Detecting *Salmonella*, *E. coli* O157:H7 and non-O157 STEC in Leafy Greens from a Single Enrichment

Introduction

To demonstrate the convenience and robustness of the BAX® System, an internal study was performed to evaluate a method to detect *Salmonella*, *E. coli* O157:H7 and non-O157 STEC (*E. coli* O26), in leafy green vegetables. Twenty 25 g portions each of fresh spinach, iceberg lettuce, and red leaf lettuce were enriched in BAX® System MP media and evaluated with the appropriate BAX® System Real-Time PCR assays.

The results of this study demonstrate that the BAX® System can effectively detect *Salmonella*, *E. coli* O157:H7 and non-O157:H7 STEC from a single sample after enrichment in BAX® System MP media, using the following enrichment protocols:
- For spinach, incubate samples for 8-20 hours. No regrowth is necessary.
- For iceberg lettuce, incubate samples for 10-20 hours. No regrowth is necessary.
- For red leaf lettuce, incubate samples for 10-20 hours. Regrowth is not recommended.

Methodology

Equipment, reagents and supplies
- BAX® System Real-Time PCR Assay for *Salmonella*
- BAX® System PCR Assay for *Salmonella* 2
- BAX® System Real-Time PCR Assay for *E. coli* O157:H7
- BAX® System Real-Time PCR Assay for STEC Screening
- BAX® System Real-Time PCR Assay for STEC Panel 1 - *E. coli* O26, O111, O121
- BAX® System Q7 instrument and peripherals
- BAX® System Q7 equipment and supplies
- BAX® System MP media
- Brain-heart Infusion (BHI) broth

Sample Inoculation and Preparation

One strain of *Salmonella* Newport, *E. coli* O157:H7 and *stx-* and *eae*-containing *E. coli* O26 were selected from the N&H Culture Collection to artificially spike samples (see Table 1). A pure culture of each strain was inoculated into BHI broth and incubated at 35-37°C overnight, then serially diluted in additional BHI broth to create low-level spikes.

Table 1: Test Strains and Inoculation Information

Strain Name	Strain ID	Strain Source	Inoculation Level (cfu/sample)
Salmonella Newport	DD 707	Food poisoning	1.12
E. coli O157:H7	DD 1450	Human feces	0.70
E. coli O26	DD 1831	Unknown	1.04

Spinach, iceberg lettuce and red leaf lettuce were obtained from a commercial producer or local retailers for use in this study. Each product was divided into 20 test portions weighing 25 g each. Ten portions of each sample type were inoculated with low levels of all three target strains (see Table 1), then the portions were re-combined and mixed thoroughly. An additional three portions of each sample type were inoculated with low levels of only one of the three selected test strains, then portions were re-combined and mixed thoroughly. The final test portion of each sample type was left uninoculated to serve as a negative control. All test portions were held at 4°C for two days to stress the target organisms, then spiked portions were re-divided into 25 g portions before enrichment.

Sample Enrichment

Each 25 g test portion was combined with 225 mL pre-warmed (42°C) BAX® System MP media and swirled to mix as described in the FDA-BAM reference method for produce. Portions were incubated at 42°C for a total of 20 hours, with aliquots removed after 8, 10, 12, and 20 hours of enrichment.

Red leaf lettuce samples were tested both directly from this primary enrichment and after a regrowth step to evaluate the potential benefit of a secondary enrichment on diluting certain photosynthetic compounds found in this sample type that could inhibit PCR. Regrowth was performed before testing by transferring 10 µL primary enrichment to 500 µL pre-warmed (37°C) BHI broth, then incubating at 37°C for 3 hours before testing with the BAX® System method.

BAX® System Method

For *E. coli* O157:H7 and STEC testing, 20 µL enrichment was added to 200 µL prepared lysis reagent (150 µL protease and 12 mL lysis buffer) in one set of cluster tubes. Cluster tubes were heated for 20 minutes at 37°C and 10 minutes at 95°C. Lysates were cooled for at least 5 minutes in a cooling block at 2-8°C, then 30 µL of each lysate was used to hydrate a PCR tablet for the BAX® System Real-Time *E. coli* O157:H7 assay, BAX® System Real-Time STEC Screening assay and BAX® System Real-Time STEC Panel 1 assay. All PCR tubes were processed in the BAX® System Q7 instrument.

For *Salmonella* testing, 5 µL enrichment was added to 200 µL prepared lysis reagent in cluster tubes. Cluster tubes were heated for 20 minutes at 37°C and 10 minutes at 95°C. Lysates were cooled for at least 5 minutes in a cooling block at 2-8°C, then 30 µL of each lysate was used to hydrate a PCR tablet for the BAX® System Real-Time *Salmonella* assay, and an additional 50 µL of each lysate was used to hydrate a PCR tablet for the BAX® System *Salmonella* 2 assay. All PCR tubes were processed in the BAX® System Q7 instrument.

