

**Validation of the Microbial-Vac System for Use in Food
Safety Monitoring Activities within Beef Abattoirs**

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Abstract

The Microbial-Vac System (M-Vac) is a novel wet vacuum surface sampling device with numerous applications within food processing facilities. A broad spectrum of validations, including environmental, carcass/primal, and beef trim sampling, have been conducted to determine whether the M-Vac is capable of producing bacterial recoveries equivalent, or better, than currently utilized sampling techniques. Pre-operational environmental sampling testing showed the M-Vac was able to recover significantly higher ($P < 0.05$) levels of *Listeria* and *Salmonella* from environmental surfaces (simulated) than cellulose sponge and cotton swab. Surfaces tested include stainless steel, ceramic tile, ultra high molecular weight polyethelene (UHMWP) cutting board, and conveyor belt.

Carcass/primal surfaces were simulated using brisket and flank meat from freshly slaughtered carcasses, with inoculated pieces being held for 18 hours at 4°C prior to sampling. Under these conditions the M-Vac was able to more frequently recover detectable levels of *E. coli* O157:H7, inoculated at low levels (1 – 5 CFU), than cellulose sponge. The difference between the M-Vac and sponge in this testing was significant at the 5% probability level.

Trim testing was conducted in two phases with the first phase being three separate plant staged field trials. The M-Vac and excision techniques were used to sample sites on the same pieces of trim with samples being tested for aerobic plate count (APC), total coliforms (TC), and generic *E. coli*. In all head to head sampling, no significant differences existed between M-Vac and excision, excepting TC results in plant C where M-Vac recovered significantly more than excision ($P < 0.05$).

Phase 2 of the trim testing was conducted in the laboratory and involved low level (1 – 5 CFU) *E. coli* O157:H7 inoculated onto pre and post rigor mortis brisket and flank meat. Following inoculation, pieces of meat were held at 4°C for 18 hours prior to sampling. Following sampling, all samples were pre-enriched and processed via real-time PCR for presence/absence of the target organism. The M-Vac showed recoveries that were not significantly different than excision regardless of the condition of the meat at the time of inoculation.

Throughout all three validations (environmental monitoring, carcass/primal sampling, and trim sampling) the M-Vac was able to produce bacterial recoveries that were either significantly better than or not different than the evaluated conventional methods. These results suggest the M-Vac is a suitable alternative to current sampling techniques for food safety monitoring activities.

Introduction

The Microbial-Vac System (M-Vac) is a novel pathogen sampling device which utilizes wet-vacuum principles to aggressively sample surfaces of interest. The system consists of a durable Support Equipment Case (SEC) which allows for the generation of vacuum and a means for delivering pressurized (15 PSI) sterile solution required for collecting samples. The SEC ([Figure 1](#)) is factory calibrated to provide over 2,000 hours of repeatable, service free operation when basic maintenance recommendations are followed. The SEC is available in multiple formats to accommodate the electrical and other needs of users. In addition to the SEC an M-Vac Kit ([Figure 2](#)) is also required to collect samples. The M-Vac Kit consists of a handheld sampling head connected, via co-extruded tubing, to an air/liquid separator with an attached plastic bottle in which a liquid sample is collected/retained for analyses in the laboratory. The sampling head consists of a solution control (On/Off) switch and a flexible surface contact ring. The solution control switch gives the operator the ability to start and stop the spray of solution onto the surface being sampled. The flexible ring allows the sampling head to interface with surfaces of interest. Within this ring is a nozzle, which produces a consistent spray for application of liquid to a surface, and a vacuum port around the circumference of the ring. With most uses of the M-Vac, solution is sprayed while vacuum is simultaneously used to aspirate the dispensed solution. The co-extruded tubing connecting the sampling head to the air/liquid separator consists of two attached tubes. One of the tubes serves as a supply line for delivering sterile liquid to the sampling head while the other tube is used to carry vacuumed air/liquid/particles from the surface being sampled to the sample collection bottle. When a sample has been collected in the sample collection bottle, the

bottle is unthreaded from the air/liquid separator. The bottle is sealed with a cap and is delivered to a laboratory for further analyses of the sample. The M-Vac is capable of collecting samples from a diverse array of surfaces including hard/nonporous surface such as stainless steel and UHMWP to pliable/irregular surfaces like carcasses/primals (carcass), and beef trimmings or porous surfaces like brick and carpet.

