**BEEF INDUSTRY SAFETY SUMMIT**
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**Project Title:** **Development and Application of a Green Fluorescent Protein (GFP) Expressing *E. coli* O103 Surrogate for Tracking Contamination through Grinding and Identifying Persistent Points of Contamination**

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**Objective:** To 1.) develop and validate an easily trackable *E. coli* O157:H7/non-O157 STEC surrogate that can be detected to the same level of sensitivity as *E. coli* O157:H7; and 2.) apply the trackable surrogate to model contamination passage through grinding and identify points where contamination may persist.

**Experimental Design & Analysis:**

A series of *E. coli* serogroups O103, O26 and O145 lacking virulence genes were transformed with a GFP expression vector. Transformants that stably expressed the GFP were selected and characterized for recovery of 3 CFU in 325g ground beef following storage at 4°C for 14 days and storage at -20°C for 6 weeks using direct plating, immunomagnetic separation (IMS) and PCR. One strain of GFP-O103 *E. coli* was then used to inoculate a combo of beef trim. The inoculated combo and four non-inoculated combos were used sequentially to produce 4 lb. loaves of ground beef. The presence of the GFP-O103 was monitored through the ground beef loaves in three replicate experiments using a high inoculation dose (6 log CFU/combo) and one replicate using a low inoculation dose (4 log CFU/combo) of GFP-O103. Every 20$^{th}$ loaf was tested to identify approximate end points of GFP-O103 passage, then every 10$^{th}$ and 5$^{th}$ loaf around the approximate end point were tested in turn to identify the last GFP-O103 containing loaf. After each experimental repetition, surface and residual meat samples were collected from all equipment through or across which the inoculated trim and subsequently produced ground beef had passed.

**Key Results:**

A non-pathogenic strain of *E. coli* serogroup O103 was developed for use as an *E. coli* O157:H7 surrogate. The GFP surrogate had a similar growth curve and surface adherence (measured by biofilm forming ability) as strains of *E. coli* O157:H7. The GFP-O103 could be easily identified in ground beef to a limit of detection of 1-5 CFU/325g (the same level as *E. coli* O157:H7 typically

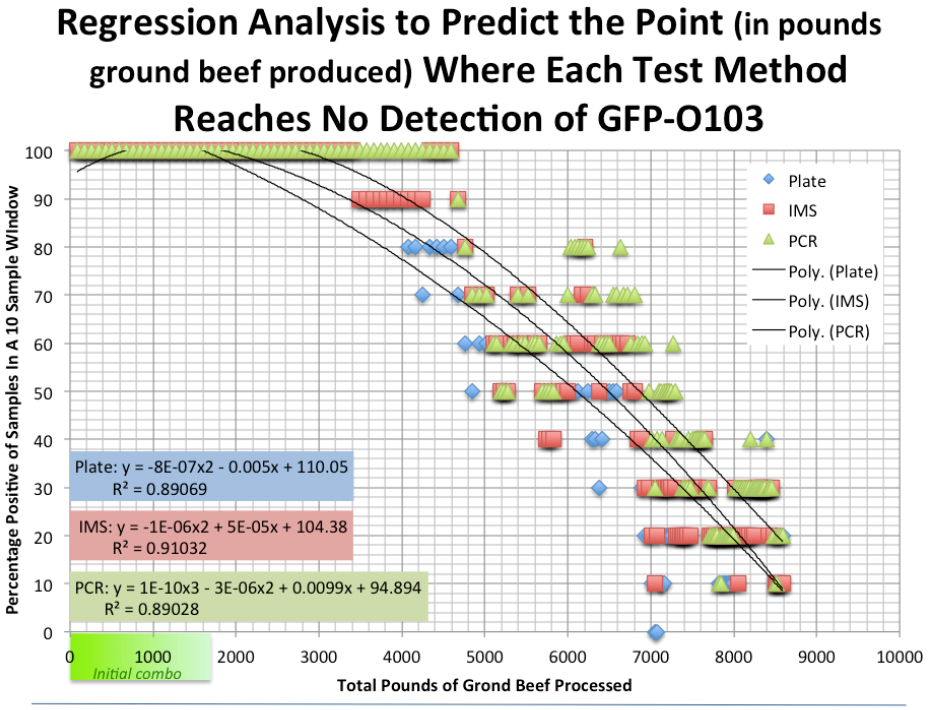
is), and could be recovered from ground beef stored at 4°C and -20°C.

The grinding study showed that neither the high nor the low dose inoculum was cleared completely through production after the fourth non-inoculated combo had been processed. Combos ranged in size from 1,000 to 1,700 lbs in different experimental repetitions. Regression analysis estimated that under the conditions of this investigation (processing equipment types, line configuration and complexity) an additional 8,542 ± 919 lbs of beef trim is required to clear a contaminant to a non-detectable level. Post-experiment surface samples and residual meat still present on belts, augers and VMAG horn identified points where the GFP-O103 persisted through the production of ground beef.

**How can this information be applied in the industry?**

An easily detectable non-pathogenic *E. coli* O157:H7 surrogate with unique markers for direct, IMS and PCR detection (GFP and O103 surface antigen) is available for use. Ground beef producers and regulators are currently basing disposition and recall decisions on data citing one combo/batch before and after an affected combo/batch in grinding as required to clear contamination. Thus, these results emphasize the need for each facility to perform their own studies to identify the point(s) at which a contaminant may clear their equipment and to take into considerations other GMP related factors to reduce cross-contamination of meats.

**Table or graph with critical information related to the results:**




Direct plating predicted to reach zero after an additional **7,313 ± 985** pounds processed
IMS predicted to reach zero after an additional **8,542 ± 919** pounds processed
PCR predicted to reach zero after an additional **7,997 ± 1,064** pounds processed