

# 802 Project Summary

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## Introduction

This paper summarizes the consulting that was done for our assigned STAT 802 group. For more information on the experiment, the data, or any other files used in this paper see our [Github page](https://github.com/maksudatoma/Stat-802-Project) which can be found at <<https://github.com/maksudatoma/Stat-802-Project>>. The coding languages used in the paper are R and SAS. The corresponding code can be found in *Appendix A - R Code* and *Appendix B - SAS Code* respectively.

## Initial Meetings

The first meeting with our clients was on September 13th. We discussed their project and what kind of data they were going to be looking at. They detailed to us their project, which is looking at the levels of Salmonella in beef jerky at different inoculations and thicknesses. Prior to the meeting they sent us what their variables would be, which gave us a good idea of what might be the best experimental design. The group informed us they were avoiding a completely randomized design (CRD) at the request of their professor. With that in mind, we suggested other possible models.

Later, after receiving feedback from Dr. Howard and several PhD students within the statistics department, we suggested adding a time component to the experiment as well as creating multiple batches to replicate each treatment combination. This lead to us suggested a mixed model for the analysis approach.

In both the initial meeting and the follow-up session the clients were more than happy to implement our suggestions. In the end the experiment involved two thickness levels (one-fourth and one-eighth of an inch), two inoculation methods (dry and wet), and five evenly spaced time points were measurements were taken (weeks 1-5) creating twenty entries per batch. The exact number of batches would not be known until after the power analysis, found in the *Power Analysis* section. We provided the client an example dataset we created to give them a better idea of what the end product may look like. This dataset had five batches.

## Study Objectives and Proposed Model

The clients were most interested in the effect of the thickness levels, the inoculation method, and their interaction had on the Salmonella levels. In the final model we included the week effect and subsequent interactions as well. These variables are the fixed effects in the proposed mixed model.

The other variable included in the experiment is the batch number. This is therefore treated as a random variable. As mentioned above, the exact number of batches needed was unknown prior to the power analysis, but five was used as a starting value.

Overall the study employs a  $2 \times 2$  factorial design with two main factors: Inoculation Method (Dry, Wet) and Thickness (1/4-inch, 1/8-inch). Repeated measurements are taken over five equally spaced time points (Weeks 1 to 5), allowing the analysis of both main effects, their interaction, and changes over time.

The model can be written in the form

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \tau_k + (\alpha\beta)_{ij} + (\alpha\tau)_{ik} + (\beta\tau)_{jk} + (\alpha\beta\tau)_{ijk} + u_l + e_{ijkl}$$

Here,  $Y_{ijkl}$  is the Salmonella level and  $\mu$  is the overall mean. The fixed effects are represented by  $\alpha_i$  for the effect of the  $i$ th inoculation method,  $\beta_j$  for the effect of the  $j$ th thickness level, and  $\tau_k$  for the effect of the  $k$ th week. The interaction effect of the  $i$ th inoculation method and the  $j$ th thickness level is represented by  $(\alpha\beta)_{ij}$ , with the other two-way interactions following this form. The three-way interaction between all fixed effects is represented as  $(\alpha\beta\tau)_{ijk}$ . The random effect for batches is represented by  $u_l$ , which we assume are distributed as  $u_l \sim N(0, \sigma_u^2)$ . Lastly, the residuals are represented by  $e_{ijkl}$ , which we assume can be distributed as  $e_{ijkl} \sim N(0, \sigma^2)$ .

## Power Analysis

Before power analysis, We reached out to the client later on in the process to determine what contrasts they were most interested in testing. They expressed they wanted to see the difference between the two levels of the inoculation method, the two levels of the thickness, and the orthogonal contrasts these variable. This resulted in six contrasts being tested.

To determine the necessary number of batches needed to increase the likelihood of detecting a true treatment effect, we performed a power analysis. To do this, probable treatment mean estimates across all five weeks and variance estimates were needed. The clients provided these metrics from Brown et al. (2024). We then used these metrics to create a dataset with five batches where the response variable was identical across the batches. This dataset was then evaluated to determine the power. The results of the power analysis performed in SAS are shown below.

