

METHODS FOR HANDLING MISSING DATA DUE TO A LIMIT OF DETECTION IN
LONGITUDINAL LOGNORMAL DATA

by

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Abstract

In animal science, challenge model studies often produce longitudinal data. Many times the lognormal distribution is useful in modeling the data at each time point. *Escherichia coli* O157 (*E. coli* O157) studies measure and record the concentration of colonies of the bacteria. There are times when the concentration of colonies present is too low, falling below a limit of detection. In these cases a zero is recorded for the concentration. Researchers employ a method of enrichment to determine if *E. coli* O157 was truly not present. This enrichment process searches for bacteria colony concentrations a second time to confirm or refute the previous measurement. If enrichment comes back without evidence of any bacteria colonies present, a zero remains as the observed concentration. If enrichment comes back with presence of bacteria colonies, a minimum value is imputed for the concentration. At the conclusion of the study the data are \log_{10} -transformed. One problem with the transformation is that the log of zero is mathematically undefined, so any observed concentrations still recorded as a zero after enrichment can not be log-transformed. Current practice carries the zero value from the lognormal data to the normal data. The purpose of this report is to evaluate methods for handling missing data due to a limit of detection and to provide results for various analyses of the longitudinal data. Multiple methods of imputing a value for the missing data are compared. Each method is analyzed by fitting three different models using SAS. To determine which method is most accurately explaining the data, a simulation study was conducted.

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CHAPTER 1 - Introduction

Longitudinal challenge model studies are common in animal sciences. The data used in this report come from a longitudinal challenge model study conducted by the Departments of Diagnostic Medicine and Pathobiology, Animal Sciences and Industry, and Clinical Sciences at Kansas State University (K-State) in 2007 and is described in Chapter 2. The study was testing for the effects of certain diets on the fecal shedding of *E. coli* O157 in orally inoculated calves. Data collected were on the concentrations of colonies of the *E. coli* O157 bacteria. These data are believed to be lognormal, so a \log_{10} transformation is applied to the data set before methods for repeated measured are used to analyze the data. However, some samples show little or no evidence of bacterial colonies because either no bacteria were present or because the concentration was below some limit of detection at the time of measurement. In these cases, an enrichment process is used to be certain that no bacteria is present in the sample. If this enrichment process does not show any signs of bacteria, then a concentration of zero is recorded. If the enrichment process does show the presence of bacteria a value of one is recorded as the concentration. When the data is log transformed these zero values become problematic since the log of zero is undefined.

This report explores methods of analyzing longitudinal lognormal data that contain values below a limit of detection. These methods are presented and explained Chapter 3. Before analyses are compared, values below the limit of detection – which are initially recorded as zeros – in the data set need to be addressed. Several methods are proposed and can be organized into two groups: before enrichment and after enrichment. The before enrichment strategies do not utilize any information obtained from the enrichment process. The data sets from this group have approximately 28% of their observations recorded as zeros. For the strategies in this group a positive value was imputed for all of the recorded zeros in the data set. The after enrichment strategies utilize the information provided by the enrichment process. This information is interpreted as identifying the true zeros from a group of all recorded zeros by looking at the results of the enrichment as positive or negative. Negative results mean the enrichment process found no bacterial colonies present, thus it can be concluded that the recorded zero is a true zero. Positive results mean that the enrichment process found some bacterial colonies and the recorded

zero was a false zero. The after enrichment strategies only impute a value for the false zeros. The true zeros are left in the data set for analysis.

After using each of the proposed methods on a preexisting lognormal longitudinal data set, collected from an *Escherichia Coli* O157 challenge study conducted at K-State, the methods for addressing the missing values due a limit of detection are compared. Each of the strategies was then fit with three different models using methods for repeated measures and longitudinal data within SAS's MIXED procedure. Use of a spatial power structure for the covariance seemed most appropriate for the data due to the unequal time spacing between measurements.

To assess which strategy provided the most appropriate results, a simulation study is performed, details of which are presented in Chapters 4 and 5. Data were generated in R under three conditions for the daily concentration means, two population variances, and two sample sizes with a common spatial power covariance structure used for all simulations. Type I error rates and power comparisons are used to interpret results.

CHAPTER 2 - The Experiment and the Data

Escherichia coli O157 (*E. coli* O157) is one of many *Escherichia coli* bacteria strains. The *E. coli* O157 bacterium produces a type of toxin called Shiga-toxin and is the most common type of Shiga-toxin producing *E. coli* in North America. The bacteria primarily affects humans who contract *E. coli* O157 from livestock infected with the bacteria (“Distillers Grains” 2008). The challenge study conducted at K-State discussed in this report used cattle to assess the prevalence of *E. coli* O157 in fecal samples over time when given one of two diets. The diets of interest were high grain based diets with either 25% dried distiller's grain (DDG) or, serving as the control, 0% DDG.

DDG is a type of distillers grains. Distillers grains is a feed supplement used in diets of various livestock such as dairy, beef, swine, and poultry. It is created as a by-product during ethanol production (“By-Products” 2007). It has previously been shown that when used in diets for cattle, distillers grains increases daily weight gain (Jacob et al 2008). This benefit, along with the rise of ethanol production in the United States, will likely lead to increased usage of distillers grains in cattle diets (“From Niche” 2006). Thus, the potential effects of distillers grains diets on various diseases is being explored. The challenge study performed at K-State is a step in this direction. An overview of the study follows.

From a total sample of ten Holstein bull calves, five were randomly assigned to the control group and the other five to the treatment group. The control diet was a steam-flaked corn based high grain diet with 0% DDG; the treatment diet had 25% DDG. The calves were adjusted to their diets over a three week period, during which time they were housed in two separate pens, one for each group. After the calves were adjusted to their diets, they were moved to individual pens, where they stayed for one week before being orally inoculated with *E. coli* O157. The study continued for six weeks after inoculation. At the conclusion of the study, the calves were euthanized and necropsied (Jacob et al 2008).

During the six week period, measurements were taken to assess the prevalence of *E. coli* O157 in the calves through fecal samples. A sample was taken for each calf before inoculation, to be certain that the calves did not have *E. coli* O157 prior to inoculation. That information was

not used in the analyses of this paper. The remaining measurements were taken three times a week for each of the ten calves. The control calves were always sampled before the DDG-supplemented group. Fecal samples were taken from the individual pens, weighed to approximately one gram, then diluted and plated three times. The plates were incubated overnight and the following day the colonies of bacteria were counted for each of the three plates. A final response was computed by averaging the number of colonies found on the plates and taking into account other measures such as sample weight and dilution of the plate. If few or no colonies were visible after the initial incubation, the sample underwent a secondary incubation period, referred to as enrichment.

After enrichment, the sample is again inspected for bacteria colonies. If the sample is negative (no bacteria colonies present), then a zero is recorded for the count. If the sample is positive (bacteria colonies are present), then the minimum number of colonies that *could* have been present the previous day is recorded, one. When the mean for the final response is computed from the three plates the imputed values of zero or one are used. The entering of zero or one following enrichment serves two purposes: it identifies false zeros and it serves as a method of imputation which addresses the missing data.

A final response is computed using the values imputed after enrichment and the colony counts observed. The final response is the concentration measures in colony forming units per gram (CFU). Three counts of bacteria colonies from each sample are averaged; then sample weight and plate dilution are used to compute the final CFU value.

After either incubation period, the resulting CFU values for each day are assumed to follow a lognormal distribution, thus the data are commonly \log_{10} transformed so analyses can model a normal response (logCFU). This transformation can cause a mathematical problem. If the original recorded value for a calf is zero, then it is not possible to log transform the value because $\log_{10}(0)$ is undefined. The current method for handling this is to carry the zero from the lognormal concentrations over to the log-transformed concentrations. This method inaccurately portrays the actual trend of the response. A value of 0 in the \log_{10} transformed data implies a value of 1 in the original CFU-value, which is incorrect.

Under the assumption that the log-transformed data are normal, the data are analyzed using methods for repeated measures with SAS's PROC MIXED. In order to use PROC MIXED, a covariance structure must be specified; the current analysis assumes a first order

autoregressive covariance structure. A first order autoregressive structure is commonly used to model the correlation in a data set where values closer together in time are more highly correlated than values further apart in time (Littell et al 2006).

The researchers are looking for a difference in the amount of *E. coli* O157 present between calves in the treatment group and cows in the control group over time. The results from their analysis show that the treatment by study date interaction is significant at the $\alpha=0.10$ level and almost significant at the $\alpha=0.05$ level ($P=0.07$). During the first several days of the study, the daily means for both treatment groups are very similar, which is expected by researchers. Early in the study the different treatments have not had time to have an effect, if any, on the amount of *E. coli* O157 shed. The resulting overlap may be concealing a difference in treatments (see Figure 1). Since the interaction effect is nearly significant, looking at the treatment effect for each day may provide some additional information. The study does this analysis and finds that the two treatment groups differ on each of the last four days of the study, with the *E. coli* O157 concentrations higher for the calves fed DDG (Jacob et al 2007).

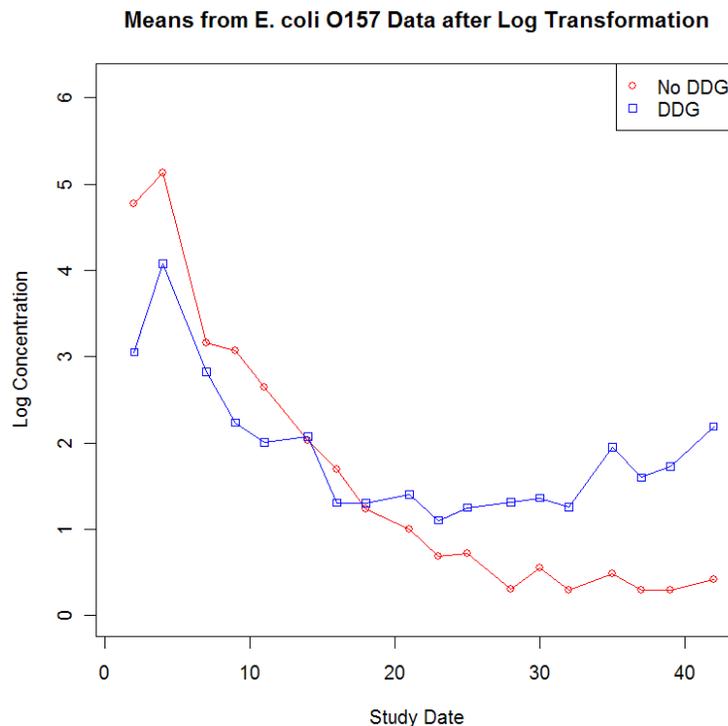


Figure 1: Log10 Transformed Treatment Means

In addition to the model fit by Jacob et al, two other models are fit in this paper. The original model used by Jacobs treats the study date as a qualitative variable. The two additional models treat study date as a continuous variable, in the first with a linear effect and in the second with both a linear and quadratic effect. Preliminary graphs, such as Figure 1, seem to suggest that a quadratic relationship between Study Date and Treatment over the eighteen days is plausible.

CHAPTER 3 - Limit of Detection and the Proposed Analyses

A limit of detection (LOD) is the minimum value of a variable that can be measured. During data collection, values less than the LOD can be viewed as missing values or as left-censored values, but it is known that these values will be small. In addition, some of the observations left-censored by the LOD may actually be zero, not just a small undetectable value. In the *E. coli* O157 study, counts of bacteria colonies are considered left-censored by the LOD and were recorded as zero (notice that this does not necessarily mean the CFU value computed for that sample is zero).

To determine if the missing value is indeed a zero, the enrichment process is used to identify which recorded zeros are true zeros and which are false zeros. The subsequent filling in of zero or one for the count of one of the three plates from a sample is a form of imputation that addresses the missing values caused by the LOD. This method is examined along with other methods of imputation which are based on already established procedures for use when a limit of detection is the cause of missing data.

Often the LOD is claimed to be known. However, there was no LOD reported in the K-State study, but Van Baale et al (2003) report a LOD for a similar study. The Van Baale et al study also measures the concentration of *E. coli* O157 in fecal samples of cattle over time. The value of the LOD reported in this study may not be the same value as the LOD present in the K-State study due to laboratory differences in techniques and resources, but it is evidence to support the methods chosen in this paper which follow the same structure as methods currently practiced by researchers and analysts.

When data are missing due to a LOD, it is known that the missing data are less than some value. Choosing an appropriate method for handling the missingness should utilize this information by imputing a value that is small, relative to the observed data. The method currently used by the researchers is one such method, as are two methods proposed by Hornung and Reed (1990).

Hornung and Reed (1990) suggest substituting $LOD/2$ or $LOD/\sqrt{2}$ for missing values. In a longitudinal study, creating a histogram of the observed data for each time point data was

collected will help in choosing which method, dividing by 2 or $\sqrt{2}$, will be more appropriate. Hornung and Reed conclude that if a histogram shows a distribution where the frequencies start small, rapidly increase, and then gradually decrease, dividing by $\sqrt{2}$ will provide better results. But if the frequencies start out high, then gradually decrease, dividing by 2 would provide better results. In the data set discussed in this paper, the sample size is too small to accurately assess the shape of a histogram of the observed data for each day. Thus, both methods are evaluated. Since the LOD is unknown in the longitudinal *E. coli* O157 study the minimum observed value is used in its place. Note that this value may be much larger than the actual LOD.

There are many ways to go about handling missing data in a longitudinal study. From listwise deletion to using computer programs to perform multiple imputations, each method has advantages and disadvantages. One of the simplest approaches is listwise deletion, also known as complete-case analysis. If a subject has missing data, then the subject is ignored and not used in any of the analyses done on that data set. This is fine as long as the data are missing completely at random (MCAR) (Allison 2001), meaning the probability of missing data does not depend on the value of the missing data itself nor the value at any other time point for that subject (Little and Rubin 2002). Essentially, listwise deletion will result in a random sample of the population if missing data is MCAR. But, as is the case with a LOD, missing data are not likely to be MCAR since the missingness depends on the value of the missing data. Two other common missing data mechanisms are missing at random (MAR) and not missing at random (NMAR). If the probability of missing data doesn't depend on the value of the missing data itself only when the other variables are controlled, then data are said to be MAR (Gadbury et al 2003). If the probability of missing data depends on the value of the missing data itself then the missing data are said to be NMAR (Gadbury et al 2003). The *E. coli* O157 data under examination in this paper is NMAR. When using listwise deletion and missing data are MAR or NMAR, the results obtained are often biased. If used on these data, it will likely produce means which are too large and variances which are too small at each time point. Another disadvantage of listwise deletion is that it reduces the sample size which results in larger standard errors (Allison 2001). Since this study has five calves per treatment group with 28% of the data missing before the enrichment process, there would likely not be enough observations to analyze the data if listwise deletion was used.

Other ways to handle missing data involve imputing a single value for the missing data and analyzing the completed data set; these methods are called single imputation methods. If a zero is chosen as the imputed value (as is currently done), then estimates of the mean will be too low. But leaving the values as missing will lead to estimates of the mean that are too high since the small undetectable values are not included (Hornung and Reed 1990). Over time, methods have been proposed and theoretical arguments made for values other than zero for use with single imputation.

Mean imputation, or marginal mean imputation, computes the mean for a variable, or time point, using the observed responses and imputes the missing data with the calculated mean (Little and Rubin 2002). This method is not used for the data in this paper since the data is missing due to a LOD. It is known that the values are smaller than the minimum observed, imputing the mean would not be the best way to employ this knowledge. A more appropriate method would utilize the information provided by a known LOD, or if the LOD is unknown, the minimum observed value.

A disadvantage to any method which imputes a single value for missing data is the variance estimates tend to be low (Allison 2001, Gadbury et al 2003, PROC MI 2003). More complicated methods, such as multiple imputation, do not have this problem, but are more complex to perform. Multiple imputation is a method which imputes a set of possible values for each missing observation (Little & Rubin 2002). This creates multiple completed data sets which undergo the same analyses. The results are then combined to produce more accurate estimates. Multiple imputation utilizes the variability among the completed multiple data sets to account for some of the sampling variability overlooked by single imputation (Allison 2001). Multiple imputation using SAS's MI procedure was attempted but proved unsuccessful. See Chapter 6 for more information.

The methods considered for imputing the false zeros in this paper are variations of single imputation methods based on what was done in the *E. coli* O157 study and the methods proposed by Hornung and Reed. The methods considered can be organized into two groups, before enrichment and after enrichment, and are used to create multiple data sets for analysis. The lognormal response on the original scale is colony forming units per gram (CFU); the \log_{10} -transformed response is logCFU. Since the data are highly skewed, \log_{10} was used to transform the data instead of the more common natural logarithm. Three models with the normal response

logCFU are fit to each to data set created under the proposed methods for handling the missing data. As explained previously, the LOD is unknown so the minimum observed response was used in its place (CFU = 2.4762, logCFU = 0.39378).

In some of the proposed methods, a portion of the CFU values remain zero. When the log-transformation is attempted, the issue of computing the $\log_{10}(0)$ is again encountered. It does not seem appropriate to simply ignore these values or to carry the zeros to the \log_{10} -transformed data set (although both methods are tested) as the information they provide will not properly be used in either case. Instead, any data set containing at least one zero was shifted in the positive direction such that no zeros remained in the data. The addition of a fixed value δ to every observation in the original CFU data effects the mean, but not the variance. However, the magnitude of the fixed value can have an effect on the results after the \log_{10} -transformation (Osborne 2002). When working with the log-transformation, adding a fixed value less than one would influence the transformation more than adding a fixed value greater than one. This is due to the inherent properties of the log-function; the slope is much steeper for values below 1 than it is for values greater than one. This paper looks at the effect of adding a small fixed value $\delta_1=0.1$ and a larger fixed value $\delta_2=1$ to all values in any data set where zeros are present before transformation. When imputing the missing data, the order stated by Hornung and Reed (1990) was followed: CFU values are imputed before being transformed.