A sampling screen tool ([Figure 3](#)) has been developed to allow sampling personnel to easily sample pliable surfaces such as beef carcasses and trimmings. This tool may also include a hook, as shown in Figure 3, for the user's convenience. The hook portion of this tool is similar to the meat hooks which are ubiquitous in abattoirs and allow for ease in moving meat around as needed. The stainless steel screen and handle are configured to allow the user to easily place the screen on the surface of the meat to be sampled. A screen between the meat and the sampling head prevents the vacuum from pulling meat into the sampling head and blocking air flow. The spacing of the sampling screen grid used in the validations was approximately 1 cm x 1 cm. Using this tool also provides a template for users to ensure the same surface area is covered at every site. The sampling screen tool is easily decontaminated by rinsing in 180°F water as is commonly done with other stainless steel devices (knives, meat hooks, etc.) currently used within beef processing facilities.

Objective

Several studies were done to compare the effectiveness of the M-Vac sampling technique to the techniques currently used in environmental monitoring, carcass/primal sampling, and beef trim sampling. Each of these studies represents potential applications for the M-Vac sampling system within food safety monitoring activities for food processors.

For environmental monitoring and carcass sampling studies, the M-Vac was compared to cellulose sponges. The objective in the environmental monitoring study was to show how the M-Vac compared to sponging in quantitative recovery of bacteria from simulated pre-operational environmental surfaces. A simulated carcass sampling study was performed to determine qualitatively if the M-Vac provided comparable results to sponging in recovering detectable levels of *E. coli* O157:H7. For beef trim sampling, two studies were conducted which compared the M-Vac to the excision technique, as currently utilized by several facilities within the beef industry. The first of these two studies consisted of multiple plant staged field trials which evaluated both sampling techniques for recovery of total aerobic bacteria, total coliforms, and generic *E. coli*. The second trim related study was conducted in the laboratory and evaluated the two sampling techniques for recovery of low level (1 – 5 CFU) *E. coli* O157:H7 contamination. This low level testing was conducted both with freshly slaughtered (pre-rigor) meat and meat that had been chilled at least 18 hours at 4°C (post-rigor) at the time of inoculation. These two variables (pre-rigor/post-rigor) were evaluated since both modes of trim contamination are possibilities in the beef fabrication process. Results for this study were qualitative (+/-).

Procedures

Environmental Sampling

Two strains were examined for recovery in this study: *Salmonella enterica* (ATCC#: 700720) and *Listeria innocua* (ATCC#: 49595). Continuous cultures of these bacterial strains were kept by transferring a loop full of a 48 hour culture into a fresh test tube of sterile broth. *Salmonella* was cultured in 10 mL Tryptic Soy Broth (TSB) and *Listeria* was cultured in 10 mL Demi-Fraser Broth (DFB). Both cultures were cultured at 37° C for 24 hours prior to use in inoculation.

Four surfaces were examined in this study: stainless steel, ceramic tile, cutting board, and conveyor belt. 100 cm² (10cm x 10cm) squares were outlined on each of the surfaces to be the designated area to inoculate and sample. This surface area was sufficient for accomplishing the desired objective while also being convenient to accommodate in the lab. One square represented one sample in the study. Stainless steel coupons, ceramic tiles, and the conveyor belt were autoclaved at 121° C for 20 minutes and allowed to cool to room temperature prior to inoculation. The cutting boards were cleaned using 70% EtOH and allowed to dry prior to inoculation.

Three sampling techniques were examined in this study: M-Vac, Sponge, and Swab (*Salmonella* only). Sterile surfaces were inoculated with 0.2 mL of the stock culture in broth (~10⁹ CFU/mL). Inoculum was spread evenly over the surface using an L-spreader. Surfaces were allowed to dry completely before sampling (~30 minutes).

Upon drying, the inoculated *Salmonella* squares were divided equally and sampled with each of the three techniques. Dried *Listeria* squares were divided evenly and sampled with M-Vac and sponge.

M-Vac samples were collected using Butterfield's Buffer with 0.1% Tween 80 and 0.05% antifoam (mBB) as the sterile rinse solution. Sampling was performed by passing the M-Vac sampling head with the vacuum and solution turned ON over the entire surface slowly using 5 strokes, and then again in the other direction. Solution was then turned off and the M-Vac sampling head was run quickly back over the surface with only the vacuum turned ON to collect residual solution, if any, remaining on the surface. The sample collection bottle was removed, capped, and hand shaken for 5 seconds.

