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SHIGA TOXIN-PRODUCING ESCHERICHIA COLI IN MEAT: A PRELIMINARY SIMULATION STUDY ON DETECTION CAPABILITIES FOR THREE SAMPLING METHODS

Julie Couton

University of Nebraska-Lincoln, julie.couton@huskers.unl.edu

David Marx

University of Nebraska-Lincoln

John Luchaansky

USDA-ARS Wyndmoor, PA

Randall Phebus

Kansas State University

Anna Porto-Fett

USDA-ARS Wyndmoor, PA

See next page for additional authors

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Author Information

Julie Couton, David Marx, John Luchaansky, Randall Phebus, Anna Porto-Fett, Nicholas Severt, Manpreet Singh, and Harshavardhan Thippareddi

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Julie Couton, Department of Statistics, University of Nebraska- Lincoln
David Marx, Department of Statistics, University of Nebraska- Lincoln
John Luchansky, Eastern Regional Research Center, USDA-ARS, Wyndmoor, PA
Randall Phebus, Food Science Institute, Kansas State University
Anna Porto-Fett, Eastern Regional Research Center, USDA-ARS, Wyndmoor, PA
Nicholas Severt, Food Science Institute, Kansas State University
Manpreet Singh, Department of Food Sciences, Purdue University
Harshavardhan Thippareddi, Department of Poultry Science, University of Georgia

ABSTRACT

Contamination by Shiga Toxin-producing *Escherichia coli* (STEC) is a continuing concern for meat production facility management throughout the United States. Several methods have been used to detect STEC during meat processing, however the excessive experimental cost of determining the optimal method is rarely feasible. The objective of this preliminary simulation study is to determine which sampling method (Cozzini core sampler, core drill shaving, and N-60 surface excision) will better detect STEC at varying levels of contamination present in the meat. 1000 simulated experiments were studied using a binary model for rare occurrences to find the optimal method. We found that for meat contamination levels less than 0.1% or greater than 10% all sampling methods perform equally. At moderate levels of contamination (between 0.1% and 10%) core drill shaving and N-60 perform significantly better than Cozzini core sampler. However, there does not appear to be a significant difference between core drill shaving and N-60. This project was supported by an Agriculture and Food Research Initiative Competitive Grant no. 2012-68003-30155 from the USDA National Institute of Food and Agriculture.

Key Words: simulation study; binary response variable; rare occurrence model; STEC

INTRODUCTION

Contamination by Shiga Toxin-producing *Escherichia coli* (STEC) is a continued concern among meat production facilities throughout the United States. A variety of methods have been developed to detect STEC during meat processing, however full experimental implementation is costly.

This simulation study focuses on the detection capabilities of three of these methods: Cozzini core sampler, core drill shaving, and N-60 surface excision. While all three methods perform well when high percentages of contaminated meat are present, results differs across methods as the percentage of contaminated meat decreases. The primary research question is which of the three methods is best able to detect STEC with varying levels of contamination present among the meat.

MEAT PROCESSING AND SAMPLING

Typically in production, the carcass is processed and cuts of meat are sent down a conveyor belt then collected in large bins, called combos. Combos are selected at random to be tested. One of the sampling methods is used to collect cuts of meat which are then ground together and enriched before undergoing contamination analysis. If STEC is found to be present in the sample, all of the meat in the combo cannot be processed for human consumption and the bin is considered to be contaminated (1) otherwise it is considered to not be contaminated (0). Contamination is typically found on the surface of the individual cuts of meat.

Loading Process- Simulation

In this simulation study, all meat was considered to be the same size (2000cm³) with varying orientation determined at random. See Figure 1 in the Appendix.

Meat was loaded into a combo (80 cm long × 80 cm wide × 100 cm tall) layered from the bottom until the combo was filled. The randomly assigned orientations acted as a packing mechanism to ensure that all pieces of generated meat would fit within the combo. Once filled the combos held on average 225-250 cuts of meat. Figure 2 in the Appendix shows a transverse view of the combo from above once it had been filled.