The before enrichment methods, which do not utilize information provided by the enrichment process, treat all of the unobserved values after the initial incubation process as missing positive values below the LOD. The before enrichment methods create five data sets, one under each the following settings. The methods are all implemented on the original concentrations (CFU) before the transformation.

1. Denoting all zero concentrations as missing values.
2. Leaving zero concentrations as zero then adding:
 - a) $\delta_1=0.1$ to all values in data set.
 - b) $\delta_2=1$ to all values in data set.
3. Imputing $\frac{\min CFU}{2}$ for all zero concentrations.
4. Imputing $\frac{\min CFU}{\sqrt{2}}$ for all zero concentrations.

The after enrichment methods use the information provided by the enrichment process to determine which of the recorded zeros are true zeros. In the data sets resulting from these methods, the unobserved values still remaining after the enrichment process are treated as missing positive values due to a LOD and are the only values imputed. The true zeros remain in the data set. The method of adding a fixed value δ to all values in the data set is used in all the methods in this category, since all resulting data sets will contain zeros before the log-transformation. The smallest possible concentration referred to in method 6 refers to The after enrichment methods create eight data sets, one under each of the following settings. The methods are all implemented on the original concentrations (CFU) before the transformation.

5. Denoting the false zeros as missing values and adding:
 - a) $\delta_1=0.1$ to all values in data set.
 - b) $\delta_2=1$ to all values in data set.
6. Imputing the false zeros with the smallest possible concentration and adding:
 - a) $\delta_0=0$ (this is the approach currently used by researchers).
 - b) $\delta_1=0.1$ to all values in data set.
 - c) $\delta_2=1$ to all values in data set.
7. Imputing $\frac{\min CFU}{2}$ for the false zeros and adding:
 - a) $\delta_1=0.1$ to all values in data set.
 - b) $\delta_2=1$ to all values in data set.
8. Imputing $\frac{\min CFU}{\sqrt{2}}$ for the false zeros and adding:
 - a) $\delta_1=0.1$ to all values in data set.
 - b) $\delta_2=1$ to all values in data set.

Since the distribution of the response is assumed to be approximately normal after a \log_{10} -transformation, SAS's MIXED procedure can be used without violating the requirement of a normal response. PROC MIXED allows for modeling a covariance structure, which is necessary for a repeated measures or longitudinal study. Another lesser known feature of PROC MIXED is that it allows missing data. When analyzing a data set such as the *E. coli* O157 data set where

there are multiple observations for each subject over time, PROC MIXED uses all the data available for each subject to analyze the data. If subject j has a missing value at time t_i but an observed value at time t_{i+1} , PROC MIXED will not use subject j for analysis at time t_i , but will use subject j for analysis at time t_{i+1} .

Under the current analysis used by researchers, the covariance structure modeled is first order autoregressive. A first order autoregressive model assumes that observations closer in time will be more highly correlated than observations further apart in time, and it requires equal spacing of the measurements (Littell et al 2006). If σ^2 is the overall variance and observations one time unit apart have correlation ρ , then the covariance between observations one time unit apart is $Cov(y_{t_i}, y_{t_{i+1}}) = \sigma^2 \rho$, two time units apart $Cov(y_{t_i}, y_{t_{i+2}}) = \sigma^2 \rho^2$, and so on. However, in the *E. coli* O157 study data were collected on Monday, Wednesday, and Friday during the 6 weeks of data collection. Therefore measurements are not equally spaced, instead they are spaced by either two or three days. A more appropriate choice for the covariance structure would be a spatial covariance structure, such as spatial power law. A spatial power law model is similar to an autoregressive model in that observations closer in time are more correlated than observations farther apart, but it does not require the time between observations to be equal. If σ^2 is the overall variance and ρ is an autoregressive parameter, the covariance between two observations y_{t_i} and y_{t_j} is given by (Littell et al 2006):

$$Cov(y_{t_i}, y_{t_j}) = \sigma^2 \rho^{|t_i - t_j|}$$

A slightly better fit is achieved when covariance is modeled with the spatial power law instead of the first-order autoregressive structure. Table 1 shows the fit statistics provided by SAS for each. Data were analyzed using the same code as currently used by researchers with a first-order autoregressive structure, then with a spatial power law structure. The method of imputation used was the one currently used by researchers. The AIC, AICC, and BIC are all computed from the log likelihood value presented first, the smaller these values are the better the fit. They include a penalty for adding parameters which do not help the goodness of fit.

<i>Fit Statistic</i>	<i>Covariance Structure</i>	
	<i>AR(1)</i>	<i>SP(POW)</i>
-2 Res Log Likelihood	395.1	391.0
AIC (smaller is better)	399.1	395.0
AICC (smaller is better)	399.2	395.1
BIC (smaller is better)	399.7	395.6

Table 1: Fit Statistics for Comparing Covariance Structures

Use of a new SAS procedure called GLIMMIX was considered. PROC GLIMMIX does not require the response to have a normal distribution. Any response belonging to the exponential family, conditional on random effects, can be modeled by specifying the distribution it follows (SAS GLIMMIX 2006). This means PROC GLIMMIX can model lognormal distribution, so that a log-transformation of the data would not be required and all encounters with $\log(0)$ would be avoided. However, for a lognormal response PROC GLIMMIX models $\log(\text{response})$ as normal. Thus if an observation equals zero GLIMMIX will ignore the observation in its analyses, because the $\log(0)$ is undefined. This does not offer any information that could not be obtained from PROC MIXED, therefore PROC GLIMMIX was not used.

No matter which method of handling the missing values is used, the purpose of the study is to detect a difference between the two treatment groups over time, if a difference exists. To do this three models were fit for each of the proposed methods. Model 1 is a repeated measures model and it mimics the model currently used by researchers. This model treats the time (SDate) as a categorical variable. The variable trt is the categorical variable for treatment group, trt = 0 (No DDG), 1 (DDG).

In models 2 and 3, the study date is treated as a continuous variable, in model 1 with a linear effect and in model 3 with both linear and quadratic effects. The response,

$\underline{y}_i = (y_{it_1}, y_{it_2}, \dots, y_{it_{18}})$, is the $\log_{10}\text{CFU}$ for each cow where $i = 1, 2, \dots, n$ and t_1, t_2, \dots, t_{18} are the study dates. In the *E. coli* O157 study, $n = 10$ calves and $(t_1, t_2, \dots, t_{18}) = (2, 4, 7, 9, 11, 14, 16, 18, 21, 23, 25, 28, 30, 32, 35, 37, 39, 42)$. The model then fit is:

$$\underline{y}_i = X_i \underline{\beta} + \epsilon_i$$

Where in model 2,

$$X_i = \begin{pmatrix} 1 & t_1 & trt_i & t_1 \cdot trt_i \\ 1 & t_2 & trt_i & t_2 \cdot trt_i \\ \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot \\ 1 & t_{18} & trt_i & t_{18} \cdot trt_i \end{pmatrix}$$

and in model 3,

$$X_i = \begin{pmatrix} 1 & t_1 & t_1^2 & trt_i & t_1 \cdot trt_i & t_1^2 \cdot trt_i \\ 1 & t_2 & t_2^2 & trt_i & t_2 \cdot trt_i & t_2^2 \cdot trt_i \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ 1 & t_{18} & t_{18}^2 & trt_i & t_{18} \cdot trt_i & t_{18}^2 \cdot trt_i \end{pmatrix}$$

Where $\underline{\epsilon}_1, \dots, \underline{\epsilon}_{18}$ are iid multivariate normal errors with mean $\underline{0}$ and covariance matrix Σ . The covariance is the same for all three models and is given by the matrix below. Note that σ^2 is the overall variance, ρ is an autoregressive parameter explaining the correlation between responses at different time points, and $d_{i,j} = |t_i - t_j|$ is the time lag (Davidian 2007),

$$\Sigma_i = \sigma^2 \begin{pmatrix} 1 & \rho^{d_{1,2}} & \cdot & \rho^{d_{1,18}} \\ & 1 & \cdot & \rho^{d_{2,18}} \\ & & \cdot & \cdot \\ & & & 1 \end{pmatrix}.$$

Using the *E. coli* O157 data, each model was fit using each of the imputed data sets. The resulting p-values for the main effects and interaction are presented in Tables 2, 3, and 4. Time (SDate) was significant in every analysis (p-value < 0.0001), as was the quadratic effect of time (SDate2) fit in model three (p-value < 0.0001). The p-values from the F-tests for SDate and SDate2 have been omitted from the tables. P-values showing significance at the $\alpha = 0.05$ level are in blue, at the $\alpha = 0.10$ level in green.

Estimate	Method for Handling Missing Data													
	1	2a	2b	3	4	5a	5b	6a	6b	6c	7a	7b	8a	8b
Trt	0.7851	0.0456	0.1872	.2227	0.2760	0.0169	0.0903	0.2279	0.0652	0.2315	0.0640	0.2155	0.0661	0.2229
Trt*SDate	0.3617	0.0246	0.0594	0.0724	0.0842	0.0176	0.0628	0.0735	0.0348	0.0694	0.0394	0.0694	0.0404	0.0714

Table 2: P-values for Model 1 (SDate as factor)

<i>Estimate</i>	<i>Method for Handling Missing Data</i>													
	<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	0.0095	0.0154	0.0513	0.0157	0.0150	0.0073	0.0074	0.0145	0.0123	0.0140	0.0140	0.0149	0.0136	0.0148
Trt*SDate	0.0013	0.0002	0.0007	0.0090	0.0011	<.0001	0.0002	0.0008	0.0003	0.0008	0.0003	0.0008	0.0003	0.0008

Table 3: P-values for Model 2 (SDate as continuous, linear)

<i>Estimate</i>	<i>Method for Handling Missing Data</i>													
	<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	0.0106	0.0236	0.0094	0.0094	0.0084	0.0058	0.0043	0.0061	0.0060	0.0050	0.0081	0.0065	0.0072	0.0059
Trt*SDate	0.0905	0.1533	0.0954	0.0976	0.0925	0.0367	0.0404	0.0704	0.0543	0.0594	0.0684	0.0716	0.0622	0.0667
Trt*SDate2	0.4563	0.8923	0.6580	0.6547	0.6210	0.4741	0.4146	0.5554	0.5409	0.5039	0.6118	0.5617	0.5798	0.5383

Table 4: P-values for Model 3 (SDate as continuous, linear and quadratic)

Model 1 is the model currently fit by the researchers to the *E. coli* O157 data. It treats SDate as a categorical variable. This model is the more conservative than model 2, finding a significant interaction at the $\alpha = 0.05$ level in six of the fourteen proposed imputation methods, as opposed to all, and finding treatment significant at the $\alpha = 0.05$ level only when method 5a is used.

Model 2 results are less varied. Model 2 fits SDate as a continuous variable with a linear effect only. Results from this model always find the interaction between time and treatment to be significant at the $\alpha = 0.05$ level of significance, and using all methods except for 2b find the treatment main effect significant at this level as well. Although results from this model may be the most attractive to researchers, a linear relationship is not evidenced in Figure 1 (page 5).

Results from model 3 remain relatively consistent across the proposed methods. The quadratic interaction (Trt*SDate2) is not significant $\alpha = 0.05$ level with any method, meaning the two treatments do not have a differing effect on the quadratic coefficient in the model. The quadratic time term is significant (p-value < 0.0001) for all methods, so there is evidence of a quadratic relationship over time, but it does not change with the treatment groups. The p-values for the treatment by time interaction (Trt*SDate) show significance at the $\alpha = 0.05$ level of significance for only two proposed methods, 5a and 5b. These are both after enrichment methods where the false zeros are treated as missing values. In 5a, $\delta_1 = 0.1$ is added to all non-missing values before the log-transformation. In 5b $\delta_2 = 1$ is added. The treatment effect (Trt) is significant in every method proposed at the $\alpha = 0.05$ level of significance.

With the addition of either $\delta_1 = 0.1$ or $\delta_2 = 1$, a pattern appears. When fitting model 1, methods 2a, 5a, 6b, 7a, and 8a, all of which add $\delta_1 = 0.1$ reach the same conclusion about the treatment by time interaction, it is significant at the $\alpha = 0.05$ level. The only other method to reach this conclusion is method 1. This means that the addition of a small constant, such as $\delta_1 = 0.1$, or a larger constant, such as $\delta_2 = 1$, has a large impact upon the conclusions drawn. The small constant shows significance more often than the large constant. The simulation study may help to determine which is more appropriate.

Examining fit statistics helps in determining which model is more appropriate. By comparing any one fit statistic provided in the PROC MIXED output across all models the most adequate model can be identified. For each of the five fit statistics provided, the conclusions drawn were the same. Therefore, only one criterion is reported in Table 5 below. Corrected Akaike Information Criterion (AICC) converges to AIC as sample size increases and is recommended for use when sample sizes are small (Hurvich and Tsai 1991). Since the sample sizes in the *E. coli* O157 study are small, AICC is presented.

<i>AICC</i>	<i>Method for Handling Missing Data</i>													
	<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Model 1	285.4	488.9	414.5	409.5	400.2	333.9	315.7	395.1	431.7	389.3	433.8	396.0	431.3	393.3
Model 2	363.8	570.4	488.0	482.0	471.9	411.5	392.5	465.1	502.7	459.2	506.2	467.3	503.1	464.1
Model 3	368.7	569.6	485.3	479.3	469.2	416.2	394.3	462.1	500.0	456.0	503.9	464.3	500.7	461.0

Table 5: AICC for all models and methods

When interpreting the AICC values provided by SAS, the lower the value the better the model fits the data. So, from looking at the values in Table 5, it is easy to see that the AICC values for Model 1 are smaller than the AICC values for Models 2 and 3. This would mean that Model 1 is providing a better fit to the data than models 2 and 3, which supports the analysis currently done in the *E. coli* O157 study. It can also be seen that almost every AICC value from Model 3 is less than its corresponding AICC value for Model 2; the model where a continuous study date has both a quadratic and linear effect is a better fit than the model without the quadratic effect. This should be expected since the data do not appear to follow a linear trend.

It is hard to draw conclusions based on the information from this one data set alone. The simulation results should help to identify which of the methods for handling the data missing due to a LOD provide the best results.

CHAPTER 4 - Simulation Study

To help support and draw conclusions from analyses performed on the *E. coli* O157 data, a simulation study was conducted. Data were generated using R to have similar daily means and variances with a similar covariance structure to the the *E. coli* O157 data (see Appendix A for code). Three models for daily means were considered, along with two variances, two sample sizes, and a common covariance structure. Missingness due to an LOD was simulated before each of the proposed methods was applied to create a new data set for analysis. The same three models were fit to the generated data as were to the *E. coli* O157 data.

When modeling the daily means to use for generating data, piecewise linear functions were created that were similar to the patterns and values of the actual data. They model the response which is assumed to follow a lognormal distribution. The overall shape of each model is a simplified version of what is occurring in the actual data; means start high, then drop off quickly before leveling out.

Under the null hypothesis, there is no difference in means of the treatment groups. Daily means were generated using the following model, a plot of which can be seen in Figure 2. The study dates (t_i) used to generate the means are identical to the study dates used in the *E. coli* O157 study where $(t_1, t_2, \dots, t_{18}) = (2, 4, 7, 9, 11, 14, 16, 18, 21, 23, 25, 28, 30, 32, 35, 37, 39, 42)$.

$$\begin{aligned}\mu_i &= 5.4 - 0.2 \cdot t_i, & \text{if } t_i < 17 \\ & 2.84 - 0.04 \cdot t_i, & \text{if } 17 \leq t_i < 29 \\ & 1.804 - 0.003 \cdot t_i, & \text{if } 29 \leq t_i\end{aligned}$$

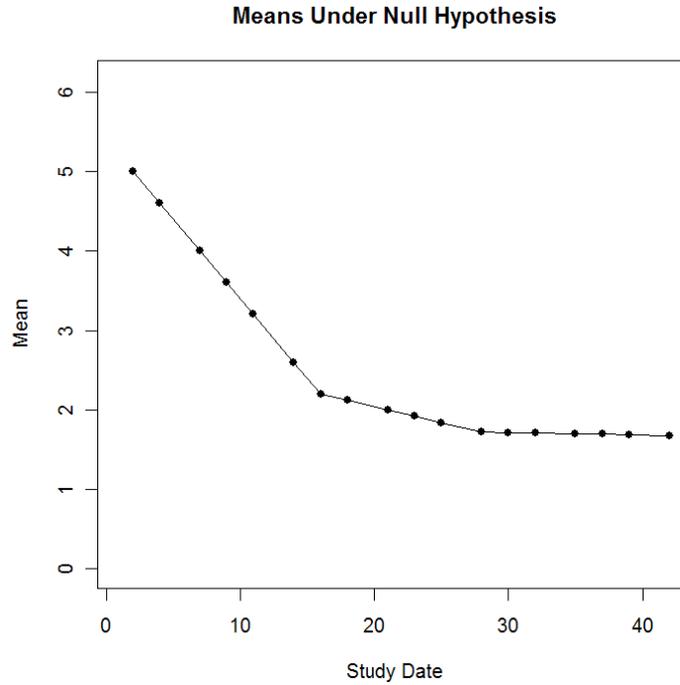


Figure 2: Daily Means Under Null Hypothesis

Under the parallel alternative hypothesis, the difference between the means of the treatment groups is constant throughout the study. This does not reflect the actual pattern seen in the *E. coli* O157 study, but the constant difference in treatment means between groups should make it easier to detect if the proposed methods are performing as should be expected. The difference between the two treatment groups is similar to the difference seen in the second half of the *E. coli* O157 study. This is the part of the study where the No DDG treatment group begins to separate from the DDG treatment group. The mean difference between treatment groups from study dates 23 through 42 is 1.08, the difference used under the null hypothesis is 1.18. Means for the parallel hypothesis were generated under the following model for the No DDG treatment (Figure 3).

