Method Sheet 30

Statistical analysis of bacterial growth kinetics data

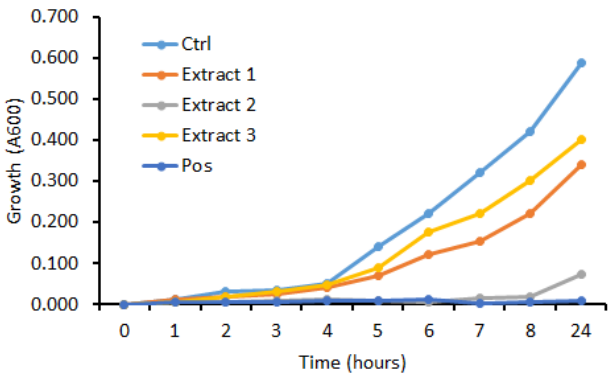
Overview

This method sheet explains how to perform a statistical analysis of bacterial growth kinetics data using two way repeated measures ANOVA. This test will help to establish whether any of the tested hit extracts significantly slow the growth of a bacterial strain of interest relative to a control condition of DMSO treatment. This type of test is similar to the two way ANOVA used previously, but the data must be arranged differently to enable the test to work properly using Jamovi software.

Background correction of data

1) Copy and paste the data from the primary plate reads at every timepoint into a single Excel file, with the data from each experiment within its own, separate worksheet.
2) For example, if you have 8 timepoints and 4 experiments, your Excel file will have 4 worksheets and each sheet will have 8 tables of data in 96-well plate format.
3) Perform a background correction of the data for every timepoint by subtracting the t=0 values (i.e. the very first measurement) from every plate, as per Method sheet 18.
4) Repeat that process in each of the following separate worksheets.
5) There is no requirement to normalise the data to a percentage for this type of analysis, instead we will work with raw absorbance values.
6) Now calculate the mean absorbance for each of the 4 replicate wells for each treatment (remembering to include the negative and positive controls too).
7) Collate the data from each experiment so that you have a single row for each timepoint (the values in this table will be the means of measurements of the 4 replicate wells of one treatment on one plate).
8) Insert the number of hours for each timepoint in the first cell of each row.
9) Insert the number of the experiment (e.g. Exp 1) above every column as an additional column header for this data table.
10) The data for each individual experiment (each plate) should now look something like the table shown at left, and charting the data with a line graph should give a chart something like the one shown at right:

	A	B	C	D	E	F
1						
2		Exp 1	Exp 1	Exp 1	Exp 1	Exp 1
3		Ctrl	Extract 1	Extract 2	Extract 3	Pos
4	0	0.000	0.000	0.000	0.000	0.000
5	1	0.011	0.011	0.005	0.007	0.005
6	2	0.030	0.020	0.004	0.020	0.004
7	3	0.035	0.025	0.010	0.032	0.005
8	4	0.052	0.040	0.012	0.047	0.008
9	5	0.140	0.070	0.008	0.090	0.009
10	6	0.220	0.120	0.007	0.175	0.011
11	7	0.320	0.155	0.014	0.220	0.003
12	8	0.420	0.220	0.020	0.301	0.005
13	24	0.588	0.340	0.072	0.400	0.008



11) Repeat the formation of this summary table for each of the other worksheets (one table per experiment).
12) Use Excel to calculate the average and SD for every timepoint and treatment, over all four experiments.
13) You can use this data to prepare a line chart to insert into your dissertation with error bars showing the SD values.
14) In this example, the chart suggests that the tested extracts may affect the rate of bacterial growth, but it is not clear if these results are statistically significant.
15) We should test this using a two way repeated measures ANOVA.

