Detecting *Salmonella* in Environmental Samples

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BACKGROUND

Environmental monitoring is an important part of a HACCP control plan to minimize the risk of *Salmonella* in foods. Evidence has shown that *Salmonella* can survive and persist in a food manufacturing environment. Environmental samples can pose risks for detection due to presence of inhibitors and/or the presence of high levels of background microbial flora that can mask detection of low levels of *Salmonella*. Environmental samples are often prepared using hydration buffers that contain an aryl sulfonate complex that can inhibit testing. In addition environmental surfaces are often contaminated with cleaning residues, food debris, oil or grease from equipment, dust, biofilms, or other substances that could inhibit test methods for pathogen detection.

PURPOSE

To evaluate a new and simplified workflow for environmental testing of *Salmonella* species using real-time PCR.

METHODS

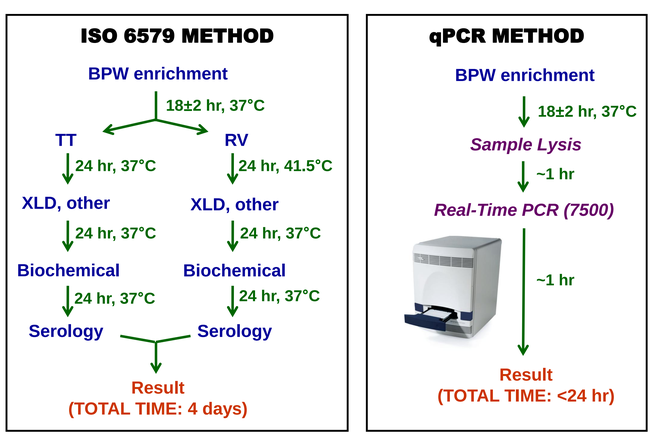
Environmental Samples and Enrichment
Environmental sponges were hydrated with 10 mL of Neutralizing Buffer or Dey-Engley Neutralizing Broth. For some studies environmental samples were spiked with increasing concentrations of background microbial flora followed by addition of low levels of *Salmonella* and stored overnight at 4°C. In another study environmental sponges were used to contact environmental surfaces at a manufacturing facility for ready-to-eat foods, shipped overnight with icepacks, spiked with low levels of *Salmonella* that was also stored overnight at 4°C, followed by storage of the environmental samples for an additional 2-3 hour at 4°C. All environmental samples were combined with 100 mL of buffered peptone water (BPW), mixed by hand massage for about 10 seconds and incubated at 37°C for 16 to 24 hours.

Cultures
Salmonella strains used in this study consist of *Salmonella* Poona (FS-304), and *Salmonella* Montevideo (SARB 30). Background microbial flora for challenge studies consist of *Enterobacter cloacae* (ATCC 35030), *Klebsiella pneumonia* (ATCC 4352), and *Citrobacter fruendii* (ATCC 43864).

Confirmation
All samples were tested for presence of *Salmonella* by culture according to ISO 6579. Cultures presumptive for *Salmonella* on selective agar media were confirmed by streaking selective colonies on triple sugar/iron agar (TSI agar).

Real-Time PCR Detection
Real-time was run on the 7500 Fast instrument using standard conditions (95 ºC for 2 min; 40 cycles at 95 ºC for 3 seconds and 60 ºC for 30 seconds). Analysis settings for reviewing real-time PCR results were as follows: Manual Ct with the FAM™ dye threshold set to 0.5 and the VIC® dye threshold set to 0.3, and Auto Baseline for both FAM™ dye and VIC® dye. FAM™ dye Ct values below 35.69 are considered positive for *Salmonella*.

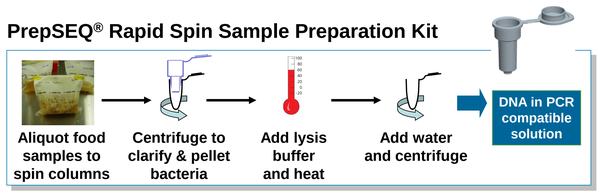
Figure 1. Workflow for Testing Environmental Samples for *Salmonella*



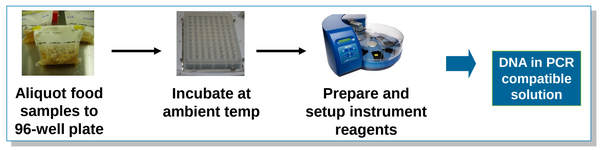
RESULTS

Figure 2. Sample Lysis for Real-Time PCR Detection

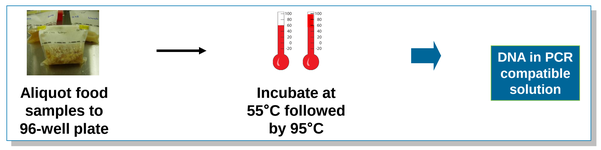
PrepSEQ® Rapid Spin Sample Preparation Kit