$$\begin{aligned} \mu_i &= 4.636 - 0.2 \cdot t_i, & \text{if } t_i < 17 \\ & 2.076 - 0.04 \cdot t_i, & \text{if } 17 \leq t_i < 29 \\ & 1.04 - 0.003 \cdot t_i, & \text{if } 29 \leq t_i \end{aligned}$$

And the following model for the DDG treatment:

$$\mu_i = \begin{cases} 5.816 - 0.2 \cdot t_i, & \text{if } t_i < 17 \\ 3.256 - 0.04 \cdot t_i, & \text{if } 17 \leq t_i < 29 \\ 2.22 - 0.003 \cdot t_i, & \text{if } 29 \leq t_i \end{cases}$$

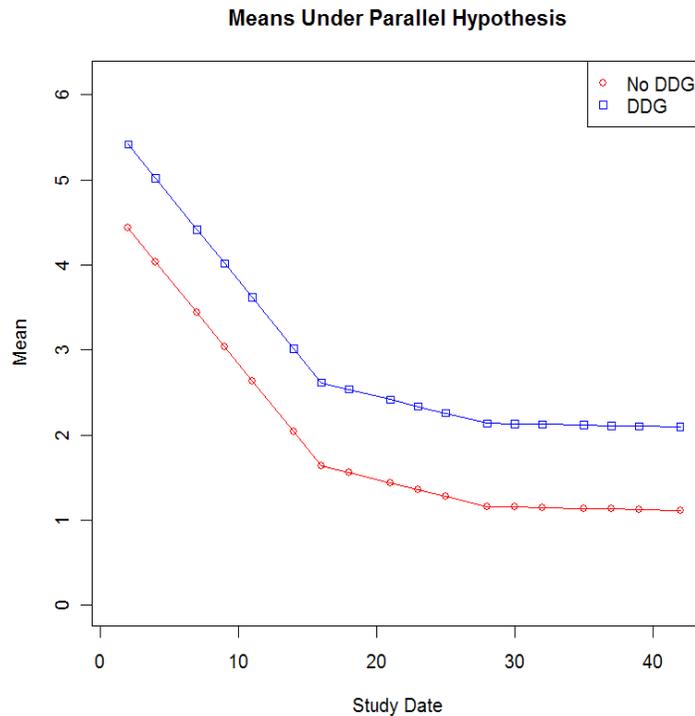


Figure 3: Daily Means Under Parallel Hypothesis

The second alternative hypothesis, the nonparallel hypothesis, more closely resembles the pattern seen in the *E. coli* O157 data. The difference between the means of the two treatment groups at the end of the study is equal to the difference seen in the parallel hypothesis, however the difference is not constant each day of the study. The treatment groups start out the same then as time progresses, the difference between the treatment means grows. This reflects what could be logically expected. Before treatments have a chance to take an effect, the response from each group should be similar. If there is a treatment difference, it should become more evident as time passes. Daily means for each treatment group under the nonparallel hypothesis were generated under the following model for the No DDG treatment group (see Figure 4):

$$\begin{aligned} \mu_i &= 5.4 - 0.2 \cdot t_i, & \text{if } t_i < 15 \\ &= 3.66 - 0.084 \cdot t_i, & \text{if } 15 \leq t_i < 29 \\ &= 1.52 - 0.01 \cdot t_i, & \text{if } 29 \leq t_i \end{aligned}$$

And the following conditions for the DDG treatment:

$$\begin{aligned} \mu_i &= 5.4 - 0.2 \cdot t_i, & \text{if } t_i < 15 \\ &= 2.852 - 0.018 \cdot t_i, & \text{if } 15 \leq t_i < 29 \\ &= 2.404 - 0.002 \cdot t_i, & \text{if } 29 \leq t_i \end{aligned}$$

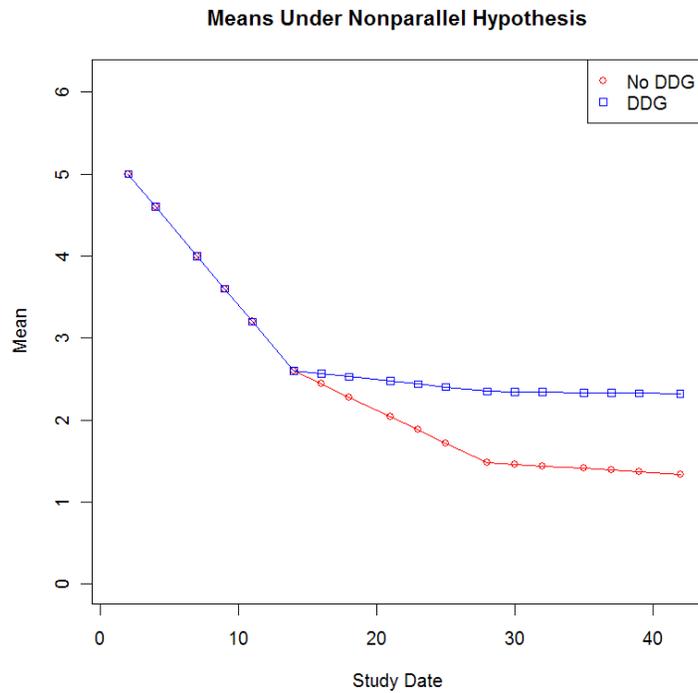


Figure 4: Daily Means Under Nonparallel Hypothesis

The variance was assumed constant for both treatment groups and over all time points. Two variances were compared, a small variance, $\sigma_S^2=0.4$ and a large variance, $\sigma_L^2=1.5$. The values chosen were based on the variances computed each day from the actual data for each treatment group.

Data were also generated under two sample sizes. A small sample of five subjects per treatment mimics the sample sizes in the *E. coli* O157 study. A larger sample of fifteen subjects

per treatment group was also used to see if the conclusions drawn for small samples were consistent with those for larger samples. The sample sizes chosen are consistent with similar studies as well.

The same covariance structure was used for each simulation scenario. A spatial power correlation structure with $\rho=0.95$ was used to compute the covariance matrix required for R. The value chosen for ρ reflects the highest observed correlation in the *E. coli* O157 data. Multivariate normal data representing the logCFU were generated 1000 times under each of the twelve condition combinations. It is assumed that the original CFU data follow a lognormal distribution, therefore the generated data were exponentiated (base e) before the proposed strategies were imposed after which the response underwent a \log_{10} -transformation for analyses.

In order to simulate missing data due to a LOD, a value for the LOD had to be set. The *E. coli* O157 data were used to determine the value used for the generated data. In the actual data, approximately 28% of the observations were less than the LOD before enrichment. Therefore, the 28th percentile was computed under each hypothesis, the average found and used as the LOD for the generated data. Once set, the same LOD was used for every method and model.

To model the enrichment process as accurately as possible, two issues had to be addressed: 1) which values less than the LOD are true zeros and 2) what values should be imputed for the false zeros. To determine which values less than the LOD were true zeros, random Bernoulli data were generated, where the probability of success depended on the study date. A success means the lognormal value generated may be a true zero. The actual data provided reason to believe that as the study progressed, the chances of a data value being less than the LOD increased. Each random Bernoulli value generated was paired with one value from the generated multivariate normal (MVN) data. If the MVN data value was less than the LOD and the Bernoulli value was 1, the MVN value was a true zero. If the MVN value was less than the LOD but the Bernoulli value was 0, the MVN value was a false zero, i.e. a positive missing value below the LOD. If the MVN value was greater than the LOD, no matter what the Bernoulli value, the MVN value remained as the observed value. This resulted in approximately 8% of the data being recorded as a true zero after the enrichment process, which is consistent with the actual *E. coli* O157 data.

The second issue to be addressed is what value to impute. The enrichment process in the *E. coli* O157 study showed that the CFU values computed from the imputed enrichment values were distributed $N(0.5, 0.008)$ after the log transformation and were not correlated to the study date. Since the generated data produced values slightly larger than the original *E. coli* O157 data, the mean of the enrichment values needed to be shifted as well. Therefore random values generated under a $N(0.873, 0.008)$ distribution were used to represent the enrichment values in the generated data set. It is worth noting that although the methods chosen to estimate the enrichment process are based on the patterns seen in the actual data, the fact that the enrichment process is only estimated in the generated data means one should interpret results with caution.

The tables in Appendix B show the results of the simulations. Provided are the Type I error rates and the power of each of the three models using each of the proposed methods for each of the twelve generation conditions. Chapter 5 describes the results in more detail.

CHAPTER 5 - Results

When looking at the Type I error rates and power from the simulations, it is important to understand what should be expected as a result of the models under which the data were generated and analyzed. Under all hypotheses, there is a significant time (SDate) and, where applicable, time by time interaction (SDate2). For data generated under the null hypothesis the same mean model was used for each treatment group. Therefore, it is known that there is no significant treatment main effect (Trt), no significant treatment by time interaction (Trt*SDate), and, where applicable, no significant treatment by time by time interaction (Trt*SDate2). This should be reflected in the Type I error rates. With an $\alpha = 0.05$ level of significance, non-significant effects should have Type I error rates of approximately 0.05.

For data generated under the parallel hypothesis a different mean model was used to generate data for each treatment group. It is known that the treatment main effect is significant, along with the time main effect and time quadratic effect. But the interactions, treatment by time and treatment by time by time, are still not significant. So if a test is performing well, it will find the significant effects while maintaining a Type I error rate of approximately 0.05 for the non-significant effects.

Since the data generated under the nonparallel hypothesis also used different mean models it is known there is a significant treatment main effect, time main effect, and time quadratic effect, as well as a significant treatment by time interaction. The only effect which should not show as significant is the quadratic interaction of treatment and time. Therefore, a test that is performing well will find the effects known to be significant, while keeping the Type I error rate of the non-significant term near 0.05.

The selected tables and discussion presented in this chapter provide a summary of all three models fit, under each of the methods proposed. The complete tables containing the Type I error rates and power are provided in Appendix B. All values are color coded throughout the tables. Blue values are used for Type I error rates which are smaller than expected, or conservative. Red values are used for Type I error rates which are larger than expected, or liberal. Green values are used to show the tests with the highest power. It should be noted that

error rates for the variable SDate and SDate2 are omitted from the tables. These two effects were consistently significant, as is expected. In the pages that follow, the proposed methods will be compared by each model and then across all three models fit.

To determine if observed Type I error rates were statistically different than the expected 0.05, a 95% margin of error was computed. Type I error rates within the margin of error were deemed acceptable. Value too low are liberal and too high are conservative. To determine if two powers were statistically different or not, a hypothesis test for two population proportions was conducted at the $\alpha = 0.05$ level of significance.

Model 1

At the $\alpha = 0.05$ level of significance, the observed Type I error rates under the null hypothesis should be 0.050. If results show error rates greater than this, the method used is said to be liberal and will not be considered as a choice for use with the longitudinal lognormal data analysis. Tables 6 through 11 show the Type I error rates from fitting Model 1, where SDate is a categorical variable. This is the model currently fit by researchers.

Null

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt,	σ_S^2	0.043	0.127	0.122	0.087	0.072	0.039	0.038	0.058	0.059	0.053	0.095	0.080	0.077	0.064
	σ_L^2	0.037	0.114	0.095	0.066	0.058	0.039	0.039	0.046	0.048	0.044	0.071	0.060	0.060	0.051
Trt*	σ_S^2	0.023	0.053	0.045	0.028	0.028	0.035	0.023	0.038	0.035	0.037	0.024	0.027	0.025	0.031
SDate	σ_L^2	0.017	0.043	0.034	0.024	0.025	0.035	0.018	0.034	0.033	0.035	0.024	0.026	0.022	0.025

Table 6: Model 1, Under Null Hypothesis, n=5 per treatment

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt,	σ_S^2	0.041	0.114	0.104	0.079	0.067	0.033	0.034	0.040	0.040	0.031	0.076	0.073	0.064	0.057
	σ_L^2	0.034	0.111	0.089	0.056	0.045	0.035	0.030	0.027	0.033	0.025	0.055	0.049	0.044	0.038
Trt*	σ_S^2	0.039	0.064	0.055	0.038	0.041	0.038	0.034	0.047	0.044	0.051	0.042	0.037	0.039	0.039
SDate	σ_L^2	0.038	0.047	0.043	0.043	0.039	0.041	0.034	0.045	0.045	0.047	0.040	0.039	0.040	0.037

Table 7: Model 1, Under Null Hypothesis, n=15 per treatment

For data generated under the null hypothesis, it can be seen that none of the methods are too liberal in detecting significant interactions. This is expected, as data were generated such that no interaction existed. When looking at the Type I error rates for testing significant treatment effect, the different methods start to show how well they actually perform. Methods 2a and 2b consistently have high Type I error rates under both sample sizes and variances. These methods treat all the missing values as zeros, then add either $\delta_1 = 0.1$ (2a) or $\delta_2 = 1$ (2b). This poor performance shows these methods are not good alternatives to the method already used and will not be considered further.

The method maintaining an acceptable Type I error rate is 8 in which the true zeros were kept, the false zeros were imputed with $\min\text{CFU}/\sqrt{2}$, then $\delta_2 = 1$ was added to all values. This method provided Type I error rates near 0.05 for treatment effect under both sample sizes and variances.

Six other methods also provided acceptable Type I error rates when fitting model 1 on data generated under the null hypothesis: 1, 5a, 5b, 6a, 6b, and 6c. Methods 6a, 6b, and 6c are variations on the enrichment process already used. Simulating what is currently done is method 6a; adding $\delta_1 = 0.1$ is method 6b; adding $\delta_2 = 1$ is method 6c. All six of these methods provide Type I error rates near 0.05 with small sample size, but can be conservative when the sample size is large. Since it is known that this is actually the case, these methods will still be considered in the power study.

Of the five remaining models fit under model 1, three may still be reasonable methods to use. The two that are most questionable are methods 3 and 7a. Both of these methods have high Type I error rates for small sample sizes with either variance and also for large sample size with small variance. Therefore these two methods will also be removed from consideration. That leaves methods 4, 7b, and 8a. With small sample size and small variance, these three methods were liberal. With large variance all three methods have Type I error rates near 0.05. Their inconsistent performance makes it hard to remove them from consideration. Therefore, these methods will still be considered in the power analysis.

Parallel

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.765	0.930	0.938	0.910	0.878	0.700	0.741	0.680	0.544	0.642	0.854	0.884	0.782	0.834
	σ_L^2	0.222	0.502	0.466	0.382	0.323	0.217	0.216	0.231	0.199	0.217	0.333	0.332	0.275	0.286
Trt*	σ_S^2	0.037	0.239	0.097	0.072	0.072	0.195	0.074	0.341	0.258	0.345	0.033	0.045	0.043	0.075
SDate	σ_L^2	0.028	0.041	0.027	0.042	0.049	0.055	0.030	0.083	0.068	0.089	0.028	0.044	0.035	0.050

Table 8: Model 1, Under Parallel Hypothesis, n=5 per treatment

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.999	1.000	1.000	1.000	1.000	0.997	0.999	0.996	0.998	0.998	1.000	1.000	0.999	1.000
	σ_L^2	0.728	0.927	0.913	0.867	0.837	0.713	0.704	0.721	0.699	0.702	0.852	0.842	0.816	0.805
Trt*	σ_S^2	0.090	0.630	0.199	0.069	0.207	0.153	0.126	0.897	0.834	0.923	0.063	0.098	0.162	0.290
SDate	σ_L^2	0.068	0.058	0.041	0.056	0.095	0.064	0.060	0.270	0.251	0.291	0.056	0.082	0.087	0.114

Table 9: Model 1, Under Parallel Hypothesis, n=15 per treatment

For data generated under the parallel hypothesis, there were problems in detecting a significant interaction too often, i.e. high Type I error rates for treatment by time interaction. Data were generated so that there was a treatment effect, but not an interaction between treatment and time. The extremely high Type I error rates for some of the methods make it easy to remove them from consideration, in particular, methods 6a, 6b, and 6c are all performing very poorly; their Type I error rates are near 0.90.

After removing methods 2a, 2b, 3, 7a, and methods 6a, 6b, and 6c (the methods shaded in gray), the power of the remaining methods can be compared. Even though for the large sample size and small variance almost all methods had powers close to 1.000, under other conditions a few stand out. Under both variances and sample sizes, methods 4 and 7b provide the highest power. Method 8b performs just as well as 4 and 7b when sample size and variance are small, but not as well otherwise. The Type I error rates for the treatment by time interaction are large for all three methods, with 0.098 (from 7b) as the smallest.

Methods 1, 5a, 5b, and 8a have good power, but they are not consistently as high as methods 4, 7b, and 8b.

Nonparallel

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.345	0.420	0.387	0.373	0.366	0.312	0.326	0.125	0.327	0.336	0.344	0.351	0.339	0.346
	σ_L^2	0.060	0.269	0.212	0.141	0.117	0.053	0.048	0.069	0.075	0.065	0.146	0.121	0.115	0.107
Trt*	σ_S^2	0.340	0.307	0.367	0.386	0.387	0.346	0.328	0.107	0.321	0.346	0.354	0.366	0.359	0.365
SDate	σ_L^2	0.036	0.092	0.074	0.053	0.052	0.031	0.031	0.045	0.042	0.042	0.054	0.051	0.044	0.051

Table 10: Model 1, Under Nonparallel Hypothesis, n=5 per treatment

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.658	0.979	0.956	0.884	0.802	0.648	0.617	0.390	0.346	0.342	0.859	0.833	0.763	0.724
	σ_L^2	0.198	0.521	0.422	0.305	0.256	0.127	0.142	0.133	0.152	0.125	0.306	0.265	0.261	0.225
Trt*	σ_S^2	0.764	0.947	0.948	0.952	0.938	0.813	0.749	0.471	0.395	0.430	0.933	0.931	0.891	0.905
SDate	σ_L^2	0.142	0.275	0.250	0.252	0.241	0.091	0.108	0.115	0.086	0.112	0.213	0.233	0.185	0.215

Table 11: Model 1, Under Nonparallel Hypothesis, n=15 per treatment

For data generated under the nonparallel hypothesis, both treatment effect and the interaction effect should be significant. After removing the methods shaded in gray from consideration, those methods with the highest power can be seen. Consistent with data generated under the parallel hypothesis, methods 4 and 7b are consistently providing the highest power. Although, under various conditions other methods perform just as well. For small sample size, each of the remaining methods performs similarly, with 4, 7b, 8a, and 8b providing powers that are not significantly different from each other. Looking at the larger sample sizes a more detailed picture can be seen.

