

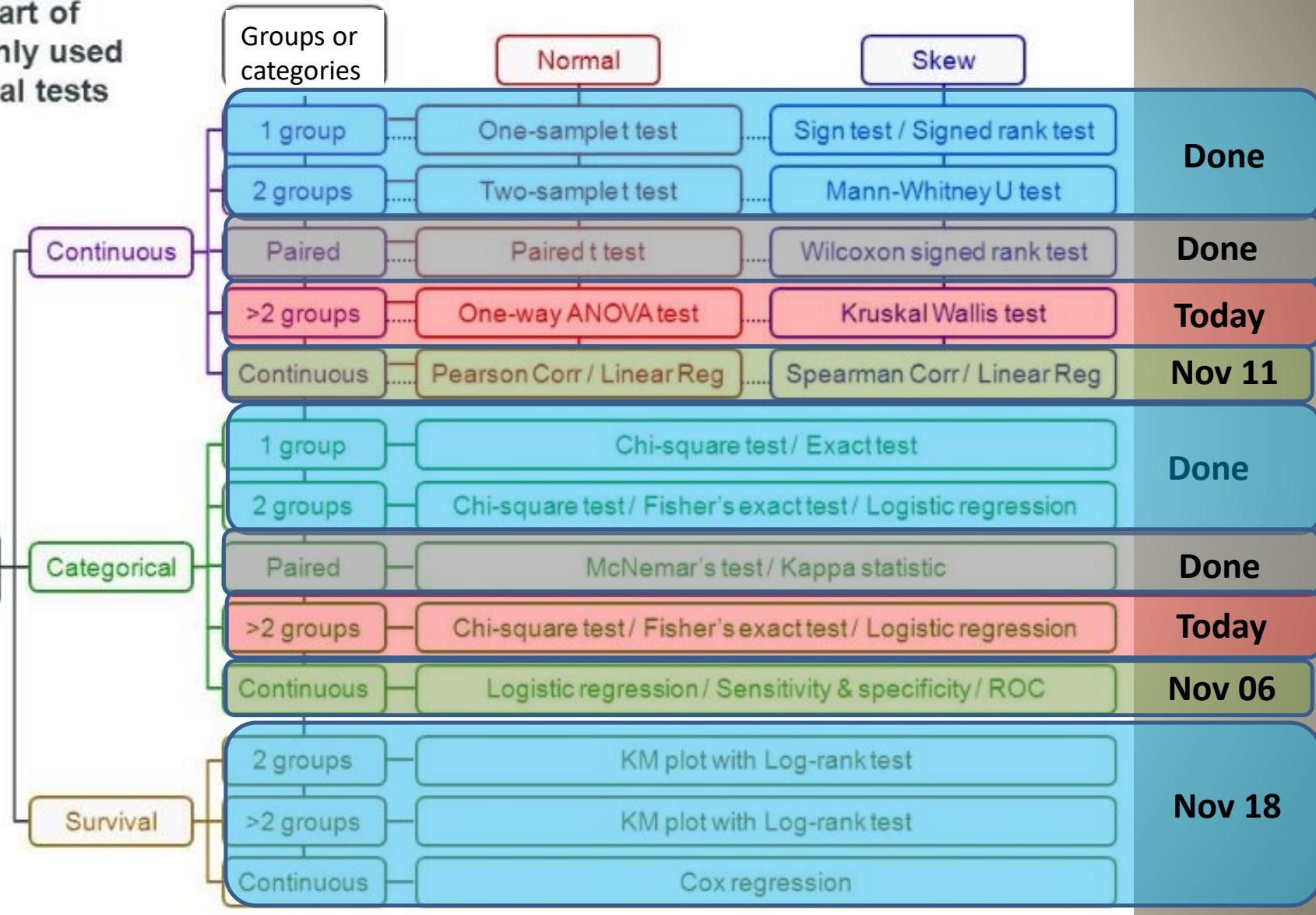
# Applied Statistics: Tests on >2 groups

