

## Guidelines for MAFMA Final Report

Final Reports due 3 months after completion of project  
(4-5 pages)

Project Title Development of a Novel Method to Detect Enterohemorrhagic *Escherichia coli* in Ground Beef

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### Please complete all questions below and attached form

#### 1. Objective Summary (1-2 sentence summary)

The long-term goal of this project was the development of a highly selective and sensitive laboratory protocol for the detection of EHEC in ground beef. Specifically, we evaluated the investigated protocol that included acid shock and plating onto selective media for recovering EHEC strains in single and mixed cultures of non-pathogenic *Enterobacteriaceae* and we compared the selectivity and sensitivity of the proposed protocol with the USDA official *E. coli* O157:H7 method to detect EHEC from inoculated ground beef samples.

#### 2. Objective Accomplishments

(If objectives were not met, what extenuating circumstances contributed to that factor?)

Convey all of your progress on this project including that obtained with the industry and other matching funds.

For the most part, all of the objectives originally planned for this project were fulfilled and a very promising protocol capable of identifying *E. coli* O157, O26 and O111 has been developed.

The summary of results follows:

The objective of this project was to develop a specific and sensitive protocol for the isolation of EHEC serotypes from ground beef utilizing a combination of glutamate-based acid shocks and plating on a selective agar containing ceftazidime and potassium tellurite. A series of lab experiments in which pure cultures of 203 different EHEC strains, 152 other *E. coli*, 25 enterobacteria, and 14 Gram-positive bacteria were used in different formats. All strains were streaked onto tryptic soy agar (TSA) containing ceftazidime and potassium tellurite (S-TSA) and classified based on their ability to grow on the medium. Single and mixed culture experiments were performed to determine the recovery rate of EHEC after glutamic acid shock (GAS) and plating on S-TSA. EHEC strains were inoculated into ground beef to compare the protocol that included enrichment in EC broth, GAS, and plating onto S-TSA, referred to as selective acid shock (SAS), with an USDA-approved method. S-TSA significantly reduced the recovery of *E. coli* other than EHEC. The data suggested that GAS and S-TSA yielded significant recovery rates of EHEC strains while greatly reducing background flora. The sensitivity and specificity of this method in detecting *E. coli* O157 in ground beef was comparable to an USDA-approved method. (Tables 1 to 3) SAS was also effective in recovering other EHEC serotypes. The SAS protocol could serve as a basis for routine detection of EHEC serotypes from ground beef.

TABLE 1. Comparison of AOAC-approved method and SAS<sup>a</sup> protocol with randomly chosen E. coli O157 strains.

<b>Challenge Study</b>								
<b>Inoculated Ground Beef</b>								
Method <sup>b</sup>	Inoc. Level <sup>c</sup>	# Tested	Presum <sup>d</sup> Pos	Conf <sup>e</sup> Pos	Spec <sup>f</sup>	Sens. <sup>g</sup>	False Neg <sup>h</sup>	False Pos <sup>i</sup>
RapidChek®	1-10	115	92	88	89.5%	99.1%	12	1
	Control	21	0	--	100%	100%	0	0
SAS <sup>b</sup>	1-10	150	95	93	96.5%	99%	7	2
	Control	35	0	--	100%	100%	0	0

<sup>a</sup> SAS protocol consisted of enrichment in EC medium, GAS, and plating on S-SMAC.

<sup>b</sup> CT-SMAC was used for RapidChek® protocol and S-SMAC was used for SAS.

<sup>c</sup> CFU/25 g

<sup>d</sup> Presumptive positives,

<sup>e</sup> Confirmed positives

<sup>f</sup> Specificity

<sup>g</sup> Sensitivity

<sup>h</sup> False negatives

<sup>i</sup> False positives

TABLE 2. Specificity, sensitivity, false negatives and false positives for SAS<sup>a</sup> with E. coli O26 strains using S-SMAC and S-RMAC media.

<b>Challenge Study</b>								
<b>Inoculated Ground Beef</b>								
Media <sup>b</sup>	Inoc. Level <sup>c</sup>	# Tested	Presump <sup>d</sup> Pos	Conf <sup>e</sup> Pos	Spec <sup>f</sup>	Sens <sup>g</sup>	False Neg <sup>h</sup>	False Pos <sup>i</sup>
S-SMAC	1-10	72	34	22	100%	83.1%	0	12
	Control	9	0	--	100%	100%	0	0
S-RMAC	1-10	72	25	22	100%	96%	0	3
	Control	5	0	--	100%	100%	0	0

TABLE 3. Specificity, sensitivity, false negatives and false positives for SAS<sup>a</sup> with E. coli O111 strains.

<b>Challenge Study</b>								
<b>Inoculated Ground Beef</b>								
Media <sup>b</sup>	Inoc. Level <sup>c</sup>	# Tested	Presump <sup>d</sup> Pos	Conf <sup>e</sup> Pos	Spec <sup>f</sup>	Sens <sup>g</sup>	False Neg <sup>h</sup>	False Pos <sup>i</sup>
S-SMAC	1-10	81	41	29	97.5%	85%	2	12
	Control	7	0	--	100%	100%	0	0

3. Unexpected findings, if any

NONE

4. Practical impacts of research efforts. Include: implementation of accomplishments by industry partners (if any), identification of economic impacts, and any further pursuit by PI of research in area of this project whether MAFMA or not.

a. Short Term Impacts

The principle of the developed protocol has been taken as a prototype to develop a rapid method for detection of all EHEC in collaboration with the industry partner (Paradigm Diagnostics, Inc.). Work is currently underway to shorten the detection period.

The current protocol is no being tested in collaboration with the Minnesota Department of Health to determine if it can enhance the recovery of EHEC from clinical samples.

Currently as many as 30% of all non-O157 EHEC infections cannot be traced to a specific isolates because of lack of sufficiently differential methods. This method has a great potential to improve this recovery.

b. Long Term Impacts

Once a rapid method that takes less than a day to complete is developed it will be patented and most likely commercialized.

5. If you are also making reports to other funding agencies in the course of this research work, please include a copy of that report.

6. If any publications resulted from the research, a copy must be included. Please note we were notified by the USDA/CSREES National Program Leader for the Midwest Advance Food Manufacturing Alliance (MAFMA) that all publications resulting from research that was funded by MAFMA must include the following wording **“The project was supported by the USDA Cooperative State Research, Education and Extension Service, special research grant number 200X-34328-xxxxx.**

**This work was presented at the 2006 International Association for Food Protection in Calgary, Alberta:**

Kuruc, J., A. Olstein, and F. Diez-Gonzalez. 2006. The application of acid shock as a selective step to isolate Enterohemorrhagic *Escherichia coli*. IAFP Annual Meeting, August 13-1, Calgary, Alberta.

In addition, a paper has been prepared ready for submission, but because of potentially patentable material, it has not been sent for publication.