Arranging the data for analysis in Jamovi

1) Create a new worksheet in your Excel data analysis file, then copy and paste as values all the data tables from each of the four experiments so that they are all side by side with no gaps.
2) The new data table containing the results from all the experiments should now look something like this:

		Exp 1	Exp 1	Exp 1	Exp 1	Exp 1	Exp 2	Exp 2	Exp 2	Exp 2	Exp 2	Exp 3	Exp 3	Exp 3	Exp 3	Exp 3	Exp 4	Exp 4	Exp 4	Exp 4	Exp 4
		Ctrl	Extract 1	Extract 2	Extract 3	Pos	Ctrl	Extract 1	Extract 2	Extract 3	Pos	Ctrl	Extract 1	Extract 2	Extract 3	Pos	Ctrl	Extract 1	Extract 2	Extract 3	Pos
0		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1		0.011	0.011	0.005	0.007	0.005	0.009	0.009	0.004	0.006	0.004	0.011	0.011	0.005	0.007	0.005	0.011	0.011	0.005	0.007	0.005
2		0.030	0.020	0.004	0.020	0.004	0.025	0.017	0.003	0.017	0.003	0.031	0.021	0.004	0.021	0.004	0.029	0.019	0.004	0.019	0.004
3		0.035	0.025	0.010	0.032	0.005	0.029	0.021	0.008	0.027	0.004	0.036	0.026	0.010	0.033	0.005	0.034	0.024	0.010	0.031	0.005
4		0.052	0.040	0.012	0.047	0.008	0.044	0.034	0.010	0.039	0.007	0.054	0.042	0.012	0.049	0.008	0.050	0.038	0.012	0.045	0.008
5		0.140	0.070	0.008	0.090	0.009	0.118	0.059	0.007	0.076	0.008	0.146	0.073	0.008	0.094	0.009	0.134	0.067	0.008	0.086	0.009
6		0.220	0.120	0.007	0.175	0.011	0.185	0.101	0.006	0.147	0.009	0.229	0.125	0.007	0.182	0.011	0.211	0.115	0.007	0.168	0.011
7		0.320	0.155	0.014	0.220	0.003	0.269	0.130	0.012	0.185	0.003	0.333	0.161	0.015	0.229	0.003	0.307	0.149	0.013	0.211	0.003
8		0.420	0.220	0.020	0.301	0.005	0.353	0.185	0.017	0.253	0.004	0.437	0.229	0.021	0.313	0.005	0.403	0.211	0.019	0.289	0.005
24		0.588	0.340	0.072	0.400	0.008	0.494	0.286	0.060	0.336	0.007	0.612	0.354	0.075	0.416	0.008	0.564	0.326	0.069	0.384	0.008

3) Note that the experiment numbers are in the top row, the treatment names are in the second row and the timepoint hours are given in the first column.
4) Now copy the whole table by pressing Ctrl and C, and then ‘Paste Special’ in an empty area further down the sheet choosing the options of ‘Values’ and ‘Transpose’
5) Your table should now be rotated by 90º, which is the correct orientation for use in Jamovi, as shown below:

			0	1	2	3	4	5	6	7	8	24
Exp 1	Ctrl		0.000	0.011	0.030	0.035	0.052	0.140	0.220	0.320	0.420	0.588
Exp 1	Extract 1		0.000	0.011	0.020	0.025	0.040	0.070	0.120	0.155	0.220	0.340
Exp 1	Extract 2		0.000	0.005	0.004	0.010	0.012	0.008	0.007	0.014	0.020	0.072
Exp 1	Extract 3		0.000	0.007	0.020	0.032	0.047	0.090	0.175	0.220	0.301	0.400
Exp 1	Pos		0.000	0.005	0.004	0.005	0.008	0.009	0.011	0.003	0.005	0.008
Exp 2	Ctrl		0.000	0.009	0.025	0.029	0.044	0.118	0.185	0.269	0.353	0.494
Exp 2	Extract 1		0.000	0.009	0.017	0.021	0.034	0.059	0.101	0.130	0.185	0.286
Exp 2	Extract 2		0.000	0.004	0.003	0.008	0.010	0.007	0.006	0.012	0.017	0.060
Exp 2	Extract 3		0.000	0.006	0.017	0.027	0.039	0.076	0.147	0.185	0.253	0.336
Exp 2	Pos		0.000	0.004	0.003	0.004	0.007	0.008	0.009	0.003	0.004	0.007
Exp 3	Ctrl		0.000	0.011	0.031	0.036	0.054	0.146	0.229	0.333	0.437	0.612
Exp 3	Extract 1		0.000	0.011	0.021	0.026	0.042	0.073	0.125	0.161	0.229	0.354
Exp 3	Extract 2		0.000	0.005	0.004	0.010	0.012	0.008	0.007	0.015	0.021	0.075
Exp 3	Extract 3		0.000	0.007	0.021	0.033	0.049	0.094	0.182	0.229	0.313	0.416
Exp 3	Pos		0.000	0.005	0.004	0.005	0.008	0.009	0.011	0.003	0.005	0.008
Exp 4	Ctrl		0.000	0.011	0.029	0.034	0.050	0.134	0.211	0.307	0.403	0.564
Exp 4	Extract 1		0.000	0.011	0.019	0.024	0.038	0.067	0.115	0.149	0.211	0.326
Exp 4	Extract 2		0.000	0.005	0.004	0.010	0.012	0.008	0.007	0.013	0.019	0.069
Exp 4	Extract 3		0.000	0.007	0.019	0.031	0.045	0.086	0.168	0.211	0.289	0.384
Exp 4	Pos		0.000	0.005	0.004	0.005	0.008	0.009	0.011	0.003	0.005	0.008

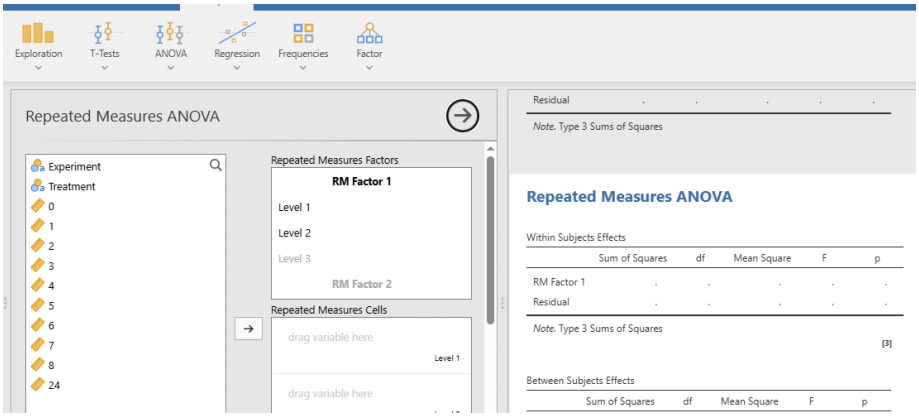
Copying the data into Jamovi

1) For additional help on how to handle data in Jamovi, refer to the earlier advice in Method sheet 25.
2) Unlike the previous format used for a standard two way ANOVA where all the results are stacked in one column, Repeated Measures in Jamovi requires the data to be arranged in a 'wide format'.
3) Column A will contain the experimental replicate number (e,g. Exp 1, Exp 2, etc.)
4) Column B will contain the names of each treatment (e.g., Control, Extract 1, etc.)
5) Column C will contain the data from the first time point, which was t=0.
6) Column D will contain the data from the second time point, which was t=1, and so on in subsequent columns until the final timepoint (typically t=24).
7) Copy the whole table except for the first row containing the time point values, and paste it into cell A1 of Jamovi.
8) Rename the heading for column A to be 'Experiment'
9) Rename the heading for column B to be 'Treatment'
10) Rename the headings for the subsequent columns to be the respective time point numbers (i.e. 0, 1, 2, ... 24).
11) While renaming the column headings, make sure the data type for the first two columns is 'Nominal' and for all the others 'Continuous' (be careful, the zero time column may automatically be incorrectly assumed by Jamovi to be Nominal).
12) Your table should now look like this in Jamovi:

	Experiment	Treatment	0	1	2	3	4	5	6	7	8
1	Exp 1	Ctrl	0	0.011	0.030	0.035	0.052	0.140	0.220	0.320	0.420
2	Exp 1	Extract 1	0	0.011	0.020	0.025	0.040	0.070	0.120	0.155	0.220
3	Exp 1	Extract 2	0	0.005	0.004	0.010	0.012	0.008	0.007	0.014	0.020
4	Exp 1	Extract 3	0	0.007	0.020	0.032	0.047	0.090	0.175	0.220	0.301
5	Exp 1	Pos	0	0.005	0.004	0.005	0.008	0.009	0.011	0.003	0.005
6	Exp 2	Ctrl	0	0.009	0.025	0.029	0.044	0.118	0.185	0.269	0.353
7	Exp 2	Extract 1	0	0.009	0.017	0.021	0.034	0.059	0.101	0.130	0.185
8	Exp 2	Extract 2	0	0.004	0.003	0.008	0.010	0.007	0.006	0.012	0.017
9	Exp 2	Extract 3	0	0.006	0.017	0.027	0.039	0.076	0.147	0.185	0.253
10	Exp 2	Pos	0	0.004	0.003	0.004	0.007	0.008	0.009	0.003	0.004
11	Exp 3	Ctrl	0	0.011	0.031	0.036	0.054	0.146	0.229	0.333	0.437
12	Exp 3	Extract 1	0	0.011	0.021	0.026	0.042	0.073	0.125	0.161	0.229
13	Exp 3	Extract 2	0	0.005	0.004	0.010	0.012	0.008	0.007	0.015	0.021
14	Exp 3	Extract 3	0	0.007	0.021	0.033	0.049	0.094	0.182	0.229	0.313
15	Exp 3	Pos	0	0.005	0.004	0.005	0.008	0.009	0.011	0.003	0.005
16	Exp 4	Ctrl	0	0.011	0.029	0.034	0.050	0.134	0.211	0.307	0.403
17	Exp 4	Extract 1	0	0.011	0.019	0.024	0.038	0.067	0.115	0.149	0.211
18	Exp 4	Extract 2	0	0.005	0.004	0.010	0.012	0.008	0.007	0.013	0.019
19	Exp 4	Extract 3	0	0.007	0.019	0.031	0.045	0.086	0.168	0.211	0.289
20	Exp 4	Pos	0	0.005	0.004	0.005	0.008	0.009	0.011	0.003	0.005

Performing the two way repeated measures ANOVA

1) After naming all of the column headings correctly, click on the 'Analyses' tab at the very top ribbon, then click the 'ANOVA' button at the top of the page and select 'Repeated Measures ANOVA'
2) A new interface appears which should look like this:



3) In the ‘Repeated Measures Factors’ box, double click on the text saying ‘RM Factor 1’, and rename it to ‘Time’.

