FERN Multi-laboratory Evaluation of MicroSEQ® *Salmonella* spp. Detection Kit in Comparison with an FDA Rapid Screening qPCR Method

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ABSTRACT

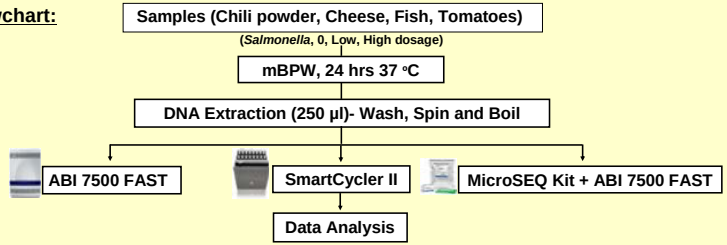
Introduction: *Salmonella* spp. are the most frequently reported cause of food borne illness worldwide. To augment time-consuming conventional culture methods, the FDA developed a qPCR method for rapid screening purposes. This method has been shown to be reliable and accurate and has been validated by the FERN (Food Emergency Response Network) in a multi-laboratory validation study using both ABI 7500 FAST and SmartCycler II systems. The method is used routinely by the FERN and in FDA mobile laboratory deployments. To expand the repertoire of available molecular diagnostic tests for *Salmonella* in foods, the FERN has conducted a similar multi-laboratory validation study to evaluate the performance of the MicroSEQ® *Salmonella spp.* Detection Kit (Life Technologies, Inc.). **Purpose:** To compare the sensitivity and specificity of the MicroSEQ® *Salmonella* spp. to those performance parameters previously validated for the FDA qPCR rapid screening method for *Salmonella*. **Method:** Four food types (chili powder, soft cheese, fish and tomatoes) were inoculated at three levels (six replicates for each) – uninoculated, low (1-5 cfu/25g) and high (10-50 cfu/25g). All samples were tested for *Salmonella* using the 24-hr qPCR method which utilizes modified Buffered Peptone Water (mBPW) as the sole enrichment medium. Eighteen samples for each food type were independently analyzed by the participating laboratories using the MicroSEQ® *Salmonella* spp. Detection Kit in parallel with the qPCR method. **Results:** For all food types, the specificity is 1/288 for FDA qPCR and 2/288 for MicroSEQ®. The sensitivity is 513/576 for FDA qPCR and 515/576 for MicroSEQ® indicating that there was no significant difference (p ≥0.05) statistically between the MicroSEQ® *Salmonella* spp. Detection Kit method and the corresponding reference method. **Significance:** The consistent results among 12 laboratories support the utility of the MicroSEQ® *Salmonella spp.* Detection Kit as an alternative method for detecting *Salmonella* in food.

MATERIALS & METHOD

TABLE 1 Spiking serotype, spiking dosage and aging condition

Spiking / Food Matrix	*Salmonella* serotype	Low dose (cfu/25 g, or ~100 g of tomatoes)	High dose (cfu/25 g, or ~100 g of tomatoes)	Aging	
				Temp (°C)	Time (Days)
Chili Powder	S. Weltervreden	3	30	ambient	2
Soft Cheese	S. Typhymurium	3.8	38	4	2
Fish	S. Sentenberg	2	20	-20	2
Tomatoes	S. Newport	2.4	24	4	2

Flowchart:



RESULTS

TABLE 2. Overall Comparison between MicroSEQ and the reference method

Food	Samples	Positive Rate (%) [a]			Sensitivity [b] (%)		False Negative [c] (%)		Specificity [d] (%)		False Positive [e] (%)	
		SCII	ABI	MicroSEQ	SCII	ABI	SCII	ABI	SCII	ABI	SCII	ABI
Chili Powder	Non-spiked	0 (0/72)	0 (0/72)	0 (0/72)	—	—	—	—	100	100	0	0
	Low-spiked	57 (41/72)	61 (44/72)	67 (48/72)	117	110	0	0	—	—	—	—
	High-spiked	100(72/72)	100(72/72)	100(72/72)	100	100	0	0	—	—	—	—
Soft Cheese	Non-spiked	0 (0/72)	1 (1/72)	0 (0/72)	—	—	—	—	100	100	0	0
	Low-spiked	100(72/72)	100(72/72)	100(72/72)	100	100	0	0	—	—	—	—
	High-spiked	100(72/72)	100(72/72)	100(72/72)	100	100	0	0	—	—	—	—
Fish	Non-spiked	0 (0/72)	0 (0/72)	3 (2/72)[f]	—	—	—	—	96	96	3	3
	Low-spiked	83 (60/72)	83 (60/72)	81 (58/72)	98	98	3	3	—	—	—	—
	High-spiked	100(72/72)	100(72/72)	97 (70/72)	97	97	3	3	—	—	—	—
Tomato	Non-spiked	0 (0/72)	0 (0/72)	0 (0/72)	—	—	—	—	100	100	0	0
	Low-spiked	65 (47/72)	68 (49/72)	68 (49/72)	105	100	0	0	—	—	—	—
	High-spiked	100(72/72)	100(72/72)	100(72/72)	100	100	0	0	—	—	—	—

a: Positive Rate –The portion of the inoculated samples (for each food matrix) distributed to each participant that gave a positive result for each method.
b: Sensitivity(%) – (positive number of inoculated samples from MicroSEQ method / positive number of inoculated samples from the validated method) X 100%.
c: False Negative (%) – The percentage that the inoculated sample was positive from the validated method but was negative from MicroSEQ method.
d: Specificity (%) – (negative number of Uninoculated samples from MicroSEQ method / negative number of Uninoculated samples from the validated method) X 100 %.
e: False Positive (%) – The percentage that the Uninoculated sample was negative from the validated method but was positive from MicroSEQ method.
f: False positive results from uninoculated sample using MicroSEQ were reported by two collaborators

Summary of the Results:

- The relative sensitivity and specificity of the MicroSEQ® *Salmonella* spp. Detection Kit method for all food types indicate that there was no significant difference (p ≥0.05) statistically between this method and the corresponding validated method. McNemar's test was used to determine the significant difference.

CONCLUSIONS

In addition to its equivalent performance compared to the reference methods, the MicroSEQ kit offers several advantages including commercial availability, ease of use, and software guided process (from PCR setup to data analysis). Based on the results of this multi-laboratory study, we conclude that the MicroSEQ® *Salmonella* spp. Detection Kit method offers a consistent and efficient alternative to the reference method of rapid screening of *Salmonella* in chili powder, soft cheese, fish and tomato.

REFERENCES

1. Cheng, C.-M., W. Lin , K. T.Van, L. Phan, N. T. Tran and D. Farmers. 2008. Rapid Detection of Salmonella in Foods using Real-Time PCR. J. Food. Prot. 71:2436-2441.
2. Cheng, C.-M., K. T.Van, W. Lin, R. M. Ruby. 2009. Inter-Laboratory Validation of a Real-Time PCR 24-Hour Rapid Method for Detection of Salmonella in Foods. J. Food. Prot. 72:945-95
3. Balachandran P, Cao Y, Wong L, Furtado MR, Petrauskene OV, Tebbs RS. 2011.Evaluation of applied biosystems MicroSEQ real-time PCR system for detection of Salmonella spp. in food. J. AOAC int. 94(4):1106-16.

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