Sponge samples were obtained using a sterile, dehydrated cellulose sponge (3M; St. Paul, MN) that was pre-moistened with 10 mL Butterfield's Buffer (BB). Each square designated to be sampled using a sponge was sampled by passing the sponge over the surface 10 times in one direction and 10 times in a perpendicular direction. The sponge was then placed back into its pouch. 15 mL sterile BB was added to each sponge and the pouch was stomached for 2 minutes.

Swab samples were obtained using a sterile, cotton tipped swab (Puritan Medical Products; Guilford, ME) that was dipped into BB for pre-moistening. Each square designated to be sampled using a swab was sampled by passing the swab over the entire surface in one direction and then again in a perpendicular direction. The swab was then placed in a test tube containing 10 mL BB. Each test tube containing a swab was vortexed 12 times for 10 seconds each time.

For all samples, an appropriate dilution was prepared in BB. This dilution was thoroughly mixed and plated in duplicate onto XLD plates (Becton Dickinson; Sparks, MD) for *Salmonella* samples and OLA plates (Becton Dickinson) for *Listeria* samples.

Plates were incubated at 37°C overnight followed by enumeration via direct plate count with results converted to log₁₀ values.

Carcass Sampling

The recovery efficiency of the M-Vac and Sponge was compared at low levels of simulated carcass contamination with *E. coli* O157:H7. Pre-rigor flank and brisket pieces of beef were obtained from a local slaughter facility and transported to the laboratory within 2 hours from slaughter for this study. Collected pieces had been dehided, eviscerated, and exposed to a 2% lactic acid wash by the manufacturer prior to transportation to the lab. Sampling sites for this study were established by using a template to outline 100 cm² squares. Each square represented one sample. Two squares were outlined on each piece of meat – one designated to be sampled using the M-Vac and one designated to be sampled using a sponge. The samples were inoculated with 1-5 CFU *E. coli*. Each square was labeled with the type of sampling technique that was to be exercised.

A 10⁻⁸ dilution of a 24 hour *E. coli* O157:H7 (ATCC#: 700728) culture was prepared and 0.2 mL of the dilution was used to inoculate the squares. Inoculated meat was placed in a plastic tote and a wet paper towel was added to the tote to provide a source of humidity to prevent desiccation of the organisms on the meat. A lid was then placed on each tote and chilled at 4°C for 24 hours.

After refrigeration, sponge samples were obtained using a sterile, cellulose sponge pre-moistened with 10 mL of Butterfield's Buffer. Each square designated to be sampled using a sponge was sampled by passing the sponge over the surface of the meat 10 times in one direction and 10 times in a perpendicular direction. The sponge was then placed

back into its pouch. 25 mL 1X mEHEC broth was added to the pouch followed by stomaching (Weber Scientific; Hamilton, NJ), at 240 RPMs for 2 minutes. An additional 75 mL 1X mEHEC broth was added to the pouch and the sample was incubated at 42° C for 18 hours.

M-Vac samples were collected by first placing a sampling screen template on the surface of the inoculated square. The sampling head was then moved back and forth over the template twice with vacuum ON but no buffer spraying. Solution spray, Butterfield's Buffer (BB), was then turned ON and the head was again moved back and forth twice for a total of 2 seconds. After 2 seconds of sampling, the spray of BB was stopped, and an identical procedure was performed for a strip immediately adjacent to the first strip. A third strip was also sampled the same way so the entire square had been covered. This sample was weighed and 5X mEHEC broth was added to each sample at a ratio of 1:5 of 5X mEHEC to the final weight of sample and mEHEC. Samples were incubated at 42°C for 18 hours.

Following incubation, samples were prepared for PCR analysis in the BioControl Genetic Detection System (GDS) using *E. coli* O157:H7 test kits (BioControl Part #: 61007-100) according to manufacturers written instructions by appropriately trained laboratory personnel. Results were reported as +/- for the target organism.

