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Practical Protocol 03 – Non-parametric tests
Biostatistics, M1 Biology
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Mann–Whitney U test

1. Objective

The aim of this section is to compare two independent groups using the nonparametric Mann–Whitney U test in SPSS.

2. Biological Context

A research team studied the effect of two organic soil treatments (A) and (B) on plant dry biomass (g).

Data:

- Treatment A: 2, 6, 9, 10, 12, 15, 18 ($n = 7$)
- Treatment B: 4, 7, 11, 14, 16, 17, 19, 20 ($m = 8$)

3. Hypotheses

H_0 : The two distributions are identical

H_1 : The two distributions are different

4. Step 1: Data Entry in SPSS

- Open SPSS
- Create two variables:
 - **Biomass**
 - **Groups (treatments)**
- Code the groups:
 - 1 = Treatment A
 - 2 = Treatment B

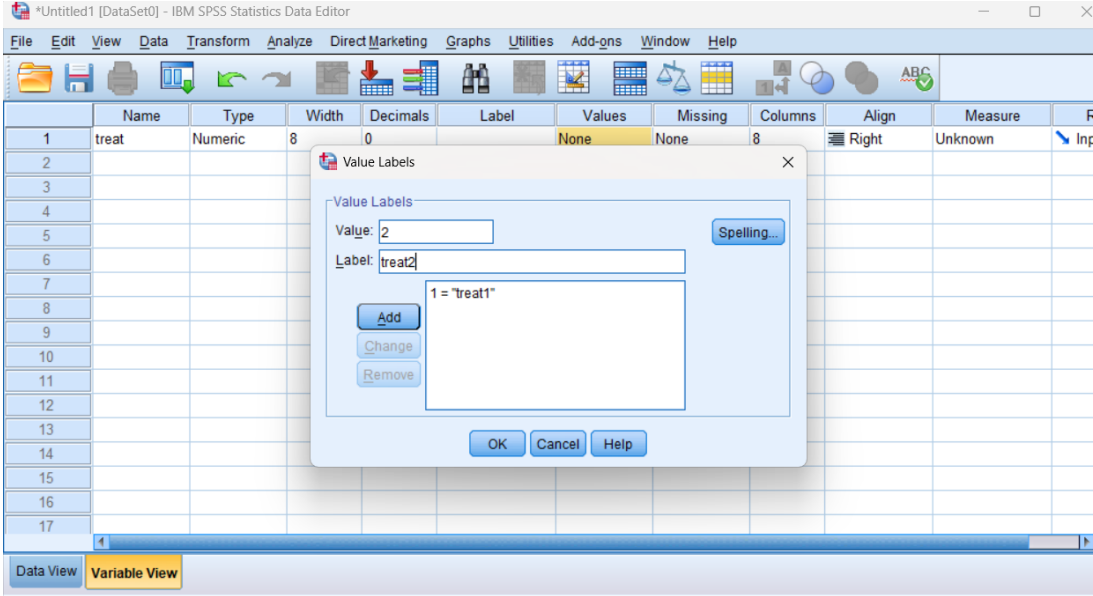


Figure 1: Create two variables

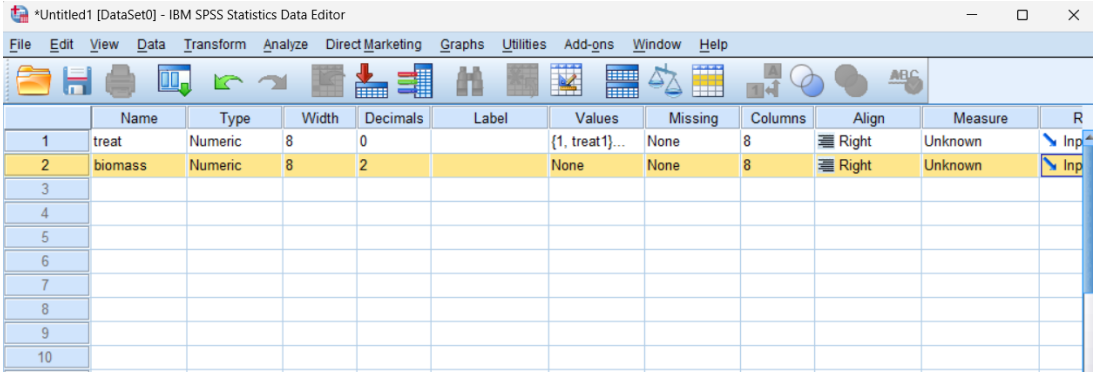


Figure 2: Create two variables

15 : treat		2
	treat	biomass
1	treat1	.
2	treat1	.
3	treat1	.
4	treat1	.
5	treat1	.
6	treat1	.
7	treat1	.
8	treat2	.
9	treat2	.
10	treat2	.
11	treat2	.
12	treat2	.
13	treat2	.
14	treat2	.
15	treat2	.
16	treat1	.
17	treat2	.