Obs	Label	NumDF	DenDF	FValue	ProbF	Effect	ncparm	alpha	fcrit	power
1	Dry vs Wet	1	76	9.23	0.0033		9.226	0.05	3.96676	0.85058
2	1/4 vs 1/8 inches	1	76	103.28	<.0001		103.276	0.05	3.96676	1.00000
3	Dry vs Wet at 1/4 Inches	1	76	5.70	0.0195		5.695	0.05	3.96676	0.65406
4	Dry vs Wet at 1/8 Inches	1	76	44.65	<.0001		44.651	0.05	3.96676	1.00000
5	1/4 vs 1/8 inches for Dry inoculation	1	76	137.36	<.0001		137.364	0.05	3.96676	1.00000
6	1/4 vs 1/8 inches for Wet inoculation	1	76	7.03	0.0097		7.031	0.05	3.96676	0.74475
7		1	76	9.23	0.0033	Inoculation_Method	9.226	0.05	3.96676	0.85058
8		1	76	103.28	<.0001	Thickness	103.276	0.05	3.96676	1.00000
9		1	76	41.12	<.0001	Inoculatio*Thickness	41.120	0.05	3.96676	0.99999
10		4	76	7.82	<.0001	Week	31.280	0.05	2.49205	0.99659
11		4	76	13.77	<.0001	Inoculation_Met*Week	55.092	0.05	2.49205	1.00000
12		4	76	20.78	<.0001	Thickness*Week	83.132	0.05	2.49205	1.00000
13		4	76	4.04	0.0050	Inocula*Thickne*Week	16.162	0.05	2.49205	0.89525

Figure 1: Results of power analysis.

The first six rows of the table correspond to the contrasts the clients were interested in testing, while the bottom seven rows are measuring the fixed effects of the model. Many of the terms have more than 80% power. Specifically the fixed effects were all high enough for both the clients and ourselves to feel comfortable using five batches. Two of the orthogonal contrasts, **Dry vs Wet at 1/4 Inches** and **1/4 vs 1/8 inches for Wet inoculation** did have lower power scores, but after talking with both the clients and Dr. Howard about them, we felt comfortable to proceed.

## Simulating Data

After finding the necessary number of batches, which was five, we proceeded with simulating the data. The estimated treatment means and variances provided by the client were used in the simulation as well. We then reviewed the simulated dataset for major issues, such as negative response values, and reran the power analysis on the new data set to ensure everything was working properly. After finding no problems with the dataset, we sent it to the clients. Note, the simulation was performed in SAS.

## Data Analysis

### Summary Statistics

As part of the project, we analyzed the simulated dataset. Before fitting out model to the dataset, we first wanted to explore some of the variables. Figure 2 shows the mean values and

standard deviations for each treatment combination. We can see the changes in mean values are small, so further exploration and analysis are needed.

Inoculation Method	Thickness	Week	Mean Response	SD Response	Count
Dry	1/4-inch	1	4.199	0.187	5
Dry	1/4-inch	2	4.137	0.163	5
Dry	1/4-inch	3	4.187	0.294	5
Dry	1/4-inch	4	4.306	0.272	5
Dry	1/4-inch	5	4.645	0.178	5
Dry	1/8-inch	1	4.801	0.173	5
Dry	1/8-inch	2	4.801	0.238	5
Dry	1/8-inch	3	4.596	0.235	5
Dry	1/8-inch	4	4.425	0.206	5
Dry	1/8-inch	5	4.876	0.177	5
Wet	1/4-inch	1	4.240	0.307	5
Wet	1/4-inch	2	4.523	0.236	5
Wet	1/4-inch	3	4.534	0.173	5
Wet	1/4-inch	4	4.357	0.421	5
Wet	1/4-inch	5	4.299	0.200	5
Wet	1/8-inch	1	4.697	0.181	5
Wet	1/8-inch	2	4.809	0.327	5
Wet	1/8-inch	3	4.530	0.215	5
Wet	1/8-inch	4	4.223	0.361	5
Wet	1/8-inch	5	4.151	0.295	5

Figure 2: Summary of response variable across factors and weeks

### Distribution of response variable

Before continuing our investigation into the relationships among the treatment variables, we want to look into the response variable (Salmonella levels). Specifically, we want to see how it is distributed. Figure 3 shows a histogram and Q-Q plot of the response variable in the left and right plots respectively. While the histogram shows a slight potential skew, this is not enough for us say the distribution is non-normal. Furthermore, the Q-Q plot indicates the response variable follows a relatively normal distribution.