As stated, methods 4 and 7b are consistently doing well, but these methods also have higher Type I error rates than may be acceptable. However the methods with the closest power to 4 and 7b are methods 8a and 8b, which do not have problems with liberal Type I error rates. The amount of power gained by using either method 4 or 7b seems to outweigh the loss of accuracy from the Type I error rates suggesting that these methods will provide better results than the others.

Under the small sample size, small variance condition Type I error rates for 4 and 7b are 0.072 and 0.080 respectively. Their corresponding power under the parallel hypothesis are 0.878 and 0.884 (two of the highest), and under the nonparallel hypothesis the corresponding powers are 0.802 and 0.833 (two of the highest). For model 8b, where the Type I error rate is not liberal at 0.064, the power under the parallel hypothesis is 0.834 and under the nonparallel the power is 0.346. The difference in Type I error rates, approximately 0.01, is not very large considering the increase in power, approximately 0.04 (parallel) and 0.50 (nonparallel).

When fitting model 1, using either method 4 and 7b appears to provide the highest power with the reasonable Type I error rates. Method 4 imputes $\min\text{CFU}/2$ for all zero concentration, method 7b imputes $\min\text{CFU}/2$ for false zeros then adds $\delta_2 = 1$ to all values. If using one of these methods, it would be wise to use a slightly lower level of significance for tests to account for the seemingly liberal nature of these methods. If the Type I error rate issue can not be compromised, method 8b will provide reliable results.

Model 2

For model 2, the performance of all 14 methods is relatively similar. For data generated under the null hypothesis, Type I error rates are only above what should be expected for two methods, 6b and 6c, occurring only when the variance and sample size are large. For the rest of the cases, Type I Error rates are near 0.05 or lower. For data generated under the parallel hypothesis, some methods are too liberal in detecting a significant interaction, specifically methods 6a, 6b, and 6c. Methods 4, 7b, and 8b had relatively high power under all conditions for model 2. For data generated under the nonparallel hypothesis, the performance of all methods was relatively similar. Complete results for fitting model 2 are presented as tables in Appendix B. Despite the results, this model is believed to be inadequate for the data which were not generated to follow a single linear trend, as is suggested by fitting model 2.

Model 3

When fitting model 3, where time is a continuous variable having both linear and quadratic effects, there are multiple methods performing poorly. Tables 12 through 17 show the results from fitting this model with data generated under each of the three hypotheses.

Null

Estimate	Method For Handling Missing Values														
	1	2a	2b	3	4	5a	5b	6a	6b	6c	7a	7b	8a	8b	
Trt	σ_S^2	0.086	0.000	0.002	0.038	0.076	0.073	0.094	0.142	0.129	0.154	0.039	0.065	0.065	0.094
	σ_L^2	0.089	0.000	0.017	0.067	0.096	0.073	0.088	0.136	0.122	0.143	0.059	0.088	0.083	0.107
Trt*	σ_S^2	0.050	0.014	0.010	0.025	0.040	0.049	0.053	0.034	0.035	0.043	0.023	0.032	0.035	0.044
SDate	σ_L^2	0.049	0.013	0.014	0.034	0.053	0.050	0.046	0.076	0.068	0.082	0.033	0.043	0.043	0.058
Trt*	σ_S^2	0.042	0.019	0.017	0.025	0.023	0.064	0.050	0.022	0.024	0.026	0.031	0.027	0.033	0.030
	SDate2	σ_L^2	0.025	0.018	0.018	0.024	0.029	0.038	0.028	0.037	0.041	0.040	0.026	0.026	0.032

Table 12: Model 3, Under Null Hypothesis, n=5 per treatment

Estimate	Method For Handling Missing Values														
	1	2a	2b	3	4	5a	5b	6a	6b	6c	7a	7b	8a	8b	
Trt	σ_S^2	0.069	0.000	0.002	0.026	0.054	0.053	0.072	0.108	0.099	0.120	0.023	0.044	0.048	0.075
	σ_L^2	0.078	0.000	0.015	0.057	0.080	0.070	0.087	0.135	0.130	0.142	0.056	0.073	0.077	0.096
Trt*	σ_S^2	0.039	0.007	0.009	0.016	0.026	0.037	0.045	0.029	0.027	0.036	0.016	0.018	0.022	0.028
SDate	σ_L^2	0.035	0.010	0.014	0.030	0.043	0.039	0.039	0.064	0.066	0.075	0.033	0.039	0.043	0.049
Trt*	σ_S^2	0.038	0.016	0.018	0.022	0.025	0.048	0.040	0.017	0.022	0.020	0.027	0.026	0.036	0.028
	SDate2	σ_L^2	0.039	0.013	0.014	0.027	0.032	0.044	0.040	0.042	0.045	0.046	0.031	0.030	0.035

Table 13: Model 3, Under Null Hypothesis, n=15 per treatment

Under the null hypothesis it is known that the quadratic effect for each treatment is the same (Trt*SDate2 is not significant), the linear interaction (Trt*SDate) is not significant, and the treatment effect (Trt) is not significant. Therefore the Type I error rates for all three should be approximately 0.05. None of the methods have Type I error rates too high when testing for significant quadratic interaction, but a few do for the Trt*SDate interaction term. They are the three methods simulating the currently used enrichment methods (6a, 6b, and 6c) when the variance is large. These three methods also provide the highest Type I error rates for the

treatment main effect under both sample sizes and variances. If model 3 will be fit, these methods should not be used.

Additionally, methods 1, 5b, and 8b show high Type I error rates for both sample sizes and variances. Although not as liberal as the three methods discussed above, these methods are not performing well enough to be taken into consideration, therefore they will be removed from the power study.

Of the eight remaining methods, those maintaining the expected Type I error rates are methods 2a, 2b, and 7a. These methods are not liberal under any of the tested conditions. Method 3 is borderline. It is conservative when sample size is large and variance is small ($\alpha = 0.026$), it is liberal when sample size is small and variance is large ($\alpha = 0.067$), and it is maintaining an expected Type I error rate in the other two conditions ($\alpha = 0.038, 0.057$). Since the Type I error rate is not very large this model will still be considered in the power study.

Four methods which are slightly liberal, but may have high enough power to make them contenders are methods 4, 5a, 7b, and 8a. For all four, they are liberal under every condition except for small variance with large sample size, where all are meeting expectations. Although liberal, the Type I error rates are not as liberal as other methods which are performing poorly. These methods will not be removed from consideration. Their performance under the other two hypothesis will be considered first.

Parallel

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.748	0.006	0.374	0.746	0.815	0.631	0.734	0.866	0.795	0.867	0.678	0.794	0.728	0.825
	σ_L^2	0.354	0.031	0.205	0.352	0.414	0.283	0.342	0.497	0.438	0.506	0.326	0.385	0.350	0.428
Trt*	σ_S^2	0.043	0.276	0.067	0.022	0.039	0.050	0.040	0.337	0.318	0.408	0.025	0.040	0.052	0.110
SDate	σ_L^2	0.049	0.039	0.016	0.047	0.081	0.064	0.065	0.208	0.197	0.232	0.049	0.071	0.093	0.121
Trt*	σ_S^2	0.068	0.140	0.050	0.028	0.032	0.163	0.054	0.117	0.149	0.160	0.039	0.035	0.062	0.053
Sdate2	σ_L^2	0.027	0.035	0.011	0.025	0.041	0.065	0.046	0.115	0.145	0.133	0.050	0.045	0.075	0.070

Table 14: Model 3, Under Parallel Hypothesis, n=5 per treatment

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	1.000	0.050	0.972	0.999	1.000	0.997	1.000	1.000	1.000	1.000	0.998	1.000	1.000	1.000
	σ_L^2	0.780	0.119	0.600	0.778	0.820	0.759	0.786	0.882	0.878	0.887	0.773	0.810	0.812	0.841
Trt*	σ_S^2	0.075	0.661	0.175	0.013	0.088	0.065	0.048	0.745	0.742	0.832	0.020	0.054	0.115	0.236
SDate	σ_L^2	0.056	0.081	0.016	0.069	0.141	0.066	0.079	0.383	0.391	0.430	0.083	0.122	0.151	0.203
Trt*	σ_S^2	0.238	0.308	0.096	0.028	0.019	0.200	0.147	0.256	0.302	0.334	0.028	0.023	0.048	0.065
SDate2	σ_L^2	0.029	0.049	0.015	0.036	0.058	0.038	0.027	0.164	0.187	0.199	0.050	0.060	0.075	0.088

Table 15: Model 3, Under Parallel Hypothesis, n=15 per treatment

For data generated under the parallel hypothesis, it is known that the only effect which should be significant is the treatment effect. The other two estimates should have Type I error rates near 0.05. The three most problematic methods are again methods 6a, 6b, and 6c. They have consistently high Type I error rates for treatment by time interaction for both sample sizes and variances, further showing that these are not the optimal methods. In addition, method 8b is also performing poorly. The Type I error rates seen from method 8b are not as high as for 6a, 6b, and 6c, but are still too high to consider. In addition, when variance is small, the Type I error rates from methods 2a and 2b are too high. Methods 5b and 8a are also liberal under various conditions. None of these methods will be considered.

From the remaining methods, methods 4 and 7b appear to be consistently having the best power. But recall that the Type I error rate from methods 4 and 7b are higher than should be expected. Even though these methods have a higher power, if the Type I error rate can not be adjusted, then it may not be best to use these methods. If Type I error rates can not be comprised methods 3 and 7a can be used. These two methods produce the highest power among methods while maintaining acceptable Type I error rates.

Method 1, which is consistently liberal under the null hypothesis, has mixed results for data generated under the nonparallel hypothesis. When sample size is large and variance is small, the Type I error rate is much larger than the acceptable 0.05. The power of method 1 varies under different sample size and variance combinations, this method is not worth considering since it is not clear whether it out performs the other methods. Method 5a performs much better when the sample size is small then when it is large. When sample size is large, method 5a is liberal with moderately high power. When sample size is small, method 5a is still

slightly liberal, but the power does not compete with other methods. It is not the worst choice, but it is clear that better methods exist.

Nonparallel

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.052	0.021	0.034	0.045	0.050	0.038	0.051	0.082	0.048	0.058	0.033	0.048	0.040	0.052
	σ_L^2	0.078	0.004	0.024	0.050	0.071	0.059	0.074	0.128	0.112	0.138	0.048	0.066	0.065	0.093
Trt*	σ_S^2	0.153	0.069	0.111	0.134	0.140	0.107	0.122	0.064	0.097	0.111	0.093	0.112	0.098	0.114
SDate	σ_L^2	0.055	0.033	0.039	0.057	0.063	0.054	0.055	0.059	0.054	0.063	0.051	0.059	0.058	0.064
Trt*	σ_S^2	0.040	0.028	0.029	0.034	0.035	0.046	0.037	0.025	0.042	0.037	0.044	0.040	0.044	0.039
SDate2	σ_L^2	0.032	0.024	0.024	0.032	0.035	0.036	0.032	0.029	0.031	0.030	0.031	0.033	0.034	0.034

Table 16: Model 3, Under Nonparallel Hypothesis, n=5 per treatment

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6ca</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.098	0.003	0.020	0.061	0.085	0.076	0.098	0.089	0.085	0.113	0.054	0.083	0.070	0.094
	σ_L^2	0.107	0.002	0.027	0.078	0.096	0.079	0.100	0.143	0.133	0.148	0.075	0.096	0.096	0.112
Trt*	σ_S^2	0.547	0.253	0.306	0.388	0.393	0.410	0.485	0.179	0.149	0.173	0.305	0.346	0.294	0.344
SDate	σ_L^2	0.144	0.065	0.068	0.120	0.137	0.101	0.130	0.111	0.083	0.114	0.090	0.121	0.094	0.128
Trt*	σ_S^2	0.168	0.027	0.020	0.038	0.060	0.139	0.139	0.031	0.054	0.047	0.042	0.040	0.055	0.055
SDate2	σ_L^2	0.048	0.021	0.019	0.027	0.032	0.046	0.051	0.032	0.030	0.035	0.028	0.034	0.030	0.038

Table 17: Model 3, Under Nonparallel Hypothesis, n=15 per treatment

The nonparallel hypothesis generates data under a model that resembles the actual *E. coli* O157 more closely. Results drawn from this data will provide the strongest recommendations for what could be done with the actual data. The only effect which should not be significant is the quadratic interaction (Trt*SDate2). The only time the Type I error rate is too high for this effect is under methods 1, 5a, and 5b, when variance is small and sample size is large. Every other condition and model provides acceptable, or conservative, Type I error rates.

In general, the power results from fitting model 3 under the nonparallel hypothesis are quite low. Method 4 consistently provides the highest power under all conditions tested. But, the issue again arises of the high Type I error rate. As long as it is reasonable to perform tests at a lower level of significance, method 4 should be used. The same is true of method 7b, a liberal

method with relatively high power. If the level of significance remains at the customary 0.05, then method 3 should be used, otherwise methods 4 and 7b perform well.

All Models

The performance of the methods when fitting model 1 is practically opposite of the performance of the methods when fitting model 3. Methods 2a and 2b performed well under model 3, but poorly under model 1. Method 8b performed poorly under model 3, but well under model 1. Despite this contradiction, methods 4 and 7b perform relatively well under each model. Method 4 imputes $\text{minCFU}/\sqrt{2}$. Method 7b adds $\delta_2 = 1$ to all observations after imputing $\text{minCFU}/2$ for the false zeros.

There isn't a single method which is consistently high in power for all three methods with acceptable Type I error rates. This means that the method used will vary depending on the model to be fit. To summarize, for Model 1 the following methods are inadequate for use: 2a, 2b, 3, 6a, 6b, 6c, and 7a, while method 8b performs well. For Model 3 the following methods are not adequate: 1, 2a, 2b, 5b, 6a, 6b, 6c, 8a and 8b, while method 3 performs well. For both models, methods 4 and 7b also perform relatively well, although these methods have slightly liberal observed Type I error rates.

As long as one is willing to accept slightly high Type I error rates and account for them by using a lesser level of significance, models 4 and 7b perform relatively well under all conditions tested. The methods performing best in each of model 1 and model 3 perform poorly in the other model, thus unless it is known what model will be fit recommending just one model to use is difficult

As for the appropriateness of adding a constant, it is not clear whether adding $\delta_1 = 0.1$ or $\delta_2 = 1$ is more appropriate. Under various models and conditions, the two approaches outperform each other. However, since method 7b ($\delta_2 = 1$) is a relatively good performer over its counterpart 7a ($\delta_1 = 0.1$), one may tend to believe that adding 1 is more appropriate than adding 0.1 to all values in the data set.

CHAPTER 6 - Discussion

Results from the simulation study help in determining which method is the best for each of the models fit and which method is best regardless of the model fit. When fitting model 1, with time as a categorical variable, method 8b is the best in terms of both Type I error rate and power. Method 8b imputes $\min\text{CFU}/\sqrt{2}$ for the false zeros then adds $\delta_2 = 1$ to all values in the data set. Methods 4 and 7b are liberal in terms of Type I error rates, but have high power. Method 4 imputes $\min\text{CFU}/\sqrt{2}$ for all zero concentrations. Method 7b imputes $\min\text{CFU}/2$ for false zeros then adds $\delta_2 = 1$ to all values in the data set. When fitting model 3, with time as a continuous variable with both linear and quadratic effect, method 3 is the best in terms of both Type I error rate and power. Method 3 imputes $\min\text{CFU}/2$ for all zero concentration. Similarly to fitting model 1, methods 4 and 7b are liberal in terms of Type I error rates, but have high power for this model.

Methods 6a, 6b, and 6c, the three methods simulating on the enrichment process currently used, showed to be among the worst. Method 6a simulates the process currently used by researchers. Method 6b simulates the current process then adds $\delta_1 = 0.1$ to all values in the data set and method 6c adds $\delta_2 = 1$ to all values in the data set. When fitting Model 1, these three methods had high Type I error rates for the treatment by time interaction under the parallel hypothesis and low power under the nonparallel hypothesis. When fitting Model 3, these three methods had high Type I error rates under both the null and parallel hypothesis. However, since these simulations are based on a strict set of conditions for the data, it would not be correct to reject the current enrichment process. All that can be said, is that under the conditions used for the data generation, manipulation, and analysis, the three methods attempting to simulate the current enrichment process perform poorly. Under different conditions, this may not be the case.

According to the actual *E. coli* O157 data, it seems that Model 1 provides the best fit. Therefore, the recommended method to be used is Method 8b. Table 18 shows the resulting p-values for model 1 under all 14 methods (as did Table 2). All methods besides the best three have been shaded. At the $\alpha = 0.05$ level of significance, none of the top three methods show a significant interaction, although at the $\alpha = 0.10$ level of significance, all three do. Also, none of

the three recommended methods show a significant treatment effect. The identical conclusions reached by these three methods help to support adequacy of each.

