National Institute of Allergy and Infectious Diseases

Practical Training on GraphPad Prism for Statistical Testing

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Outline

Dependent variable is Continuous

- Unpaired t-test vs paired t-test vs Mann-Whitney test
- One-way ANOVA vs Repeated measures one-way ANOVA vs Kruskal-Wallis and Friedman tests
  - Multiple comparison

Dependent variable is Categorical

- Chi-square test vs Fisher exact test

T-test and related nonparametric - Overall

T tests, and related nonparametric tests compare **two sets** of measurements (data expressed using an interval or ratio scale).

- Paired or not paired?
- Parametric or nonparametric?
- Equal standard deviation or not?

Checklist: unpaired t-test, paired t-test, Mann-Whitney test
Kolmogorov-Smirnov test, Wilcoxon matched pairs test…

Unpaired or paired?

Treatment A				Treatment B			
ID	preA	postA	ΔA	ID	preB	postB	ΔB
1	63	77	14	11	81	101	20
2	69	88	19	12	87	103	16
3	76	90	14	13	77	107	30
4	78	95	17	14	80	114	34
5	80	96	16	15	76	116	40
6	89	96	7	16	86	116	30
7	90	102	12	17	98	116	18
8	92	104	12	18	87	120	33
9	103	110	7	19	105	120	15
10	112	115	3	20	69	127	58

ID: individual identification, preA, preB: before the treatment A or B, postA, postB: after the treatment A or B, ΔA, ΔB: difference between before and after the treatment A or B.

- If we want to compare ΔA and ΔB, is this unpaired or paired experimental design?

Unpaired.

Example Source

Unpaired and paired? Cont

Treatment A					Treatment B			
ID	preA	postA	ΔA		ID	preB	postB	ΔB
1	63	77	14		1	73	103	30
2	69	88	19		2	74	104	30
3	76	90	14		3	76	107	31
4	78	95	17		4	84	108	24
5	80	96	16	wash out	5	84	110	26
6	89	96	7		6	86	110	24
7	90	102	12		7	92	113	21
8	92	104	12		8	95	114	19
9	103	110	7		9	103	118	15
10	112	115	3		10	115	120	5

ID: individual identification, preA, preB: before the treatment A or B, postA, postB: after the treatment A or B, ΔA, ΔB: difference between before and after the treatment A or B.

- If we want to compare ΔA and ΔB, is this unpaired or paired experimental design?

Paired.

[Read more] Experimental design: unpaired or paired

Parametric or nonparametric?

If the data in both groups **follow Gaussian** distribution: use parametric test.

Parametric test:

The **unpaired t test** compares the means of two **unmatched** groups, assuming that the values follow a Gaussian distribution.

The **paired t test** compares the means of two **matched** groups, assuming that the distribution of the before-after differences follows a Gaussian distribution.

Parametric or nonparametric? Cont

If the data does not follow Gaussian distribution?

1. Data transformation
2. Use t-test anyway, given that t-test is robust when sample size is large.
3. Use nonparametric test.

The **Mann-Whitney** and the **Kolmogorov-Smirnov** compares the distributions of two **unmatched** groups. [**Nonparametric**]

The **Wilcoxon matched pairs test** compares two **paired** groups. [**Nonparametric**]

Nonparametric tests does not require Gaussian but have less power. Deciding when to use a nonparametric test is not straightforward.

[Read more] Assume Gaussian distribution? Choose test?

Let's practice in Prism

- Use sample data *provided by Prism* for hands on
- Perform unpaired, paired t-test and related nonparametric tests.

Interpreting result:
Unpaired t-test, paired t-test, Mann-Whitney test, Kolmogorov-Smirnov test, Wilcoxon matched pair test.

One-way ANOVA and related nonparametric test

One-way ANOVA and related nonparametric test compare **three or more sets** of measurements (data expressed using an interval or ratio scale).

- Paired or not paired (Matching/Repeat Measure)?
- Parametric or nonparametric?
- Equal standard deviation or not? Sphericity?

Checklist: ordinary one-way ANOVA, RM one-way ANOVA, Kruskal-Wallis test, Friedman test…

Unpaired or paired?

A study is designed to test whether there is a difference in mean daily calcium intake in adults with normal bone density, adults with osteopenia (a low bone density which may lead to osteoporosis) and adults with osteoporosis.