Note: Because the BAX® System Real-Time assays for *E. coli* O157:H7, STEC Screening and STEC Panel 1 use the same cycling parameters during sample processing, these assays can be combined into a single run on the Q7 instrument. The BAX® System Real-Time *Salmonella* and BAX® System *Salmonella* 2 assays were run individually due to different cycling conditions.

Results and Discussion

The results for spinach samples are summarized in Table 2. The results for all assays at each time point tested were identical to the confirmation results, with the exception of one data point each for samples spiked with all three target organisms and tested at 12 and 20 hours with the BAX® System STEC Screening assay. Based on previous studies, it is suggested that the longer enrichment times resulted in increased interference from certain phenolic and pigmented compounds contained in the sample.

However, swirling samples instead of stomaching before enrichment should reduce this interference. All negative control samples returned negative results at all time points tested.

The results for iceberg lettuce samples are summarized in Table 3. The results for all assays at each time point tested were identical to the confirmation results, with the exception of one sample spiked with all three target organisms and tested at 20 hours with the BAX® System STEC Screening assay, one sample spiked with *E. coli* O26 and tested at 10 hours with the STEC Panel 1 assay, and one sample spiked with *Salmonella* and tested at 8 hours with the BAX® System Real-Time *Salmonella* assay. Upon examination, the discrepant result with the BAX® System STEC Screening assay was determined to be a weak positive result, most likely due to a crosscontamination event. All negative control samples returned negative results at all time points tested.

The results for red leaf lettuce samples are summarized in Tables 4 and 5. For samples tested directly (without regrowth), the results for all assays at each time point tested were identical to the confirmation results, with the exception of one sample spiked with *E. coli* O157:H7 and tested at 8 hours with the BAX® System Real-Time *E. coli* O157:H7 assay and one sample spiked with all three target organisms and tested at 8 hours with the BAX® System Real-Time *Salmonella* assay.

For samples tested after a regrowth step, a total of six samples returned results discrepant from the culture confirmation when tested at 10 hours or less. All negative control samples returned negative results at all time points tested. Although additional research would be needed to determine the exact cause of these discrepant results, one possibility is that a competing, faster-growing organism was present, inhibiting the growth of *Salmonella* during the non-selective secondary enrichment phase.

Conclusion

The results of this study demonstrate that the BAX® System can effectively detect *Salmonella*, *E. coli* O157:H7 and non-O157:H7 STEC from a single sample after enrichment in BAX® System MP media, using the following enrichment protocols:
- For spinach, incubate samples for 8-20 hours. No regrowth is necessary.
- For iceberg lettuce, incubate samples for 10-20 hours. No regrowth is necessary.
- For red leaf lettuce, incubate samples for 10-20 hours. Regrowth is not recommended.

In addition, it is suggested that customers whose sample types differ from those tested internally validate their own produce samples before using the BAX® System method.

Table 2: Results for Spinach Samples

Spiking Organism(s)	Samples	8 Hours Enrichment	10 Hours Enrichment	12 Hours Enrichment	20 Hours Enrichment	Confirmed
Real-Time STEC Screening Assay						
Multiple*	10	8	8	8	7	8
Salmonella	3	—	—	—	—	—
E. coli O157:H7	3	3	3	3	3	3
E. coli O26	3	1	1	1	1	1
Real-Time STEC Panel 1 Assay						
Multiple*	10	4	4	4	4	4
Salmonella	3	—	—	—	—	—
E. coli O157:H7	3	—	—	—	—	—
E. coli O26	3	1	1	1	1	1
Real-Time *E. coli* O157:H7 Assay						
Multiple*	10	7	7	7	7	7
Salmonella	3	—	—	—	—	—
E. coli O157:H7	3	3	3	3	3	3
E. coli O26	3	—	—	—	—	—
Real-Time *Salmonella* Assay						
Multiple*	10	5	5	5	5	5
Salmonella	3	1	1	1	1	1
E. coli O157:H7	3	—	—	—	—	—
E. coli O26	3	—	—	—	—	—
***Salmonella* 2 Assay**						
Multiple*	10	5	5	5	5	5
Salmonella	3	1	1	1	1	1
E. coli O157:H7	3	—	—	—	—	—
E. coli O26	3	—	—	—	—	—