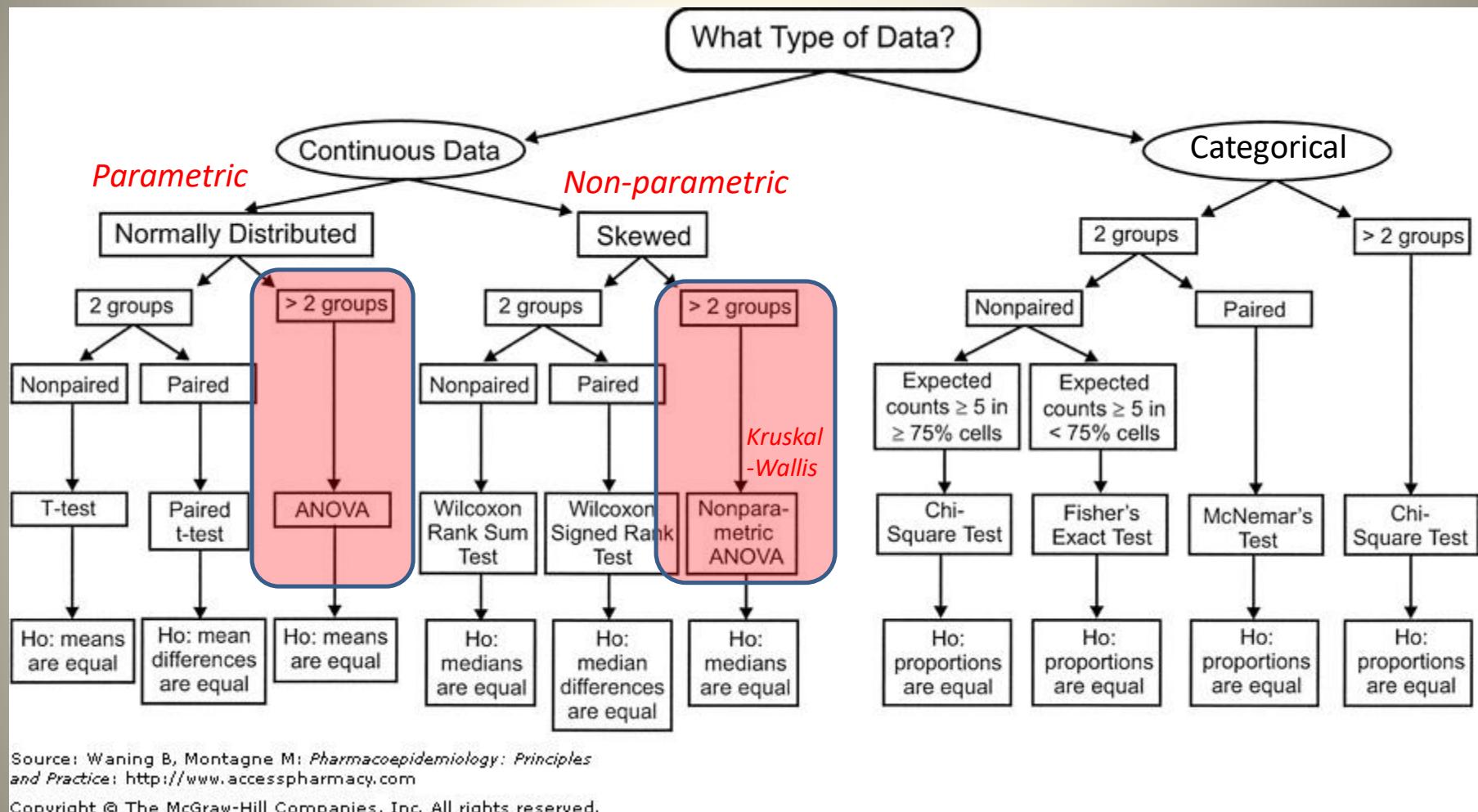
## One-way and Two-way ANOVAs

## and the Kruskal-Wallis test

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## Flow chart of commonly used statistical tests





# Objectives

- Learn the differences between and one-way and a two-way ANOVA
- Learn the assumptions of each ANOVA
- Learn how perform and interpret both ANOVAs using Prism
- Learn to use the non-parametric one-way ANOVA equivalent, the Kruskal-Wallis test

# ANOVA Basics

## ANalysis Of VAriance (ANOVA)

One categorical variable (“factor”; >2 groups)

AKA the **independent\*** variable

Type of diet (diet1, diet2, diet3)

One continuous variable

AKA the **dependent\*** variable

the variable we want to know if it differs between groups of the categorical independent variable

Weight loss

\*This terminology comes from linear regression where the independent variable “predicts” or determines levels of the dependent variable

weight loss = diet (diet predicts weight loss)

# ANOVA Family

One way ANOVA

One Categorical  
(Independent) Variable  
(AKA one factor)

