# Microbial Mapping III. Determining Microbiological Counts on Beef Subprimal Cuts During/Following Fabrication with and without Microbiological Decontamination Treatments

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**SUMMARY**

This study evaluated the efficacy of decontamination treatments, used alone and in combination, in lowering, or retarding proliferation of, microbiological populations on fabrication table surfaces and beef subprimal cuts during carcass fabrication. Microbiological populations, especially total coliform counts (TCC) and *Escherichia coli* counts (ECC), on carcasses immediately entering fabrication were very low, with non-detectable counts on 89.5 and 93.8% of samples, respectively. Lactic acid solution rinsing of carcasses reduced ($P < 0.05$) total plate counts (TPC), and increased the percentage of non-detectable TCC and ECC from 87.6% and 92.4% to 92.4% and 94.3%, respectively. Lactic acid solution rinsing of fabrication tables resulted in lower microbiological populations on table surfaces, while top sirloin butt rinsing reduced TPC, TCC, and ECC, when applied alone, and reduced ECC when applied in combination with carcass and fabrication table rinsing.

**Key Words:** Microbiological populations, Beef carcasses, Fabrication, Decontamination

**INTRODUCTION**

In 1996, a study conducted by Colorado State University, known as Microbial Mapping I, involved collection of microbiological data from cattle and carcasses. The objective was to characterize then current status of microbiological populations on beef carcasses as they were harvested, after subsequent slaughter and dressing procedures plus the application of decontamination treatments but before initial carcass chilling, and following carcass chilling. Data produced suggested that beef packers are succeeding in efforts to control the extent of microbiological contamination on carcasses.

However, beef can be subsequently contaminated, and inasmuch as handling, fabricating, packaging, distributing and retailing can cause increased contamination with, and/or proliferation of, bacteria, a sequel study—Microbial Mapping II—was conducted. Conclusions of Microbial Mapping II included the observed increase in microbiological populations during fabrication of carcasses into subprimal cuts.

In order to address the increase in microbiological populations observed during carcass fabrication, Microbial Mapping III was designed to evaluate applied decontamination treatments, applied solely and in combination, for their efficacy in reducing or controlling the proliferation of microbiological populations on beef carcasses and subprimal cuts, as well as on table surfaces, during the fabrication process.

**MATERIALS AND METHODS**

Samples (N = 805) were collected during six weeks (A through F) from beef carcasses, top sirloin butts, and table surfaces, before and during the fabrication process in a ConAgra Red Meat Company fed beef packing plant.

Microbiological decontamination treatments evaluated for their individual and collective efficacy in improving microbiological quality were: (a) post-chilling lactic acid solution (1.3 to 2.9%, 29.5°C (85°F), 182 kPa, ~2.8 sec) carcass rinsing, immediately after entering the fabrication area but before the chilled carcass scale; (b) lactic acid solution (1.3 to 2.9%, 29.5°C (85°F)) table rinsing, applied as a constant mist via a spray bar on the fabrication table, and; (c) lactic acid solution (1.3 to 2.9%, 29.5°C (85°F)) rinsing of top sirloin butts, applied manually in two passes using a pump sprayer to product removed from the end of the loin fabrication table.

Sampling occurred before and during the fabrication process at five different in-plant sampling locations, which were: (1) carcass (site 1), immediately after entering the fabrication area but before lactic acid solution carcass rinsing; (2) carcass (site 2), immediately after lactic acid solution carcass rinsing but before passing the chilled-carcass scale; (3) table surface (site 3), at the end of the loin fabrication table; (4) subprimal cut (site 4), immediately after being removed from the end of the loin fabrication table but before lactic acid solution subprimal rinsing; and; (5) subprimal cut (site 5), immediately following lactic acid solution subprimal rinsing but before vacuum packaging.

During each week of sampling (Monday through Friday), at each in-plant sampling site, samples (n = 7) were taken each day every half-hour, for three hours, beginning at the start of the first shift. The sampling protocol for week A included the collection of 35 samples each from sites 1, 3, and 4 with no decontamination treatments applied, in order to develop a complete baseline-data subset of microbiological populations on carcasses, table surfaces, and subprimal cuts.