Figure 3: Data view

	treat	biomass	
1	treat1	2,00	
2	treat1	6,00	
3	treat1	9,00	
4	treat1	10,00	
5	treat1	12,00	
6	treat1	15,00	
7	treat1	18,00	
8	treat2	4,00	
9	treat2	7,00	
10	treat2	11,00	
11	treat2	14,00	
12	treat2	16,00	
13	treat2	17,00	
14	treat2	19,00	
15	treat2	20,00	
16			

Figure 4: Data view

5. Step 2: Running the Test

- Click: **Analyze**

- Then: **Nonparametric Tests**
- Then: **Legacy Dialogs**
- Choose: **2 Independent Samples**

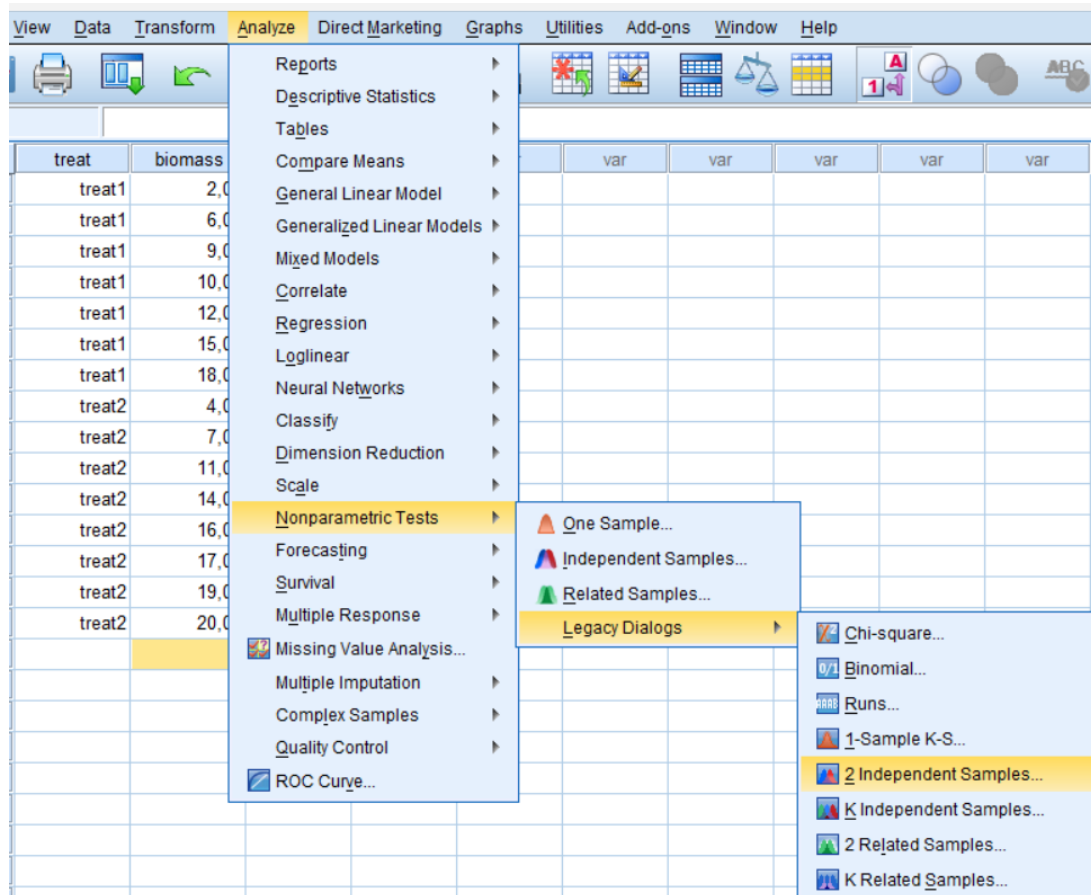


Figure 5: Running the Test

- Move **Biomass** to Test Variable
- Move **Treat** to Grouping Variable
- Click **Define Groups**
- Enter:
 - treat 1: 1
 - treat 2: 2