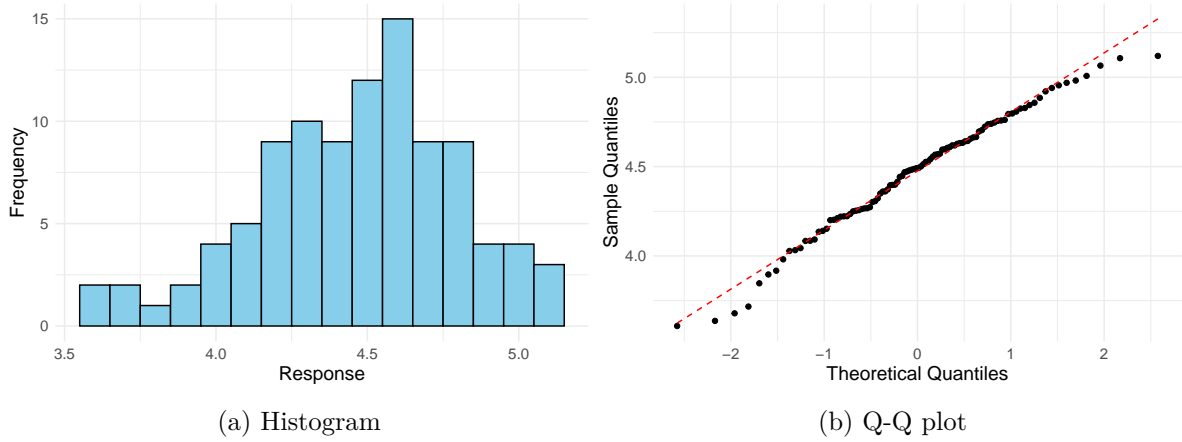


Figure 3: Plots to see the distribution of the response variable.

## Exploring the Data

Now we will graphically look at how the different variables of the model impact the response variable. Figure 4 shows how the mean response variables we saw in Figure 2 change over time, while also controlling for thickness and inoculation method. Note the y-axis of plot does not start at the origin. We can see the mean values of samples that used a wet inoculation method (blue lines) tended decrease over time, while samples with the dry inoculation method (red lines) were more of a mixed bag but saw sharp increases between weeks four and five. The samples cut to 1/8 inches thick (dashed lines) were very similar for most weeks, but diverged near the end of the experiment, while samples cut to 1/4 inches thick (solid lines) did not seem as similar.