One continuous  
(dependent)  
variable  
measured in  
different  
groups

Repeated  
measures /  
Within  
subjects

Different  
participants

Same  
participants

Factorial ANOVA

More than One  
Categorical Variable

Two  
way

Three  
way

Four  
way

One-way ANOVA

Weight loss by type of diet  
Extension of independent (unpaired) t-test  
for >2 groups

Two-way ANOVA

Weight loss by type of diet and gender  
Can include tests for *interaction* between  
factors

## One-way ANOVA compared to an independent t-test

A two sample t-test assuming equal variance and an ANOVA comparing only two groups will give you the exact same p-value (for a two-sided hypothesis).

$$H_0: \mu_1 = \mu_2$$

$$H_A: \mu_1 \neq \mu_2$$

One-way ANOVA

F-statistic

$$H_0: \mu_1 = \mu_2$$

$$H_A: \mu_1 \neq \mu_2$$

t-test assuming equal variance

t-statistic

But the t-test is more flexible

can run a t-test assuming unequal variance if you are not sure that the two populations have the same standard deviations.

## One-way ANOVA: Null and Alternative Hypotheses

Example: categorical variable has three groups

$$H_0: \mu_1 = \mu_2 = \mu_3$$

$H_A$ : at least group one is different from one other

$$H_A: \mu_1 = \mu_2 \neq \mu_3$$

-- or --

$$H_A: \mu_1 \neq \mu_2 = \mu_3$$

-- or --

$$H_A: \mu_1 \neq \mu_2 \neq \mu_3$$

-- or --

$$H_A: \mu_1 = \mu_3 \neq \mu_2$$

## ANOVA Assumptions - basically the same as the t-test *Normality, homoscedasticity, independence*

The continuous variable should be approximately normally distributed for *each group* of the categorical variable

More important if groups have unequal n (unbalanced ANOVA)

The more unbalanced, the greater the problem

If all groups are skewed in the same direction, some skew is OK

Deviations from normality less important with large sample sizes

Homoscedasticity between the groups of the categorical variable

The variance (SD) of the continuous variable is equal in each group of the categorical variable (use <2 rule)

More important if groups have unequal n (unbalanced)

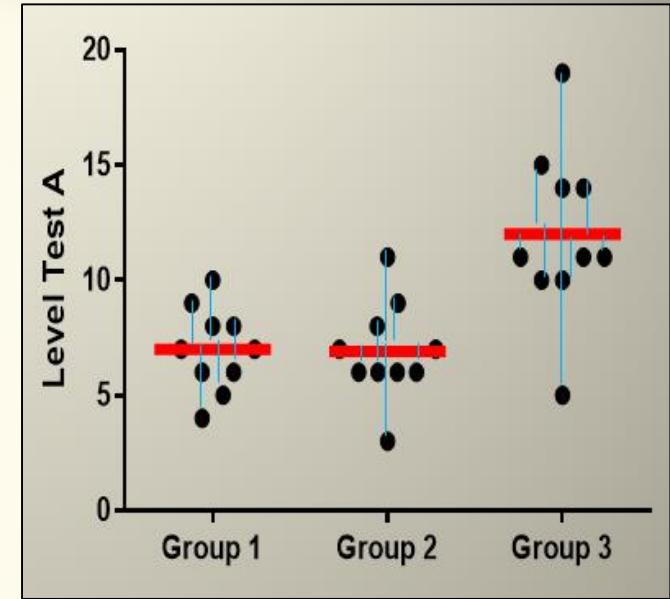
Categorical groups are independent

# ANOVA Assumptions: Test on residuals

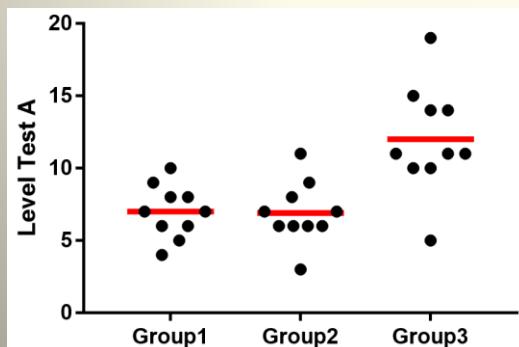
Assumption of normality and homoscedasticity relate to the residuals (AKA errors) of the model and not to the continuous data itself

However, for the ANOVA, they are equivalent

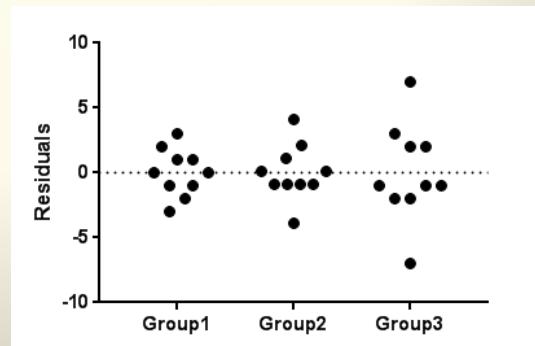
Much easier to test the continuous variable than to have to first compute the residuals and then test them.



