Thermo Fisher Scientific Salmonella Rapid Culture Method Granted PTM Status

Thermo Fisher Scientific
Salmonella Rapid Culture Method Using ONE Broth-Salmonella and Brilliance™ Salmonella
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Brilliance™ Salmonella is a novel chromogenic medium for the detection and identification of *Salmonella* spp. in food. When used in conjunction with ONE Broth-Salmonella (the combination is referred to as the Salmonella Rapid Culture Method), it reduces *Salmonella* detection time from 3–4 to two days. This study evaluated and compared the performance of the Salmonella Rapid Culture Method to the reference U.S. Department of Agriculture-Food Safety and Inspection Service (USDA/FSIS) and U.S. Food and Drug Administration's *Bacteriological Analytical Manual* (FDA/BAM) methods for the detection of *Salmonella* spp. in the following selected food types: raw ground beef, ground chicken, lettuce, shrimp, and shell eggs. Results demonstrate the Salmonella Rapid Culture Method to be superior to the reference methods in incubation time and ease of identification.

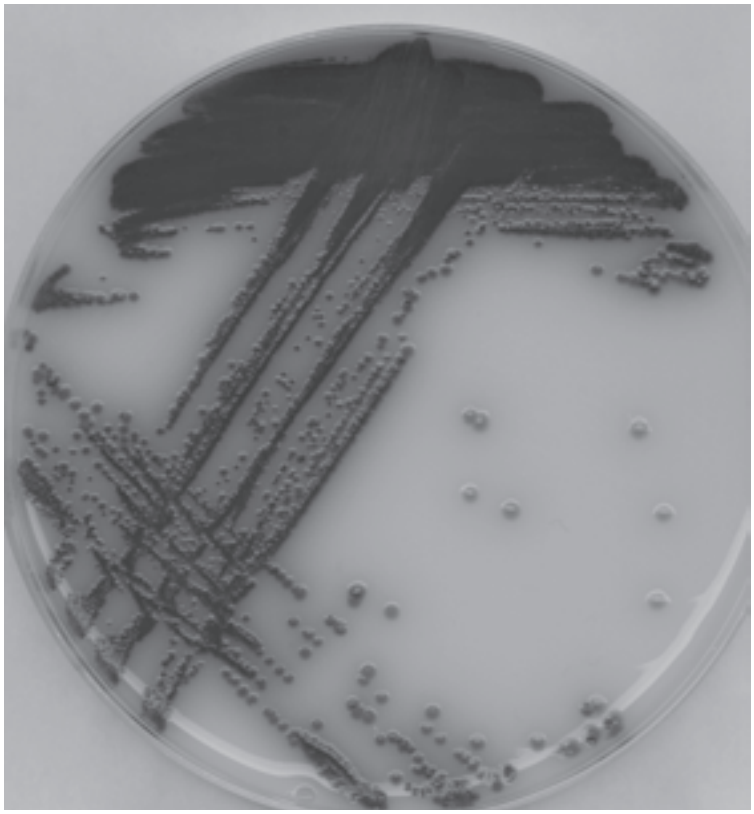

Figure 1. Mixed culture grown on Brilliance Salmonella plate.

Principle of the Test

The Salmonella Rapid Culture Method involves a single enrichment with ONE Broth-Salmonella, followed by plating on Brilliance Salmonella chromogenic medium, taking a total of two days or less to generate an initial result.

ONE Broth-Salmonella is a highly nutritious enrichment broth specifically formulated to ensure excellent recovery of stressed and damaged *Salmonella*

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Major Modification of BAX® System PCR Assay for Genus *Listeria* Granted PTM Status

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TABLE 4. RESULTS OF EXTERNAL STUDY

				Positive samples			BAX system				
Sample	Sample type	MPN/25 g	Total	BAX positive[a]	BAX confirmed	Reference method	Chi square[b]	Sensitivity rate[c], %	False negative[d], %	False positive[e], %	Specificity rate[f], %
Frankfurters	Spiked	0.58	20	7	7	8	0.10	100	0	0	100
	Control		5	0	0	0				0	

[a] Results were identical between BAX original and Q7 instruments.
[b] Calculated using the Mantel-Haenszel Chi-square analysis.
[c] Sensitivity is calculated as 100% – false-negative rate.
[d] False-negative rate is calculated as BAX (–) reference (+) BAX enrichment samples/total reference (+) samples.
[e] False-positive rate is calculated as BAX (+) reference (–)/total reference (–) samples.
[f] Specificity is calculated as 100% – false-positive rate.