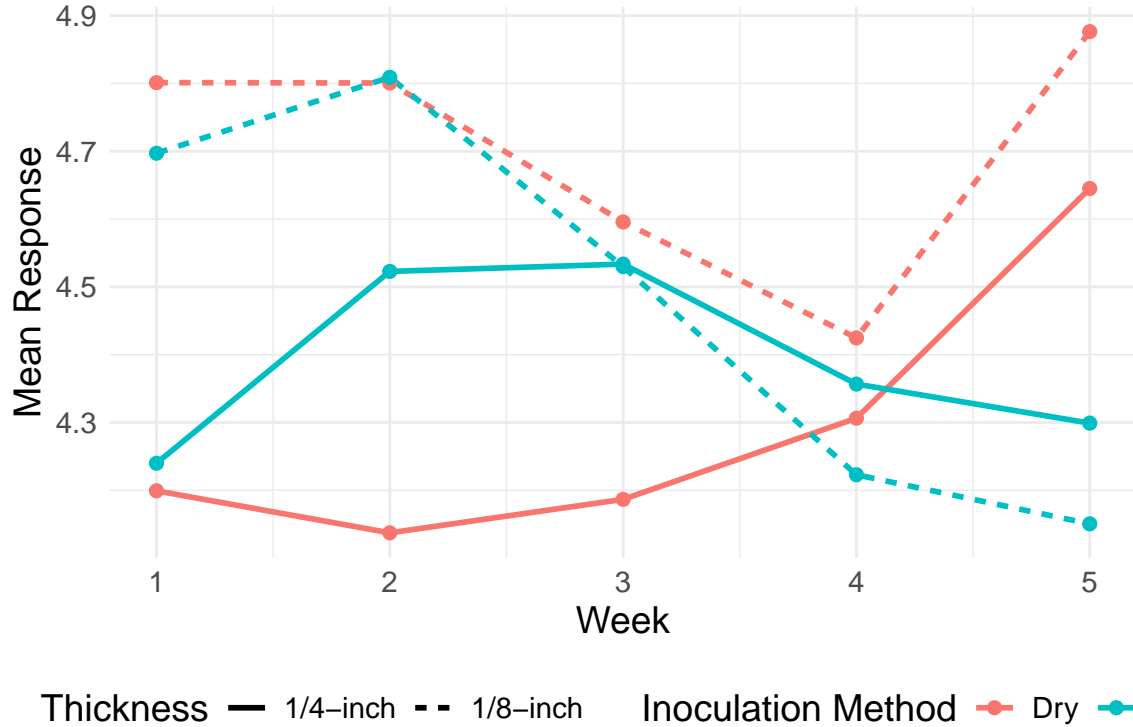


Figure 4: Line plot of response variable over time, controlling for thickness and inoculation method.

Another element to consider is correlation over time. Since this is a repeated measures experiment we need to account for this correlation by selecting a type of covariance structure. There are several types of structures such as variance components (VC), unstructured (UN), compound symmetry (CS) and heterogeneous compound symmetry (CSH),  $p$ -order auto-regressive ( $AR(p)$ ) and heterogeneous auto-regressive ( $ARH(p)$ ),  $p$ -order ante-dependence ( $ANTE(p)$ ), and Toeplitz (TOEP) among others. For more information on these see Lipka and Tyner (2004) and USDA (n.d.). The  $AR(p)$  structure fits data that is ordered through time an equally spaced. For that reason, our initial plan was to use this as the covariance structure with  $p = 1$ .

To see if this first-order auto-regressive structure might fit the data, let's consider the table and plot in Figure 5. The correlation matrix (left) and plot (right) show the relationships between repeated measurements over weeks one to five. Strong correlations are observed between adjacent weeks (e.g., Week 1 vs. Week 2,  $r=0.69$ , Week 2 vs. Week 3,  $r=0.74$ ), indicating temporal dependency. Correlations weaken as the time gap increases (e.g., Week 1 vs. Week 5,  $r=0.29$ ), leading us to believe orders of  $p > 1$  are not necessary. This can be seen visually in the plot which uses circle size and color to model the correlation metrics seen in the matrix.

We can see as the gap between weeks increases, the circles become smaller and lighter. This pattern supports the use of models like AR(1).

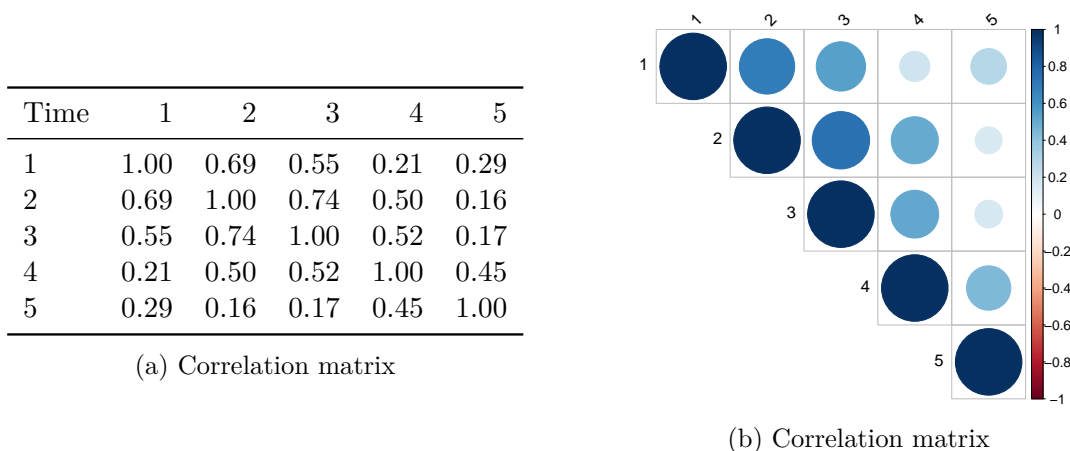


Figure 5: Table and plots to see the correlation across time.