<i>Estimate</i>	<i>Method for Handling Missing Data</i>													
	<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	0.7851	0.0456	0.1872	.2227	0.2760	0.0169	0.0903	0.2279	0.0652	0.2315	0.0640	0.2155	0.0661	0.2229
Trt*SDate	0.3617	0.0246	0.0594	0.0724	0.0842	0.0176	0.0628	0.0735	0.0348	0.0694	0.0394	0.0694	0.0404	0.0714

Table 18: P-values for Model 1 (SDate as factor)

Table 19 below shows the results of fitting Model 3 after applying each of the 14 proposed methods (as does Table 4). The three remaining unshaded methods correspond to the top three methods found in the simulation study. Even though this method did not provide the lowest fit statistics (per Chapter 3), it is of interest to see how the three methods chosen perform. All three methods show that the quadratic interaction is not significant, the interaction between treatment and time is significant at the $\alpha = 0.10$ level of significance, but not at the $\alpha = 0.05$ level, and the treatment effect is significant at the $\alpha = 0.01$ level of significance. Again, the conclusions drawn from all three methods are identical, supporting the adequacy of each model.

<i>Estimate</i>	<i>Method for Handling Missing Data</i>													
	<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	0.0106	0.0236	0.0094	0.0094	0.0084	0.0058	0.0043	0.0061	0.0060	0.0050	0.0081	0.0065	0.0072	0.0059
Trt*SDate	0.0905	0.1533	0.0954	0.0976	0.0925	0.0367	0.0404	0.0704	0.0543	0.0594	0.0684	0.0716	0.0622	0.0667
Trt*SDate2	0.4563	0.8923	0.6580	0.6547	0.6210	0.4741	0.4146	0.5554	0.5409	0.5039	0.6118	0.5617	0.5798	0.5383

Table 19: P-values for Model 3 (SDate as continuous, linear and quadratic)

Recall that in Figure 1 (page 11) there appeared to be a quadratic relationship between treatment and time, not simply a linear relationship. Model 3 was fit to see if this model could stand up to the model currently used by the researchers by taking advantage of the quadratic pattern. Results from the simulation suggest that the answer is no, observed power from methods fit under Model 3 do not have as high of power as methods fit under Model 1. Perhaps this is due to the way the data were generated. To see if Model 3 would perform well under more extreme conditions, a second short simulation was conducted. Data were generated under a

second nonparallel hypothesis where the difference in the treatment groups was double what it originally was by the end of the study. Figure 5 below shows a plot of the model used for to generate the means of each treatment group. Sample size was fixed at 15 subjects per treatment group. The same two variances were used as the previous simulations as was the same covariance matrix.

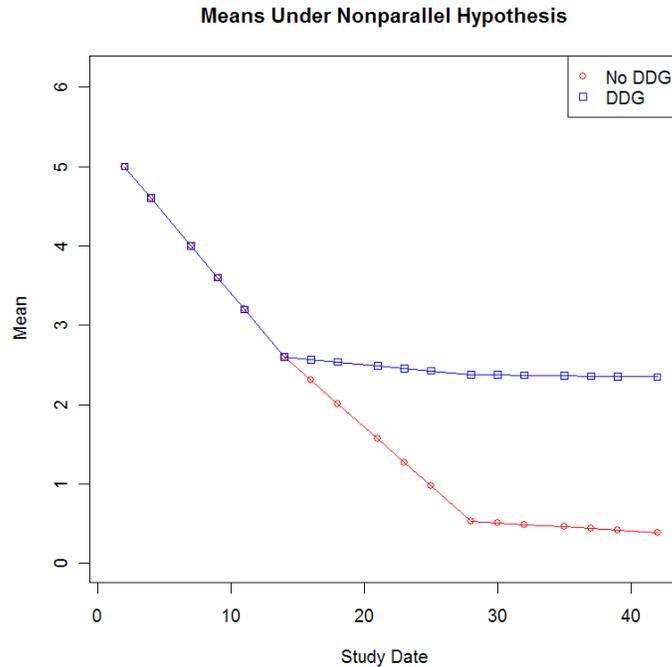


Figure 5: Means under Second Nonparallel Hypothesis

Tables 20 and 21 below show the resulting Type I error rates and power after fitting models 1 and 3 only, model 2 is not of interest. The methods removed from consideration were the ones deemed inadequate in the initial simulation, and are shaded in gray. The red values are liberal, the blue values conservative, and the green values show the methods with highest power. After generating the data, the same LOD was applied and the same proposed methods were used to create fourteen data sets for analysis. After analyzing the data, it was found that model 1 still had higher power than model 3, although the power achieved in model 3 is higher than it was under the initial simulation. Table 20 can be compared to table 11 on page 13, and table 21 to table 13.

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.748	1.000	1.000	0.990	0.972	0.830	0.790	0.483	0.431	0.433	0.989	0.979	0.961	0.952
	σ_L^2	0.370	0.864	0.758	0.571	0.483	0.243	0.272	0.239	0.261	0.220	0.568	0.509	0.492	0.436
Trt*	σ_S^2	0.977	1.000	1.000	1.000	0.997	0.982	0.982	0.669	0.579	0.646	1.000	0.997	0.992	0.990
SDate	σ_L^2	0.267	0.687	0.643	0.571	0.502	0.172	0.212	0.213	0.163	0.189	1.486	0.511	0.407	0.416

Table 20: Model 1, Under Second Nonparallel Hypothesis

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.074	0.113	0.114	0.102	0.107	0.057	0.088	0.105	0.082	0.113	0.085	0.109	0.082	0.110
	σ_L^2	0.096	0.009	0.043	0.088	0.106	0.079	0.096	0.148	0.146	0.157	0.084	0.102	0.103	0.124
Trt*	σ_S^2	0.813	0.896	0.876	0.812	0.740	0.322	0.466	0.222	0.188	0.215	0.677	0.723	0.552	0.622
SDate	σ_L^2	0.244	0.229	0.233	0.259	0.259	0.178	0.218	0.147	0.121	0.150	0.206	0.241	0.194	0.236
Trt*	σ_S^2	0.031	0.052	0.062	0.111	0.126	0.316	0.069	0.057	0.050	0.053	0.075	0.088	0.085	0.095
SDate2	σ_L^2	0.067	0.034	0.036	0.051	0.061	0.057	0.065	0.051	0.043	0.060	0.044	0.052	0.048	0.060

Table 21: Model 3, Under Second Nonparallel Hypothesis

This second simulation reinforces some of the conclusions drawn from the previous simulation. When fitting model 1, the power achieved is near 1 for almost all methods, but methods 6a, 6b, and 6c have power near 0.400 and 0.600 for the treatment and treatment by time interaction, respectively, both of which are considerably lower than the other methods. When fitting model 3, a similar trend is seen, although methods 5a and 5b are also performing poorly in this case. In summary, if the difference in treatments is exaggerated, then model 3 performs better than when the difference in treatments is relatively small, yet it is still outperformed by model 1.

The type of challenge model study reflected in these simulations is common in animal science, where the data collected are assumed lognormal and small values are undetectable due to a limit of detection. What this simulation study has shown is that the method currently used by researchers in conjunction with their enrichment process is not the most appropriate method. Although, the model being fit to the data is appropriate when the covariance structure is changed, imputing a 0 when no bacteria colonies are found after enrichment or a 1 if colonies are found does not accurately explain what is happening. If this model is to be fit in the future and if the

enrichment process is still going to be performed, then a better method to use would be to impute $\min\text{CFU}/2$ for samples showing positive enrichment. The simulation study evidenced that this method performs well under various sample sizes and variances.

A Second *E. coli* O157 Study

Studies such as the *E. coli* O157 challenge model study performed at K-State in 2007 are common. Many times these studies have small sample sizes due to the high cost of the subjects involved. Having a reliable method for analyzing the data is crucial to maximizing the efficiency of such studies. In an attempt to illustrate the use of the recommendations above, a second study was analyzed. One year following the *E. coli* O157 study, a similar study was conducted at K-State. The second study compared four treatments and the response was also the *E. coli* O157 concentrations found in fecal sample of cattle. There were eight subjects in three of the treatment groups and seven in the fourth. A plot of the means for each treatment group throughout the study can be seen in Figure 6.

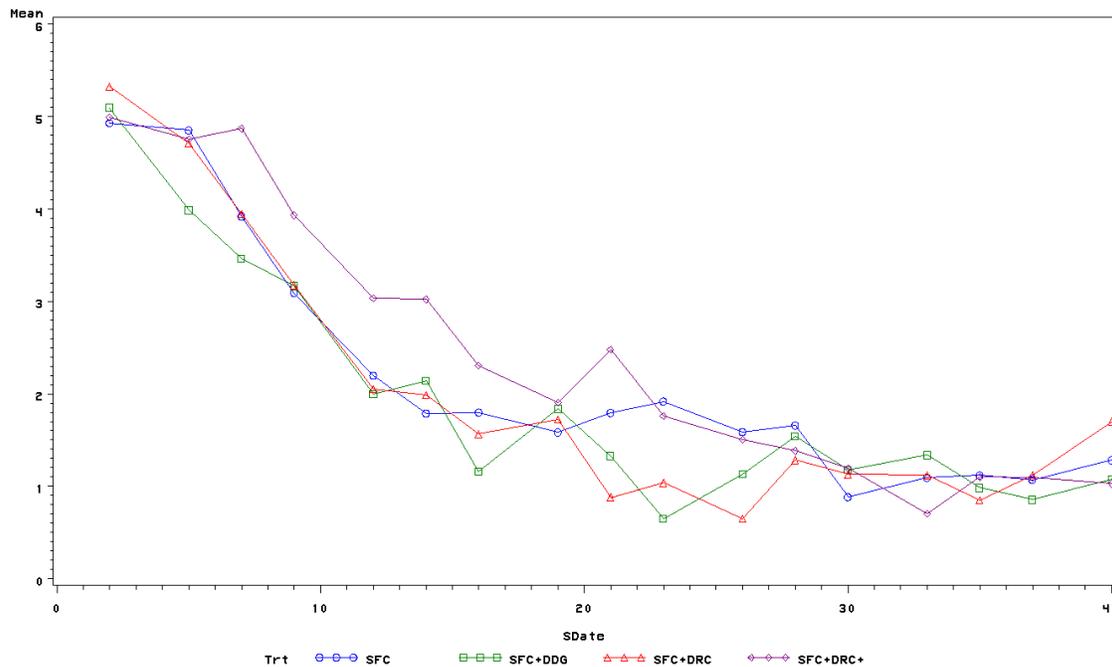


Figure 6: Log10 Transformed Means by Treatment Group for Second Study

After fitting the models and methods proposed in this report, none of the methods found significance in the treatment main effect, in the treatment by time linear interaction, nor in the

treatment by time by time quadratic interaction. This is expected as the plot above does not suggest a difference in treatments.

Future Work

There are still many other approaches that can be taken to explore the information provided in the *E. coli* O157 data and other longitudinal lognormal data sets similar to it. Fitting the data with a nonlinear mixed model composed of a lognormal distribution and a discrete distribution may provide a more accurate fit to the data. One of the underlying assumptions of these types of studies is that the data collected at each day follow a lognormal distribution. A data set with a lognormal distribution can not contain zeros. This contradicts another assumption of this report, that it is possible to have no *E. coli* O157 bacteria present in a sample. A logical way to think of how both of these assumptions can be valid is to think of the data at each time point as having a zero-inflated lognormal distribution. A zero-inflated lognormal distribution is a mixture of two distributions, a continuous lognormal distribution and a discrete distribution where the probability of an observation equaling zero is one. In order to fit a model to a response with a zero-inflated lognormal distribution, a procedure in SAS called NLMIXED could be used. It allows the conditional distribution of the data, given the random effects, to be specified (NLMIXED 1999). In fact, the programming required for NLMIXED was attempted, but without success. Perhaps the sample sizes are too small to use this procedure to adequately fit a nonlinear mixed model.

Another model which may more accurately represent the trends seen in a longitudinal challenge model study would weight the responses taken after a specific time point more heavily than those before. It seems logical that a difference in treatments would not appear in the beginning of a study because the treatments have not had time to take effect, but would appear towards the end, as displayed under the nonparallel hypotheses. Knowing when the differences occur in the treatment groups would aid greatly in building such a model. Further research could be done to explore where the treatments begin to differ, then analyze the data accordingly. This would make it easier to find any differences in the treatment groups since the initial period where all treatments are behaving similarly would be weighted less heavily.

There exist other methods for handling missing data due to a LOD not considered in this paper, not just other models. For example, if the first missing response is at time t_i , the average

of the responses at times t_{i-1} and t_{i+1} could be imputed for the missing value. If the data in the t_{i+1} location is also missing, then a zero would be used to compute the average. This method would help account for the variability of the responses for each subject. A problem is that if one of the values being averaged is not a zero, then the mean imputed will likely be a value that is greater than the actual missing response. Knowing the data is missing due to a LOD, we should expect the missing value to be smaller than its neighboring responses.

Another method that could be used is multiple imputation. Multiple imputation involves imputing a set of plausible values for a missing variable instead of just one value. By imputing a set of say five plausible values for each missing value, five different complete data sets are created. Each data set can then undergo the same analysis. The variability between the results for each analyzed data set is used to account for the sampling variability that would have naturally occurred if the values were observed. Using appropriate methods, the five results are combined to provide a more reliable set of estimates for the problem at hand. Multiple imputation requires more time and more work, but results in estimates that are consistent, asymptotically efficient, and asymptotically normal when data are MAR (Allison 2001). However, it is known that the data of concern in this report is NMAR. Results reached from multiple imputation methods may be as consistent and efficient as if data were MAR.

Originally, multiple imputation was to be one of the methods proposed, but the combination of small sample size, missing data, and correlation present in the *E. coli* O157 data set did not allow for it. The SAS procedures MI and MIANALYZE were to be used to impute and combine the results received from PROC MIXED. PROC MI uses the inputted data and the Expectation-Maximization (EM) algorithm to create initial parameter estimates, one of which is covariance (PROC MI 2003). The *E. coli* O157 data were not sufficient to develop a reliable estimate of covariance. During the EM process the covariance matrix computed was singular and even though the imputation was carried out, a small simulation study proved the results to be unreliable. Thus, a method involving multiple imputation was not examined.

An issue not addressed in the simulations, but important to drawing conclusions is the proportion of data missing. The *E. coli* O157 data set analyzed in this report had approximately 28% of its data missing before the enrichment process was undergone, and only 8% deemed missing after the true zeros were determined. If these percentages change, then it is likely that

results from simulations will change. Further work should explore various percents of missingness.

CHAPTER 7 - Conclusion

In challenge model studies, the data collected is often longitudinal data assumed to follow a lognormal distribution. The study used in this paper measures the *E. coli* O157 concentrations of cattle over a period of one and half months. The longitudinal lognormal data has missing data due to a limit of detection (LOD). This issue is not unique to the data set addressed in this paper, it is a common method of imputation is currently used to address the problems caused by the missing data. In this paper, new strategies for addressing missing data due to a LOD have been explored through use of a simulation study and application to real data sets. These strategies include different methods for handling the missing values and different models for fitting the data after using one of the proposed methods.

Three models were fit. Model 1 is a repeated measures model which treats time as a categorical variable. This is the model currently used by researchers to analyze longitudinal lognormal data. Models 2 and 3 fit time as a continuous variable. In Model 2, time has only a linear effect. In Model 3, time has both a linear and quadratic effect. After analysis, it has been shown that the most appropriate of these three models is model 1. It provides the best fit statistics for the *E. coli* O157 data and the highest power for the simulations. Model 2 does not adequately represent the trend seen in the data and its use is not recommended. Model 3, although more appropriate than model 2, does not represent the data as well as model 1. Model 1 provided the most adequate fit of the three models tested.

When fitting Model 1, one method of addressing the missing values consistently performed better than, or as good as, the rest, method 8b. This method imputes $\min\text{CFU}/\sqrt{2}$ for the false zero then adds $\delta_2 = 1$ to all values in the data set prior to taking a \log_{10} transformation. The simulations showed that this method produced Type I error rates near 0.05 under almost all conditions tested and provided power as high or higher than the other methods tested.

When fitting Model 3, simulation results show the most appropriate method to use to address the missing values is method 3. This method does not require the researchers to go through the enrichment process. Method 3 simply imputes $\min\text{CFU}/2$ for all recorded zero

concentrations. This method produces the most consistent Type I error rates and highest power for model 3.

Two methods performed relatively well across all models in terms of power, but had moderately liberal Type I error rates. These were methods 4 and 7b. Method 4 imputes $\text{minCFU}/\sqrt{2}$ for all zero concentrations. Method 7b imputes $\text{minCFU}/2$ for the false zeros then adds $\delta_2 = 1$ to all values in the data set. If these methods are used, selecting a smaller level of significance is suggested, to accommodate the more liberal results.

Under model 1, the three suggested methods came to the same conclusions for the *E. coli* O157 data, the treatment by time interaction was not significant at the $\alpha = 0.05$ level of significance, but was significant at the $\alpha = 0.10$ level. The treatment effect was not significant. Under model 3, the three suggested methods also came to the same conclusions for the *E. coli* O157 data. The treatment by time by time quadratic interaction was not significant, the treatment by time interaction was not significant at the $\alpha = 0.05$ level of significance, but was significant at the $\alpha = 0.10$ level, and the treatment effect was significant at the $\alpha = 0.01$ level of significance, accommodating for the liberal Type I error rates of methods 4 and 7b.

In conclusion, simulation results show that to achieve the best power with the most accurate results, longitudinal lognormal data with missing values due to a LOD should be analyzed using a repeated measures model that treats time as categorical. To help alleviate problems caused by data missing due to a LOD, the current process of enrichment can be done to determine which recorded values of zero at true zeros and which are false. Instead of imputing a 1 for samples with positive enrichment results, the minimum observed response divided by $\sqrt{2}$ should be imputed for the missing CFU values. Samples with negative enrichment results should be left as zero. Then before the analysis is done, $\delta_2 = 1$ should be added to all values prior to the \log_{10} transformation. This shift of the data will allow the \log_{10} transformation to be correctly applied.