Normal Bone Density	Osteopenia	Osteoporosis
1200	1000	890
1000	1100	650
980	700	1100
900	800	900
750	500	400
800	700	350

Unpaired

Example Source

Unpaired or paired? Cont

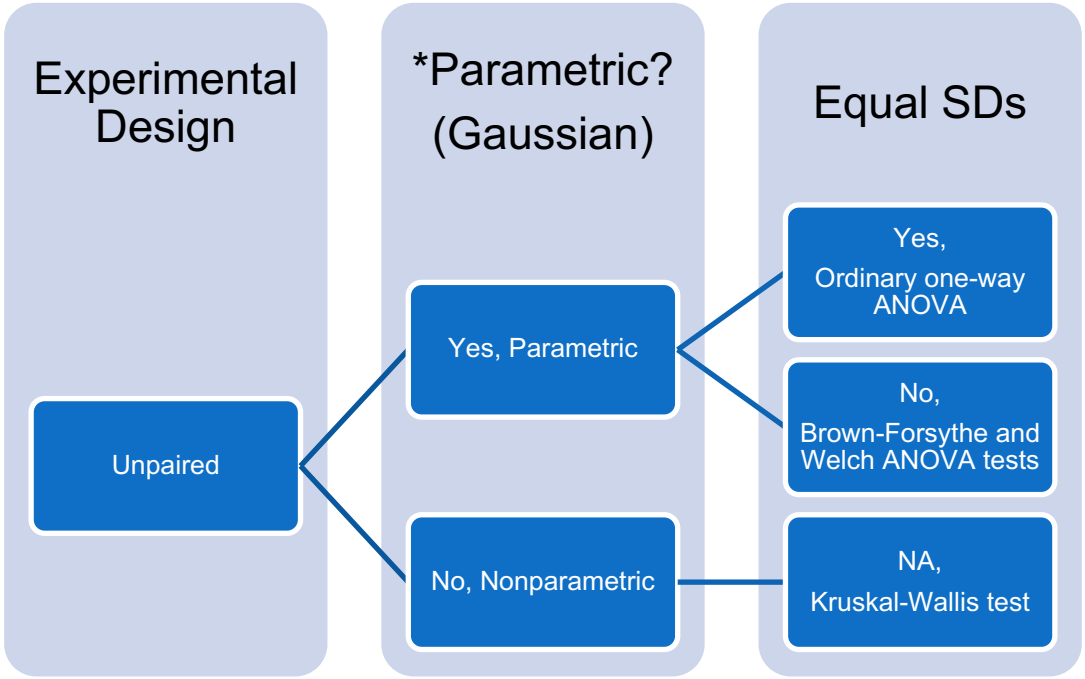
The four columns represent four sequential treatments. Each row represents a different subject (or a different set of matched data).

		Group A	Group B	Group C	Group D
		Control	Treatment 1	Treatment 2	Treatment 3
		Y	Y	Y	Y
1	GS	54	43	78	111
2	JM	23	34	37	41
3	HM	45	65	99	78
4	DR	31	33	36	35
5	PS	15	25	30	26

Paired
(Repeat Measures)

