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Recovery of Microbial Contamination from Simulated Food Manufacturing Pre-Operation Environmental Surfaces

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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 polyethylene (UHMWP) cutting board, and conveyor belt.

Objective

Compare the effectiveness of the M-Vac sampling technique to cellulose sponges and cotton tipped swabs for environmental monitoring in pre-operation environments.

Procedures

Two strains were examined for recovery in this study: *Salmonella enterica* (ATCC#: 700720) and *Listeria inocua* (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 cm^2 (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 (~10^9 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

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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 $\log_{10}$ values.

Statistical Analysis

Data was imported into ProStat (v. 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.

Results

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. Given the performance of swabs in the *Salmonella* testing, they were not included in the *Listeria* trial.

Conclusions

The M-Vac produces bacterial recoveries which are superior to those produced by the sponge/swab technique. Utilizing the M-Vac for environmental monitoring 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.

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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 Log_{10} 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.