Plot of raw data with means



Plot of residuals



## Bartlett's and Brown and Forsythe tests for homoscedasticity: *Advice from the creators of Prism*

Bartlett's test works great if the data really are sampled from Gaussian distributions. But if the distributions deviate even slightly from the Gaussian ideal, Bartlett's test may report a small P value even when the differences among standard deviations is trivial.

Conclude there is a difference when there is really not one  
For this reason, many do not recommend that test.

The Brown and Forsythe test has the same goal as the Bartlett's test, but is less sensitive to minor deviations from normality.

***We suggest that you pay attention to the Brown-Forsythe result, and ignore Bartlett's test (left in to be consistent with prior versions of Prism).***

*But remember the lack of meaning in small and large sample sizes*

If you conclude that the data are not normal or are heteroscedastic, you have some choices:

Transform the data to get a normal distribution and/or equalize the standard deviations, and then rerun the ANOVA.

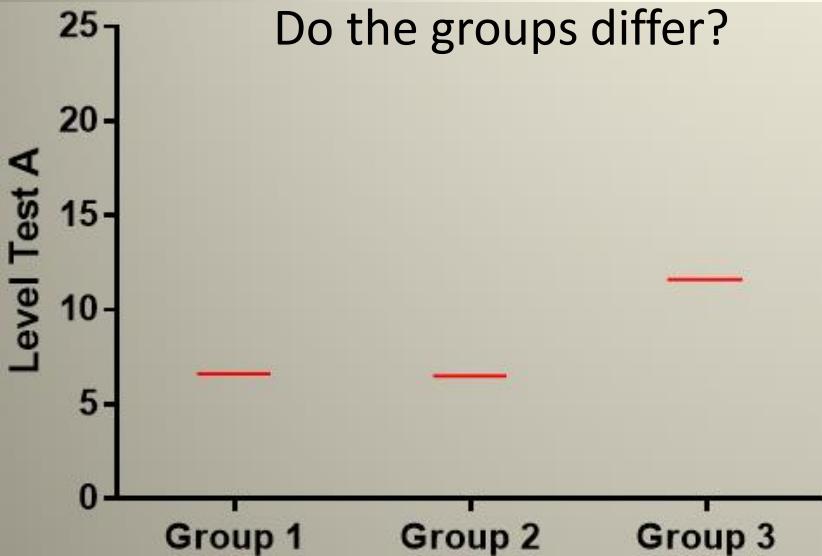
Converting values to their reciprocals or logarithms will often equalize the standard deviations and make the distributions more normal.

Use the nonparametric Kruskal-Wallis test

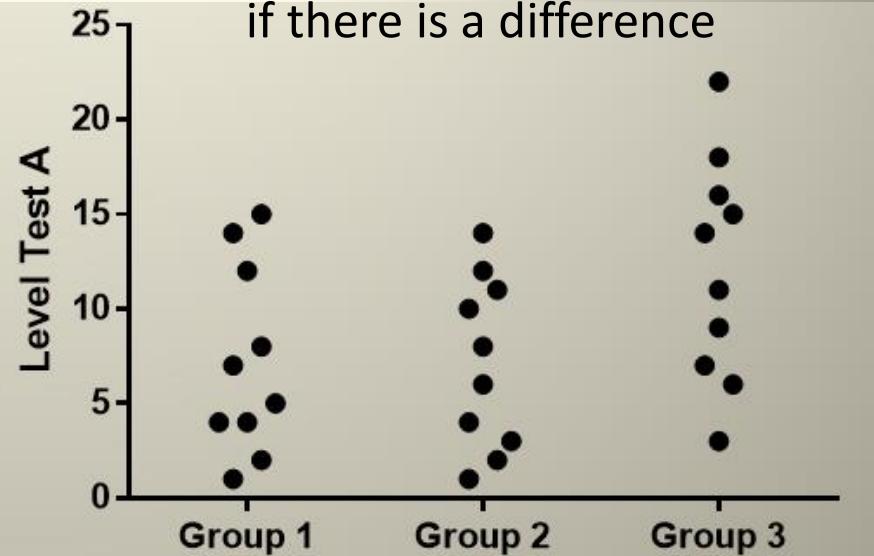
Or use the ANOVA anyway knowing it is not that sensitive to deviations from the normal distribution (very robust) and unequal variances (not quite as robust), especially if groups have equal n and the sample size is large (~30 or greater).