The sampling protocol for week B included the collection of 35 samples each from sites 1, 2, 3, and 4 with lactic acid solution rinsing of carcasses (treatment a) in order to determine the contribution of this decontamination treatment to the microbiological quality of carcasses, table surfaces, and subprimal cuts.

The sampling protocol for week C included the collection of 35 samples each from sites 1, 3, and 4 with the application of lactic acid solution table rinsing (treatment b) in order to determine the contribution of this decontamination treatment to the microbiological quality of table surfaces and primal cuts.

The sampling protocol for week D included the collection of 35 samples each from sites 1, 3, 4, and 5 with lactic acid solution rinsing of subprimal cuts (treatment c) in order to determine the contribution of this decontamination treatment to the microbiological quality of subprimal cuts.

The sampling protocol for week E included the collection of 35 samples each from sites 1, 2, 3, and 4 with lactic acid solution rinsing of carcasses and tables (treatments a and b) in order to determine the collective contribution of the decontamination treatments to the microbiological quality of table surfaces and subprimal cuts.

The sampling protocol for week F included the collection of 35 samples each from sites 1, 2, 3, 4, and 5 with lactic acid solution rinsing of carcasses, tables, and subprimal cuts (treatments a, b, and c) in order to determine the collective contribution of all decontamination treatments to the microbiological quality of subprimal cuts.

Sponge sampling was performed following procedures described in the USDA-FSIS Meat and Poultry Inspection regulations (FSIS-USDA, 1996).

Sampling of carcass sides (sites 1 and 2) was achieved using a 100 $cm^2$ disposable, sterile template (USDA Template, International BioProducts) at each of three anatomical locations for a total sampling area of 300 $cm^2$.

Sampling of table surfaces (site 3) and subprimal cuts (sites 4 and 5) was achieved using a 100 $cm^2$ template (USDA Template, International BioProducts). Table surfaces were sampled (100 $cm^2$) at the end of the loin fabrication line, while subprimal cuts (top sirloin butts) were sampled (100 $cm^2$) on the surface associated with external adipose tissue.

Sampling was performed aseptically using sterile, latex gloves (International BioProducts) which, in addition to the template, were changed between samples.

All samples were boxed and transported to Warren Analytical Laboratory (Greeley, CO), for analyses of TPC, TCC, and ECC.

Sponge sampling data (TPC, TCC and ECC) were transformed to $log_{10}$ CFU/100 $cm^2$ for statistical analyses. Minimum detection limits for TPC, TCC and ECC were 2.2, 0.9 and 0.9 log CFU/100 $cm^2$, respectively, for carcass sponge samples, and 2.7, 1.4, and 1.4 log CFU/100 $cm^2$, respectively, for fabrication table and top sirloin butt sponge samples, based on the maximum sensitivity of the tests with no further dilution of the samples beyond the original buffer volume of 25 ml. TPC, TCC and ECC falling below the minimum detection limit were entered as 2.2, 0.9, and 0.9 log CFU/100 $cm^2$, respectively, for carcass samples, and 2.7, 1.4, and 1.4 CFU/100 $cm^2$, respectively, for fabrication table and top sirloin butt samples, so that statistical analysis could be performed. Values for the mean log and standard deviation of each set of bacterial counts were calculated on the assumption of a log-normal distribution of microorganisms (Brown and Baird-Parker, 1982; Gill *et al.*, 1996; Gill and Bryant, 1997; Kilsby and Pugh, 1981). Data were evaluated with analysis of variance (AOV) using the model $y = a + x_1 + x_2 + x_1x_2$ and least-squares means were computed for TPC, TCC and ECC by week ($x_1$), in-plant sampling location ($x_2$), and week x in-plant sampling location ($x_1x_2$) fixed effects using the General Linear Models procedure of SAS® (SAS, 1995). When AOV detected effects ($P \leq 0.05$), least-squares means were separated using Fisher's protected LSD and the pairwise t-test of SAS® (SAS, 1995).