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TABLE 1. DETECTION OF *SALMONELLA* SPECIES IN VARIOUS FOOD MATRIXES USING THE SALMONELLA RAPID CULTURE METHOD COMPARED TO MEDIA USED IN THE REFERENCE METHOD (USDA/FSIS, FDA/BAM)

					Reference method		Test kit		Test kit performance			
Matrix	Inoculating organism	Level	MPN/25 g	No. test portions	Positive	Presumptive positive	Confirmed positive	Chi square[a]	Sensitivity[b], %	False negative, %	Specificity[c],%	False positive, %
Ground beef	*Salmonella* Muenchen	Low	0.9	20	10	8	8	0.394	80	20	—	—
		Control	0	5	0	0	0	—	—	—	100	0
Ground chicken	*Salmonella* Montevideo	Low	2.3	20	17	15	15	0.609	88	12	—	—
		Control	0	5	0	0	0	—	—	—	100	0
Lettuce	*Salmonella* Heidelberg	Low	5.75	20	15	14	14	0.122	93	7	—	—
		Control	0	5	0	0	0	—	—	—	100	0
Shrimp	*Salmonella* Choleraesuis	Low	<0.75	20	9	10	10	0.098	111	0	—	—
		Control	0	5	0	0	0	—	—	—	100	0
Shell eggs	*Salmonella* Typhimurium	Low	0.9	20	8	11	11	0.880	138	0	—	—
		Control	0	5	0	0	0	—	—	—	100	0

[a] Chi-square values >3.84 indicate the two methods differ significantly ($P < 0.05$).
[b] Sensitivity, % = Confirmed positive inoculated sample (test method)/confirmed positive inoculated sample (reference method) x 100.
[c] Specificity, % = Confirmed positive controls (test method)/total number of controls.

cells, while inhibiting the growth of competing microorganisms. This highly effective medium allows enrichment to be performed in a single 18–24 hours incubation, eliminating the need for a secondary enrichment.

Following the single enrichment, the culture is plated onto Brilliance Salmonella, the first in a new class of novel chromogenic culture media to utilize Inhibigen™ technology. This new technology improves the recovery and differentiation of *Salmonella* by selectively reducing background flora (growth of other competing flora such as *Proteus* spp. and pseudomonads), allowing clearer visualization of target colonies in mixed cultures, while specifically inhibiting growth of *Escherichia coli.* Chromogens within the medium enable differentiation of *Salmonella* colonies (bright purple) from any remaining organisms that are able to grow, such as *Klebsiella* (blue/dark blue colonies) and *Enterobacter* (clear colonies to no growth), thus reducing the number of false positives requiring confirmation (Figure 1).

Method Validation

An independent laboratory evaluated the Salmonella Rapid Culture Method compared to standard USDA/FSIS and FDA/BAM methods for the detection of *Salmonella* spp. from fresh cultures. Food samples (raw ground beef, ground chicken, lettuce, shrimp, and egg shells) were inoculated with low (~1 CFU/25 g) or high (~1.1 CFU/g) levels of microorganisms. The final inoculum concentrations were confirmed by MPN (most probable number). Uninoculated negative controls were also tested in parallel.

A total of 122 strains of *Salmonella*, representing over 100 different serovars (including lactose- and sucrose-positive strains), were inoculated using standard methods into ONE Broth-Salmonella, incubated at 42 ± 1ºC for 18 ± 2 hours followed by plating onto Brilliance Salmonella chromogenic media. [Equivalent inoculations were performed in reference method media (as indicated in USDA/FSIS and FDA/BAM publications).] Following incubation at 37ºC for 24 ± 2 hours, Brilliance Salmonella plates were observed for purple colonies, indicating the presence of *Salmonella*. In total, 98 of 102 (96.1%) of the strains produced a positive purple-colored colony. The remaining four showed no growth (either in ONE Broth-Salmonella or on Brilliance Salmonella plates), and three of these four could be enriched in nonselective media and plated directly onto Brilliance Salmonella to demonstrate the typical purple colonies. In addition, both lactose- and sucrose-positive serovars demonstrated typical growth using the Salmonella Rapid Culture Method.

Results are summarized in Table 1 and clearly demonstrate that *Salmonella* was detected in all matrixes tested, even at low MPNs of <0.75/25 g (average). In addition, all colonies presumed to be positive on Brilliance Salmonella plates were confirmed positive when tested using USDA/FSIS or FDA/BAM guidelines (reference method). The results obtained for ground beef, ground chicken, lettuce, shrimp, and shell eggs demonstrate that the Salmonella Rapid Culture Method provides results equivalent to the reference method in the foods tested. In fact, in two of five matrixes, more positive (inoculated) *Salmonella* samples were detected with the Salmonella Rapid Culture Method when compared to the reference method. Another advantage was that the results for the Salmonella Rapid Culture Method were obtained in two days, while the reference method required 3–4 days to obtain results. As indicated in Table 1, all Chi-square values are <1, indicating no significant difference between the Salmonella Rapid Culture Method and the reference method. Sensitivity was calculated by comparing the number of inoculated samples detected by the Salmonella Rapid Culture Method to the number of samples confirmed to be positive by the reference method. Overall sensitivity was >98%. Specificity was 100% as calculated by examining uninoculated control samples for growth of organisms by the test method.