Trim Sampling

Plant Staged Field Trials

Three identical trials were conducted in separate beef processing facilities. Within each facility combo bins were pulled from the fabrication process and moved to a separate area where samples could be collected using both M-Vac and excision sampling

techniques. From each bin, 12 individual pieces of trim were selected for sampling. For the excision technique, an excision tool designed for surface N-60 testing (G-R Manufacturing; Manhattan, KS) with a diameter of 3.8 cm was used to cut circular sites in each of the 12 selected pieces of trim. A scalpel was then used to cut the circular sites of meat from the pieces of trim at a thickness of 1/8". The 12 excised sites were then combined in one sterile Whirl-pak pouch (Weber Scientific), labeled, and placed in a cooler for transportation to a laboratory. In actual use in industry, this excision tool has been shown to produce 60 excision disks with a combined weight of 375 ± 37 g and is part of standard trim sampling protocols within several beef processing establishments in the United States. Since this tool allows users to accurately and reproducibly obtain a consistent surface area, it was employed in this study as a suitable technique for excision sampling.

The M-Vac was used to sample a site, of equivalent surface area, in close proximity to the excised site on each of the 12 pieces of trim. M-Vac sites were sampled by first placing a sampling screen on the surface of the trim. The sampling head was then moved back and forth over the template with vacuum and BB spray ON for 2 seconds. After 2 seconds of sampling, the spray of buffer was stopped, the screen was removed and the sampling head quickly dabbed over the sampled site to remove any remaining moisture from the surface of the meat. The same procedure was then used to sample the 11 remaining pieces of trim. For each sample of n=12 sites, a sterile M-Vac Kit was used.

Following transportation to the laboratory, excision samples had 100 mL of Butterfield's phosphate diluent added. Samples were then hand massaged for 2 minutes

to promote release of organisms into the surrounding solution. M-Vac samples were hand shaken for five seconds to mix the contents of each sample. A 1.0 mL aliquot of each sample was then deposited on different Petrifilm (3M) for total aerobic plate count (APC), total coliforms (TC), and presence/absence of generic *E. coli* (EC). Petrifilm were then processed according to manufacturer's written instructions and incubated at 37°C for 24 hours. Following incubation, Petrifilm were enumerated, again following manufacturer's written instructions, by direct counting and visual observation.

Low Level *E. coli* O157:H7 Trim Contamination Testing

Pre-rigor mortis contamination testing:

Several pieces of brisket and flank meat from freshly slaughtered, dehided, eviscerated, and lactic acid washed carcasses were obtained from a local slaughter facility. Collected pieces were transported to the laboratory within 2 hours from slaughter. In the laboratory, each piece of meat was laid in a foil lined plastic tote with the carcass external side of the meat facing up. Meat branding ink was used to outline specific sites on each piece of meat for inoculation. Five sites were outlined for both excision and M-Vac sampling techniques per sample. For excision sites, 1.5" diameter circles were outlined using a disposable template dipped in the ink and then "stamped" on the meat. M-Vac sites were rectangular in shape with dimensions of 1 3/8" x 4".

A strain of *E. coli* O157:H7 (ATCC#: 700728) was cultured in Brain Heart Infusion (BHI) broth (Becton Dickinson) for 48 hours at 37°C. The culture was transferred a minimum of 3 times prior to use in testing. On the day of inoculation, a 48 hour culture was diluted to 10⁻⁸ and labeled as Working Inoculation Solution (WIS). For

each set of 5 outlined sites a 0.2 mL aliquot of WIS was deposited drop-wise over the 5 sites (all 5 sites contained a total of 0.2 mL of WIS). In between every two inoculations of WIS onto the meat, two test tubes were also inoculated with 0.2 mL of WIS. One of the test tubes contained BHI while the other contained Buffered Peptone Water (BPW). Following inoculation all of the meat and test tubes were transferred to cold storage at 4°C for 24 hours. A water saturated paper towel was placed inside each tote containing inoculated meat during refrigeration to provide a source of humidity and prevent desiccation of the inoculated cells.

Following refrigeration, inoculated test tubes were filtered through individual 0.45 µ membranes (VWR; West Chester, PA). Membranes were then transferred to Tryptic Soy Agar (TSA) plates (Becton Dickinson) and incubated at 37°C for 24 hours. Plates were then enumerated by direct count of the membranes to establish an average number of cells inoculated on the sites for each sample on the day of inoculation.

Totes were removed one at a time from refrigeration and sampled via excision or M-Vac. For excision samples, a flamed scalpel was used to cut around the outlined sites on the meat and each site was removed at a thickness of 1/8". All five excised sites were combined in a sterile Whirl-pak pouch. Each pouch was then weighed and mEHEC broth (BioControl), pre-warmed to 42°C, was added at a 1:5 dilution of sample weight to total weight of sample plus media followed by two minutes of hand massaging.