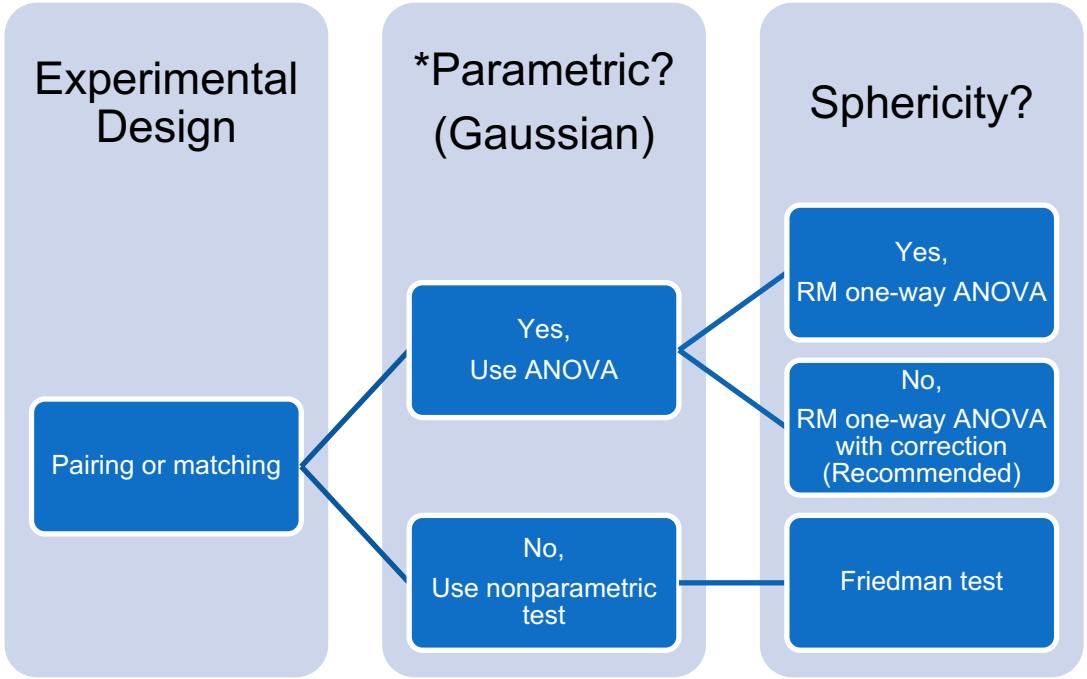
Example source: Prism 8

Ordinary one-way ANOVA and related nonparametric test



* Deciding when to use a nonparametric test is not straightforward.

Repeated Measure One-way ANOVA and related nonparametric test



Read more: 1 2

** Under this experimental design, if there is missing values, mixed effects model will be performed instead of ANOVA. Result only meaningful if the values are missing for random reasons.

Multiple Comparison

Prism provide an option of doing multiple comparison option when customizing your ANOVA analysis. It is usually noted as a post doc test in ANOVA, but however they could be performed independently.

[**Definition**] Multiple comparisons arises when a statistical analysis involves multiple simultaneous statistical tests, each of which has a potential to produce a "discovery."

[**Methods**] Multiple comparisons corrected by statistical hypothesis testing; controlling the false discovery rate, don't correct. If using a familywise definition of alpha, the significance level doesn't apply to each comparison, but rather to the entire family of comparisons. In general, this makes it harder to reach significance.

Let's practice in Prism

- Use *bone density and calcium intake data* to perform ordinary one-way ANOVA
- Use the *sample data provided by Prism* to perform RM one-way ANOVA

Interpreting result:
Ordinary one-way ANOVA, RM one-way ANOVA, mixed model one-way, Kruskal-Wallis test, Friedman test.

Challenging question

Exercise Intervention data: A 6-month exercise-training intervention where six subjects had their fitness level measured on three occasions: pre-, 3 months, and post-intervention. Their data is shown below along with some initial calculations:

Subjects	Pre-	3 Months	6 Months
1	45	50	55
2	42	42	45
3	36	41	43
4	39	35	40
5	51	55	59
6	44	49	56

Questions:

1) Is there any difference in fitness level on these three occasions?
2) If differences exist, compare group differences in 3 months intervention and 6 months intervention with the pre- group.

Test for data with categorical data

	Patient ID	Smoking status	Lung cancer
	1	Yes	Case
Smo	2	No	Control
	3	Yes	Case
Yes	4	No	Control
	5	Yes	Control
No	6	No	Control
Tota	7	No	Control
	8	No	Case
	9	Yes	Control
	...	...	...

What is the appropriate test for seeing whether smoking is associated with lung cancer in a case-control study?

Chi-square test and Fisher's exact test

Chi-square test and fisher's exact test are commonly used to access associations between **two categorical variables,** assuming individual observations are independent.

H_0: Two variables are independent. H_a: They are not independent.

In Prism, the input data should be "**contingency**" form. Prism could not cross tabulate data.

Chi-square test is usually used with **large or well** balanced sample. Fisher's exact test uses with **small data** and it calculates exact p-value.

Chi-square test OR Fisher's exact test?

Why not always Fisher's exact test?

Fisher's test is based on assuming that the row and column totals are fixed by the experiment, which is not satisfied in the design of a retrospective case-control study and a cross-sectional experiment.

In Prism, if you enter huge numbers (the sum is greater than 1,000,000), it will perform the chi-square test even if you chose Fisher's test.

Let’s practice in Prism

- Use sample data provided by Prism
- Perform Chi-square test and Fisher’s exact test to evaluate association.

Interpreting result:
Chi-square test and Fisher’s exact test

More tests for categorical data

Cochran-Armitage trend test is used for study association between a variable with **two categories and an ordinal variable with k categories** (e.g. dose-response studies).

McNemar's test is used for comparing categorical responses for two samples that are **statistically dependent**.

Cochran-Mantel-Haenszel test for the analysis of **stratified or matched** categorical data.

…….

In the end

Statistics is a lot of fun! Continue to attend our workshop in future!

We try to improve! Please finish the survey and let us know your thoughts!

Check out our Github page, more related material has been uploaded.