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Appendix -A Simulation Code

Code used for data generation in R can be found in section A.1. The SAS code used for model fitting can be found in section A.2.

A.1: R code

```
sdate<-c(2,4,7,9,11,14,16,18,21,23,25,28,30,32,35,37,39,42) #vector of collection days
n<-1000 # n is number of simulations
cows<-5 # cows is number of cows per trt group

#####
#           Setting the values for the means under the different hypotheses           #
#####

#this function computes the values to use for the mean under the null hypothesis
nullmeans<-function(x){
  means<-0
  for (i in 1:length(x)) {
    if (x[i]<17) {means[i]<-(5.4-.2*x[i])}
#depending on the time into the study, different lines are used
    else if (x[i]<29) means[i]<-(2.84-.04*x[i])
    else means[i]<- {(1.804-.003*x[i])}
  }
  m0<<-means
#m0 is the vector of means to be outputted and used by SAS
  return(means)
}

#this function computes the values to use for the mean under the alternative hypothesis with parallel lines
parmeans<-function(x){
  means1<-0
  means2<-0
  for (i in 1:length(x)) {
    if (x[i]<17) {means1[i]<-(5.816-.2*x[i])}
    else if (x[i]<29) means1[i]<-(3.256-.04*x[i])
    else means1[i]<- {(2.22-.003*x[i])}
  }
  for (i in 1:length(x)) {
    if (x[i]<17) {means2[i]<-(4.636-.2*x[i])}
    else if (x[i]<29) means2[i]<-(2.076-.04*x[i])
    else means2[i]<- {(1.04-.003*x[i])}
  }
  m1<<-means1
  m2<<-means2
#m1 is the vector of means for the DGS group, m2 is the means for the No DGS group
```

```

}

#this function computes the values to use for the mean under the alternative hypothesis with nonparallel lines
altmeans<-function(x){
  means3<-0
  means4<-0
  for (i in 1:length(x)) {
    if (x[i]<15) {means3[i]<-(5.4-.2*x[i])}
    else if (x[i]<29) means3[i]<-(2.852-.018*x[i])
    else means3[i]<- {(2.404-.002*x[i])}
  }
  for (i in 1:length(x)) {
    if (x[i]<15) {means4[i]<-(5.4-.2*x[i])}
    else if (x[i]<29) means4[i]<-(3.66-.084*x[i])
    else means4[i]<- {(1.52-.01*x[i])}
  }
  m3<<-means3
  m4<<-means4
#m3 is the vector of means for the DGS group, m4 is the means for the No DGS group
}

#creates m0 to use for data generation under null
nullmeans(sdate)
#creates m1 and m2 to use for data generation under parallel alt, DGS and noDGS groups
parmeans(sdate)
#creates m3 and m4 to use for data generation under nonparallel alt, DGS and noDGS groups
altmeans(sdate)

#####
#           Generating the Data for the Simulations           #
#####

library(mvtnorm)

#correlation matrix for all sims with spatial power law format
spcorr<-
matrix(c(1.000,0.950,0.857,0.815,0.774,0.698,0.663,0.630,0.569,0.540,0.513,0.463,0.440,0.418,0.377,0.358,0.
341,0.307,0.950,1.000,0.903,0.857,0.815,0.735,0.698,0.663,0.599,0.569,0.540,0.488,0.463,0.440,0.397,0.377,
0.358,0.324,0.857,0.903,1.000,0.950,0.903,0.815,0.774,0.735,0.663,0.630,0.599,0.540,0.513,0.488,0.440,0.41
8,0.397,0.358,0.815,0.857,0.950,1.000,0.950,0.857,0.815,0.774,0.698,0.663,0.630,0.569,0.540,0.513,0.463,0.4
40,0.418,0.377,0.774,0.815,0.903,0.950,1.000,0.903,0.857,0.815,0.735,0.698,0.663,0.599,0.569,0.540,0.488,0.
463,0.440,0.397,0.698,0.735,0.815,0.857,0.903,1.000,0.950,0.903,0.815,0.774,0.735,0.663,0.630,0.599,0.540,
0.513,0.488,0.440,0.663,0.698,0.774,0.815,0.857,0.950,1.000,0.950,0.857,0.815,0.774,0.698,0.663,0.630,0.56
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99,0.569,0.540,0.488,0.569,0.599,0.663,0.698,0.735,0.815,0.857,0.903,1.000,0.950,0.903,0.815,0.774,0.735,0.
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0.698,0.663,0.630,0.569,0.513,0.540,0.599,0.630,0.663,0.735,0.774,0.815,0.903,0.950,1.000,0.903,0.857,0.81
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03,0.815,0.774,0.735,0.663,0.440,0.463,0.513,0.540,0.569,0.630,0.663,0.698,0.774,0.815,0.857,0.950,1.000,0.
950,0.857,0.815,0.774,0.698,0.418,0.440,0.488,0.513,0.540,0.599,0.630,0.663,0.735,0.774,0.815,0.903,0.950,
1.000,0.903,0.857,0.815,0.735,0.377,0.397,0.440,0.463,0.488,0.540,0.569,0.599,0.663,0.698,0.735,0.815,0.85
7,0.903,1.000,0.950,0.903,0.815,0.358,0.377,0.418,0.440,0.463,0.513,0.540,0.569,0.630,0.663,0.698,0.774,0.8
15,0.857,0.950,1.000,0.950,0.857,0.341,0.358,0.397,0.418,0.440,0.488,0.513,0.540,0.599,0.630,0.663,0.735,0.

```

```
774,0.815,0.903,0.950,1.000,0.903,0.307,0.324,0.358,0.377,0.397,0.440,0.463,0.488,0.540,0.569,0.599,0.663,
0.698,0.735,0.815,0.857,0.903,1.000),18,18,byrow=T)
```

```
#####
#                               Using Small Varinace                               #
#####
```

```
svari<-.4                               #value of variance (constant for all days), repeat with 1.5
ssigma<-svari*spcorr                    #sigma is the covariance matrix
```

```
#empty matrix that will store all the generated values under the null hypothesis
log_h0mxs<-matrix(0,2*cows*length(sdate),n)
for (i in 1:n)
log_h0mxs[,i]<-matrix(rmvnorm(n=10,mean=m0,sigma=ssigma),2*cows*length(sdate),1,byrow=T)
h0mxs<-exp(log_h0mxs)
```

```
#for DGS group under parallel alt
log_h1mxs<-matrix(0,cows*length(sdate),n)
for (i in 1:n)
log_h1mxs[,i]<-matrix(rmvnorm(n=5,mean=m1,sigma=ssigma),cows*length(sdate),1,byrow=T)
```

```
#for No DGS group under parallel alt
log_h2mxs<-matrix(0,cows*length(sdate),n)
for (i in 1:n)
log_h2mxs[,i]<-matrix(rmvnorm(n=5,mean=m2,sigma=ssigma),cows*length(sdate),1,byrow=T)
log_hpmxs<-rbind(log_h1mxs,log_h2mxs)
#matrix of values under parallel alt
hpmxs<-exp(log_hpmxs)
```

```
#matrix of values for mean under nonparallel alt for DGS group
log_h3mxs<-matrix(0,cows*length(sdate),n)
for (i in 1:n)
log_h3mxs[,i]<-matrix(rmvnorm(n=5,mean=m3,sigma=ssigma),cows*length(sdate),1,byrow=T)
#matrix of mean values under nonparallel alt for No DGS group
log_h4mxs<-matrix(0,cows*length(sdate),n)
for (i in 1:n)
log_h4mxs[,i]<-matrix(rmvnorm(n=5,mean=m4,sigma=ssigma),cows*length(sdate),1,byrow=T)
log_hnpmxs<-rbind(log_h3mxs,log_h4mxs)
#matrix of values for mean under nonparallel alt
hnpmxs<-exp(log_hnpmxs)
```

```
#study data vector for null
SDV0<-
matrix(c(rep(2,2*cows),rep(4,2*cows),rep(7,2*cows),rep(9,2*cows),rep(11,2*cows),rep(14,2*cows),rep(16,2
*cows),rep(18,2*cows),rep(21,2*cows),rep(23,2*cows),rep(25,2*cows),rep(28,2*cows),rep(30,2*cows),rep(3
2,2*cows),rep(35,2*cows),rep(37,2*cows),rep(39,2*cows),rep(42,2*cows)),2*cows*length(sdate),1)
```

```
#study date vector for alternatives
SDVA<-
matrix(rep(c(rep(2,cows),rep(4,cows),rep(7,cows),rep(9,cows),rep(11,cows),rep(14,cows),rep(16,cows),rep(18
,cows),rep(21,cows),rep(23,cows),rep(25,cows),rep(28,cows),rep(30,cows),rep(32,cows),rep(35,cows),rep(37,
cows),rep(39,cows),rep(42,cows)),2),2*cows*length(sdate),1)
```

```

#treatment vector for use with null
TRTV0<-matrix(rep(c(rep(1,cows),rep(0,cows)),length(sdate)),2*cows*length(sdate),1)
#treatment vector for use with both alts
TRTVA<-matrix(c(rep(1,cows*length(sdate)),rep(0,cows*length(sdate))),2*cows*length(sdate),1)
#id vector for null
IDV0<-matrix(c(rep(1:(2*cows),length(sdate))),2*cows*length(sdate),1)
#id vector for alternatives
IDVA<-matrix(c(rep(1:cows,length(sdate)),rep((1+cows):(2*cows),length(sdate))),2*cows*length(sdate),1)
#this matrix will be used for null hypothesis
Predictors0<-cbind(IDV0,SDV0,TRTV0)
#this matrix will be used for alternative hypotheses
PredictorsA<-cbind(IDVA,SDVA,TRTVA)

```

```

#creating enrichment results to be used for all hypotheses.

```

```

p<-sdate/100
Enrich<-matrix(1,2*cows*length(sdate),n)
for (i in 1:n)
Enrich[,i]<-rbinom(2*cows*length(sdate),1,p)

```

```

Data0s<-cbind(Predictors0,h0mxs,Enrich)
DataPs<-cbind(PredictorsA,hpmxs,Enrich)
DataNPs<-cbind(PredictorsA,hnpmxs,Enrich)

```

```

write.table(Data0s[,1:(n+3)],file="E data under null small var.txt",quote=F,row.names=F)
write.table(DataPs[,1:(n+3)],file="E data under parallel atl small var.txt",quote=F,row.names=F)
write.table(DataNPs[,1:(n+3)],file="E data under nonparallel alt small var.txt",quote=F,row.names=F)

```

```

#####
#                               Creating Data Set for Post-Enrichment Use                               #
#####
#                               Finding the LOD                               #
#####

```

```

#since 28% of data was missing before enrichment, looked for 28th percentile of generated data
mine0s<-apply(Data0s[,4:(n+3)],2,sort)
fifty0s<-mine0s[round(.28*2*cows*length(sdate),0),]
LOD0s<-mean(fifty0s)

```

```

mineps<-apply(DataPs[,4:(n+3)],2,sort)
fiftyps<-mineps[round(.28*2*cows*length(sdate),0),]
LODps<-mean(fiftyps)

```

```

minenps<-apply(DataNPs[,4:(n+3)],2,sort)
fiftynps<-minenps[round(.28*2*cows*length(sdate),0),]
LODnps<-mean(fiftynps)

```

```

LODs<-mean(c(LOD0s,LODps,LODnps))
#using mean of all 3 LOD's as LOD
LOD<- 4.8

```

```

#####
#                               Under Null Hypothesis                               #
#####

```

#we need to create a data set where the true values are present
 #do this by checking the enrichment values. If Enrichment = 1 and Generated Value is less than LOD, the value is a true zero.

```
Data0Es<-Data0s[,1:(n+3)]
for (i in 1:2*cows*length(sdate)){
for (j in 4:(n+3)){
k<-j+n
if(Data0s[i,j]<LOD && Data0s[i,k]==1) Data0Es[i,j]<-0
} }
write.table(Data0Es,file="E data under null post enrichment small var.txt",quote=F,row.names=F)
```

```
#####
#                               Under Parallel Hypothesis                               #
#####
```

```
DataPEs<-DataPs[,1:(n+3)]
for (i in 1:2*cows*length(sdate)){
for (j in 4:(n+3)){
k<-j+n
if(DataPs[i,j]<LOD && DataPs[i,k]==1) DataPEs[i,j]<-0
} }
write.table(DataPEs,file="E data under parallel alt post enrichment small var.txt",quote=F,row.names=F)
```

```
#####
#                               Under Nonparallel Hypothesis                               #
#####
```

```
DataNPEs<-DataNPs[,1:(n+3)]
for (i in 1:2*cows*length(sdate)){
for (j in 4:(n+3)){
k<-j+n
if(DataNPs[i,j]<LOD && DataNPs[i,k]==1) DataNPEs[i,j]<-0
} }
write.table(DataNPEs,file="E data under nonparallel alt post enrichment small var.txt",quote=F,row.names=F)
```

A.2: SAS code

For *E.coli* O157 data

```
ods listing;
*****
**                               Original E. Coli Data                               **
*****;

libname Ecoli "c:\SAS\library";
data Ecoli.smallpre;
Z='09'X;
infile "C:\...\smallunequaldate.txt" firstobs=2 dlm=Z dsd;
input Trt $ Day ID $ LgCFU Enr $ LgEnr CFU SDate;
SDate1=SDate;
SDate2=SDate*SDate;
run;
```

```

proc print data=Ecoli.smallpre (where=(SDate>37));
run;

*****
**                               Creating the data sets                **
**                               with the proposed methods            **
*****;

title "1: All Zeros as missing";
data small_allmissing;
set ecoli.smallpre;
if LgCFU=0 then LgCFU=".";
keep Trt ID LgCFU SDate SDate1 SDate2;
run;
*proc print data=smallpre_allmissing (where=(SDate>37));
*run;

title "2a: All Zeros Stay";
data small_allzerosstayA;
set Ecoli.smallpre;
LgCFU=log10(CFU+.1);
keep Trt ID LgCFU SDate SDate1 SDate2;
run;

title "2b: All Zeros Stay";
data small_allzerosstayB;
set Ecoli.smallpre;
LgCFU=log10(CFU+1);
keep Trt ID LgCFU SDate SDate1 SDate2;
run;

title "3: All 0's with minCFU/2";
proc means data=small_allminover2;
var LgCFU;
run;
*we can see that the miniumum observed LgCFU is 0.39378;
data small_allminover2;
set small_allmissing;
if LgCFU="." then LgCFU=log10((10**.39378)/2);
keep trt ID LgCFU SDate SDate1 SDate2;
run;

title "4: All zero's with minCFU/sqrt 2";
data small_allminoversqrt2;
set small_allmissing;
if LgCFU="." then LgCFU=log10((10**.39378)/sqrt(2));
keep trt ID LgCFU SDate SDate1 SDate2;
run;

title "5a: False missing";
data small_falsemissingA;
set Ecoli.smallpre;
if CFU=0 and Enr='+' then CFU=.;
if CFU=0 and Enr='-' then CFU=0;
else CFU=CFU;
CFU=CFU+.1;
LgCFU=log10(CFU);
keep Trt ID LgCFU SDate SDate1 SDate2;
run;

title "5b: False missing";
data small_falsemissingB;

```

```

set Ecoli.smallpre;
if CFU=0 and Enr='+' then CFU=.;
if CFU=0 and Enr='-' then CFU=0;
else CFU=CFU;
CFU=CFU+1;
LgCFU=log10(CFU);
keep Trt ID LgCFU SDate SDate1 SDate2;
run;

title "6: False Enrichement";
data small_falseenriched;
set Ecoli.smallpre;
if LgCFU=0 and Enr="+" then LgCFU=LgEnr;
if LgCFU=0 and Enr="-" then LgCFU=0;
else LgCFU=LgCFU;
keep Trt ID LgCFU SDate SDate1 SDate2;
run;

title "6a: False Enrichement";
data small_falseenrichedA;
set Ecoli.smallpre;
if CFU=0 and Enr='+' then CFU=10**LgEnr;
if CFU=0 and Enr='-' then CFU=0;
else CFU=CFU;
CFU=CFU+.1;
LgCFU=log10(CFU);
keep Trt ID LgCFU SDate SDate1 SDate2;
run;

title "6b: False Enrichement";
data small_falseenrichedB;
set Ecoli.smallpre;
if CFU=0 and Enr='+' then CFU=10**LgEnr;
if CFU=0 and Enr='-' then CFU=0;
else CFU=CFU;
CFU=CFU+1;
LgCFU=log10(CFU);
keep Trt ID LgCFU SDate SDate1 SDate2;
run;

title "7a: False minCFU/2";
data small_falseminover2A;
set Ecoli.smallpre;
if CFU=0 and Enr='+' then CFU=(10**.39378/2);
if CFU=0 and Enr='-' then CFU=0;
else CFU=CFU;
CFU=CFU+.1;
LgCFU=log10(CFU);
keep Trt ID LgCFU SDate SDate1 SDate2;
run;

title "7b: False minCFU/2";
data small_falseminover2B;
set Ecoli.smallpre;
if CFU=0 and Enr='+' then CFU=(10**.39378/2);
if CFU=0 and Enr='-' then CFU=0;
else CFU=CFU;
CFU=CFU+1;
LgCFU=log10(CFU);
keep Trt ID LgCFU SDate SDate1 SDate2;
run;

title "8a: False minCFU/sqrt2";

```

```

data small_falseminoversqrt2A;
set Ecoli.smallpre;
if CFU=0 and Enr='+' then CFU=(10**.39378/sqrt(2));
if CFU=0 and Enr='-' then CFU=0;
else CFU=CFU;
CFU=CFU+.1;
LgCFU=log10(CFU);
keep Trt ID LgCFU SDate SDate1 SDate2;
run;

```

```

title "8b: False minCFU/sqrt2";
data small_falseminoversqrt2B;
set Ecoli.smallpre;
if CFU=0 and Enr='+' then CFU=(10**.39378/2);
if CFU=0 and Enr='-' then CFU=0;
else CFU=CFU;
CFU=CFU+1;
LgCFU=log10(CFU);
keep Trt ID LgCFU SDate SDate1 SDate2;
run;

```

```

*****
**           Analysis           **
**           SDate as Class     **
*****;
ods listing close;
ods pdf file="c:\sas\Cow Data Analyses.pdf";

```

To analyze all 14 methods, copy same code as below. Change the data set name;

```

title "1: All zeros missing";
proc mixed data=small_allmissing;
class ID trt SDate;
model LgCFU=trt|SDate/ddfm=kr;
repeated/subject=id(trt) type=sp(pow) (SDate1);
run;

```

```

.
.
.