For M-Vac sampling, a 1 cm x 1 cm sampling screen was placed on an inoculated site. The sampling head was placed on the screen and moved back and forth over the site two times with vacuum ON but without solution spraying. Then with Butterfield's buffer solution spraying, the sampling head was again moved back and forth two times over the

site for a total of 2 seconds at which time the spray was stopped. The screen was removed from the site and the sampling head was dabbed on the surface of the meat to collect any residual buffer spray visually remaining on the site of meat. This procedure was repeated four more times until all five inoculated sites were sampled. The sample collection bottle was removed, weighed, and 5X concentrated mEHEC media, pre-warmed to 42°C, was added to the bottle at a dilution of 1:5 of the weight of the 5X mEHEC media to the weight of the sample plus media.

Excision and M-Vac samples were then incubated for 18 hours at 42°C. Following incubation, samples were prepared for PCR analysis in the BioControl Genetic Detection System (BioControl International; Bellevue, WA) using *E. coli* O157:H7 test kits (BioControl) according to manufacturers written instructions by appropriately trained laboratory personnel with results reported as +/- for the target organism.

Post-rigor mortis contamination testing:

For post-rigor contamination testing, the exact same procedures as detailed in the “Pre-rigor mortis contamination testing” section above were followed with one deviation. Collected pieces of flank and brisket were held at least overnight at 4°C prior to inoculation with WIS.

Statistical Analysis

All data from quantitative studies, including environmental sampling along with APC and TC from the plant staged field trials, was imported into ProStat (version 3.0) software (Poly Software International; Pearl River, NY). The data was analyzed using an un-paired T-test to determine if any significant differences exist between the different

sampling techniques utilized. EC data from plant staged field trials was analyzed using the Fisher Exact test with results interpreted at the 5% probability level. The qualitative data, including carcass sampling and both pre/post-rigor trim contamination sampling, were analyzed using a Chi Square Goodness of Fit test with significance determined at the 5% probability level.

Results

Environmental Sampling

The M-Vac recovered significantly higher levels of *Listeria* and *Salmonella* from each simulated environmental surface type ([Table 1](#)). The M-Vac samples were calculated to have an average of over 7 log CFU recovered for both *Listeria* and *Salmonella* testing across all surfaces tested. Considerably more variability was observed from sponge samples when comparing recovery for *Listeria* and *Salmonella* on identical surfaces. Given the performance of swabs in the *Salmonella* testing, they were not included in the *Listeria* trial.

Carcass Sampling

For recovery of low level *E. coli* O157:H7 contamination, the sponge technique, currently recommended for use by beef processors, recovered significantly less positive samples than the M-Vac ([Table 2](#)).

Trim Sampling

Overall results from the plant staged field trials showed the M-Vac achieved recoveries of APC and TC that were, for the most part, not significantly different from excision ([Table 3](#)). The one exception was in plant C where the M-Vac recovered significantly more ($P < 0.05$) coliforms than excision. Analysis of the EC data within each plant showed no statistically significant differences, at the 5% probability level, between the two sampling techniques. Given the variability of the EC data from plant to plant, a statistical analysis was not performed on the combined data. Raw data from the

in-plant trials ([Graphs 1 – 2](#)) also showed a definite correlation between M-Vac and excision recoveries. Additional testing in the lab demonstrated the M-Vac was able to recover low level *E. coli* O157:H7 contamination without any significant differences to excision. This was shown to be true for both pre-rigor contamination ([Table 4](#)) and post-rigor contamination ([Table 5](#)).

Conclusions

Compared to sampling techniques currently utilized in food safety monitoring procedures the M-Vac showed comparable recoveries of bacteria and in some cases statistically outperformed these techniques. Results from the environmental monitoring study showed the M-Vac produces bacterial recoveries which are superior to those produced by the sponge/swab technique. Utilizing the M-Vac for such testing would give quality control personnel a more accurate estimation of the cleanliness of surfaces throughout their facilities which are involved in the production process. More efficient bacterial recovery would also allow quality personnel to detect the presence of food pathogens when such organisms are still at relatively low levels in the processing environment. Earlier detection of pathogens in the environment would make it less likely for manufacturers to produce pathogen containing product and could make it easier for manufacturers to implement steps to eliminate the pathogens while at lower levels.