## Model Results

After exploring the data we can move on to fitting the model. While we were confident in using the AR(1), we chose to fit the model using other covariance structures as well so we could see how the fit compares. The results are shown in Figure 6. For each of the fit statistics in this table a lower score is better, even when looking at negative values. (Bobbitt (2021)). This means the model fit using an AR(1) structure had the best AIC and AICC scores and a respectable BIC score. This verifies our choice in the AR(1) covariance structure.

Model	AIC	AICC	BIC
VC	-12.25	-12.09	-13.03
UN	-12.22	-3.58	-18.47
CS	-11.60	-11.29	-12.77
AR(1)	-12.86	-12.55	-14.03
ARH(1)	-12.56	-11.01	-15.30
ANTE(1)	-8.57	-5.39	-12.48
TOEP	-7.34	-6.19	-9.68

Figure 6: Model comparison table.

After verifying the fit of the AR(1) covariance structure for the repeated measures, we needed to see if the assumptions for a linear mixed model were violated. These include the residuals being normally distributed and homogeneous. The plots below allow us to evaluate these

assumptions. To graphically test normality, we can look at both the histogram (top right) and the Q-Q plot (bottom left). These both appear approximately normal, indicating the assumption holds. The boxplot (bottom right) can also show normality as well as potential outliers. It appears there is one outlier, but the normality assumption still holds. The residual plot (top left) allows us to check if the homogeneous assumption holds, and it appears to since the points seem somewhat randomly distributed with no clear pattern. Since the assumptions are holding, we can proceed with the linear mixed model.

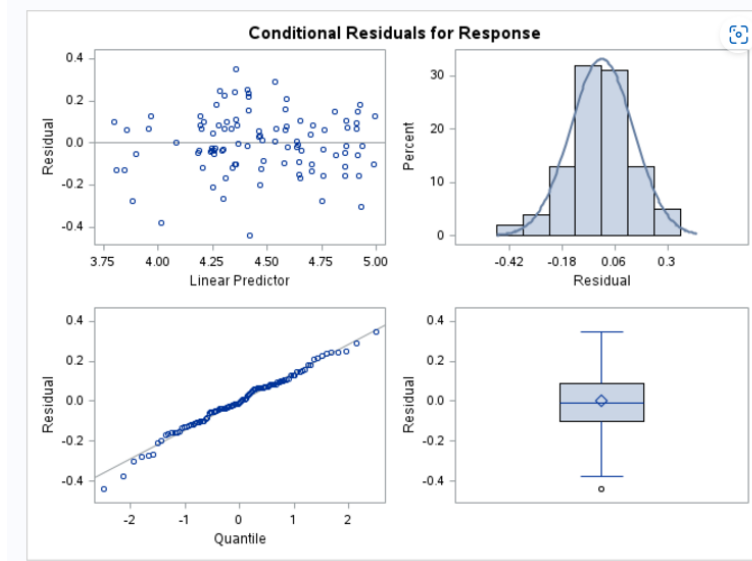


Figure 7: Residual plots for checking assumptions.

Below are three tables from the SAS output. The first is the *Fit Statistics* table, which is where the metrics used in Figure 6 come from. The *Covariance Parameter Estimates* table shows how much of the variance in the model is explained by the random terms.

### HEY TOMA - Is it ok for covariance estimate to be negative?

The *Type III Tests of Fixed Effects* table allows us to see if the fixed effects and/or their interactions are significant by looking at the p-values reported in the  $PR > F$  column. We first need to look at the interaction terms. While the three-way interaction between the inoculation method, thickness, and week is not significant, all three two-way interactions between these variables are. Therefore, we need to consider simple effects.



