

Protocol for rapid detection of *Salmonella* and *E. coli* O157:H7 in salad greens from a single 8-hour enrichment

Andrew D. Farnum, C. J. Janes, Morgan Wallace, Angeline Stoltzfus - DuPont Qualicon, Wilmington, Delaware

Abstract

Successful distribution of short shelf-life foods, like spinach and other leafy greens, requires accurate, rapid methods for the detection of pathogens. We tested the feasibility of using the BAX® system to detect both *Salmonella* and *E. coli* O157:H7 in various types of leafy greens from a single enrichment.

The study tested 14 samples of freshly picked untreated leafy greens that were shipped from a commercial producer to DuPont Qualicon. We divided each sample into four 25-g portions and spiked 3 portions with *Salmonella* and *E. coli* O157:H7 at less than 10 cfu/25g, leaving 1 portion unspiked for the control. Using BAX® system *E. coli* O157:H7 MP enrichment broth, prepared with DuPont™ StatMedia™ soluble packets, we incubated all samples at 42° C and tested them at 8, 10, 12 and 24 hours with the BAX® system.

The study demonstrated that the BAX® system can detect low levels (<10 cfu/25 g) of both *Salmonella* and *E. coli* O157:H7 in fresh salad greens after just 8 hours enrichment in MP broth.

Introduction

E. coli O157:H7 and *Salmonella*, food-borne pathogens often found in fresh produce (1,2), can cause illness at a low infectious dose. The organisms are often difficult to detect with traditional culture methods, especially where low levels of the target bacteria must be isolated from high levels of competing background flora. Rapid methods, with typical sensitivity of 10⁶ cfu/mL, can deliver false negative results on samples where competing flora reach high levels that overgrow the target.

The recent food-borne outbreaks of these pathogens underscore the need for fast and accurate methods of detection. The BAX® system, which uses DNA-based PCR technology (3), provides reliable, reproducible results after enrichment, even in the presence of competing flora, leading to quicker release of product.

Materials and Methods

1. Start overnight culture of *Salmonella dublin* strain DD3019 and *E. coli* O157:H7 strain DD1450.
2. Serially dilute overnight cultures (assumed 10⁹ cfu/mL) down to 10² cfu/mL. Plate 50 µL of 10³ cfu/mL dilution onto 2x BHI or TSA plates for cultural enumeration.
3. Weigh four 25 g/bag samples of each food into stomacher bag with filter. (1 bag unspiked + 3 bags for spiking = 4 bags X 14 produce samples = 56 total samples.)
4. Place one packet of DuPont™ StatMedia™ (BAX® System *E. coli* O157:H7 MP) in each sample bag. Add 225 mL pre-warmed (42°C) sterile water to stomacher bags and stomach for 2 minutes at 260 rpm.
5. Separate the unspiked sample bags and set aside. Add 100 µL of 10² cfu/mL dilution for *Salmonella* and for *E. coli* O157:H7 to each spiked bag. Incubate all enrichments at 42°C. Obtain test volumes at 8, 10, 12 and 24 hours.
6. Prepare two lysates per enrichment as follows: Mix lysis reagent (150 µL protease + 12 mL lysis buffer) and add 200-µL aliquots to cluster tubes. Transfer 5-µL aliquots of each enrichment to cluster tubes, and heat at 37°C for 20 minutes, then 95°C for 10 minutes. Use one lysate to hydrate PCR tablets and store second lysate at 4°C for troubleshooting.
7. Arrange triplicate racks of PCR tubes, one with *Salmonella* tablets and two with *E. coli* O157:H7 MP tablets. For each sample, use one lysate to hydrate each of the 3 PCR tablets. Load the racks into BAX® system Q7 instruments, and run a *Salmonella* program, an *E. coli* O157:H7 MP standard program (3.5-hr protocol) and an *E. coli* O157:H7 MP Express program (2.5-hr protocol).
8. Record all results and observations in a data spreadsheet. Save all BAX® system files.

Table 1. BAX® system results for *Salmonella* in salad greens

Sample Type	Inoculum	# Samples	Number of <i>Salmonella</i> Positive			
			8 hr	10 hr	12 hr	24 hr
Red Oak	7.5 cfu/25 g	3	3	3	3	3
	0	1	0	0	0	0
Lolla Rosa	7.5 cfu/25 g	3	2	1	0	2
	0	1	0	0	0	0
Spinach	7.5 cfu/25 g	3	3	3	3	3
	0	1	0	0	0	0
Green Oak	7.5 cfu/25 g	3	3	3	3	3
	0	1	0	0	0	0
Wild Arugula	7.5 cfu/25 g	3	3	3	3	3
	0	1	0	0	0	0
Green Chard	7.5 cfu/25 g	3	3	3	3	3
	0	1	0	0	0	0
Red Romaine	7.5 cfu/25 g	3	3	0	0	3
	0	1	0	0	0	0
Red Leaf	7.5 cfu/25 g	3	2	0	0	3
	0	1	0	0	0	0
Red Chard	7.5 cfu/25 g	3	3	3	3	3
	0	1	0	0	0	0
Green Romaine	7.5 cfu/25 g	3	3	3	3	3
	0	1	0	0	0	0
Tatsoi	7.5 cfu/25 g	3	3	3	3	3
	0	1	0	0	0	0
Tango	7.5 cfu/25 g	3	3	3	3	3
	0	1	0	0	0	0
Mizuna	7.5 cfu/25 g	3	3	3	3	3
	0	1	0	0	0	0
Arugula	7.5 cfu/25 g	3	3	3	2*	3
	0	1	0	0	0	0