Simulated carcass/primal sampling showed the M-Vac was capable of recovering low level O157:H7 contamination more frequently than sponging. These results suggest that processing facilities which screen carcasses for O157:H7 contamination with sponges are more likely to have false negative results leading to increased introduction of contaminated meat into the fabrication process. Utilizing the M-Vac sampling technique in the carcass screening process could have an immediate impact on lowering the incidence of contaminated meat entering downstream processing.

The M-Vac demonstrated results that were not significantly different than excision on beef surfaces; both in the field trials and in extensive laboratory testing for O157:H7. The lack of a significant difference between the two sampling techniques

suggests that, regardless of the technique utilized to sample beef surfaces, O157:H7 contamination will be collected with the same efficiency. Therefore, abattoirs that determine utilization of the M-Vac would be a better fit in their processes would still be using a sampling technique as robust as excision to validate the safety of their product.

Summary

The M-Vac is a non-destructive wet-vacuum based sampling system which is capable of aggressively collecting bacteria from a diverse array of surfaces. Many applications within food processing establishments exist for the M-Vac. In the beef industry these applications include environmental monitoring, carcass/primal sampling, and beef trimmings sampling. Validations of each of these applications have shown the M-Vac is capable of recovering bacteria as well as, or better than, techniques currently utilized for these applications within the beef industry. The M-Vac could provide significant advantages for food manufacturers by recovering bacteria more efficiently than currently utilized surface sponges/swabs. Carcass screening for O157:H7 with sponges is more likely to introduce contaminated meat into the fabrication process than the M-Vac due to the high frequency of false negative results produced by sponging. For beef processors who wish to utilize the M-Vac for trim sampling, the M-Vac has been shown to be a robust sampling technique not statistically different than excision. This was demonstrated both in plant staged field trials and in the laboratory.

Appendix

Table 1

Environmental Sampling

Recovery of Common Food Pathogen Simulants from Representative Food Processing Environmental Surfaces					
Organism	Device	Stainless Steel	Cutting Board	Ceramic Tile	Conveyor Belt
Listeria	M-Vac	7.49 a	7.07 a	7.31 a	7.51 a
	Sponge	5.65 b	5.77 b	5.52 b	5.55 b
Salmonella	M-Vac	7.29 a	7.37 a	7.05 a	7.30 a
	Sponge	6.06 b	6.57 b	5.92 b	6.03 b
	Swab	6.02 b	5.48 c	5.62 c	5.75 b

Table 1: Recovery of *Listeria* and *Salmonella* from common food processing environmental surfaces, reported as Log₁₀ values, using three sampling techniques. In all treatments M-Vac recoveries were statistically higher than those for sponge and swab. Sponge and swab results for *Salmonella* with the same letters were not significantly different ($P < 0.05$). $n = 15$ for each treatment.

Table 2*Carcass/Primal Sampling*

Recovery of Low Level (1 - 5 CFU) <i>E. coli</i> O157:H7 Contamination from Simulated Beef Carcass Surfaces				
Sampling Technique	Sample Result		Total	χ^2 Value
	Positive	Negative		
M-Vac	15	9	24	6.4
Sponge	9	15	24	

Table 2: Recovery of *E. coli* O157:H7 from simulated carcass surfaces using two sampling techniques. Differences between the two techniques were statistically significant ($\chi^2 > 3.84$).

Table 3*Plant Staged Field Trials*

Multiparameter Plant Staged Field Trials for Recovery of Bacteria from Beef Trimmings				
Plant	Technique	Average Aerobic Bacteria Recovered	Average Total Coliform Recovered	Samples E. coli +
A	M-Vac	3.23 a	0.87 a	0.0% (0/20)
	Excision	3.25 a	0.53 a	0.0% (0/20)
B	M-Vac	3.80 a	2.33 a	35.0% (7/20)
	Excision	3.69 a	1.86 a	35.0% (7/20)
C	M-Vac	4.24 a	3.41 a	25.0% (5/20)
	Excision	4.16 a	2.93 b	0.0% (0/20)
Total	M-Vac	3.76 a	2.21 a	20.0% (12/60)
	Excision	3.70 a	1.78 a	11.7% (7/60)

Table 3: Recovery of APC, TC, and generic *E. coli* from beef trimmings. APC and TC results reported as Log₁₀ values. Results with the same letter for APC and TC are not significantly different ($P < 0.05$). Using the Fisher Exact test at the 5% probability level, no significant differences were observed between techniques for recovery of *E. coli*.