Fit Statistics	
-2 Res Log Likelihood	-18.86
AIC (smaller is better)	-12.86
AICC (smaller is better)	-12.55
BIC (smaller is better)	-14.03
CAIC (smaller is better)	-11.03
HQIC (smaller is better)	-16.01
Generalized Chi-Square	2.13
Gener. Chi-Square / DF	0.03

Covariance Parameter Estimates			
Cov Parm	Subject	Estimate	Standard Error
Intercept	Batches	0.03802	0.02755
AR(1)	Batche*Inocul*Thickn	-0.2140	0.1286
Residual		0.02664	0.004423

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Inoculation_Method	1	76	4.98	0.0286
Thickness	1	76	81.92	<.0001
Inoculatio*Thickness	1	76	32.65	<.0001
Week	4	76	6.12	0.0003
Inoculation_Met*Week	4	76	17.90	<.0001
Thickness*Week	4	76	14.59	<.0001
Inocula*Thickne*Week	4	76	0.55	0.6970

Figure 8: *Fit Statistics*, *Covariance Parameter Estimates*, and *Type III Tests of Fixed Effects* tables.

Inocula*Thickne*Week Least Squares Means										
Inoculation_Method	Thickness	Week	Estimate	Standard Error	DF	t Value	Pr >  t	Alpha	Lower	Upper
Dry	1/4-inch	1	4.1992	0.1137	76	36.93	<.0001	0.05	3.9727	4.4257
Dry	1/4-inch	2	4.1373	0.1137	76	36.38	<.0001	0.05	3.9108	4.3637
Dry	1/4-inch	3	4.1866	0.1137	76	36.82	<.0001	0.05	3.9601	4.4131
Dry	1/4-inch	4	4.3063	0.1137	76	37.87	<.0001	0.05	4.0798	4.5328
Dry	1/4-inch	5	4.6451	0.1137	76	40.85	<.0001	0.05	4.4186	4.8716
Dry	1/8-inch	1	4.8012	0.1137	76	42.22	<.0001	0.05	4.5747	5.0277
Dry	1/8-inch	2	4.8007	0.1137	76	42.21	<.0001	0.05	4.5742	5.0272
Dry	1/8-inch	3	4.5957	0.1137	76	40.41	<.0001	0.05	4.3692	4.8222
Dry	1/8-inch	4	4.4247	0.1137	76	38.91	<.0001	0.05	4.1982	4.6512
Dry	1/8-inch	5	4.8764	0.1137	76	42.88	<.0001	0.05	4.6499	5.1029
Wet	1/4-inch	1	4.2399	0.1137	76	37.28	<.0001	0.05	4.0134	4.4663
Wet	1/4-inch	2	4.5228	0.1137	76	39.77	<.0001	0.05	4.2963	4.7493
Wet	1/4-inch	3	4.5336	0.1137	76	39.87	<.0001	0.05	4.3071	4.7601
Wet	1/4-inch	4	4.3566	0.1137	76	38.31	<.0001	0.05	4.1301	4.5831
Wet	1/4-inch	5	4.2990	0.1137	76	37.80	<.0001	0.05	4.0725	4.5255
Wet	1/8-inch	1	4.6971	0.1137	76	41.30	<.0001	0.05	4.4706	4.9236
Wet	1/8-inch	2	4.8091	0.1137	76	42.29	<.0001	0.05	4.5826	5.0356
Wet	1/8-inch	3	4.5298	0.1137	76	39.83	<.0001	0.05	4.3034	4.7563
Wet	1/8-inch	4	4.2229	0.1137	76	37.13	<.0001	0.05	3.9964	4.4494
Wet	1/8-inch	5	4.1505	0.1137	76	36.50	<.0001	0.05	3.9240	4.3770

Figure 9: LS Means table.