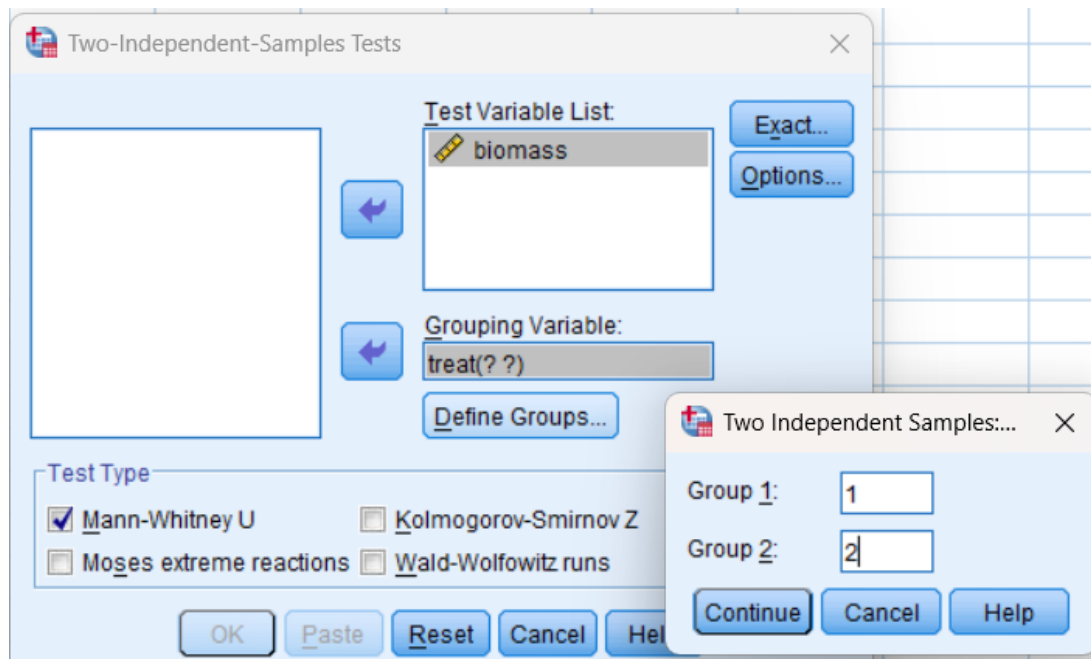


Figure 6: Running the Test

- Select **Mann–Whitney U**
- Click **OK**

6. Step 3: Output Interpretation

SPSS provides:

- Mann–Whitney U statistic
- p-value (Asymp. Sig.)

Mann-Whitney Test

treat		N	Mean Rank	Sum of Ranks
biomass	treat1	7	6,57	46,00
	treat2	8	9,25	74,00
	Total	15		

	biomass
Mann-Whitney U	18,000
Wilcoxon W	46,000
Z	-1,157
Asymp. Sig. (2-tailed)	,247
Exact Sig. [2*(1-tailed Sig.)]	,281 ^b

a. Grouping Variable: treat

b. Not corrected for ties.

Figure 7: Output tables from SPSS

7. Decision Rule

- If $p \leq 0.05 \Rightarrow$ Reject H_0
- If $p > 0.05 \Rightarrow$ Fail to reject H_0
where $p =$ Asymp. Sig.

Remark. The Exact Sig. value is also two-tailed. It provides a more accurate result for small samples.

8. Interpretation

Since $p = 0.247 > 0.05$, we fail to reject H_0 , so there is no significant difference between treatments A and B.

9. Conclusion

Final conclusion in biological terms : At the 5% significance level, there is no statistically significant difference in plant biomass between the two soil treatments.

Wilcoxon Signed-Rank Test

1. Objective

The aim of this section is to compare two **related measurements** using the nonparametric **Wilcoxon Signed-Rank Test** in SPSS.

2. Biological Context

A biologist studies the effect of a fertilizer on the growth of tomato plants. The height of the plants (in cm) is measured **before** and **after** the application of the fertilizer.

Data:

Plant	Before	After
1	12	15
2	10	13
3	14	14
4	9	11
5	13	17
6	11	12

3. Hypotheses

Since the measurements are paired, the Wilcoxon signed-rank test is appropriate.

H_0 : there is no difference between the measurements before and after treatment

H_1 : there is a difference between the measurements before and after treatment

4. Why this test?

This test is used when:

- the two samples are **dependent** or **paired**,
- the data do not necessarily follow a normal distribution,
- we want a nonparametric alternative to the paired-samples t -test.

5. Step 1: Data entry in SPSS

Open SPSS and create two variables in **Variable View**:

- **Before**
- **After**

Then enter the measurements of the 6 plants in **Data View**.

Name	Type	Width	Decimals	Label	Values	Missing	Columns	Align	Measure	Role
before	Numeric	8	0		None	None	8	Right	Unknown	Input
after	Numeric	8	0		None	None	8	Right	Unknown	Input

Figure 8: Variable view

before	after
12	15
10	13
14	14
9	11
13	17
11	12

Figure 9: Data view

6. Step 2: Running the Wilcoxon Signed-Rank Test

Follow these steps in SPSS:

1. Click on **Analyze**
2. Select **Nonparametric Tests**
3. Choose **Legacy Dialogs**
4. Click on **2 Related Samples**

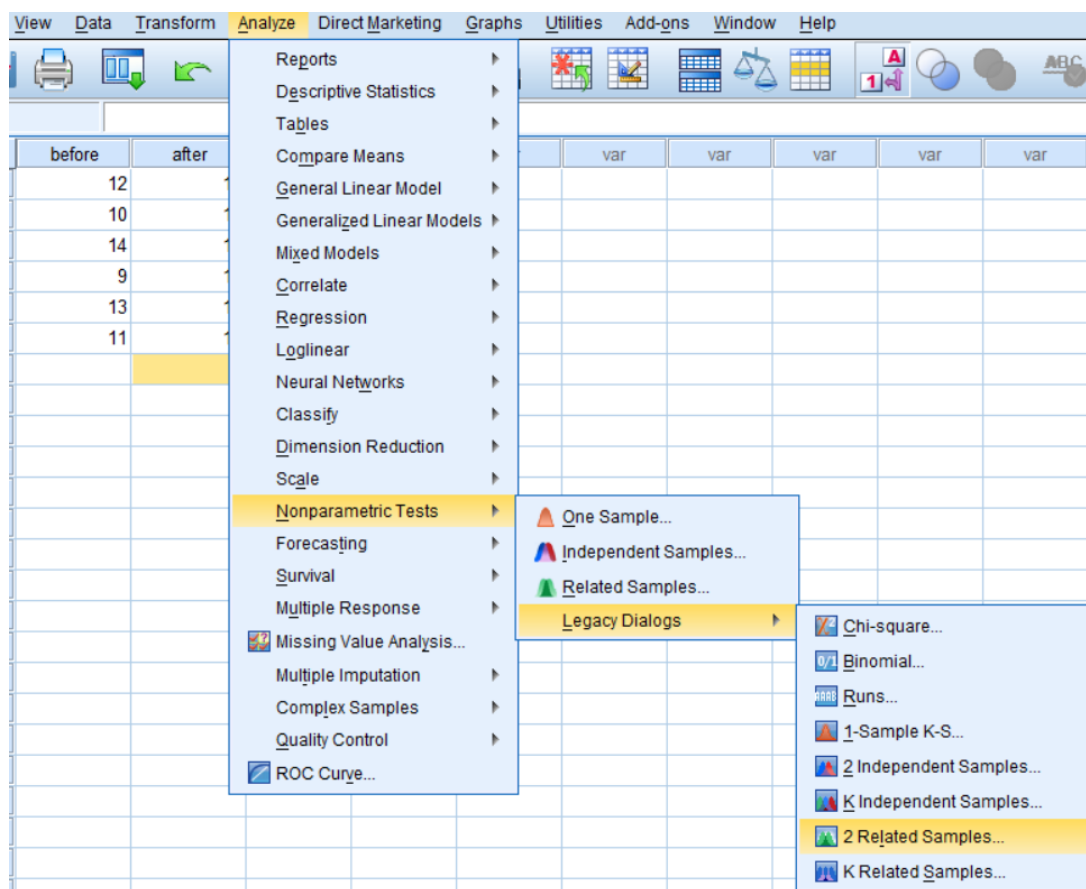


Figure 10: Running the Wilcoxon Signed-Rank Test

In the dialog box:

- Move **Before** and **After** into the test pairs box
- Select **Wilcoxon**
- Click **OK**

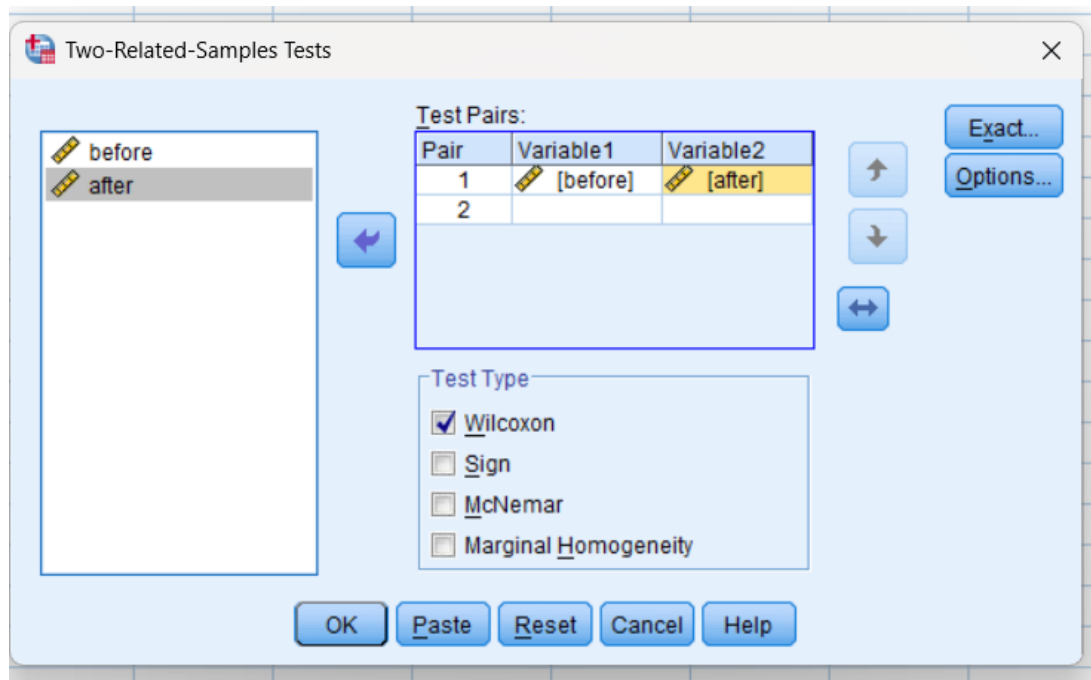


Figure 11: Running the Wilcoxon Signed-Rank Test

7. Step 3: SPSS output

SPSS usually provides:

- the number of positive ranks,
- the number of negative ranks,
- the number of ties,
- the test statistic,
- the standardized value Z ,
- the p-value (**Asymp. Sig.**).