Table 4*Pre-rigor Contamination Trim Testing*

Recovery of Low Level (1 - 5 CFU) <i>E. coli</i> O157:H7 Contamination from Beef Trimmings: Inoculated Pre-rigor						
Trial	Average Inoculum	Device	Result		Total Observations	χ^2 Value
			Positives	Negatives		
1	2.9	M-Vac	9	7	16	2.4
		Excision	6	10		
2	4.0	M-Vac	9	11	20	1.88
		Excision	12	8		
3	3.0	M-Vac	9	7	16	0.27
		Excision	10	6		
Combined	3.4	M-Vac	27	25	52	0.08
		Excision	28	24		

Table 4: Recovery of *E. coli* O157:H7 inoculated on pre-rigor mortis meat surfaces and sampled after 24 hour refrigeration. No significant differences were observed between the two techniques in individual trials or in the combined data at the 5% probability level (χ^2 value < 3.84).

Table 5*Post-rigor Contamination Trim Testing*

Recovery of Low Level (1 - 5 CFU) <i>E. coli</i> O157:H7 Contamination from Beef Trimmings: Inoculated Post-rigor						
Trial	Average Inoculum	Device	Results		Total Observations	χ^2 Value
			Positives	Negatives		
1	2.6	M-Vac	12	6	18	2.89
		Excision	7	8	15	
2	2.7	M-Vac	10	2	12	Not Calculated
		Excision	10	1	11	
Combined	2.7	M-Vac	22	8	30	0.6
		Excision	17	9	26	

Table 5: Recovery of *E. coli* O157:H7 inoculated on post-rigor mortis meat surfaces and sampled after 24 hour refrigeration. No significant differences were observed between the two techniques in the combined data at the 5% probability level (χ^2 value < 3.84).

Figures



Figure 1: Support Equipment Case (SEC)