Write down the model structure

Contrasts				
Label	Num DF	Den DF	F Value	Pr > F
Dry vs Wet	1	76	4.98	0.0286
1/4 vs 1/8 inches	1	76	81.92	<.0001
Dry vs Wet at 1/4 Inches	1	76	6.06	0.0161
Dry vs Wet at 1/8 Inches	1	76	31.56	<.0001
1/4 vs 1/8 inches for Dry inoculation	1	76	108.99	<.0001
1/4 vs 1/8 inches for Wet inoculation	1	76	5.57	0.0209

Figure 10: Contrasts table.

Conclusion

Future Work

## References

- Bobbit, Zach. 2021. “How to Interpret Negative AIC Values.” Statology. 2021. <https://www.statology.org/negative-aic/>.
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- Lipka, Alex, and Benjamin Tyner. 2004. “Repeated Measures Covariance Structure.” Statistical Consulting Service - Purdue University. 2004. <https://www.stat.purdue.edu/scs/help/method/Repeated%20measures%20analysis.pdf>.
- USDA. n.d. “Appendix e: Selecting an Appropriate Covariance Structure,” n.d., E.1–8. <https://www.ars.usda.gov/ARUserFiles/80000000/StatisticsGroupWebinars/Appendix%20E%20-%20Selecting%20a%20Covariance%20Structure.pdf>.

## **Appendix A - R Code**

## Appendix B - SAS Code

### Power Analysis

```
data rptm_means;
input Inoculation_Method $ Thickness $ @@;
do Week=1 to 5 by 1;
    input mu @@;
    output;
end;
datalines;
Dry 1/4 4.26 4.25 4.47 4.33 4.54
Dry 1/8 4.91 4.95 4.67 4.56 4.97
Wet 1/4 4.21 4.57 4.65 4.49 4.38
Wet 1/8 4.86 4.78 4.62 4.32 4.22
;

data rptm_design;
set rptm_means;
do Batches = 1 to 5; /* Creating 3 blocks (batches) */
    output;
end;
run;

proc print data=rptm_design;
run;

/* Creating Model */

proc glimmix data=rptm_design;
class Batches Inoculation_Method Thickness Week;
model mu = Inoculation_Method|Thickness|Week;
random intercept / subject=Batches;
random Week / subject=Batches*Inoculation_Method*Thickness type=ar(1) residual;
parms (.029)(0.017)(.028)/hold=1,2,3;
/* Provide 3 parameters for variance components */
lsmeans Inoculation_Method*Thickness*Week / slicediff=Week cl;
/* Define main effect contrasts */
contrast 'Dry vs Wet'
    Inoculation_Method 1 -1;
contrast '1/4 vs 1/8 inches'
```

```

        Thickness 1 -1;

/* Define interaction contrasts */
contrast 'Dry vs Wet at 1/4 Inches'
    Inoculation_Method 1 -1 Inoculation_Method*Thickness 1 0 -1 0;
contrast 'Dry vs Wet at 1/8 Inches'
    Inoculation_Method 1 -1 Inoculation_Method*Thickness 0 1 0 -1;
contrast '1/4 vs 1/8 inches for Dry inoculation'
    Thickness 1 -1 Inoculation_Method*Thickness 1 -1 0 0;
contrast '1/4 vs 1/8 inches for Wet inoculation'
    Thickness 1 -1 Inoculation_Method*Thickness 0 0 1 -1;

ods output contrasts=f_contrast tests3=f_anova;
run;

/*Power*/
data power;
    set f_contrast f_anova;
    ncparm = numdf * fvalue;
    alpha = 0.05;
    fcrit = finv(1-alpha, numdf, dendf, 0);
    power = 1 - probf(fcrit, numdf, dendf, ncparm);
run;

proc print data=power;
run;

```

## Simulation

```

/* Step 1: Define AR(1) Covariance Structure in PROC IML */
proc iml;
    n = 20; /* Number of subjects per treatment*/
    mean = {0 0 0 0 0}; /* Mean for each week */
    T = 5; /* Number of repeated measures (weeks) */
    rho = 0.2; /* AR(1) correlation parameter */
    sigma2 = {0.29 0.29 0.29 0.29 0.29}; /* Variance for each week */

    /* Construct AR(1) covariance matrix */
    cov = j(T, T, 0);
    do i = 1 to T;

