To: Dave Alburty, Ann Packingham
From: Mike Hornback, PhD
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Subject: **Reduced Incubation for Detection of *Escherichia coli* O157:H7 in Experimentally Infected Spinach Samples using the InnovaPrep Concentrating Pipette**

Introduction

This report contains the results of a trial study to develop a new method permitting the detection of *Escherichia coli* O157:H7 in experimentally infected spinach samples within a single work-shift. Current AOAC approved methods require an 8 to 24 hour enrichment period followed by a minimum of 1 hour to perform the detection protocol.

This study was funded by FDA through a Phase 1 & Phase 2 Small Business Innovation Research Grant awarded to InnovaPrep. Specifically, this work evaluates the ability to detect *Escherichia coli* O157:H7 in spinach samples using a reduced enrichment time followed by mechanical concentration of the enriched fluid using the InnovaPrep Concentrating Pipette (CP-150) and detection using real-time quantitative Polymerase Chain Reaction (qPCR). This report provides an overview of the protocol and test data.

Protocol

Two sets of tests were performed consisting of four samples each for a total of eight samples.

Test 1	3 spiked samples	1 control sample
Test 2	3 spiked samples	1 control sample

Each sample consisted of twenty-five grams of prepackaged spinach, which was weighed and put into a stomacher bag (Bag Filter 400S, Interscience). The bags were then filled with 225 mL of pre-warmed (42°C), sterile tryptic soy broth. The spinach filter bags were placed inside a stomacher (AES, Smasher) and processed for 60 seconds on the "normal" setting.

Three of the stomached spinach samples in each test were inoculated with 200 µL of diluted *E. coli* O157:H7 (50 CFU/mL), resulting in a spike of 10 CFU per sample. One sample from each test was not spiked, to serve as a negative control. The samples were placed in a 42°C incubator for 5 ½ hours to allow for bacterial enrichment.

To determine the titer of the spike, 200 µL of the *E. coli* spike dilution was also spread onto tryptic soy agar plates and incubated at 42°C overnight, at which point the plates were enumerated and recorded.

BagFilter 400S by Interscience

After incubation of the samples, pre-filtration of the spinach sample was achieved within the BagFilter 400S by passage through the integral filter membrane as pictured at right. This filter material removes particles larger than 250 microns from the enriched sample. A 100 mL of each pre-filtered sample was pipetted out of the stomacher bag and dispensed into a sterile container.

Each 100 mL, filtered sample was concentrated using the InnovaPrep Concentrating Pipette (CP) with a 0.45 µm hollow-fiber Concentrating Pipette tip (hf-CPTs item #CC08018) and eluted using InnovaPrep's TRIS elution fluid (item # HC08001). The elution volumes then were weighed and recorded.

For sample concentration, the CP settings were adjusted to provide small elution volumes that resulted in a high quality foam. The settings used are provided in the table below.

The InnovaPrep Concentrating Pipette

Advanced settings on the CP for concentration of spinach samples

Valve open time	30 milliseconds
Valve closed time	100 milliseconds
Pulse count	2
Flow buffer	3.0 seconds
Extract delay	3 seconds

Detection of *E. coli* O157:H7 in the concentrated samples was achieved by using the DuPont BAX system. In accordance with the BAX protocol, twenty microliters of concentrated spinach samples were pipetted into 200 µL of lysis solution. After lysis was complete, 30 µL of the lysed solution was used to rehydrate the *E. coli* O157:H7 real-time PCR assay and placed within the pre-heated BAX qPCR thermocycler. After approximately 1 hour, the results from BAX analysis were displayed. Results of the testing are provided in the table below.

CP run times and elution volumes for spinach samples

Test	Sample number	Concentration run time (mins)	Elution volume (microliters)	BAX detection results	*E. coli* spike (CFU average)
1	1.1	2.05	193.9	Positive	12.33
	1.2	5.82	195.7	Positive	
	1.3	12.8	227.3	Positive	
	1.4 (neg. control)	1.47	208.9	Negative	
	average	5.54	206.5		
2	2.1	3.63	176.3	Positive	10.67
	2.2	4.46	227.5	Positive	
	2.3	1.87	287.1	Positive	
	2.4 (neg. control)	2.17	155.3	Negative	
	average	3.03	211.6		
Total average		4.28	209.0		
Total stand. dev.		3.75	39.88		

The total average elution volume for concentrating 100 mL of spinach sample was 209 µL and the total average concentration time per sample was 4.28 minutes The average amount of *E. coli* spiked in test 1 (runs 1.1 - 1.3) and test 2 (runs 2.1 – 2.3), was 12.33 and 10.67 CFU per 25 g spinach sample, respectively.

Total time to result were similar for each test run:

- Preparation time for weighing of spinach, addition of media, stomaching of samples and inoculation of samples was approximately 30 minutes.
- The samples were incubated for 5 ½ hours.
- Pipetting and concentration of all samples were slightly varied, depending primarily on the time to concentrate each sample. The longest of the two test runs was 35 minutes to concentrate all 4 samples.
- These steps result in an overall total time for enrichment and concentration, including all preparation steps, of approximately 6 ½ hours.
- Sample preparation using the BAX sample lysis protocol took 45 minutes and qPCR detection required one hour.
- These steps result in an overall, un-optimized time from sample weighing through detection of approximately 8 hours and 20 minutes.

Conclusions

- Detection of as low as 10 CFU *E. coli* O157:H7 in 25 grams of spinach requiring only 5 ½ hours of incubation was demonstrated using InnovaPrep's Concentrating Pipette and the 0.45 µm disposable, hollow-fiber Concentrating Pipette tips along with the Dupont BAX platform.
- The average time from sample weighing through detection, demonstrated in this study, was 8 hours and 20 minutes.
- Further optimization of the protocol is anticipated to lead to detection times of less than 8 hours.