## RESULTS AND APPLICATION

Mean TPC and TCC on carcasses immediately upon entering the fabrication area (site 1), across all weeks, ranged from 2.9 to 4.0 and 0.9 to 1.0 log CFU/100 $cm^2$, respectively, while corresponding mean ECC were 0.9 log CFU/100 $cm^2$. Of the total (n = 210) carcass samples obtained, across all weeks, immediately after entering the fabrication area but before lactic acid solution carcass rinsing (site 1), 9.0, 89.5, and 93.8% of TPC, TCC, and ECC, respectively, were below the detection limit (Table 1).

Lactic acid solution rinsing of carcasses (weeks B, E, and F) resulted in reductions ($P < 0.05$) in TPC as the number of samples below the detection limit (during three weeks only) increased from 12.4 to 21.9%. However, TCC and ECC were not reduced significantly as the efficacy of the treatment may have been masked by the extremely high number of samples already existing below the detectable limit. Nonetheless, the percentage of non-detectable samples increased from 87.6 and 92.4% to 92.4 and 94.3% for TCC and ECC, respectively (Table 2).

Mean TPC, TCC, and ECC on fabrication table surfaces, without the intervention of a single decontamination treatment, were 5.5 and 5.5, 2.8 and 3.6, and 2.4 and 3.2 log CFU/100 $cm^2$ during weeks A and D, respectively (Table 3). Microbiological populations on fabrication table surfaces, when lactic acid solution rinsing of carcasses was applied solely as a decontamination treatment (week B), were not lower ($P > 0.05$) than surface populations without carcass rinsing (weeks A and D). Lactic acid solution rinsing of fabrication tables (week C) resulted in lower ($P < 0.05$) microbiological populations as mean TPC, TCC, and ECC were 4.6, 2.7, and 2.1 log CFU/100 $cm^2$, respectively (Table 3). Additionally, the combination of lactic acid solution rinsing of carcasses and fabrication table surfaces also resulted in lower ($P < 0.05$) microbiological populations as mean TPC, TCC, and ECC were 4.1 and 4.2, 2.1 and 2.5, and 1.8 and 2.3 log CFU/100 $cm^2$ during weeks E and F, respectively (Table 3).

Lactic acid solution rinsing of top sirloin butts, applied alone (week D), reduced ($P < 0.05$) mean TPC, TCC, and ECC. However, lactic acid solution rinsing of top sirloin butts, applied in combination (as multiple decontamination treatments) with lactic acid solution rinsing of carcasses and fabrication tables (week F), reduced ($P < 0.05$) ECC, but there were no differences ($P > 0.05$) in mean TPC and ECC on top sirloin butt surfaces sampled before and after lactic acid solution rinsing (Table 4).

Microbiological populations on carcasses entering the fabrication process did not ($P > 0.05$) depend upon sampling time, as mean TPC, TCC, and ECC at the first sampling time were 3.4, 0.9, and 0.9 log CFU/100 $cm^2$, respectively, while corresponding counts after three hours of sampling were 3.5, 1.0, and 0.9 log CFU/100 $cm^2$, respectively (Table 5). In contrast, as expected, microbiological populations on fabrication tables and top sirloin butts changed ($P < 0.05$) with sampling time. TPC, TCC, and ECC on fabrication table surfaces were 3.8, 1.9, and 1.8 log CFU/100 $cm^2$, respectively, while corresponding counts after three hours of sampling were 5.2, 3.1, and 2.7 log CFU/100 $cm^2$, respectively. TPC, TCC, and ECC on top sirloin butts were 4.5, 2.6, and 2.2 log CFU/100 $cm^2$, respectively, while corresponding counts after three hours of sampling were 5.8, 3.6, and 3.2 log CFU/100 $cm^2$, respectively (Table 5).