The combo was then chosen to have one of six levels of contamination: 0.1%, 1%, 3%, 5%, 10%, or 15%. Using the assigned level of contamination, the cuts of meat within each combo were then uniformly contaminated at random. Whether the combo contains 1 cut or 100 cuts of meat that are contaminated the entire combo is deemed to be unfit for human consumption.

This process was repeated to form 1000 combos for each level of contamination resulting in 6000 total combos used for analysis. The three sampling methods discussed in a subsequent section were used on each combo.

Assumptions

Several assumptions were made in the process of this preliminary simulation study. First, the meat is assumed to be contaminated by STEC at random; no clustering mechanism was established for cuts of meat from the same carcass. Additionally all cuts of meat were assumed to be the same size. As mentioned above, after random contamination the cuts were assumed to be uniformly contaminated. If a piece of contaminated meat was sampled, it was assumed that the contamination was captured and then perfectly detected.

Sampling Methods

1. N-60 Surface Excision

With N-60 Surface Excision (N60), 60 samples are drawn from the surface of the combo (see Figure 3). This method is commonly used in practice, because it is the simplest to implement. A variant of this method is N-90 where an additional 30 samples are drawn from the surface.

In Figure 2 in the appendix, the transect shows 40 cuts of meat. To obtain N60, 60 cuts of meat on the top of the combo were sampled, meaning that sampling continued into the second layer of meat. This is commonly done in practice.

2. Cozzini Core Sampler

The Cozzini core sampler (Cozz) is 4.4 cm in diameter and permeates to the bottom of the combo. This core samples both the surface and interior of the individual cuts of meat (see Figure 4). In this method, five cores are drawn from the combo: one from each corner and one from the center. Within each of these areas, the specific placement of the sampler is chosen at random (see Figure 5). These cores are then combined and collectively act as a sample from the combo.

3. Core Drill Shaving

The core drill shaving method (Core) utilizes a shaver that is 3.2 cm in diameter. Although the shaver is shorter in length than the Cozzini core, reaching only halfway down into the combo, it collects samples from primarily the surface area of each cut (see Figure 6). While the Cozzini permeates through the cuts of meat, the Core shaves all meats surrounding it, at times traveling horizontally rather than strictly vertically. Like the Cozzini, 5 shavings are drawn from the combo, one from each corner with an additional shaving collected in the center (see Figure 7). These shavings are combined and considered to be a single sample from the combo.

Basic Data Summary

Due to our assumptions, it is apparent that a large component of successful detection of STEC is the number of pieces of meat sampled.

Number of Pieces Sampled		
Method	Mean	Std Dev
Cozz	37.30	2.46
N60	60.00	0.00
Core	63.78	2.30

Table 1: Average number of pieces sampled within each combo.

We assumed that meat is contaminated strictly at random (without a clustering mechanism), thus the more pieces of meat that are sampled the better the detection will be. In Table 1, Cozz is sampling fewer pieces of meat on average than the other methods, while N60 and Core are performing similarly on average.

ANALYSIS AND RESULTS

Recall that the sampling method either detects contamination (1) or does not (0). Following the notation of Stroup (2013), this binary response variable was modeled as follows:

$$y_{ijk}|b(c)_{jk} \sim \text{Binary}(\pi_{ijk}), \quad \eta_{ijk} = \text{logit}(\pi_{ijk}) = \log\left(\frac{\pi_{ijk}}{1 - \pi_{ijk}}\right)$$

$$\eta_{ijk} = \eta + m_i + c_j + mc_{ij} + b(c)_{jk}, \quad b(c)_{jk} \sim \text{Gaussian}(0, \sigma_B^2)$$