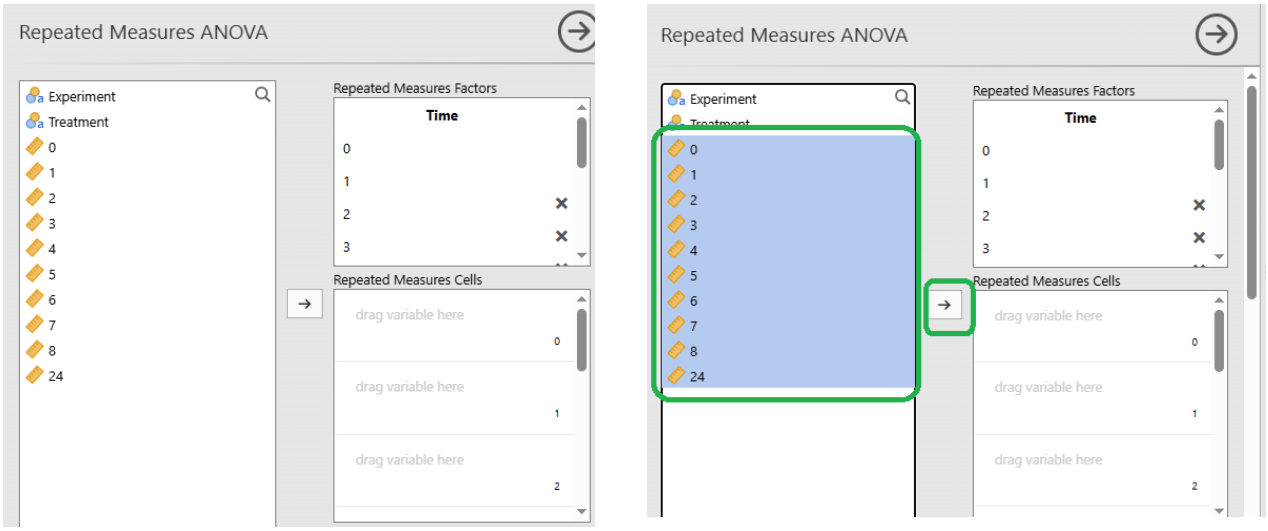
4) Within the same box, just below where it now says ‘Time’, rename the first level by double clicking on the ‘Level 1’ text, and typing the exact name of the first timepoint column heading, which should be ‘0’, then hit enter.

5) Then rename ‘Level 2’ to ‘1’, and hit enter.

6) A new level will automatically appear at the bottom of the list, rename this too with the next timepoint and repeat until you have added the names of all of the column headings in this levels box (i.e. 0, 1, 2, ... 24).

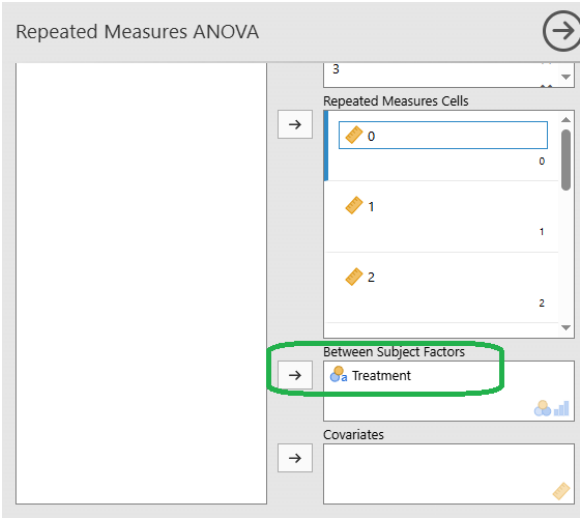
7) Note that these ‘Level’ names should match exactly the time column headings.

8) You should now see that the ‘Repeated Measures Cells’ box has all of these labels (i.e. 0, 1, 2, ... 24) in the right hand side of each row, as shown in the left hand image:



9) Now select all of the time columns in the left hand pane of the RM ANOVA dialogue box and press the right hand arrow button to drag them into the ‘Repeated Measures Cells’ section on the right hand side of the dialogue box, as shown in the right hand image.

10) Finally, drag the ‘Treatment’ column from the left hand pane into the ‘Between Subjects Factors’ box on the right (or if your screen is too small to do this easily, highlight the ‘Treatment’ column first, then scroll down the page a little and click the right hand arrow that points to the ‘Between Subjects Factors’, it should now look like this:



11) A list of p-values will appear in a new pane at the right hand side of the screen, one for each of the three main factors tested: **Treatment**, **Time** and the interaction between the two, which is **Treatment x Time**.