Overall, carcasses entered the fabrication process with very low microbiological populations on their surfaces as 9.0, 89.5, and 93.8% of the samples collected possessed non-

detectable TPC, TCC, and ECC, respectively (Table 1). Lactic acid solution rinsing of carcasses, alone as a decontamination treatment, reduced ($P < 0.05$) TPC, but there were no differences ($P > 0.05$) in TCC or ECC, although the percentage of non-detectable samples increased from 87.6 and 92.4% to 92.4 and 94.3% for TCC and ECC, respectively (Table 2). Lactic acid solution rinsing of fabrication tables, applied solely (week C) or in combination with lactic acid solution rinsing of carcasses (weeks E and F), resulted in lower ($P < 0.05$) microbiological populations on table surfaces (Table 3). Lactic acid solution rinsing of top sirloin butts reduced ($P < 0.05$) TPC, TCC, and ECC, when applied alone, but only reduced ($P < 0.05$) ECC when applied in combination with lactic acid solution rinsing of carcasses and fabrication tables (Table 4). The application of multiple decontamination treatments "upstream" in the fabrication process (lactic acid solution rinsing of carcasses and fabrication tables), may have an effect on the efficacy of lactic acid solution rinsing of top sirloin butts.

Microbiological populations entering the fabrication process, on the surface of carcasses, remained consistent over time. However, as expected, microbiological populations on fabrication table surfaces and subsequently on top sirloin butts increased with time (Table 5). While none of the decontamination strategies completely prevented contamination and/or proliferation of microbiological populations on fabrication tables and top sirloin butts, results suggest that the application of multiple decontamination treatments may result in lower microbiological populations over time, as well as retarding the rate of proliferation. Furthermore, increased microbiological quality during fabrication of carcasses into subprimal cuts might also reduce microbiological populations on beef trimmings generated during this process, although additional decontamination evaluation and research is required.

This information regarding means for lowering, or controlling the proliferation of, microbiological populations on the surfaces of fabrication tables and top sirloin butts should be useful to industry personnel as means for improving end-product microbiological quality.

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**Table 1. Least squares means ( $\bar{x}$ ) and standard deviations (s) for the log10 values of total plate counts (TPC), total coliform counts (TCC), and Escherichia coli counts (ECC) (CFU/100 cm2), and the number of samples (n (%)) in which counts were not detected, on carcasses immediately prior to fabrication for each of the sampling weeks.**

| | Microbiological Counts on Carcasses at Sampling Site 1 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total Plate Counts | | | Total Coliform Counts | | | Escherichia coli Counts | | |
| Week | $\bar{x}$ | s | n | $\bar{x}$ | s | n | $\bar{x}$ | s | n |
| A | 4.0a | 1.40 | 5 (14.3) | 0.9b | 0.10 | 32 (91.4) | 0.9 | 0.06 | 34 (97.1) |
| B | 3.1bc | 0.81 | 5 (14.3) | 1.0a | 0.56 | 29 (82.9) | 0.9 | 0.33 | 33 (94.3) |
| C | 3.3b | 0.63 | 1 (2.9) | 0.9b | 0.12 | 31 (88.6) | 0.9 | 0.01 | 33 (94.3) |
| D | 3.9a | 0.65 | 0 (0.0) | 0.9ab | 0.26 | 33 (94.3) | 0.9 | 0.26 | 33 (94.3) |
| E | 3.7a | 1.37 | 2 (5.7) | 0.9b | 0.18 | 32 (91.4) | 0.9 | 0.01 | 33 (94.3) |
| F | 2.9c | 0.67 | 6 (17.1) | 0.9b | 0.14 | 31 (88.6) | 0.9 | 0.14 | 31 (88.6) |
| Total | 3.5 | 1.00 | 19 (9.0) | 0.9 | 0.28 | 188 (89.5) | 0.9 | 0.18 | 197 (93.8) |

Number of samples analyzed during each week: 35 for each type of count; total number of samples analyzed: 210.
abc Means in the same column bearing a common superscript letter are not different ($P > 0.05$).
Detection limits for TPC, TCC, and ECC were 2.2, 0.9, and 0.9 log CFU/100 cm2, respectively, or 20, 1, and 1 CFU/ml of diluent tested, respectively.