Where η_{ijk} is the linear predictor, m_i represents the main effect of sampling method i , c_j represents the main effect of contamination level j (0.1%, 1%, 3%, 5%, 10%, or 15%), and mc_{ij} represents their interaction. Observed response y_{ijk} represents the contamination (0 or 1) which is conditioned on the random effect $b(c)_{jk}$ because the observation occurs within each combo or block, b_k , assigned a level of contamination. Here, combos are considered to be random due to the nature of the testing procedure in reality (each combo is a sample from a large population of combos) which was emulated in the simulation process. Combos are assumed to be normally distributed with mean 0 and variance σ_B^2 . All simulated replications were combined and analyzed using SAS PROC GLIMMIX™.

The analysis results can be found in the appendix in Table 2, and are plotted in Figure 8 below. Of note is that the interaction between m and c is statistically significant (p-value < 0.0001).

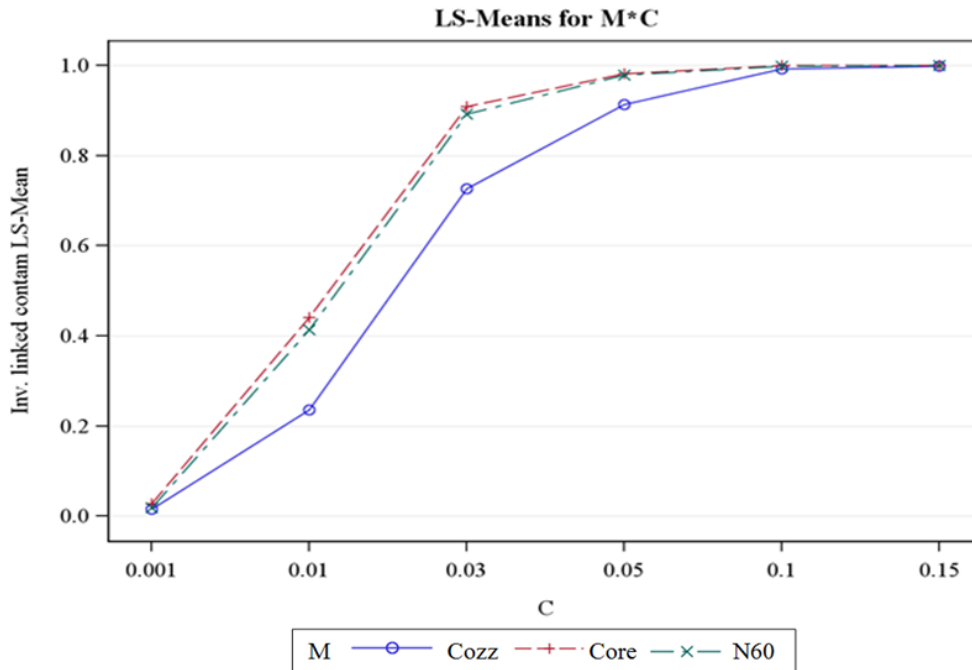


Figure 8: A plot of the mean estimates for each method considered across the different levels of contamination for the full model (e.g. 0.001 represents the case where 0.1% of meat is contaminated, etc.).

The plot makes it apparent that at the lowest level of contamination (0.001) the methods are equally ineffective at detecting contamination. At high levels of contamination (0.1 and 0.15) the methods are equally successful at detecting contamination. Thus, the most noticeable differences occur across moderate levels of contamination (0.01, 0.03, and 0.05).

Restricting the analysis to moderate levels of contamination, we again find that the interaction between m and c is statistically significant (p -value = 0.0042, Table 3 in the Appendix). These results are considered to be statistically significant, which largely occurred due to the large number of replications.

Looking at the simple effects (Table 4 in the Appendix), we can see that across all considered levels of contamination in this model, Cozz performs significantly worse than the other two methods. N60 and Core do not perform significantly different from each other.