```

```

        do j = 1 to T;
            cov[i, j] = sqrt(sigma2[i] * sigma2[j]) * rho**abs(i - j);
        end;
    end;

/* Print covariance matrix */
print "Covariance Matrix:", cov;

/* Generate simulated data using the covariance matrix */
call randseed(12349);      /* Set random seed */
x = randnormal(n, mean, cov); /* Simulate AR(1) correlated data */
cname = {"t1", "t2", "t3", "t4", "t5"};

/* Print the simulated data matrix directly */
print "Simulated Data Matrix (x):", x;
/* Print Sample mean */
samplemean = x[:,];
print samplemean n;

/* Create dataset from simulated data */
create inputdatacb from x[colname=cname];
append from x;
close inputdatacb;
quit;

/* Step 2: Display the Simulated Data as a SAS Table */
proc print data=inputdatacb label;
    title "Simulated Data with AR(1) Covariance Structure";
run;

/* Step 3: Define Treatment Structure and Random Effects */
data rptm_simulation;
    retain Subject 0;
    keep Inoculation_Method Thickness Week Batches Response;

    array weeks[5] t1-t5;

/* Define mean values for each combination of factors and week */
if _n_ = 1 then do;
    array mean_values[4,2,5] _temporary_ (
        /* Dry, 1/4 inch */
        4.26, 4.25, 4.47, 4.33, 4.54,

```

```

        /* Dry, 1/8 inch */
        4.91, 4.95, 4.67, 4.56, 4.97,
        /* Wet, 1/4 inch */
        4.21, 4.57, 4.65, 4.49, 4.38,
        /* Wet, 1/8 inch */
        4.86, 4.78, 4.62, 4.32, 4.22
    );
end;

/* Simulation parameters */
sigma_batch = sqrt(0.029); /* Batch variance */
sigma_resid = sqrt(0.017); /* Residual variance */

/* Loop through each combination of factors */
do Batches = 1 to 5; /* Number of batches */
    batch_effect = rand("Normal", 0, sigma_batch); /*Random batch effect*/

    do Inoculation_Method = "Dry", "Wet";
        do Thickness = "1/4-inch", "1/8-inch";
            Subject + 1;
            set inputdatacb;

            /* Generate response for each week with AR(1) structure */
            do Week = 1 to 5;
                Mean_Value = mean_values[
                    (Inoculation_Method="Dry")*1+(Inoculation_Method="Wet")*2,
                    (Thickness="1/4-inch")*1 + (Thickness="1/8-inch")*2,
                    Week
                ];
                Response = Mean_Value + batch_effect + weeks[Week];
                output;
            end;
        end;
    end;
end;

run;

/* Step 4: Display the Simulated Data in a Structured Format */
proc print data=rptm_simulation label;
    title "Simulated Data for 2x2 Factorial Design with Repeated Measures";
run;

```



## Analysis

```
proc glimmix data=data plots=residualpanel;
  class Batches Inoculation_Method Thickness Week;
  model Response = Inoculation_Method|Thickness|Week;
  random intercept / subject=Batches;
  random Week / subject=Batches*Inoculation_Method*Thickness type=ar(1) residual;
  lsmeans Inoculation_Method*Thickness*Week / adjust=tukey cl;

  /* Define main effect contrasts */
  contrast 'Dry vs Wet'
    Inoculation_Method 1 -1;
  contrast '1/4 vs 1/8 inches'
    Thickness 1 -1;

  /* Define interaction contrasts */
  contrast 'Dry vs Wet at 1/4 Inches'
    Inoculation_Method 1 -1 Inoculation_Method*Thickness 1 0 -1 0;
  contrast 'Dry vs Wet at 1/8 Inches'
    Inoculation_Method 1 -1 Inoculation_Method*Thickness 0 1 0 -1;
  contrast '1/4 vs 1/8 inches for Dry inoculation'
    Thickness 1 -1 Inoculation_Method*Thickness 1 -1 0 0;
  contrast '1/4 vs 1/8 inches for Wet inoculation'
    Thickness 1 -1 Inoculation_Method*Thickness 0 0 1 -1;

  ods output contrasts=f_contrast tests3=f_anova;
run;
```