# The Heart of an ANOVA: Between and Within Variability

*Between Groups:*  
Our main question –  
Do the groups differ?



*Within Each Group:*  
Variability that helps determines  
if there is a difference



Looks at variability between group means

Are the means different?

Biological variability, error (sampling,  
measurement)

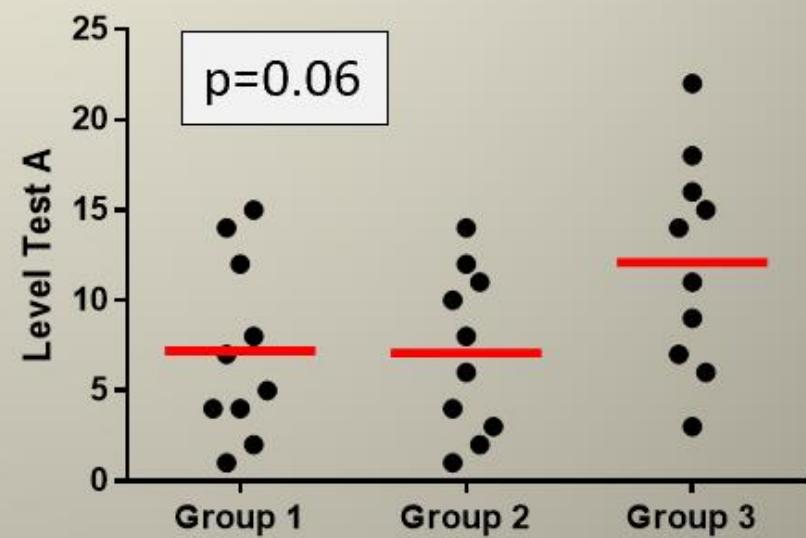
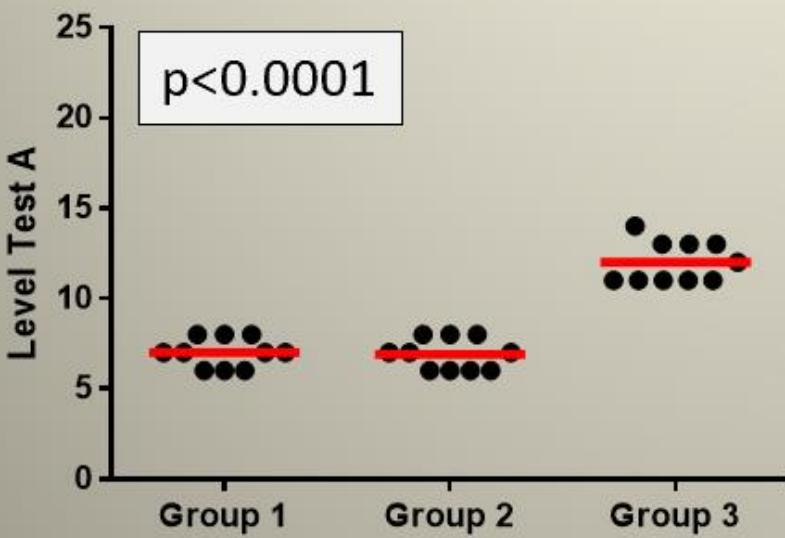
Looks at variability within each group

Is there a lot of spread (variability) in each  
group? Does the variability differ between  
groups (AKA heteroscedasticity)?