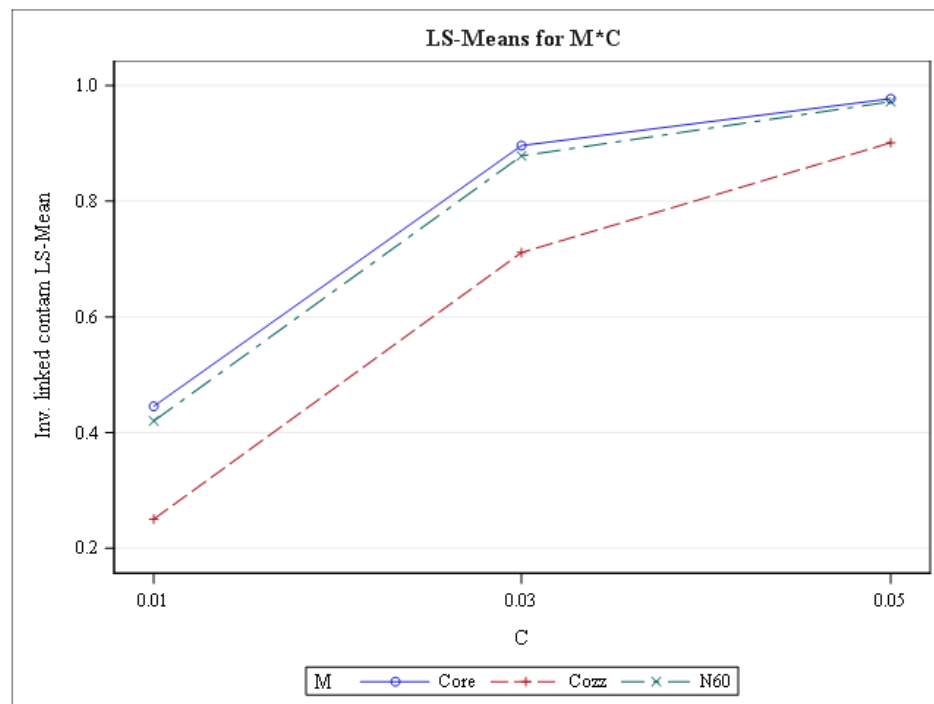


Figure 9: A plot of the mean estimates for each method considered across moderate levels of C for the full model. Because the levels for c under consideration have been reduced, this will be referred to as the reduced model (e.g. 0.01 represents the case where 1% of meat is contaminated, etc.).

The methods of analysis used thus far are sufficient to answer the initial research question. However, further analysis is warranted in regards to estimation. Because this data was simulated, we can determine how well the methods estimated the true proportion of contaminated combos. The estimation capabilities are explored in Figure 10 below.