Figure 2: M-Vac Kit

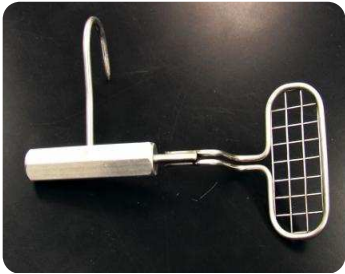
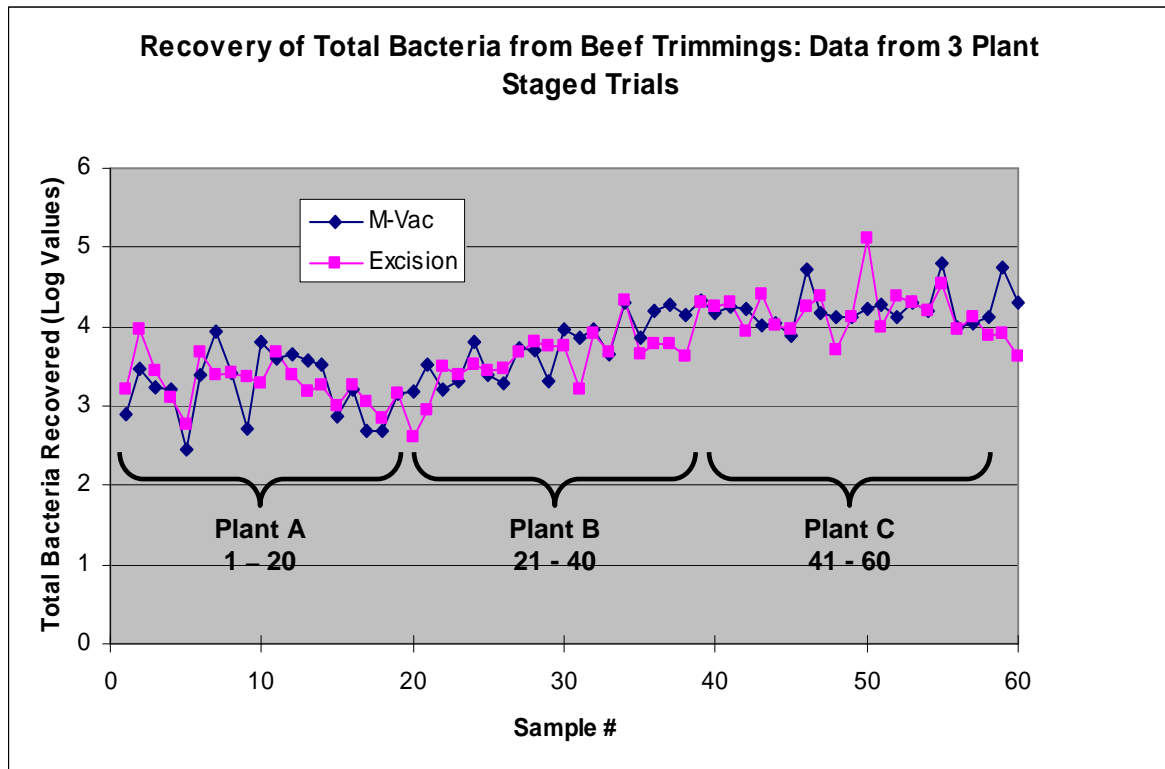
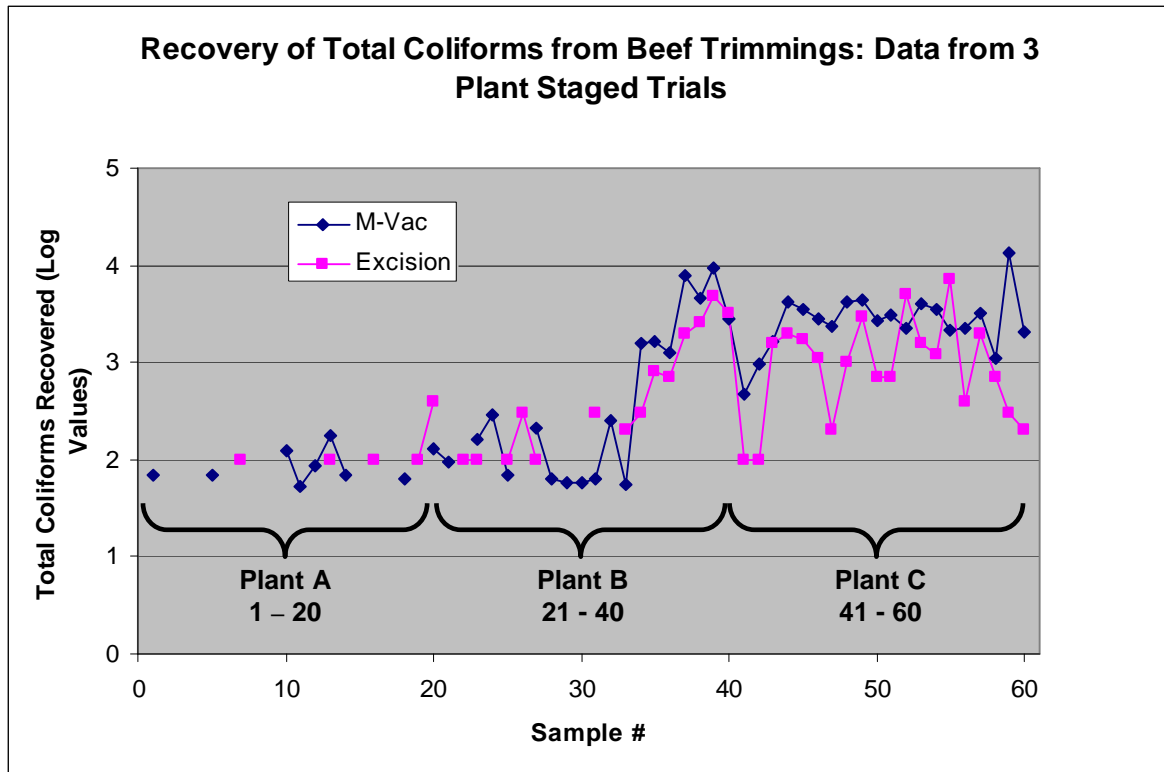


Figure 3: Sampling Hook and Screen Tool

Graphs



Graph 1: Raw data showing recovery of total aerobic bacteria from beef trimmings by M-Vac and excision in three separate in-plant trials.



Graph 2: Raw data showing recovery of total coliforms from beef trimmings by M-Vac and excision in three separate in-plant trials. Data from samples showing no recovery of coliforms was omitted to enable visualization of the overall trends.