Wilcoxon Signed Ranks Test

		Ranks		
		N	Mean Rank	Sum of Ranks
after - before	Negative Ranks	0 ^a	,00	,00
	Positive Ranks	5 ^b	3,00	15,00
	Ties	1 ^c		
	Total	6		

a. after < before

b. after > before

c. after = before

Test Statistics^a

	after - before
Z	-2,032 ^b
Asymp. Sig. (2-tailed)	,042

a. Wilcoxon Signed Ranks Test

b. Based on negative ranks.

Figure 12: SPSS output

8. Decision rule

At the significance level $\alpha = 0.05$:

- If the p-value is less than or equal to 0.05, reject H_0 .
- If the p-value is greater than 0.05, fail to reject H_0 .

9. Interpretation

Since the p-value = 0.042 is less than 0.05, then we reject H_0 .

10. Conclusion

The fertilizer significantly increases tomato plant height.

Kruskal–Wallis Test

1. Objective

The aim of this section is to compare **three independent groups** using the nonparametric **Kruskal–Wallis test** in SPSS.

2. Biological Context

We study the effect of three treatments on enzyme concentration (U/L).

Data:

Group 1 (Drug A)	Group 2 (Drug B)	Group 3 (Drug C)
27	20	34
2	8	31
4	14	3
18	36	23
7	21	30
9	22	6

3. Hypotheses

The Kruskal–Wallis test is used to compare several independent groups.

H_0 : the three groups come from identical distributions

H_1 : at least one group differs from the others

4. Why this test?

This test is appropriate when:

- we compare **more than two independent groups**,
- the data are ordinal or quantitative but do not satisfy the normality assumption,
- we want a nonparametric alternative to one-way ANOVA.

5. Step 1: Data entry in SPSS

Open SPSS and create two variables in **Variable View**:

- **Enzyme**
- **treat**

Use the following coding for the groups:

- Group = 1 for Drug A
- Group = 2 for Drug B
- Group = 3 for Drug C

Then enter all observations in **Data View**.

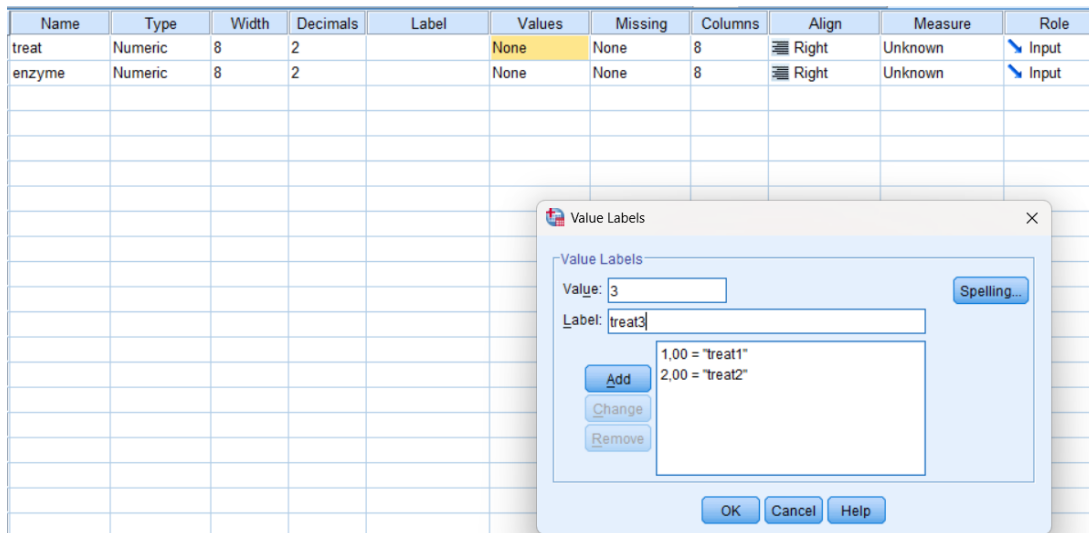


Figure 13: Variable view

treat	enzyme
treat1	27,00
treat1	2,00
treat1	4,00
treat1	18,00
treat1	7,00
treat1	9,00
treat2	20,00
treat2	8,00
treat2	14,00
treat2	36,00
treat2	21,00
treat2	22,00
treat3	34,00
treat2	21,00

6. Step 2: Running the Kruskal–Wallis Test

Follow these steps in SPSS:

1. Click on **Analyze**
2. Select **Nonparametric Tests**
3. Choose **Legacy Dialogs**
4. Click on **K Independent Samples**