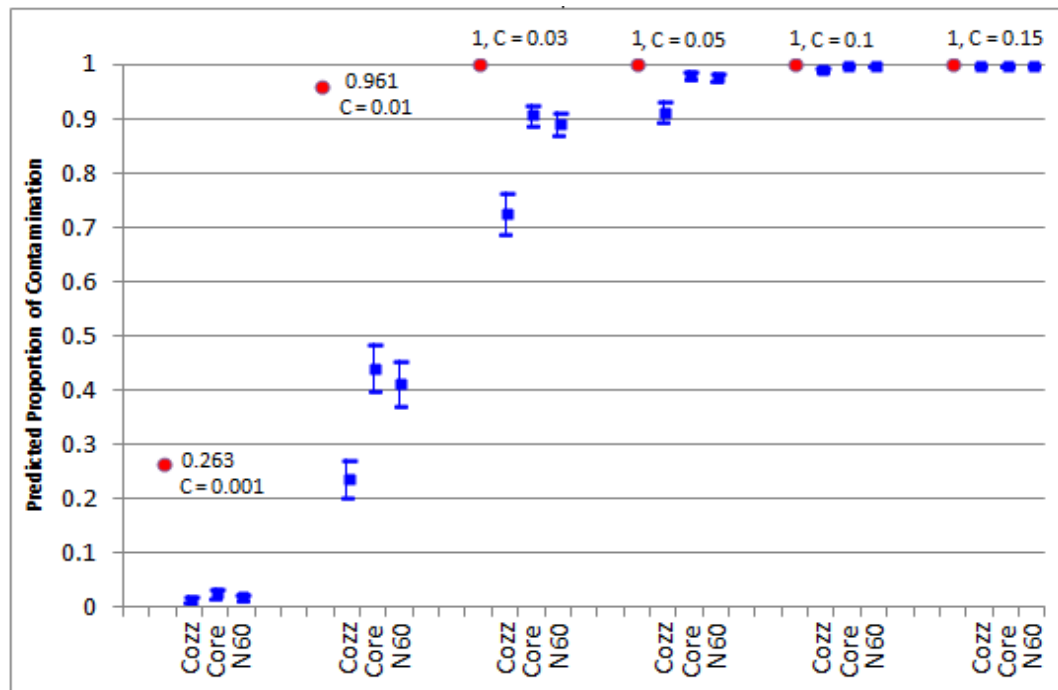


Figure 10: This plot, shows the confidence interval bounds for each method (Cozz, Core, N60) at each level of contamination, C (C=0.001=0.1% of the meat is contaminated, etc.). The value next to the dot is the true proportion of combos that contained at least one piece of contaminated meat.

These are 95% confidence intervals, using a normal approximation and the standard errors given in Table 5 in the Appendix. It is apparent that as the true percentage of contaminated meat decreases, the sampling methods ability to detect the contamination drops dramatically.

DISCUSSION

- Answering the research question.

This research shows that if the contamination is less than 0.01% all sampling methods will struggle to detect contamination and at levels greater than 10% all methods are effectively detecting the contamination. Over moderate levels of contamination (between 1% and 5%) core drill shaving and N-60 surface excision perform significantly better than Cozzini core sampler at all levels. There does not appear to be a significant difference between core drill shaving and N-60.

Revisiting the descriptive statistics from Table 1, these results are as expected. The sampling methods that collect samples from more pieces of meat will have a higher chance of detecting the contamination. Both Core and N60 were sampling significantly more pieces of meat per combo than Cozz which explains the results presented above. So we can conclude that sampling more meat will lead to higher detection capabilities.

However, this conclusion is valid for this simulation only. The result would likely change if the assumptions made in the simulation process were altered, to incorporate more realistic conditions. Specifically, the simulation warrants the incorporation of different sizes of meat and the inclusion of a clustering mechanism. In production facilities, each piece of meat is not contaminated completely at random. If there is contamination on the hide of the carcass, the contamination is more likely to occur on meat from that carcass. Inclusion of these components is paramount before any firm conclusions can be drawn in regards to these sampling methods.

- Looking beyond the research question towards estimation capabilities.

The results of this study show that as the presence of contamination ($c < 0.05$) decreases, the sampling methods ability to capture this contamination drops dramatically. It is unclear if this trend will be present as the simulation assumptions are relaxed.

By incorporating the estimation into this preliminary simulation study, we have come to realize that simply determining which method is superior is far from adequate. We must ensure that the sampling methods result in accurate determination of contamination due to the high consequences of misclassifying a combo as safe for consumption.

FUTURE RESEARCH

This study is intended as a first step in a sequence of simulation studies that further incorporate realistic scenarios with less restrictive assumptions. Specifically, incorporating various sized meats (proportional to those that are currently processed in the production facilities) and a clustering mechanism. Finally, assuming perfect detection (no false positives or false negatives) is not realistic. This should also be incorporated into subsequent simulations and analyses.

ACKNOWLEDGEMENTS

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REFERENCES

Stroup, W.W. 2013. *Generalized Linear Mixed Models*. Boca Raton, FL. CRC Press.

