* and *S. aureus*) tested were detected by the either SureTect Salmonella species PCR Assay or VIDAS™ UP Salmonella (SPT) Assay.

Workflow

Assay kit contents and equipment; the PikoReal instrument has a smaller footprint and weighs less than the mini-VIDAS™.

Refer to table 7 for a summary of the assay kit contents and equipment.

Another advantage of the SureTect Salmonella species PCR Assay is that the assay does not require calibration. Positive and negative controls do not need to be tested due to the inclusion of the Internal Amplification Control in each PCR tube, allowing the user complete confidence that each PCR run has completed successfully and the result obtained is accurate. In contrast, although VIDAS™ UP Salmonella (SPT) Assay reagent strip includes separate positive and negative controls, users cannot be one hundred percent sure that each of the individual samples are reported correctly as there is no way to see if an individual reaction was inhibited or not.

The calibration process of the VIDAS™ UP Salmonella (SPT) Assay is complex and includes multiple steps.

Calibration of the assay using the S1 calibrator provided in the kit must be performed each time a new lot of reagent is opened, after the master lot entry (MLE) has been entered, and then every 28 days. Each calibration must also be checked using the controls C1 and C2. The calibrator must be tested in duplicate and, for controls, singly.

When using the VIDAS™ UP Salmonella (SPT) Assay for the first time and before reading the MLE card, the bar code on the side of the assay kit box must scanned. This allows VIDAS™ UP Salmonella (SPT) protocol data to be transferred to the instrument software for its update.
As experienced during the study, this stage of setting up rarely worked as designed and frequent calls to the Technical Support helpline were required to for further assistance.

Primary enrichment is more standardized and requires less hands-on time for the SureTect Salmonella species PCR Assay compared to the VIDAS™ UP Salmonella (SPT) Assay

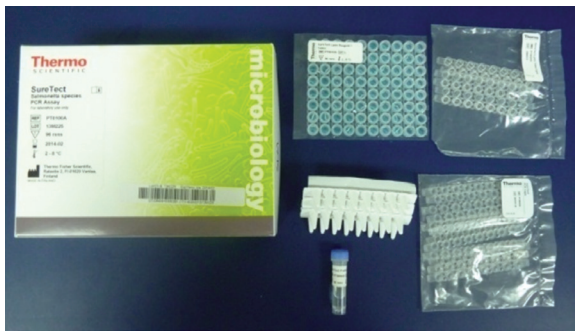
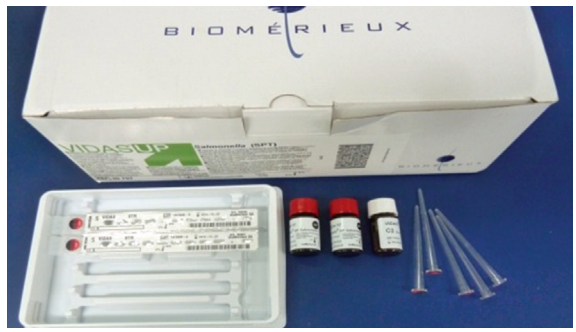
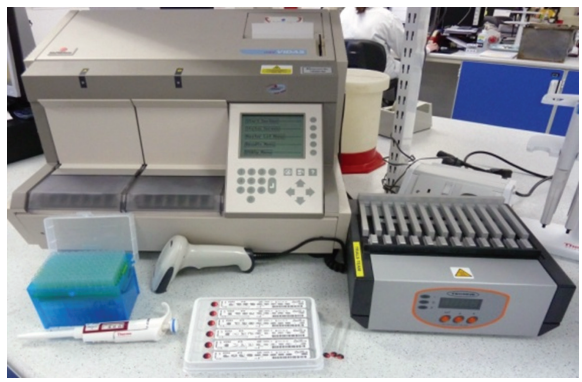
To detect *Salmonella* in food samples, both the SureTect Salmonella species PCR Assay and the VIDAS™ UP Salmonella (SPT) Assay requires a primary enrichment step. BPW (ISO) at room temperature is used for most food matrices tested with the SureTect Salmonella species PCR Assay. The enrichment protocols for the VIDAS™ UP Salmonella (SPT) Assay recommend use of BPW for all food types tested. However, when using the VIDAS™ UP Salmonella (SPT) Assay it is often necessary to add a supplement to the BPW and the BPW must be pre-warmed when testing some food matrices. This all increases the amount of manual handling and preparation the operator must perform when using the VIDAS™ UP Salmonella (SPT) Assay.

Time to result is faster with SureTect Salmonella species PCR Assay

The SureTect Salmonella species PCR Assay gives more results, faster, than VIDAS™ UP Salmonella (SPT) Assay using the miniVIDAS™. The miniVIDAS™ instrument only can process up to 12 samples at any one time, while the PikoReal instrument can process up to 24 samples on one instrument. It takes more than one hr 30 mins to get results for 24 samples using the VIDAS™ UP Salmonella (SPT) Assay on the mini VIDAS™ whereas results for 24 or more samples are available within one hr 20 mins using the SureTect Salmonella species PCR Assay on the PikoReal instrument. The Salmonella species PCR Assay also has the ability to link up to five PikoReal instruments to one computer or laptop, allowing the processing of up to 120 samples at any one time.

Confirmation of *Salmonella* is quicker when using the SureTect Salmonella species PCR Assay compared to the VIDAS™ UP Salmonella (SPT) Assay. If the results are interpreted by the PikoReal and miniVIDAS™ instruments as a positive, it is recommended to confirm these results according to the manufacturer's instructions. The VIDAS™ confirmation method takes 24-96 hrs to confirm or exclude a positive result, whereas the SureTect confirmation method can confirm or exclude a positive result in just 24-72 hrs.

Table 7. summary of SureTect Salmonella species PCR Assay and VIDAS™ UP Salmonella (SPT) Assay kit contents and equipment

	SureTect Salmonella species PCR Assay	VIDAS™ UP Salmonella (SPT) Assay
Kit contents		
Equipment		
Instrument Footprint	8" x 10"	22" x 22"
Weight of Instrument	10 kg	40 kg

Conclusions

The SureTect Salmonella species PCR Assay has proven to be equivalent or superior to the VIDAS™ UP Salmonella (SPT) Assay:

- High sensitivity, specificity and accuracy
- Greater inclusivity (33/33 *Salmonella* isolates detected compared to only 17/33 on the VIDAS™ UP Salmonella (SPT) Assay)
- Simpler, more standardized enrichment method
- Faster time-to-result
- Quicker confirmation of results
- No additional calibration required
- Smaller footprint and lighter weight

References

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2. United States Department of Agriculture, Food Safety and Inspection Service http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook
3. Bacteriological Analytical Manual http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm

Application Note

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