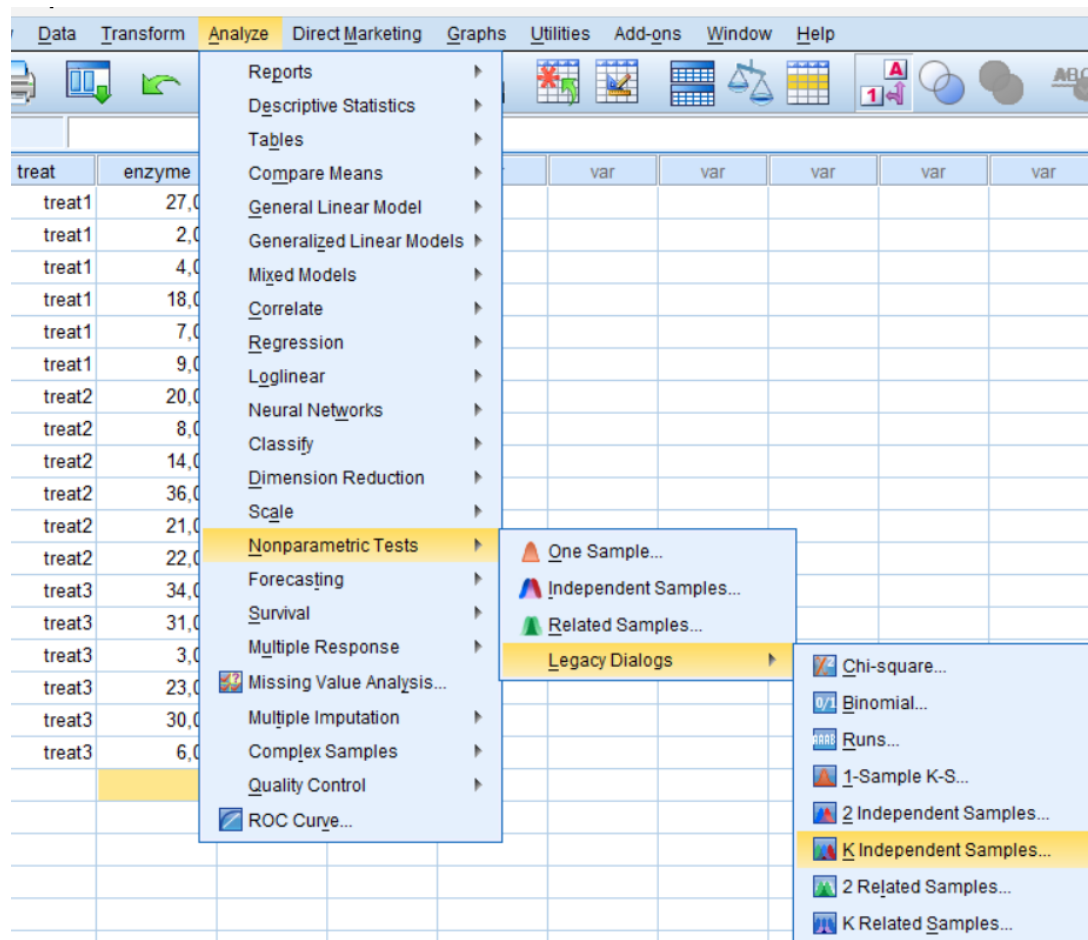


Figure 15: Running the Kruskal–Wallis Test

In the dialog box:

- Move **Enzyme** to **Test Variable List**
- Move **treat** to **Grouping Variable**
- Click on **Define Range**
- Enter:
 - Minimum = 1

- Maximum = 3
- Select **Kruskal–Wallis H**
- Click **OK**

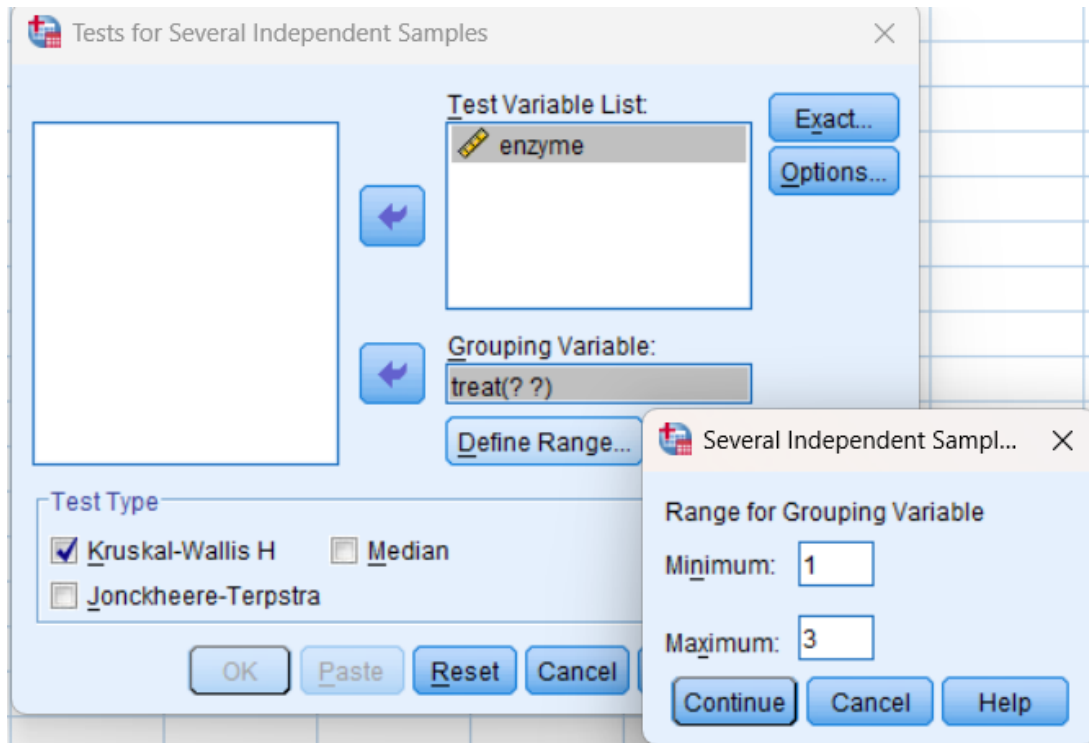


Figure 16: Running the Kruskal–Wallis Test

7. Step 3: SPSS output

SPSS generally provides:

- the number of observations in each group,
- the mean rank for each group,
- the Kruskal–Wallis test statistic H ,
- the degrees of freedom,
- the p-value (**Asymp. Sig.**).