APPENDIX

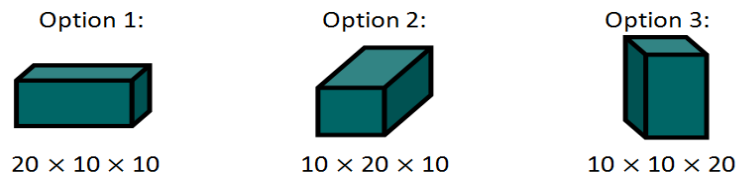


Figure 1: Each of these options represents the possible orientation assigned at random to each cut of meat.

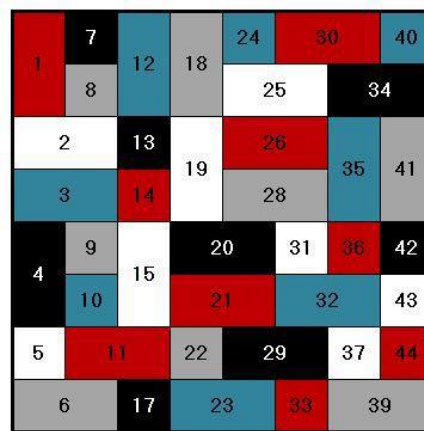


Figure 2: A transverse view of a section of the combo from above. Each rectangle or square represents a different piece of meat.

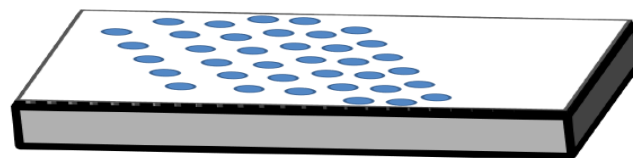


Figure 3: A simplified depiction of the surface of the combo and the N60 sampling method is shown here.

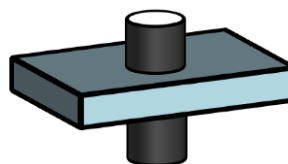


Figure 4: Here, the box is used to depict an individual cut of meat, where the Cozzini core sampler (the cylinder) is permeating through the cut.

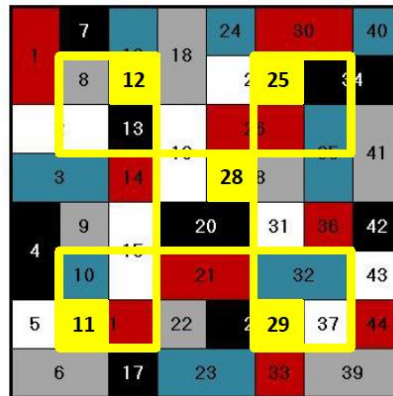


Figure 5: Here, the large yellow squares represent the areas of interest (the corners and the center). Within each location the placement of the Cozzini core sampler was chosen at random. Here the sampler would be placed above cut 11 and permeate through to the bottom of the combo. Similarly for cuts 12, 25, 28 and 29.

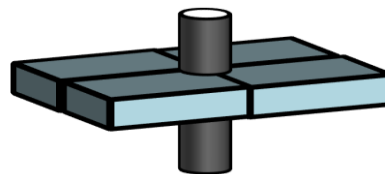


Figure 6: The core drill shaver (seen as the black cylinder) shaves all of the cuts of meat (seen as the boxes) surrounding it, obtaining a core that is primarily made up of surface area meat.