**Table 2. Difference in least squares means ($\Delta\bar{x}$) and standard deviations (s) for the log10 values of total plate counts (TPC), total coliform counts (TCC) and Escherichia coli counts (ECC) (CFU/100 cm2), the number of samples (n1 (%)) in which counts were not detected before treatment application, the number of samples (n2 (%)) in which counts were not detected after treatment application, and the associated test statistic (T), P-value, and 95% confidence interval (C.I.) for the difference in microbiological counts ($\Delta\bar{x}$) following lactic acid solution rinsing of carcasses.**

| | Decontamination Effects of Lactic Acid Solution Rinsing of Carcasses | | | | | | |
|---|---|---|---|---|---|---|---|
| Microbiological Counts On Carcasses | $\Delta\bar{x}$ | s | n1 (%) | n2 (%) | T | P-value | C.I. |
| TPC | 0.22 | 0.98 | 13 (12.4) | 23 (21.9) | 2.26 | 0.03 | 0.03 - 0.40 |
| TCC | 0.06 | 0.35 | 92 (87.6) | 97 (92.4) | 1.77 | 0.08 | -0.01 - 0.10 |
| ECC | 0.03 | 0.21 | 97 (92.4) | 99 (94.3) | 1.25 | 0.22 | -0.02 - 0.07 |

Number of samples analyzed at each of the two in-plant sampling locations: 105 for each type of count.
$\Delta\bar{x}$ is the difference in least squares means for log10 values of TPC, TCC, and ECC, respectively, between sampling sites 1 and 2 (site 1 minus site 2).
Detection limits for TPC, TCC, and ECC were 2.2, 0.9, and 0.9 log CFU/100 cm2, respectively, or 20, 1, and 1 CFU/ml of diluent tested, respectively.

**Table 3. Least squares means ($\bar{x}$) and standard deviations (s) for the log10 values of total plate counts (TPC), total coliform counts (TCC), and Escherichia coli counts (ECC) (CFU/100 cm2) on table surfaces for each of the sampling weeks.**

| | Microbiological Counts on Table Surfaces | | | | | |
|---|---|---|---|---|---|---|
| | Total Plate Counts | | Total Coliform Counts | | Escherichia coli Counts | |
| Week | $\bar{x}$ | s | $\bar{x}$ | s | $\bar{x}$ | s |
| A | 5.5a | 1.08 | 2.8b | 0.81 | 2.4c | 0.77 |
| B | 5.2a | 1.12 | 3.7a | 0.91 | 3.5a | 0.92 |
| C | 4.6b | 1.00 | 2.7b | 0.66 | 2.1de | 0.58 |
| D | 5.5a | 0.81 | 3.6a | 0.79 | 3.2b | 0.78 |
| E | 4.1c | 1.13 | 2.1c | 0.99 | 1.8e | 0.83 |
| F | 4.2bc | 1.16 | 2.5b | 0.90 | 2.3cd | 0.89 |

Number of samples analyzed during each week: 35 for each type of count.

Lactic acid solution rinsing of tables alone (as a decontamination treatment) occurred during week C, and lactic acid solution rinsing of tables, in combination (as multiple decontamination treatments) with lactic acid solution rinsing of carcasses occurred during weeks E and F.

abcd Means in the same column bearing a common superscript letter are not different ($P > 0.05$).

