Method Sheet 32

Statistical analysis of antibiotic enhancement data

Overview

This method sheet explains how to perform a statistical analysis of data from experiments testing whether natural extracts can enhance the anti-bacterial activity of established antibiotics. A two way ANOVA is the most appropriate statistical test to analyse this data, which is easily conducted using Jamovi software.

Background correction of data

1) Copy and paste the data from the primary plate reads at both timepoints into a single Excel file, with the data from each experiment within its own, separate worksheet.
2) For example, if you have completed 4 experiments, your Excel file will have 4 worksheets and each sheet will have 2 tables of data in 96-well plate format.
3) Perform a background correction of the data by subtracting the t=0 values (i.e. the plate measurement at 0 h) from the 24 h 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 2 replicate wells for each treatment combination.
7) Collate the data from each experiment so that you have a single row for the DMSO supplemented data values and a separate single row for the Extract supplemented data values (i.e. each of the values in this table will be the means of measurements of the 2 replicate wells of one treatment on one plate).
8) Arrange the data so that you have results from all 4 experiments in one table, it should now look something like this:

3		0	0.5	1	2	4	8	16	32	64	128	
4	DMSO	0.590	0.580	0.554	0.480	0.320	0.201	0.051	0.004	0.003	0.000	Exp 1
5	Extract	0.522	0.475	0.330	0.220	0.044	0.003	0.004	0.000	0.001	0.001	Exp 1
6	DMSO	0.502	0.493	0.471	0.408	0.272	0.171	0.043	0.004	0.003	0.000	Exp 2
7	Extract	0.444	0.404	0.281	0.187	0.037	0.003	0.003	0.000	0.001	0.001	Exp 2
8	DMSO	0.620	0.609	0.582	0.504	0.336	0.211	0.054	0.000	0.000	0.003	Exp 3
9	Extract	0.548	0.499	0.347	0.231	0.046	0.003	0.004	0.003	0.001	0.001	Exp 3
10	DMSO	0.649	0.638	0.609	0.528	0.352	0.221	0.056	0.001	0.002	0.001	Exp 4
11	Extract	0.574	0.523	0.363	0.242	0.048	0.003	0.004	0.001	0.000	0.004	Exp 4

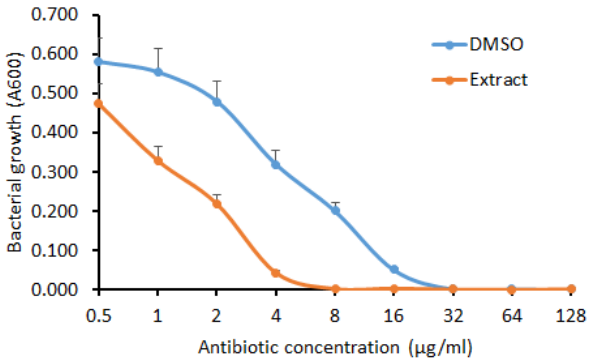
9) Calculate the mean and SD of each set of 4 replicates using formulae similar to:

`=AVERAGE(B4,B6,B8,B10)` `=STDEV(B4,B6,B8,B10)`

10) Adjust these formulae to match where your data are located in the sheet, the resulting table of means and SD values should look something like this:

14		0	0.5	1	2	4	8	16	32	64	128
15	DMSO	0.590	0.580	0.554	0.480	0.320	0.201	0.051	0.002	0.002	0.001
16	Extract	0.522	0.475	0.330	0.220	0.044	0.003	0.004	0.001	0.001	0.002
17	SD1	0.064	0.063	0.060	0.052	0.035	0.022	0.006	0.002	0.001	0.001
18	SD2	0.056	0.051	0.036	0.024	0.005	0.000	0.000	0.001	0.000	0.002

11) Plot a scatter plot of the two dose response curves using these mean and SD values - it should now look something this:



12) In this experiment, it seems likely that the antibiotic is more active in the presence of extract in comparison to when in the presence of DMSO only, but to check if this is statistically significant, we should perform two statistical tests - a Student's T-test of the MIC values and a two way ANOVA of the raw absorbance values.

13) Don't worry if your lines do not diverge as clearly as this or not at all, your results are just as valid if you can show that there is no enhancement activity of the extract.

Using a Student's T-test to analyse MIC data

1) Prepare a chart, similar to the one shown above, for each of the four individual experiments (but lacking error bars).

2) For each experiment (i.e. each plate), use the chart to find the lowest concentration of antibiotic that completely blocks bacterial growth, in other words, the point at which the line meets the x-axis.

3) This value is called the **Minimum Inhibitory Concentration** (MIC).

4) In the example chart shown above, the MIC for antibiotic in the presence of DMSO is 32 µg/ml, and the MIC for antibiotic in the presence of Extract is 8 µg/ml.

5) Do the same for both the DMSO and Extract treatments for all 4 plates.

6) You should now have 4 measurements of MIC for DMSO and 4 measurements for extract, which you should arrange in two separate columns in Excel.

7) Follow the advice given in Method Sheet 45 to use a **Student's T-test** to compare the means of the data in these two columns.

8) If the p-value for this comparison is below 0.05, we can say that the extract significantly lowers the MIC of the antibiotic, which is consistent with enhancement of antibiotic activity.

Analysis of antibiotic enhancement data by two way ANOVA

1) Follow the advice in Method Sheet 25 for how to arrange the data for analysis by two way ANOVA using Jamovi - the data table you have for this experiment can be handled in a very similar way to that shown for the previous example.
2) Record the initial p-values of the test for the effects of Treatment (DMSO or extract), Concentration (of antibiotic) and the Interaction (Concentration x Treatment).
3) You will report these p-values in your dissertation - any that are below 0.05 should be considered significant.
4) If the p-value for the Concentration x Treatment test is below 0.05, this indicates that the extract significantly enhances the activity of the antibiotic.
5) Continue following the advice in Method Sheet 25 to perform a Tukey's post-hoc test on the data, again choosing the Concentration x Treatment interaction for comparison.
6) This additional test will give you a table of p-values which will show which specific concentrations the Extract curve is significantly lower than the DMSO curve, which you can use to generate any stars necessary to add to the dose response chart in your dissertation.

Notes

- Don't worry if your extract shows no evidence of enhancing the activity of the antibiotic, your results are just as valid with this type of result and will not affect your mark so long as you complete all of the experimental and analytical work to a high standard.