```

```

*****
**           Analysis           **
**           SDate as Linear   **
*****;

```

To analyze all 14 methods, copy same code as below. Change the data set name;

```

title "1: All zeros missing";
proc mixed data=small_allmissing;
class ID trt;
model LgCFU=trt|SDate/ddfm=kr;
repeated/subject=id(trt) type=sp(pow) (SDate1);
run;

```

```

.
.
.

```

```

*****
**           Analysis           **
**           SDate as Quadratic **
*****;

```

```

To analyze all 14 methods, copy same code as below.  Change the data set name;
title "1: All zeros missing";
proc mixed data=small_allmissing;
class ID trt;
model LgCFU=trt|SDate SDate2 SDate2*Trt/ddfm=kr;
repeated/subject=id(trt) type=sp(pow) (SDate1);
run;

.
.
.

ods pdf close;
ods listing;

```

For Simulated Data

This is not the entire code. If it was all included, this paper would have over 1000 sheets. Instead, use this portion of the code provided and change file names and re-run for the datasets generated under each of the four conditions (small and large sample size; small and large variance).

Reading in the Generated Data

```

title "Under Null (small, n=5)";
  Libname ecoli "C:\SAS\EColi";
  PROC IMPORT OUT= ecoli.NULL01
    DATAFILE= "C:\...\data under null small var.txt"
      DBMS=DLM REPLACE;
    DELIMITER='20'x;
    GETNAMES=YES;
    DATAROW=2;
  RUN;
title "Null Hypothesis Post Enrichment (small, n=5)";
  Libname ecoli "C:\SAS\EColi";
  PROC IMPORT OUT= ecoli.null01E
    DATAFILE= "C:\...\data under null post enrichment small var.txt"
      DBMS=DLM REPLACE;
    DELIMITER='20'x;
    GETNAMES=YES;
    DATAROW=2;
  RUN;
title "Under Null (big, n=5)";
  Libname ecoli "C:\SAS\EColi";
  PROC IMPORT OUT= ecoli.NULL01B
    DATAFILE= "C:\...\data under null big var.txt"
      DBMS=DLM REPLACE;
    DELIMITER='20'x;
    GETNAMES=YES;
    DATAROW=2;
  RUN;
title "Null Hypothesis Post Enrichment (big, n=5)";
  Libname ecoli "C:\SAS\EColi";
  PROC IMPORT OUT= ecoli.null01Eb
    DATAFILE= "C:\...\data under null post enrichment big var.txt"
      DBMS=DLM REPLACE;
    DELIMITER='20'x;
    GETNAMES=YES;
    DATAROW=2;
  RUN;

```

```

RUN;
title "Under Parallel (small, n=5)";
Libname ecoli "C:\SAS\EColi";
PROC IMPORT OUT= ecoli.par01
  DATAFILE= "C:\...\data under parallel atl small var.txt"
  DBMS=DLM REPLACE;
  DELIMITER='20'x;
  GETNAMES=YES;
  DATAROW=2;

RUN;
title "Parallel Hypothesis Post Enrichment (small, n=5)";
Libname ecoli "C:\SAS\EColi";
PROC IMPORT OUT= ecoli.par01E
  DATAFILE= "C:\...\data under parallel alt post enrichment small var.txt"
  DBMS=DLM REPLACE;
  DELIMITER='20'x;
  GETNAMES=YES;
  DATAROW=2;

RUN;
title "Under Parallel (big, n=5)";
Libname ecoli "C:\SAS\EColi";
PROC IMPORT OUT= ecoli.par01b
  DATAFILE= "C:\...\data under parallel atl big var.txt"
  DBMS=DLM REPLACE;
  DELIMITER='20'x;
  GETNAMES=YES;
  DATAROW=2;

RUN;
title "Parallel Hypothesis Post Enrichment (big, n=5)";
Libname ecoli "C:\SAS\EColi";
PROC IMPORT OUT= ecoli.par01Eb
  DATAFILE= "C:\...\data under parallel alt post enrichment big var.txt"
  DBMS=DLM REPLACE;
  DELIMITER='20'x;
  GETNAMES=YES;
  DATAROW=2;

RUN;
title "Under NonParallel (small, n=5)";
Libname ecoli "C:\SAS\EColi";
PROC IMPORT OUT= ecoli.npar01
  DATAFILE= "C:\...\data under nonparallel alt small var.txt"
  DBMS=DLM REPLACE;
  DELIMITER='20'x;
  GETNAMES=YES;
  DATAROW=2;

RUN;
title "NonParallel Hypothesis Post Enrichment (small, n=5)";
Libname ecoli "C:\SAS\EColi";
PROC IMPORT OUT= ecoli.npar01E
  DATAFILE= "C:\...\data under nonparallel alt post enrichment small
var.txt"
  DBMS=DLM REPLACE;
  DELIMITER='20'x;
  GETNAMES=YES;
  DATAROW=2;

RUN;
title "Under NonParallel (big, n=5)";
Libname ecoli "C:\SAS\EColi";
PROC IMPORT OUT= ecoli.npar01b
  DATAFILE= "C:\...\data under nonparallel alt big var.txt"
  DBMS=DLM REPLACE;
  DELIMITER='20'x;
  GETNAMES=YES;

```

```

        DATAROW=2;
    RUN;
title "NonParallel Hypothesis Post Enrichment (big, n=5)";
Libname ecoli "C:\SAS\EColi";
PROC IMPORT OUT= ecoli.npar01Eb
    DATAFILE= "C:\...\data under nonparallel alt post enrichment big var.txt"
        DBMS=DLM REPLACE;
    DELIMITER='20'x;
    GETNAMES=YES;
    DATAROW=2;
RUN;

```

Analyzing the Data

```

/*****
**          Code for Reading, Creating, and Analysing          **
**          Data Under the Null Hypothesis                    **
**          Using small Variance                              **
*****/
    *LOD of CFU=4.8;
    *1000 reps;

/*****
**          Before Enrichment                                **
*****/
ods listing;
title "0: Under Null, no missing values";
data null01_15; *renaming the predictors;
set ecoli.E15null01;
ID=V1;
SDate=V2;
Trt=V3;
SDate1=V2;
SDate2=V2*V2;
drop V1 V2 V3;
run;
data null02_15;
set null01_15;
array fix[1000] V4-V1003;
do Sim=1 to 1000;
CFU=fix[Sim];
output;
end;
drop V4-V1003;
run;
data null02_15;
set null02_15;
lgCFU=log10(CFU);
run;
*proc print data=null02_15 (obs=10);
*run;

title "1: Under Null, All missing";
data null_allmissing_15;
set null01_15;
array test[1000] V4-V1003;
do I=1 to 1000;
if test[I]<4.8 then test[I]=.;
end;
drop I;
run;
data null_allmissing_15;
set null_allmissing_15;
array fix[1000] V4-V1003;

```

```

do Sim=1 to 1000;
CFU=fix[Sim];
output;
end;
drop V4-V1003;
run;
data null_allmissing_15;
set null_allmissing_15;
lgCFU=log10(CFU);
run;
*proc print data=null_allmissing_15 (obs=10);
*run;

title "2a: Under Null, All Zeros";
data null_allzerosstay_15;
set null01_15;
array test[1000] V4-V1003;
do I=1 to 1000;
if test[I]<4.8 then test[I]=0;
end;
drop I;
run;
data null_allzerosstay_15;
set null_allzerosstay_15;
array fix[1000] V4-V1003;
do Sim=1 to 1000;
CFU=fix[Sim];
output;
end;
drop V4-V1003;
run;
data null_allzerosstayA_15;
set null_allzerosstay_15;
lgCFU=log10(CFU+.1);
run;
*proc print data=null_allzerosstayA_15 (obs=10);
*run;

title "2b: Under Null, All Zeros";
data null_allzerosstayB_15;
set null_allzerosstay_15;
lgCFU=log10(CFU+1);
run;
*proc print data=null_allzerosstayB_15 (obs=10);
*run;

title "3: Under Null, All Min CFU/2";
data null_allminover2_15;
set null01_15;
array test[1000] V4-V1003;
do I=1 to 1000;
if test[I]<4.8 then test[I]=4.8/2;
end;
drop I;
run;
data null_allminover2_15;
set null_allminover2_15;
array fix[1000] V4-V1003;
do Sim=1 to 1000;
CFU=fix[Sim];
output;
end;
drop V4-V1003;

```

```

run;
data null_allminover2_15;
set null_allminover2_15;
lgCFU=log10(CFU);
run;
*proc print data=null_allminover2_15 (obs=10);
*run;

title "4: Under Null, All Min CFU/sqrt2";
data null_allminoversqrt2_15;
set null01_15;
array test[1000] V4-V1003;
do I=1 to 1000;
if test[I]<4.8 then test[I]=4.8/sqrt(2);
end;
drop I;
run;
data null_allminoversqrt2_15;
set null_allminoversqrt2_15;
array fix[1000] V4-V1003;
do Sim=1 to 1000;
CFU=fix[Sim];
output;
end;
drop V4-V1003;
run;
data null_allminoversqrt2_15;
set null_allminoversqrt2_15;
lgCFU=log10(CFU);
run;
*proc print data=null_allminoversqrt2_15 (obs=10);
*run;

/*****
**          After Enrichment          **
*****/

title "Under Null, With true zeros identified";
data null01E_15;
set ecoli.E15null01E;
ID=V1;
SDate=V2;
Trt=V3;      *renaming the predictors;
SDate1=V2;
SDate2=V2*V2;
drop V1 V2 V3;
run;
*proc print data=null01E_15 (obs=10);
*run;

title "5a: Under Null, False missing";
data null_falsemissing_15;
set null01E_15;
array test[1000] V4-V1003;
do I=1 to 1000;
if test[I]<4.8 and test[I]^=0 then test[I]=.;
end;
drop I;
run;
data null_falsemissing_15;
set null_falsemissing_15;
array fix[1000] V4-V1003;

```

```

do Sim=1 to 1000;
CFU=fix[Sim];
output;
end;
drop V4-V1003;
run;
data null_falsemissingA_15;
set null_falsemissing_15;
lgCFU=log10(CFU + .1);
run;
*proc print data=null_falsemissingA_15 (obs=10);
*run;

title "5b: Under Null, False missing";
data null_falsemissingB_15;
set null_falsemissing_15;
lgCFU=log10(CFU + 1);
run;
*proc print data=null_falsemissingB_15 (obs=10);
*run;

title "6a: Under Null, False Enriched Values";
data null_falseenriched_15;
set null01E_15;
array test[1000] V4-V1003;
*this array replaces the false zeros with a random normal number;
do I=1 to 1000;
if test[I]<4.8 and test[I]^=0 then test[I]=10**(.873+.008*rannor(22));
end;
run;
data null_falseenrichedA_15;
set null_falseenriched_15;
array fix[1000] V4-V1003;
do Sim=1 to 1000;
CFU=fix[Sim];
output;
end;
drop V4-V1003;
run;
data null_falseenrichedA_15;
set null_falseenriched_15;
lgCFU=log10(CFU + .1);
run;
*proc print data=null_falseenrichedA_15 (obs=10);
*run;

title "6b: Under Null, False Enriched Values";
data null_falseenrichedB_15;
set null_falseenriched_15;
lgCFU=log10(CFU + 1);
run;
*proc print data=null_falseenrichedB_15 (obs=10);
*run;

title "7a: Under Null, False Min CFU/2";
data null_falseminover2_15;
set null01E_15;
array test[1000] V4-V1003;
do I=1 to 1000;
if test[I]<4.8 and test[I]^=0 then test[I]=4.8/2;
end;
drop I;
run;

```

```

data null_falseminover2_15;
set null_falseminover2_15;
array fix[1000] V4-V1003;
do Sim=1 to 1000;
CFU=fix[Sim];
output;
end;
drop V4-V1003;
run;
data null_falseminover2A_15;
set null_falseminover2_15;
lgCFU=log10(CFU+.1);
run;
*proc print data=null_falseminLgover2A_15 (obs=10);
*run;

title "7b: Under Null, False Min CFU/2";
data null_falseminover2B_15;
set null_falseminover2_15;
lgCFU=log10(CFU+1);
run;
*proc print data=null_falseminLgover2B_15 (obs=10);
*run;

title "8a: Under Null, False Min CFU/sqrt2";
data null_falseminoversqrt2_15;
set null01E_15;
array test[1000] V4-V1003;
do I=1 to 1000;
if test[I]<4.8 and test[I]^=0 then test[I]=4.8/sqrt(2);
end;
drop I;
run;
data null_falseminoversqrt2_15;
set null_falseminoversqrt2_15;
array fix[1000] V4-V1003;
do Sim=1 to 1000;
CFU=fix[Sim];
output;
end;
drop V4-V1003;
run;
data null_falseminoversqrt2A_15;
set null_falseminoversqrt2_15;
lgCFU=log10(CFU+.1);
run;
*proc print data=null_falseminLgoversqrt2A_15 (obs=10);
*run;

title "8b: Under Null, False Min CFU/sqrt2";
data null_falseminoversqrt2B_15;
set null_falseminoversqrt2_15;
lgCFU=log10(CFU+1);
run;
proc print data=null_falseminoversqrt2B_15 (obs=10);
run;

```

```

/*****
**                               The Analyses                               **
*****/
**                               SDate as Class                               **
*****/

```

```

*****/

ods listing close;

To analyze all 14 methods, copy same code as below.
Change the data set name and the output set nams;
title "0: Null, No Missing Values";
proc sort data=null02_15;
by Sim;
proc mixed data=null02_15;
by Sim;
class ID trt SDate;
model LgCFU=trt|SDate/ddfm=kr;
repeated/subject=id(trt) type=sp(pow) (SDate1);
ods output tests3=PVal00_15 FitStatistics=fit00_15;
run;

.
.
.

/*****
**           Results of F-tests for each Analysis           **
*****/

To look at results from simulations, the p-values were summarized.
To do this for all 14 methods, copy the code below and change the data set
names and the name of the pdf created;
data PVals00_15;
set PVal00_15;
if Effect='Trt' and ProbF >.05 then ptrt95=0;
else if Effect='Trt' and ProbF <=.05 then ptrt95=1;
if Effect='SDate' and ProbF <=.05 then pSDate95=1;
else if effect='SDate' and ProbF >.05 then pSDate95=0;
if Effect='Trt*SDate' and ProbF <=.05 then pTrtbyDate95=1;
else if effect='Trt*SDate' and ProbF >.05 then pTrtbyDate95=0;
run;
ods pdf file="c:\SAS\E15PValueNullClass.pdf" startpage=never;
title "0: P-Values for Null, No Missing, Small";
proc means data=PVals00_15 mean std;
var ptrt95 pSDate95 pTrtbyDate95;
run;

.
.
.

ods pdf close;
ods listing;

/*****
**           Mixed: SDate as Continuous with Linear Effect           **
*****/

To analyze all 14 methods, copy same code as below.
Change the data set name and the output set nams;
ods listing close;
title "0: Null, No Missing Values";
proc sort data=null02;
by Sim;
proc mixed data=null02;
by Sim;

```

```

class ID trt;
model LgCFU=trt|SDate/ddfm=kr;
repeated/subject=id(trt) type=sp(pow) (SDate);
ods output tests3=PValL00 FitStatistics=fitL00;
run;
.
.
.

/*****
**                               Results of F-tests for each Analysis
**
*****/

To look at results from simulations, the p-values were summarized.
To do this for all 14 methods, copy the code below and change the data set
names and the name of the pdf created;
title "0: P-Values for Null, No Missing, Small";
data PValsL00;
set PValL00;
if Effect='Trt' and ProbF >.05 then ptrt95=0;
else if Effect='Trt' and ProbF <=.05 then ptrt95=1;
if Effect='SDate' and ProbF <=.05 then pSDate95=1;
else if effect='SDate' and ProbF >.05 then pSDate95=0;
if Effect='SDate*Trt' and ProbF <=.05 then pTrtbyDate95=1;
else if effect='SDate*Trt' and ProbF >.05 then pTrtbyDate95=0;
run;
ods pdf file="c:\SAS\E15PValueNullLinear.pdf" startpage=never;
title "0: P-Values for Null, No Missing, Small";
proc means data=PValsL00 mean std;
var ptrt95 pSDate95 pTrtbyDate95;
run;

.
.
.

ods pdf close;
ods listing;

/*****
**                               Mixed: SDate as Continuous with Quadratic
**
*****/

To analyze all 14 methods, copy same code as below.
Change the data set name and the output set nams;
ods listing close;
title "0: Null, No Missing Values";
proc sort data=null02;
by Sim;
proc mixed data=null02;
by Sim;
class ID trt;
model LgCFU=trt|SDate SDate2 trt*SDate2/ddfm=kr;
repeated/subject=id(trt) type=sp(pow) (SDate);
ods output tests3=PValQ00 FitStatistics=fitQ00;
run;

.
.
.