**Table 4. Difference in least squares means ($\Delta\bar{x}$) and standard deviations (s) for the log10 values of total plate counts (TPC), total coliform counts (TCC) and Escherichia coli counts (ECC) (CFU/100 cm2), the number of samples (n1 (%)) in which counts were not detected before treatment application, the number of samples (n2 (%)) in which counts were not detected after treatment application, and the associated test statistic (T), P-value, and 95% confidence interval (C.I.) for the difference in microbiological counts ($\Delta\bar{x}$) following lactic acid solution rinsing of top sirloin butts in each of two decontamination systems.**

| | Effects of Lactic Acid Solution Rinsing of Top Sirloin Butts by Decontamination System | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Applied as the Only Decontamination Treatment | | | | | Applied Following Rinsing of Carcasses and Tables | | | | |
| Microbiological Counts On Top Sirloins | $\Delta\bar{x}$ | s | T | P-value | C.I. | $\Delta\bar{x}$ | s | T | P-value | C.I. |
| TPC | 0.34 | 0.74 | 2.76 | 0.01 | 0.09 - 0.60 | -0.07 | 0.78 | -0.54 | 0.59 | -0.34 - 0.20 |
| TCC | 0.41 | 0.57 | 4.33 | 0.00 | 0.22 - 0.61 | 0.15 | 0.83 | 1.09 | 0.29 | -0.14 - 0.44 |
| ECC | 0.32 | 0.73 | 2.62 | 0.02 | 0.07 - 0.58 | 0.32 | 0.86 | 2.17 | 0.04 | 0.02 - 0.62 |

Number of samples analyzed at each of the two in-plant sampling locations: 105 for each type of count.

$\Delta\bar{x}$ is the difference in least squares means for the log10 values of TPC, TCC, and ECC, respectively, between sampling sites 4 and 5 (site 4 - site 5).

Lactic acid solution rinsing of top sirloin butts alone (as a decontamination treatment) occurred during week D; lactic acid solution rinsing of top sirloin butts, in combination (as multiple decontamination treatments) with lactic acid solution rinsing of carcasses and lactic acid solution rinsing of tables, occurred during week F.

**Table 5. Least squares means for the log10 values of total plate counts (TPC), total coliform counts (TCC), and Escherichia coli counts (ECC) (CFU/100 cm2) for each sampling time and by in-plant sampling location in the fabrication process.**

| | In-plant Sampling Location Prior to and During the Fabrication Process | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Carcass | | | Table | | | Top Sirloin Butt | | |
| Sampling Time (min) | TPC | TCC | ECC | TPC | TCC | ECC | TPC | TCC | ECC |
| 1 (0) | 3.4 | 0.9 | 0.9 | 3.8 | 1.9 | 1.8 | 4.5 | 2.6 | 2.2 |
| 2 (30) | 3.5 | 0.9 | 0.9 | 4.9 | 2.6 | 2.3 | 5.2 | 2.9 | 2.7 |
| 3 (60) | 3.3 | 0.9 | 0.9 | 4.9 | 3.0 | 2.6 | 5.7 | 3.2 | 2.8 |
| 4 (90) | 3.5 | 0.9 | 0.9 | 4.9 | 3.1 | 2.8 | 5.6 | 3.1 | 2.8 |
| 5 (120) | 3.5 | 0.9 | 0.9 | 5.2 | 3.3 | 2.9 | 5.7 | 3.4 | 3.1 |
| 6 (150) | 3.7 | 0.9 | 0.9 | 5.0 | 3.1 | 2.8 | 5.8 | 3.8 | 3.4 |
| 7 (180) | 3.5 | 1.0 | 0.9 | 5.2 | 3.1 | 2.7 | 5.8 | 3.6 | 3.2 |

Number of samples analyzed at each time: 30 for each type of count.

Sampling time 1 occurred with the commencement of fabrication; subsequent samples were taken every half-hour over a three-hour period of time.

Detection limits for TPC, TCC, and ECC were 2.2, 0.9, and 0.9 log CFU/100 cm2 (20, 1, and 1 CFU/ml of diluent tested), respectively, for samples taken from carcass surfaces, while detection limits for TPC, TCC, and ECC were 2.7, 1.4, and 1.4 log CFU/100 cm2 (20, 1, and 1 CFU/ml of diluent tested), respectively, for samples taken from fabrication table or top sirloin butt surfaces.