PrepSEQ® Nucleic Acid Extraction Kit (FA protocol)



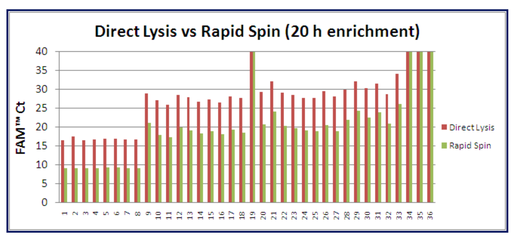
RapidFinder™ Direct Lysis Protocol

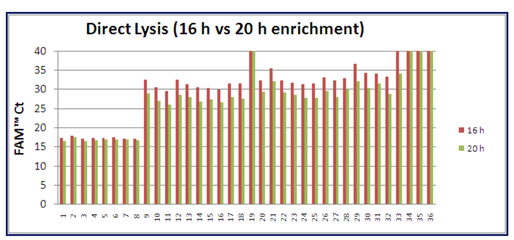


Sample preparation is often the most difficult and error-prone step for real-time PCR. Reducing steps or automating sample preparation simplifies the workflow and diminishes error. The Rapid Spin protocol is an AOAC and AFNOR certified protocol. Both the PrepSEQ® NAE Extraction method with the FA script and the RapidFinder™ Direct Lysis method are new methods evaluated in this study.

Figure 3. Real-Time PCR vs ISO 6579 in Artificially-Contaminated Samples

Sample	*Salmonella* poona	*Enterobacter cloaceae*	*Citrobacter fruendii*	*Klebsiella pneumonia*	*Aerobic Plate Count*
1-8	4 CFU	None	None	None	ND
9-16	4 CFU	1.1×10^6 CFU	1.3×10^6 CFU	1.0×10^6 CFU	3.6×10^6 CFU
17-24	4 CFU	1.1×10^7 CFU	1.3×10^7 CFU	1.0×10^7 CFU	3.6×10^7 CFU
25-32	4 CFU	1.1×10^8 CFU	1.3×10^8 CFU	1.0×10^8 CFU	3.6×10^8 CFU
33	4 CFU	1.1×10^9 CFU	1.3×10^8 CFU	1.0×10^9 CFU	3.6×10^9 CFU
34-36	None	None	None	None	ND

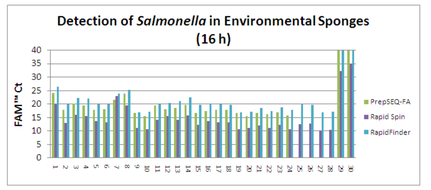


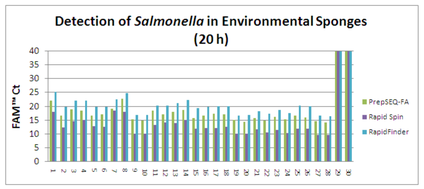


Sample	Culture	Direct Lysis 16 h	Direct Lysis 20 h
1	+	+	+
2	+	+	+
3	+	+	+
4	+	+	+
5	+	+	+
6	+	+	+
7	+	+	+
8	+	+	+
9	+	+	+
10	+	+	+
11	+	+	+
12	+	+	+
13	+	+	+
14	+	+	+
15	+	+	+
16	+	+	+
17	+	+	+
18	+	+	+
19	-	-	-
20	+	+	+
21	+	+	+
22	+	+	+
23	+	+	+
24	+	+	+
25	+	+	+
26	+	+	+
27	+	+	+
28	+	+	+
29	+	-	+
30	+	+	+
31	+	+	+
32	+	+	+
33	+	-	+
34	-	-	-
35	-	-	-
36	-	-	-

Increasing concentrations of background microflora were added to environmental sponges containing ~4 CFU of *Salmonella* Poona. The RapidFinder™ Direct Lysis method showed perfect correlation with the ISO 6579 culture reference method for detection of *Salmonella* in samples enriched for 20 hr.