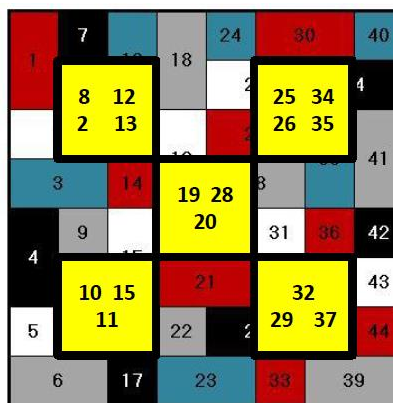


Figure 7: The core drill shaver method considers the large yellow squares as areas of interest, similar to the Cozzini. However, because the shaver captures surrounding meat placing the shaver at the center of the yellow squares leads to the collection of all surrounding pieces of meat. Thus in the top left area, cuts 2, 8, 12 and 13 are all collected. Bear in mind that this shaver only reaches halfway into the combo.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
C	5	5994	332.41	<.0001
M	2	11988	9.21	0.0001
M*C	9	11988	13.14	<.0001

Table 2: This test is for the full model analysis, showing that the interaction between contamination level and sampling method is statistically significant.

Type III Tests of Fixed Effects (Reduced)				
Effect	Num DF	Den DF	F Value	Pr > F
C	2	2997	491.48	<.0001
M	2	5994	139.7	<.0001
M*C	4	5994	3.81	0.0042

Table 3: This is for the model considered under moderate levels of contamination. This is apparent by the reduced degrees of freedom. Due to the large number of replications, we again see a significant interaction.

Simple Effect Comparisons of M*C Least Squares Means By C							
Simple Effect Level	M	_M	Estimate	Standard Error	DF	t Value	Pr > t
C 0.01	Core	Cozz	0.8769	0.1082	5994	8.11	<.0001
C 0.01	Core	N60	0.1067	0.1033	5994	1.03	0.3018
C 0.01	Cozz	N60	-0.7702	0.108	5994	-7.13	<.0001
C 0.03	Core	Cozz	1.2501	0.1235	5994	10.12	<.0001
C 0.03	Core	N60	0.1805	0.1313	5994	1.37	0.1692
C 0.03	Cozz	N60	-1.0695	0.1198	5994	-8.93	<.0001
C 0.05	Core	Cozz	1.5436	0.1895	5994	8.14	<.0001
C 0.05	Core	N60	0.1502	0.2241	5994	0.67	0.5026
C 0.05	Cozz	N60	-1.3933	0.181	5994	-7.7	<.0001

Table 4: The following shows the simple effects. Most notable is that Core and N60 always perform significantly better than Cozz, while across each contamination level, Core and N60 never perform significantly different from each other. Estimated differences and standard errors are presented on the model scale.

M*C Least Squares Means						
M	C	DF	t Value	Pr > t 	Mean*	SE Mean*
Cozz	0.001	11988	-20.64	<.0001	0.01486	0.00298
Cozz	0.01	11988	-12.28	<.0001	0.2365	0.01723
Cozz	0.03	11988	10.42	<.0001	0.7259	0.01860
Cozz	0.05	11988	19.74	<.0001	0.9135	0.00943
Cozz	0.1	11988	18	<.0001	0.9927	0.00199
Cozz	0.15	11988	9.7	<.0001	0.999	0.00072
Core	0.001	11988	-21.69	<.0001	0.02716	0.00436
Core	0.01	11988	-2.67	0.0075	0.4414	0.02170
Core	0.03	11988	19.61	<.0001	0.9084	0.00973
Core	0.05	11988	21.19	<.0001	0.9812	0.00344
Core	0.1	11988	7.61	<.0001	0.9995	0.00048
Core	0.15	11988	0.1	0.9173	1	0.00000
N60	0.001	11988	-21.16	<.0001	0.01882	0.00345
N60	0.01	11988	-3.95	<.0001	0.4135	0.02143
N60	0.03	11988	18.74	<.0001	0.8914	0.01087
N60	0.05	11988	21.43	<.0001	0.9781	0.00379
N60	0.1	11988	9.75	<.0001	0.999	0.00069
N60	0.15	11988	0.07	0.9442	1	0.00000

*Estimates presented on the data scale.

Table 5: This table shows the mean and standard error (SE) estimates based on the full model for each combination of Method (M) and level of Contamination (C). These values were used to approximate 95% confidence intervals, by multiplying each SE by two.