```

```

/*****
**
**                               Results of F-tests for each Analysis
**
*****/

To look at results from simulations, the p-values were summarized.
To do this for all 14 methods, copy the code below and change the data set
names and the name of the pdf created;
data PValsQ00;
set PValQ00;
if Effect='Trt' and ProbF >.05 then ptrt95=0;
else if Effect='Trt' and ProbF <=.05 then ptrt95=1;
if Effect='SDate' and ProbF <=.05 then pSDate95=1;
else if effect='SDate' and ProbF >.05 then pSDate95=0;
if Effect='SDate*Trt' and ProbF <=.05 then pTrtbyDate95=1;
else if effect='SDate*Trt' and ProbF >.05 then pTrtbyDate95=0;
if Effect='SDate2' and ProbF <=.05 then pSDate295=1;
else if effect="SDate2" and ProbF > .05 then pSDate295=0;
if Effect="SDate2*Trt" and ProbF <= .05 then pTrtbySDate295=1;
else if Effect="SDate2*Trt" and ProbF > .05 then pTrtbySDate295=0;
run;
ods pdf file="c:\SAS\E15PValueNullQuadratic.pdf" startpage=never;
title "0: P-Values for Null, No Missing, Small";
proc means data=PValsQ00 mean std;
var ptrt95 pSDate95 pTrtbyDate95 pSDate295 pTrtbySDate295;
run;

.
.
.

ods pdf close;
ods listing;

```

Appendix -B Tables of Results from Simulation Study

Before Enrichment Methods:

1. Denoting all zero concentrations as missing values.
2. Leaving zero concentrations as zero then adding:
 - a) $\delta_1=0.1$ to all values in data set.
 - b) $\delta_2=1$ to all values in data set.
3. Imputing $\frac{\min CFU}{2}$ for all zero concentrations.
4. Imputing $\frac{\min CFU}{\sqrt{2}}$ for all zero concentrations.

After Enrichment Methods:

5. Denoting the false zeros as missing values and adding:
 - a) $\delta_1=0.1$ to all values in data set.
 - b) $\delta_2=1$ to all values in data set.
6. Imputing the false zeros with the enrichment values and adding:
 - a) nothing (this is the approach currently used by researchers).
 - b) $\delta_1=0.1$ to all values in data set.
 - c) $\delta_2=1$ to all values in data set.
7. Imputing $\frac{\min CFU}{2}$ for the false zeros and adding:
 - a) $\delta_1=0.1$ to all values in data set.
 - b) $\delta_2=1$ to all values in data set.
8. Imputing $\frac{\min CFU}{\sqrt{2}}$ for the false zeros and adding:
 - a) $\delta_1=0.1$ to all values in data set.
 - b) $\delta_2=1$ to all values in data set.

MODEL 1: SDate as Categorical

NULL

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt,	σ_S^2	0.043	0.127	0.122	0.087	0.072	0.039	0.038	0.058	0.059	0.053	0.095	0.080	0.077	0.064
	σ_L^2	0.037	0.114	0.095	0.066	0.058	0.039	0.039	0.046	0.048	0.044	0.071	0.060	0.060	0.051
Trt*	σ_S^2	0.023	0.053	0.045	0.028	0.028	0.035	0.023	0.038	0.035	0.037	0.024	0.027	0.025	0.031
SDate	σ_L^2	0.017	0.043	0.034	0.024	0.025	0.035	0.018	0.034	0.033	0.035	0.024	0.026	0.022	0.025

Table 22: Model 1, Under Null Hypothesis, n=5 per treatment

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt,	σ_S^2	0.041	0.114	0.104	0.079	0.067	0.033	0.034	0.040	0.040	0.031	0.076	0.073	0.064	0.057
	σ_L^2	0.034	0.111	0.089	0.056	0.045	0.035	0.030	0.027	0.033	0.025	0.055	0.049	0.044	0.038
Trt*	σ_S^2	0.039	0.064	0.055	0.038	0.041	0.038	0.034	0.047	0.044	0.051	0.042	0.037	0.039	0.039
SDate	σ_L^2	0.038	0.047	0.043	0.043	0.039	0.041	0.034	0.045	0.045	0.047	0.040	0.039	0.040	0.037

Table 23: Model 1, Under Null Hypothesis, n=15 per treatment

PARALLEL

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.765	0.930	0.938	0.910	0.878	0.700	0.741	0.680	0.544	0.642	0.854	0.884	0.782	0.834
	σ_L^2	0.222	0.502	0.466	0.382	0.323	0.217	0.216	0.231	0.199	0.217	0.333	0.332	0.275	0.286
Trt*	σ_S^2	0.037	0.239	0.097	0.072	0.072	0.195	0.074	0.341	0.258	0.345	0.033	0.045	0.043	0.075
SDate	σ_L^2	0.028	0.041	0.027	0.042	0.049	0.055	0.030	0.083	0.068	0.089	0.028	0.044	0.035	0.050

Table 24: Model 1, Under Parallel Hypothesis, n=5 per treatment

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.999	1.000	1.000	1.000	1.000	0.997	0.999	0.996	0.998	0.998	1.000	1.000	0.999	1.000
	σ_L^2	0.728	0.927	0.913	0.867	0.837	0.713	0.704	0.721	0.699	0.702	0.852	0.842	0.816	0.805
Trt*	σ_S^2	0.090	0.630	0.199	0.069	0.207	0.153	0.126	0.897	0.834	0.923	0.063	0.098	0.162	0.290
SDate	σ_L^2	0.068	0.058	0.041	0.056	0.095	0.064	0.060	0.270	0.251	0.291	0.056	0.082	0.087	0.114

Table 25: Model 1, Under Parallel Hypothesis, n=15 per treatment

NONPARALLEL

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.345	0.420	0.387	0.373	0.366	0.312	0.326	0.125	0.327	0.336	0.344	0.351	0.339	0.346
	σ_L^2	0.060	0.269	0.212	0.141	0.117	0.053	0.048	0.069	0.075	0.065	0.146	0.121	0.115	0.107
Trt*	σ_S^2	0.340	0.307	0.367	0.386	0.387	0.346	0.328	0.107	0.321	0.346	0.354	0.366	0.359	0.365
SDate	σ_L^2	0.036	0.092	0.074	0.053	0.052	0.031	0.031	0.045	0.042	0.042	0.054	0.051	0.044	0.051

Table 26: Model 1, Under Nonparallel Hypothesis, n=5 per treatment

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.658	0.979	0.956	0.884	0.802	0.648	0.617	0.390	0.346	0.342	0.859	0.833	0.763	0.724
	σ_L^2	0.198	0.521	0.422	0.305	0.256	0.127	0.142	0.133	0.152	0.125	0.306	0.265	0.261	0.225
Trt*	σ_S^2	0.764	0.947	0.948	0.952	0.938	0.813	0.749	0.471	0.395	0.430	0.933	0.931	0.891	0.905
SDate	σ_L^2	0.142	0.275	0.250	0.252	0.241	0.091	0.108	0.115	0.086	0.112	0.213	0.233	0.185	0.215

Table 27: Model 1, Under Nonparallel Hypothesis, n=15 per treatment

MODEL 2: SDate as Continuous with Linear Effect

NULL

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt,	σ_S^2	0.043	0.001	0.002	0.003	0.004	0.001	0.004	0.009	0.010	0.010	0.003	0.004	0.003	0.006
	σ_L^2	0.037	0.009	0.020	0.025	0.040	0.026	0.030	0.056	0.054	0.056	0.025	0.036	0.040	0.042
Trt*	σ_S^2	0.023	0.027	0.008	0.003	0.005	0.043	0.033	0.003	0.005	0.003	0.006	0.004	0.007	0.004
SDate	σ_L^2	0.017	0.027	0.014	0.019	0.028	0.049	0.037	0.033	0.037	0.039	0.023	0.026	0.029	0.029

Table 28: Model 2, Under Null Hypothesis, n=5 per treatment

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.041	0.000	0.002	0.007	0.007	0.004	0.005	0.011	0.012	0.012	0.006	0.007	0.006	0.008
	σ_L^2	0.034	0.004	0.013	0.034	0.044	0.035	0.039	0.064	0.066	0.071	0.034	0.042	0.046	0.051
Trt*	σ_S^2	0.039	0.026	0.005	0.006	0.006	0.041	0.032	0.001	0.040	0.003	0.008	0.007	0.011	0.005
SDate	σ_L^2	0.038	0.015	0.011	0.013	0.024	0.037	0.030	0.036	0.036	0.036	0.019	0.019	0.026	0.030

Table 29: Model 2, Under Null Hypothesis, n=15 per treatment

PARALLEL

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.224	0.111	0.349	0.484	0.545	0.174	0.262	0.596	0.481	0.611	0.409	0.521	0.441	0.561
	σ_L^2	0.222	0.087	0.191	0.267	0.294	0.165	0.207	0.346	0.301	0.349	0.233	0.284	0.254	0.304
Trt*	σ_S^2	0.293	0.239	0.022	0.004	0.021	0.344	0.239	0.217	0.207	0.245	0.014	0.016	0.027	0.045
SDate	σ_L^2	0.028	0.025	0.009	0.041	0.060	0.074	0.057	0.130	0.126	0.140	0.043	0.053	0.063	0.085

Table 30: Model 2, Under Parallel Hypothesis, n=5 per treatment

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.914	0.675	0.949	0.991	0.997	0.895	0.928	0.996	0.996	1.000	0.986	0.994	0.991	0.996
	σ_L^2	0.728	0.347	0.621	0.747	0.772	0.648	0.684	0.810	0.799	0.815	0.744	0.766	0.757	0.780
Trt*	σ_S^2	0.478	0.681	0.043	0.016	0.122	0.551	0.425	0.819	0.724	0.873	0.013	0.061	0.105	0.224
SDate	σ_L^2	0.068	0.050	0.014	0.073	0.146	0.067	0.056	0.038	0.362	0.411	0.071	0.115	0.129	0.195

Table 31: Model 2, Under Parallel Hypothesis, n=15 per treatment

NONPARALLEL

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.345	0.006	0.008	0.008	0.008	0.006	0.007	0.013	0.007	0.009	0.007	0.007	0.007	0.008
	σ_L^2	0.060	0.011	0.024	0.038	0.043	0.028	0.029	0.056	0.059	0.059	0.038	0.042	0.045	0.049
Trt*	σ_S^2	0.340	0.521	0.467	0.438	0.408	0.517	0.479	0.045	0.409	0.363	0.482	0.429	0.459	0.410
SDate	σ_L^2	0.036	0.192	0.146	0.100	0.087	0.141	0.157	0.060	0.055	0.061	0.090	0.087	0.079	0.086

Table 32: Model 2, Under Nonparallel Hypothesis, n=5

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.658	0.023	0.010	0.011	0.011	0.021	0.027	0.011	0.016	0.017	0.010	0.013	0.011	0.015
	σ_L^2	0.198	0.013	0.031	0.045	0.050	0.050	0.054	0.077	0.076	0.078	0.044	0.050	0.052	0.061
Trt*	σ_S^2	0.764	0.998	0.994	0.956	0.858	0.995	0.993	0.188	0.216	0.175	0.955	0.912	0.875	0.805
SDate	σ_L^2	0.142	0.582	0.481	0.382	0.342	0.390	0.458	0.168	0.144	0.165	0.331	0.335	0.281	0.302

Table 33: Model 2, Under Nonparallel Hypothesis, n=15 per treatment

MODEL 3: SDate as Continuous with Linear and Quadratic Effect

NULL

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.086	0.000	0.002	0.038	0.076	0.073	0.094	0.142	0.129	0.154	0.039	0.065	0.065	0.094
	σ_L^2	0.089	0.000	0.017	0.067	0.096	0.073	0.088	0.136	0.122	0.143	0.059	0.088	0.083	0.107
Trt*	σ_S^2	0.050	0.014	0.010	0.025	0.040	0.049	0.053	0.034	0.035	0.043	0.023	0.032	0.035	0.044
SDate	σ_L^2	0.049	0.013	0.014	0.034	0.053	0.050	0.046	0.076	0.068	0.082	0.033	0.043	0.043	0.058
Trt*	σ_S^2	0.042	0.019	0.017	0.025	0.023	0.064	0.050	0.022	0.024	0.026	0.031	0.027	0.033	0.030
SDate2	σ_L^2	0.025	0.018	0.018	0.024	0.029	0.038	0.028	0.037	0.041	0.040	0.026	0.026	0.032	0.034

Table 34: Model 3, Under Null Hypothesis, n=5 per treatment

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.069	0.000	0.002	0.026	0.054	0.053	0.072	0.108	0.099	0.120	0.023	0.044	0.048	0.075
	σ_L^2	0.078	0.000	0.015	0.057	0.080	0.070	0.087	0.135	0.130	0.142	0.056	0.073	0.077	0.096
Trt*	σ_S^2	0.039	0.007	0.009	0.016	0.026	0.037	0.045	0.029	0.027	0.036	0.016	0.018	0.022	0.028
SDate	σ_L^2	0.035	0.010	0.014	0.030	0.043	0.039	0.039	0.064	0.066	0.075	0.033	0.039	0.043	0.049
Trt*	σ_S^2	0.038	0.016	0.018	0.022	0.025	0.048	0.040	0.017	0.022	0.020	0.027	0.026	0.036	0.028
SDate2	σ_L^2	0.039	0.013	0.014	0.027	0.032	0.044	0.040	0.042	0.045	0.046	0.031	0.030	0.035	0.039

Table 35: Model 3, Under Null Hypothesis, n=15 per treatment

PARALLEL

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.748	0.006	0.374	0.746	0.815	0.631	0.734	0.866	0.795	0.867	0.678	0.794	0.728	0.825
	σ_L^2	0.354	0.031	0.205	0.352	0.414	0.283	0.342	0.497	0.438	0.506	0.326	0.385	0.350	0.428
Trt*	σ_S^2	0.043	0.276	0.067	0.022	0.039	0.050	0.040	0.337	0.318	0.408	0.025	0.040	0.052	0.110
SDate	σ_L^2	0.049	0.039	0.016	0.047	0.081	0.064	0.065	0.208	0.197	0.232	0.049	0.071	0.093	0.121
Trt*	σ_S^2	0.068	0.140	0.050	0.028	0.032	0.163	0.054	0.117	0.149	0.160	0.039	0.035	0.062	0.053
Sdate2	σ_L^2	0.027	0.035	0.011	0.025	0.041	0.065	0.046	0.115	0.145	0.133	0.050	0.045	0.075	0.070

Table 36: Model 3, Under Parallel Hypothesis, n=5 per treatment

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	1.000	0.050	0.972	0.999	1.000	0.997	1.000	1.000	1.000	1.000	0.998	1.000	1.000	1.000
	σ_L^2	0.780	0.119	0.600	0.778	0.820	0.759	0.786	0.882	0.878	0.887	0.773	0.810	0.812	0.841
Trt*	σ_S^2	0.075	0.661	0.175	0.013	0.088	0.065	0.048	0.745	0.742	0.832	0.020	0.054	0.115	0.236
SDate	σ_L^2	0.056	0.081	0.016	0.069	0.141	0.066	0.079	0.383	0.391	0.430	0.083	0.122	0.151	0.203
Trt*	σ_S^2	0.238	0.308	0.096	0.028	0.019	0.200	0.147	0.256	0.302	0.334	0.028	0.023	0.048	0.065
SDate2	σ_L^2	0.029	0.049	0.015	0.036	0.058	0.038	0.027	0.164	0.187	0.199	0.050	0.060	0.075	0.088

Table 37: Model 3, Under Parallel Hypothesis, n=15 per treatment

NONPARALLEL

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6c</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.052	0.021	0.034	0.045	0.050	0.038	0.051	0.082	0.048	0.058	0.033	0.048	0.040	0.052
	σ_L^2	0.078	0.004	0.024	0.050	0.071	0.059	0.074	0.128	0.112	0.138	0.048	0.066	0.065	0.093
Trt*	σ_S^2	0.153	0.069	0.111	0.134	0.140	0.107	0.122	0.064	0.097	0.111	0.093	0.112	0.098	0.114
SDate	σ_L^2	0.055	0.033	0.039	0.057	0.063	0.054	0.055	0.059	0.054	0.063	0.051	0.059	0.058	0.064
Trt*	σ_S^2	0.040	0.028	0.029	0.034	0.035	0.046	0.037	0.025	0.042	0.037	0.044	0.040	0.044	0.039
SDate2	σ_L^2	0.032	0.024	0.024	0.032	0.035	0.036	0.032	0.029	0.031	0.030	0.031	0.033	0.034	0.034

Table 38: Model 3, Under Nonparallel Hypothesis, n=5 per treatment

<i>Estimate</i>		<i>Method For Handling Missing Values</i>													
		<i>1</i>	<i>2a</i>	<i>2b</i>	<i>3</i>	<i>4</i>	<i>5a</i>	<i>5b</i>	<i>6a</i>	<i>6b</i>	<i>6ca</i>	<i>7a</i>	<i>7b</i>	<i>8a</i>	<i>8b</i>
Trt	σ_S^2	0.098	0.003	0.020	0.061	0.085	0.076	0.098	0.089	0.085	0.113	0.054	0.083	0.070	0.094
	σ_L^2	0.107	0.002	0.027	0.078	0.096	0.079	0.100	0.143	0.133	0.148	0.075	0.096	0.096	0.112
Trt*	σ_S^2	0.547	0.253	0.306	0.388	0.393	0.410	0.485	0.179	0.149	0.173	0.305	0.346	0.294	0.344
SDate	σ_L^2	0.144	0.065	0.068	0.120	0.137	0.101	0.130	0.111	0.083	0.114	0.090	0.121	0.094	0.128
Trt*	σ_S^2	0.168	0.027	0.020	0.038	0.060	0.139	0.139	0.031	0.054	0.047	0.042	0.040	0.055	0.055
SDate2	σ_L^2	0.048	0.021	0.019	0.027	0.032	0.046	0.051	0.032	0.030	0.035	0.028	0.034	0.030	0.038

Table 39: Model 3, Under Nonparallel Hypothesis, n=15 per treatment