* One miss due to operator error.

Table 2. BAX® system results for *E. coli* O157:H7 MP in salad greens

Sample Type	Inoculum	# Samples	E. coli MP Standard Positive				E. coli MP Express Positive			
			8 hr	10 hr	12 hr	24 hr	8 hr	10 hr	12 hr	24 hr
Red Oak	8.0 cfu/25 g	3	3	3	3	3	3	3	3	
	0	1	0	0	0	0	0	0	0	
Lolla Rosa	8.0 cfu/25 g	3	3	3	3	3	2	1	2	
	0	1	0	0	0	0	0	0	0	
Spinach	8.0 cfu/25 g	3	3	3	3	3	3	3	3	
	0	1	0	0	0	0	0	0	0	
Green Oak	8.0 cfu/25 g	3	3	3	3	3	3	3	3	
	0	1	0	0	0	0	0	0	0	
Wild Arugula	8.0 cfu/25 g	3	3	3	3	3	3	3	3	
	0	1	0	0	0	0	0	0	0	
Green Chard	8.0 cfu/25 g	3	2	2	3	3	3	3	3	
	0	1	0	0	0	0	0	0	0	
Red Romaine	8.0 cfu/25 g	3	3	3	3	3	3	3	3	
	0	1	0	0	0	0	0	0	0	
Red Leaf	8.0 cfu/25 g	3	3	3	3	3	2	3	3	
	0	1	0	0	0	0	0	0	0	
Red Chard	8.0 cfu/25 g	3	3	3	3	3	3	3	3	
	0	1	0	0	0	0	0	0	0	
Green Romaine	8.0 cfu/25 g	3	3	3	3	3	3	3	3	
	0	1	0	0	0	0	0	0	0	
Tatsoi	8.0 cfu/25 g	3	3	3	3	3	3	3	3	
	0	1	0	0	0	0	0	0	0	
Tango	8.0 cfu/25 g	3	3	3	3	2	3	3	2	
	0	1	0	0	0	0	0	0	0	
Mizuna	8.0 cfu/25 g	3	3	3	3	2	3	3	3	
	0	1	0	0	0	0	0	0	0	
Arugula	8.0 cfu/25 g	3	3	3	2*	3	3	2*	3	
	0	1	0	0	0	0	0	0	0	

* One miss due to operator error.

DuPont Qualicon thanks Scanlan Family Farms for supplying the fresh produce for this study.

Results

- ✓ Of the 42 spiked samples, results at 8 hours were comparable to those at 24 hours for both *Salmonella* and *E. coli* O157:H7 (Tables 1 and 2).
- ✓ All 14 unspiked samples were negative for both *Salmonella* and *E. coli* O157:H7 at each testing point.
- ✓ There was no significant difference between the *E. coli* MP standard and the *E. coli* MP Express protocols.

Discussion

We observed an unusual effect for red romaine and red leaf greens, in which the number of positive results decreased at the 10 and 12 hour time points. One possible explanation could be the presence of PCR inhibitors in these fresh greens that dissipate over time when exposed to the enrichment environment. Lolla Rosa may contain inhibitors that do not disappear over time. When validating this protocol in your own lab, you may want to include a secondary enrichment with 1:50 dilution in BHI and a 3-hour regrowth at 37° C for those leafy greens.

Conclusion

The data demonstrate that the BAX® system can detect low levels (<10 cfu/25 g) of both *Salmonella* and *E. coli* O157:H7 in fresh salad greens after just 8 hours enrichment in BAX® System Media for *E. coli* O157:H7 MP.

References

- Centers for Disease Control and Prevention. 2006. Surveillance for foodborne-disease outbreaks—United States, 1998-2002. *Surveillance Summaries*. MMWR 55:1–34 (No. SS-10).
- Sivapalasingam, S. et al. 2004. Fresh Produce: A Growing Cause of Outbreaks of Foodborne Illness in the United States, 1973 through 1997. *J Food Prot*. 67:2342-53.
- Erllich, H.A. (ed.). 1989. *PCR Technology*. New York: Stockton Press.