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Time	1.4648	9	0.1628	1492	< .001
Time ✻ Treatment	0.9541	36	0.0265	243	< .001
Residual	0.0147	135	1.09e−4		

Note. Type 3 Sums of Squares

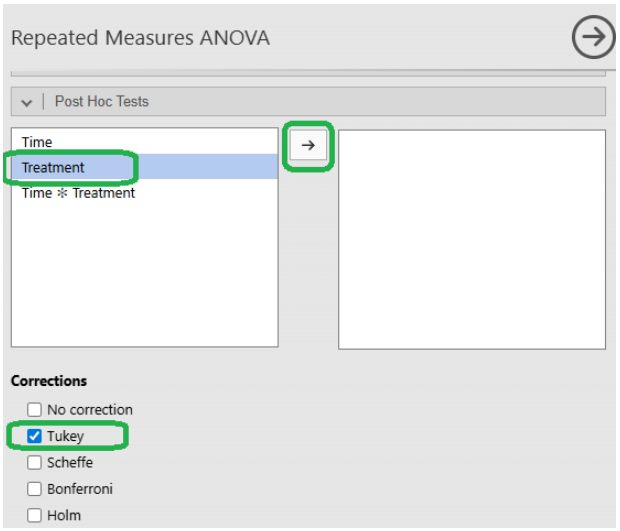
[3]

Between Subjects Effects

	Sum of Squares	df	Mean Square	F	p
Treatment	0.8352	4	0.209	234	< .001
Residual	0.0134	15	8.92e−4		

Note. Type 3 Sums of Squares

12) The **Time** p-value simply indicates whether the bacteria grew at all in any condition.
13) The **Treatment** p-value ignores any impact of time, and just tests whether there were any differences in the amount of growth overall for any of the treatments.
14) The **Treatment x Time** p-value is the one that matters most, as it gives an indication of whether the rate or pattern of growth changed over time in a manner that was different between the different treatments.
15) You will report all three of these p-values in your dissertation, but if the third one (Treatment x Time) gives a significant p-value, you will want to find out which extract(s) in particular significantly slow bacterial growth relative to the control condition.
16) To do this, you must perform a post-hoc test.
17) Scroll further down in the RM ANOVA dialogue box, and click on the Post-hoc test sub-menu.
18) Then click on the ‘Treatment’ heading and the right arrow button to move it into the right hand empty box.
19) Further down, select the ‘Tukey’ option for the correct type of post-test.



20) A table of new p-values from the post-hoc tests appears on the right hand side of the screen, it should look something like this:

Post Hoc Tests

Post Hoc Comparisons - Treatment

Comparison							
Treatment		Treatment	Mean Difference	SE	df	t	p_{tukey}
Ctrl	-	Extract 1	0.07823	0.00668	15.0	11.71	< .001
	-	Extract 2	0.15978	0.00668	15.0	23.92	< .001
	-	Extract 3	0.05030	0.00668	15.0	7.53	< .001
	-	Pos	0.16877	0.00668	15.0	25.27	< .001
Extract 1	-	Extract 2	0.08155	0.00668	15.0	12.21	< .001
	-	Extract 3	−0.02792	0.00668	15.0	−4.18	.006
	-	Pos	0.09055	0.00668	15.0	13.56	< .001
Extract 2	-	Extract 3	−0.10948	0.00668	15.0	−16.39	< .001
	-	Pos	0.00900	0.00668	15.0	1.35	.668
Extract 3	-	Pos	0.11847	0.00668	15.0	17.74	< .001

21) The p-values in the right hand column opposite the ‘Ctrl’ label in the left hand column (shown in green highlight) will tell you which extracts significantly slowed bacterial growth (i.e. $p<0.05$) and those that did not (if $p>0.05$).

22) The p-values shown in the green highlight in this example are all comparing the results against the control condition, which is the only comparison you should report in your dissertation, so you do not have to take a note of the other p-values.

Notes

- If you are struggling with the basics of ANOVAs in general, or how to input and handle data in Jamovi, please see the earlier advice given in Method sheet 25.
- In your post-hoc table, the positive control (e.g. Ampicillin) should ideally show a p-value of < 0.001 against the DMSO control - this acts as a 'quality check' to prove that the assay was sensitive enough to detect growth inhibition.