Kruskal-Wallis Test

Ranks

treat		N	Mean Rank
enzyme	treat1	6	6,50
	treat2	6	10,83
	treat3	6	11,17
	Total	18	

Test Statistics^{a,b}

	enzyme
Chi-Square	2,854
df	2
Asymp. Sig.	,240

a. Kruskal Wallis
Test

b. Grouping
Variable: treat

Figure 17: SPSS output

8. Interpretation

Since the p-value is greater than 0.05, then we fail to reject H_0 .

9. Conclusion

At the 5% significance level, there is no statistically significant difference in enzyme concentration among the three treatments.

Friedman Test

1. Objective

The aim of this section is to compare **three related measurements** using the nonparametric **Friedman test** in SPSS.

2. Biological Context

A biologist wants to study the evolution of the concentration of a protein in blood after applying an experimental treatment. The same 6 laboratory mice are followed for 3 consecutive weeks.

The measured concentrations are given below.

Week 1	Week 2	Week 3
27	20	34
2	8	31
4	14	3
18	36	23
7	21	30
9	22	6

We want to know whether there is a significant difference between Weeks 1, 2, and 3 at the significance level

$$\alpha = 0.05.$$

3. Hypotheses

Since the same subjects are measured repeatedly over time, the Friedman test is appropriate.

H_0 : there is no difference between Week 1, Week 2, and Week 3

H_1 : at least one week differs from the others

4. Why this test?

This test is used when:

- the samples are **related** or **paired**,
- the same subjects are observed under several conditions or times,
- the data do not necessarily satisfy the normality assumption,
- we want a nonparametric alternative to repeated measures ANOVA.

5. Step 1: Data entry in SPSS

Open SPSS and create three variables in **Variable View**:

- **Week1**
- **Week2**
- **Week3**

Then enter the measurements of the 6 mice in **Data View**, one mouse per row.

Name	Type	Width	Decimals	Label	Values	Missing	Columns	Align	Measure	Role
week1	Numeric	8	2		None	None	8	Right	Scale	Input
week2	Numeric	8	2		None	None	8	Right	Scale	Input
week3	Numeric	8	2		None	None	8	Right	Scale	Input

Figure 18: Variable View

	week1	week2	week3
	27,00	20,00	34,00
	2,00	8,00	31,00
	4,00	14,00	3,00
	18,00	36,00	23,00
	7,00	21,00	30,00
	9,00	22,00	6,00

Figure 19: Data View

6. Step 2: Running the Friedman Test

Follow these steps in SPSS:

1. Click on **Analyze**
2. Select **Nonparametric Tests**
3. Choose **Legacy Dialogs**
4. Click on **K Related Samples**

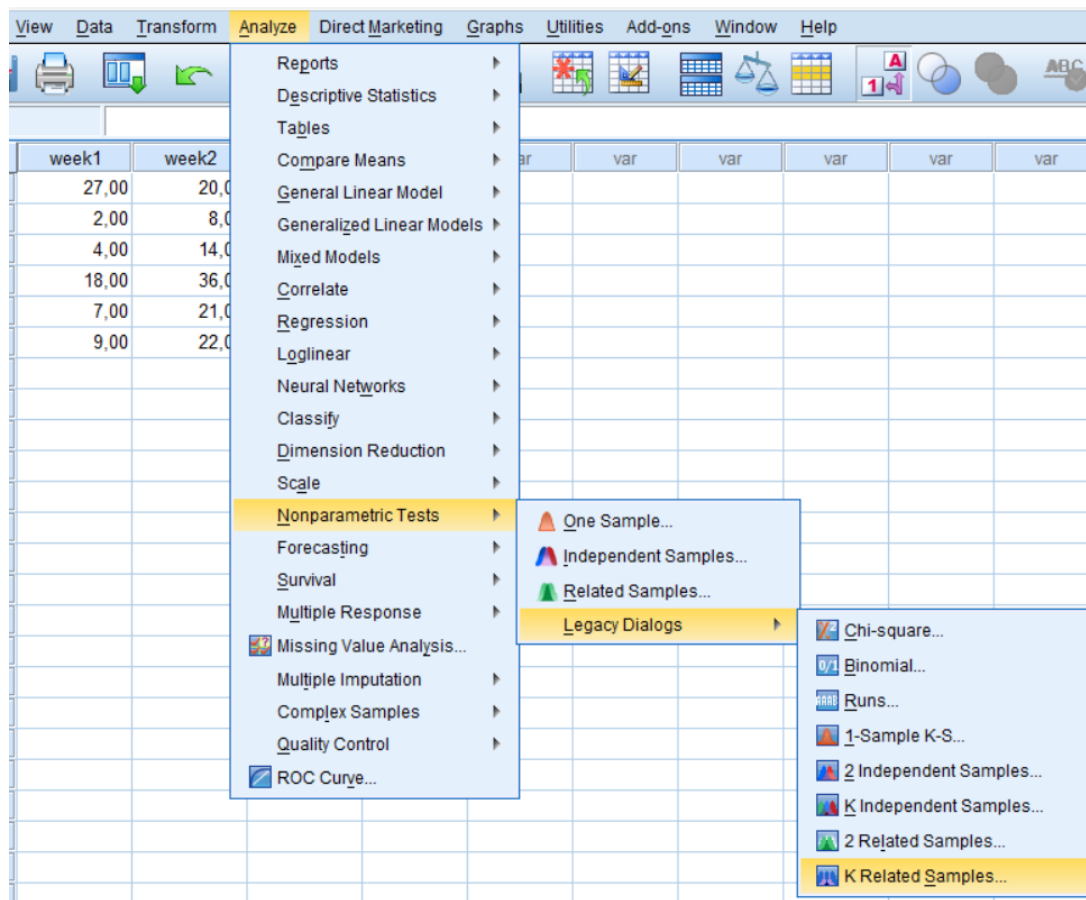


Figure 20: Running the Friedman Test

In the dialog box:

- Move **Week1**, **Week2**, and **Week3** into the **Test Variables** box
- Select **Friedman**
- Click **OK**

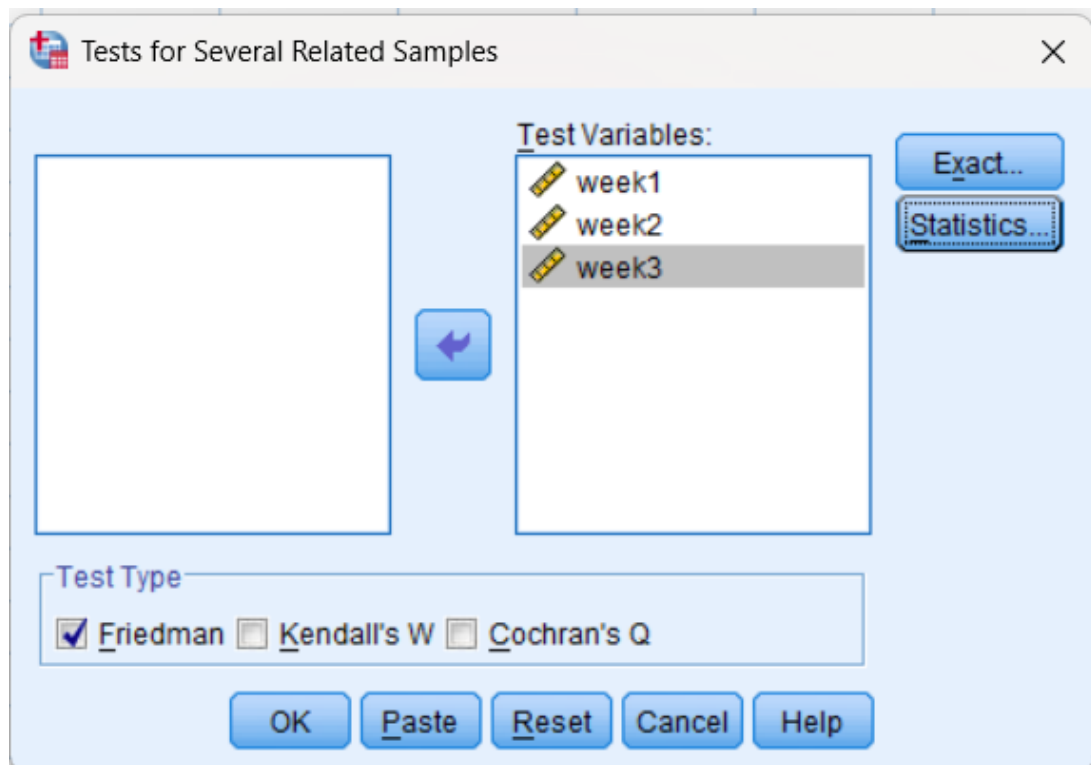


Figure 21: Running the Friedman Test

7. Step 3: SPSS output

SPSS generally provides:

- the number of subjects,
- the mean rank for each week,
- the Friedman chi-square statistic,
- the degrees of freedom,
- the p-value (**Asymp. Sig.**).

Friedman Test

Ranks

	Mean Rank
week1	1,50
week2	2,33
week3	2,17

Test Statistics^a

N	6
Chi-Square	2,333
df	2
Asymp. Sig.	,311

a. Friedman Test

10. Interpretation

Since the p-value is greater than 0.05, then we fail to reject H_0 .

11. Conclusion

No significant change in protein concentration was detected over the three weeks.