To evaluate the ability of the Salmonella Rapid Culture Method to differentiate between *Salmonella* spp. and non-*Salmonella* strains, a total of 30 isolates not belonging to the genus *Salmonella* were obtained (a combination of bacteria and yeast strains). Strains were confirmed by the bioMérieux Vitek® identification system. Organisms included four *Citrobacter* species, four *Escherichia coli* strains, *Staphylococcus aureus, Shigella, Candida,* and *Pseudomonas*. All non-Salmonella strains showed "atypical" or no growth when cultured in ONE Broth-Salmonella and plated onto Brilliance Salmonella. When enriched overnight in BHI and then plated onto Brilliance Salmonella chromogenic media, only *Enterobacter sakazakii* demonstrated 'typical' growth (positive purple-colored colonies) on the chromogenic medium. All other strains demonstrated atypical or no growth (Table 2).

The Salmonella Rapid Culture Method was also evaluated to confirm consistency of *Salmonella* detection on manufactured lots by performing ruggedness and stability tests. ONE Broth-Salmonella and Brilliance Salmonella were subjected to temperature fluctuations of 40–44 and 33–41˚C, respectively, with no effect on the detection of positive color reactions (purple) indicating the presence of *Salmonella.* In addition, Brilliance Salmonella plates were incubated from 21–27 hours with no effect on interpretation of results. Stability studies were performed on three independent lots at three different time points. Consistent results were obtained with all lots at all time points demonstrating reproducibility of 100%.

TABLE 2. EXCLUSIVITY RESULTS

Organism	Source	Origin	Result
Rhodococcus equi	ATCC 6939	Foal lung abscess	No growth
Alcaligenes faecalis	ATCC 35655	Unknown	No growth
Pseudomonas aeriginosa	ATCC 27853	Unknown	Atypical growth
Shigella sonnei	ATCC 9290	Walter Reed	Atypical growth
Morganella morganii	ATCC 25829	Stool, infant	Atypical growth
Shigella flexneri	ATCC 9199	Walter Reed	Atypical growth
Klebsiella pneumoniae	ATCC 13883	NCTC	Atypical growth
Streptococcus pneumoniae	ATCC 6303	Unknown	No growth
Candida albicans	ATCC 10231	Human	No growth
Lactobacillus fermentum	ATCC 9338	Unknown	No growth
Proteus hauseri	ATCC 13315	NCTC	No growth
Enterobacter aerogenes	ATCC 13048	CDC	Atypical growth
Edwardsiella tarda	ATCC 15947	Human feces	No growth
Enterobacter sakazakii	ATCC 51329	Unknown	Typical growth[a]
Citrobacter farmeri	Clinical	Unknown	Atypical growth
Citrobacter braakii	Clinical	Unknown	Atypical growth
Citrobacter diversus	Clinical	Unknown	Atypical growth
Citrobacter freundii	Clinical	Unknown	Atypical growth
Staphylococcus aureus	ATCC 6538	Human lesion	No growth
Pantoea agglomerans	Clinical	Unknown	No growth
Bacillus cereus	ATCC 13061	FDA	No growth
Enterobacter cloacae	ATCC 700323	Unknown	Atypical growth
Proteus vulgaris	ATCC 33420	Clinical isolate	Atypical growth
Listeria monocytogenes	ATCC 19115	Human	Atypical growth
Micrococcus luteus	ATCC 10240	Air isolate	No growth
Microbacterium testaceum	ATCC 15829	Paddy	No growth
E. coli 0157:H7	Food	Beef	Atypical growth
E. coli 055:H7	USDA	Unknown	Atypical growth
E. coli (generic)	51813	Unknown	Atypical growth
E. coli 0145:NM	USDA	Unknown	Atypical growth

[a] Isolate confirmed as 99% *E. sakazakii* by Vitek ID. *Note:* No growth observed when cultured in ONE Broth-Salmonella and plated onto Brilliance Salmonella.

Conclusions

The internal and independent method comparison evaluations of the Salmonella Rapid Culture Method clearly demonstrated that the method is equivalent to the USDA/FSIS reference method for the detection and presumptive identification of *Salmonella* spp. at low (1–9 CFU/25 g) and high (1.1 CFU/g) spike levels in the following selected foods: ground beef, ground chicken, lettuce, shrimp, and shell eggs. In addition, it was found to be superior to the reference method in incubation time and ease of identifying typical colonies. The inclusivity data demonstrated that the Salmonella Rapid Culture Method detected essentially all species and serovars of *Salmonella* tested. The exclusivity data confirmed that the method was able to discriminate *Salmonella* spp. from non-*Salmonella* microorganisms. Lot-to-lot comparability and stability data, along with ruggedness information, verified that the Salmonella Rapid Culture Method was robust and can provide reproducible results over a range of culture conditions (time and temperature).

Based on these results, the Salmonella Rapid Culture Method is recommended as the first line culture method for rapid detection of *Salmonella* spp. contamination in food products, saving time and making identification easier than the reference method. ■

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Note: ONE Broth-Salmonella and Brilliance Salmonella can be ordered through Remel (United States) and Oxoid (Europe/ROW), both part of Thermo Fisher Scientific. For more information, visit www.remel.com/salmonella.

Mention of trade names or commercial products is for identification only and does not constitute preference over similar ones not mentioned. If you are interested in submitting an article regarding a test kit that has been granted Performance-Tested Method℠ status, contact Zerlinde Johnson at zjohnson@aoac.org.