Figure 4. Real-Time PCR vs ISO 6579 in Samples from a Manufacturing Facility





Sample	Background Flora*	APC	Coliform	Inoculum	Average spike level	Culture	AOAC-certified Rapid Spin 16 h	AOAC-certified Rapid Spin 20 h	PrepSEQ NAE FA Method 16 h	PrepSEQ NAE FA Method 20 h	RapidFinder Direct Lysis 16 h	RapidFinder Direct Lysis 20 h
1	High	3.3×10^5	1×10^4	Montevideo	4.3 CFU	+	+	+	+	+	+	+
2	High	1×10^2	$<10^3$	Montevideo	4.3 CFU	+	+	+	+	+	+	+
3	High	5.2×10^3	$<10^3$	Montevideo	4.3 CFU	+	+	+	+	+	+	+
4	High	3×10^2	$<10^3$	Montevideo	4.3 CFU	+	+	+	+	+	+	+
5	High	9.4×10^3	1×10^3	Montevideo	4.3 CFU	+	+	+	+	+	+	+
6	High	1.7×10^4	$<10^3$	Montevideo	4.3 CFU	+	+	+	+	+	+	+
7	High	20×10^6	$>10^5$	Poona	4.1 CFU	+	+	+	+	+	+	+
8	High	1.2×10^6	$>10^5$	Poona	4.1 CFU	+	+	+	+	+	+	+
9	High	2×10^2	$<10^3$	Poona	4.1 CFU	+	+	+	+	+	+	+
10	High	2×10^2	$<10^3$	Poona	4.1 CFU	+	+	+	+	+	+	+
11	High	5.5×10^3	$<10^3$	Poona	4.1 CFU	+	+	+	+	+	+	+
12	High	5.3×10^5	1×10^4	Poona	4.1 CFU	+	+	+	+	+	+	+
13	Low	1.1×10^4	1×10^3	Montevideo	4.3 CFU	+	+	+	+	+	+	+
14	Low	1×10^3	$<10^3$	Montevideo	4.3 CFU	+	+	+	+	+	+	+
15	Low	1×10^2	$<10^3$	Montevideo	4.3 CFU	+	+	+	+	+	+	+
16	Low	5×10^2	$<10^3$	Montevideo	4.3 CFU	+	+	+	+	+	+	+
17	Low	2×10^2	$<10^3$	Montevideo	4.3 CFU	+	+	+	+	+	+	+
18	Low	<100	$<10^3$	Montevideo	4.3 CFU	+	+	+	+	+	+	+
19	Low	6×10^2	$<10^3$	Poona	4.1 CFU	+	+	+	+	+	+	+
20	Low	1×10^2	$<10^3$	Poona	4.1 CFU	+	+	+	+	+	+	+
21	Low	4×10^2	$<10^3$	Poona	4.1 CFU	+	+	+	+	+	+	+
22	Low	2×10^2	$<10^3$	Poona	4.1 CFU	+	+	+	+	+	+	+
23	Low	1×10^3	$<10^3$	Poona	4.1 CFU	+	+	+	+	+	+	+
24	Low	1×10^2	$<10^3$	Poona	4.1 CFU	+	+	+	+	+	+	+
25	Pos control	NA	NA	Montevideo	4.3 CFU	+	+	+	NA	+	+	+
26	Pos control	NA	NA	Montevideo	4.3 CFU	+	+	+	NA	+	+	+
27	Pos control	NA	NA	Poona	4.1 CFU	+	+	+	NA	+	+	+
28	Pos control	NA	NA	Poona	4.1 CFU	+	+	+	NA	+	+	+
29	Neg control	NA	NA	NA	NA	-	+	-	-	-	-	-
30	Neg control	NA	NA	NA	NA	-	+	-	-	-	-	-

*Descriptions for High and Low background are based on previous history of environmental testing.

Sponges used for environmental testing at a manufacturing facility were shipped overnight to Life Technologies and spiked with a low inoculums of *Salmonella* Poona or *Salmonella* Montevideo prior to processing for *Salmonella* detection. The RapidFinder™ Direct Lysis method and the automated PrepSEQ® NAE FA method showed perfect correlation with the ISO 6579 culture reference method for detection of *Salmonella* in the environmental samples enriched for 16 and 20 hr.

SUMMARY

Real-time PCR showed detection of *Salmonella* in the presence of high background concentrations of microflora artificially spiked onto environmental sponge samples. Furthermore, real-time PCR showed excellent detection *Salmonella* in all 24 environmental sponge samples collected from a manufacturing facility. No PCR inhibition was observed for any of the samples. Real-time PCR only requires 16 to 20 hours of enrichment and offers results within 24 hours from start of enrichment, whereas the reference method required 3 days to observe presumptive positive results on selective agar plates. Simple sample preparation methods with hands-on-time typically less than 15 minutes (RapidFinder™ Direct Lysis) or 30 minutes (PrepSEQ® NAE FA protocol) provide easy workflows for Real-time PCR detection of *Salmonella*.

CONCLUSIONS

- The MicroSEQ® real-time PCR workflow allows detection of *Salmonella* in less than 24 hrs (2 to 3 hr post enrichment) whereas ISO 6579 requires 4 days to confirm *Salmonella*.
- The MicroSEQ® real-time PCR workflow detected *Salmonella* in the presence of high concentrations (up to 10^9 CFU) of background microbial content.
- The simplified sample preparation workflows evaluated here offers the advantage of ease-of-use with good detection for samples enriched for 20 hr.

For testing of Food and Environmental samples only.