*Samples were co-spiked with *Salmonella*, *E. coli* O157:H7 and *E. coli* O26

Table 3: Results for Iceberg Lettuce Samples

Spiking Organism(s)	Samples	8 Hours Enrichment	10 Hours Enrichment	12 Hours Enrichment	20 Hours Enrichment	Confirmed
Real-Time STEC Screening Assay						
Multiple*	10	2	2	2	3	2
Salmonella	3	—	—	—	—	—
E. coli O157:H7	3	1	1	1	1	1
E. coli O26	3	1	1	1	1	1
Real-Time STEC Panel 1 Assay						
Multiple*	10	0	0	0	0	0
Salmonella	3	—	—	—	—	—
E. coli O157:H7	3	—	—	—	—	—
E. coli O26	3	1	0	1	1	1
Real-Time *E. coli* O157:H7 Assay						
Multiple*	10	2	2	2	2	2
Salmonella	3	—	—	—	—	—
E. coli O157:H7	3	1	1	1	1	1
E. coli O26	3	—	—	—	—	—
Real-Time *Salmonella* Assay						
Multiple*	10	2	2	2	2	2
Salmonella	3	0	1	1	1	1
E. coli O157:H7	3	—	—	—	—	—
E. coli O26	3	—	—	—	—	—
***Salmonella* 2 Assay**						
Multiple*	10	2	2	2	2	2
Salmonella	3	1	1	1	1	1
E. coli O157:H7	3	—	—	—	—	—
E. coli O26	3	—	—	—	—	—

*Samples were co-spiked with *Salmonella*, *E. coli* O157:H7 and *E. coli* O26

Table 4: Results for Red Leaf Lettuce Samples - Direct Testing

Spiking Organism(s)	Samples	8 Hours Enrichment	10 Hours Enrichment	12 Hours Enrichment	20 Hours Enrichment	Confirmed
Real-Time STEC Screening Assay						
Multiple*	10	4	4	4	4	4
Salmonella	3	—	—	—	—	—
E. coli O157:H7	3	3	3	3	3	3
E. coli O26	3	0	0	0	0	0
Real-Time STEC Panel 1 Assay						
Multiple*	10	3	3	3	3	3
Salmonella	3	—	—	—	—	—
E. coli O157:H7	3	—	—	—	—	—
E. coli O26	3	0	0	0	0	0
Real-Time *E. coli* O157:H7 Assay						
Multiple*	10	1	1	1	1	1
Salmonella	3	—	—	—	—	—
E. coli O157:H7	3	2	3	3	3	3
E. coli O26	3	—	—	—	—	—
Real-Time *Salmonella* Assay						
Multiple*	10	0	1	1	1	1
Salmonella	3	0	0	0	0	0
E. coli O157:H7	3	—	—	—	—	—
E. coli O26	3	—	—	—	—	—
***Salmonella* 2 Assay**						
Multiple*	10	1	1	1	1	1
Salmonella	3	0	0	0	0	0
E. coli O157:H7	3	—	—	—	—	—
E. coli O26	3	—	—	—	—	—

*Samples were co-spiked with *Salmonella*, *E. coli* O157:H7 and *E. coli* O26

Table 5: Results for Red Leaf Lettuce Samples - Testing After Regrowth

Spiking Organism(s)	Samples	8 Hours Enrichment	10 Hours Enrichment	12 Hours Enrichment	20 Hours Enrichment	Confirmed
Real-Time STEC Screening Assay						
Multiple*	10	4	3	3	4	4
Salmonella	3	—	—	—	—	—
E. coli O157:H7	3	0	0	3	3	3
E. coli O26	3	0	0	0	0	0
Real-Time STEC Panel 1 Assay						
Multiple*	10	3	3	3	3	3
Salmonella	3	—	—	—	—	—
E. coli O157:H7	3	—	—	—	—	—
E. coli O26	3	0	0	0	0	0
Real-Time *E. coli* O157:H7 Assay						
Multiple*	10	1	1	1	1	1
Salmonella	3	—	—	—	—	—
E. coli O157:H7	3	0	3	3	3	3
E. coli O26	3	—	—	—	—	—
Real-Time *Salmonella* Assay						
Multiple*	10	0	1	1	1	1
Salmonella	3	0	0	0	0	0
E. coli O157:H7	3	—	—	—	—	—
E. coli O26	3	—	—	—	—	—
***Salmonella* 2 Assay**						
Multiple*	10	0	1	1	1	1
Salmonella	3	0	0	0	0	0
E. coli O157:H7	3	—	—	—	—	—
E. coli O26	3	—	—	—	—	—

*Samples were co-spiked with *Salmonella*, *E. coli* O157:H7 and *E. coli* O26