Biological variability, error

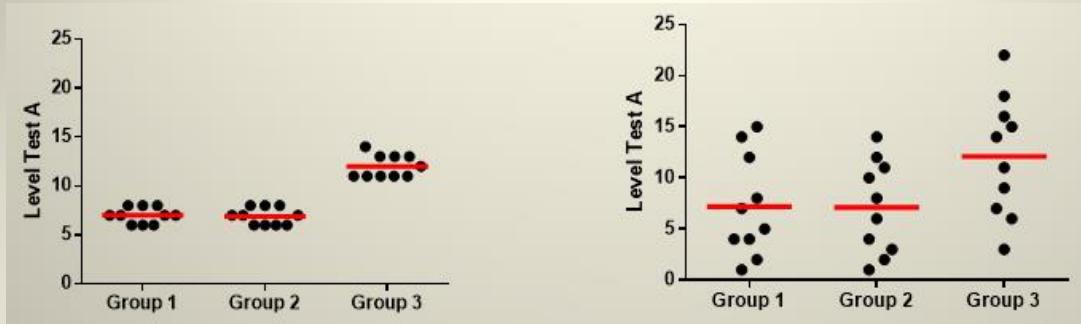
# The Heart of an ANOVA: Between Groups and Within Groups

Mean Values for Test A



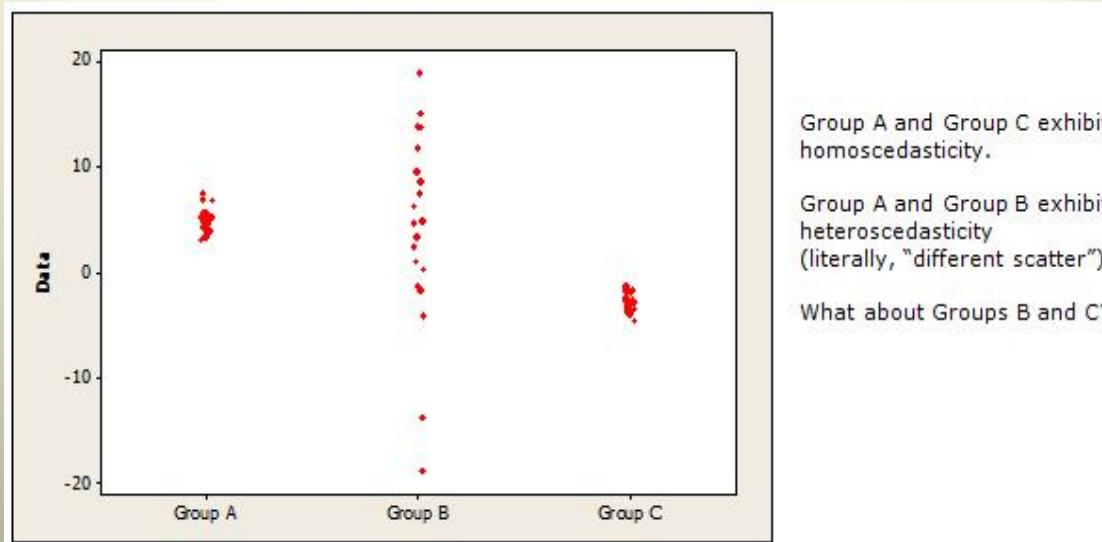
Between groups difference is what you are interested in, but within group variation can get in the way

# One-Way ANOVA: test of means for >2 groups



The name analysis of *variance* comes from the way the test uses variability to decide whether the means are different.

This is why homoscedasticity (equality of variances in groups) is important



## One-Way ANOVA: test of means for >2 groups

ANOVA tests for one overall effect only (AKA omnibus or global test)

Can tell you if there is a difference between groups, but not which ones

Avoids the problem of multiple testing and reduces Type I error

Uses the entire sample size

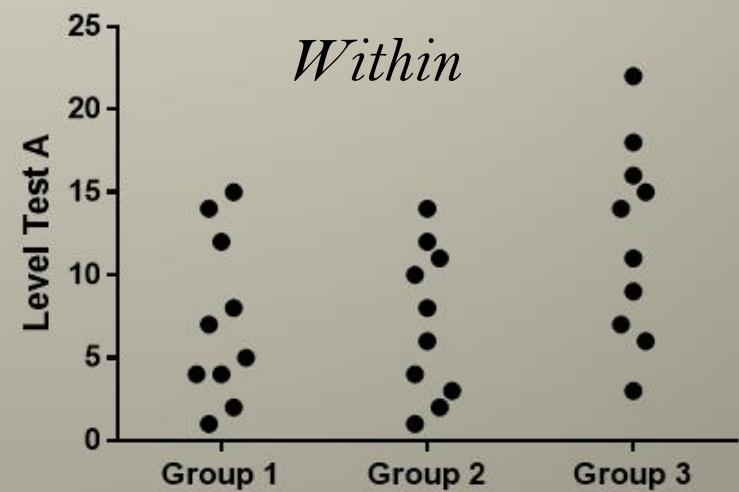
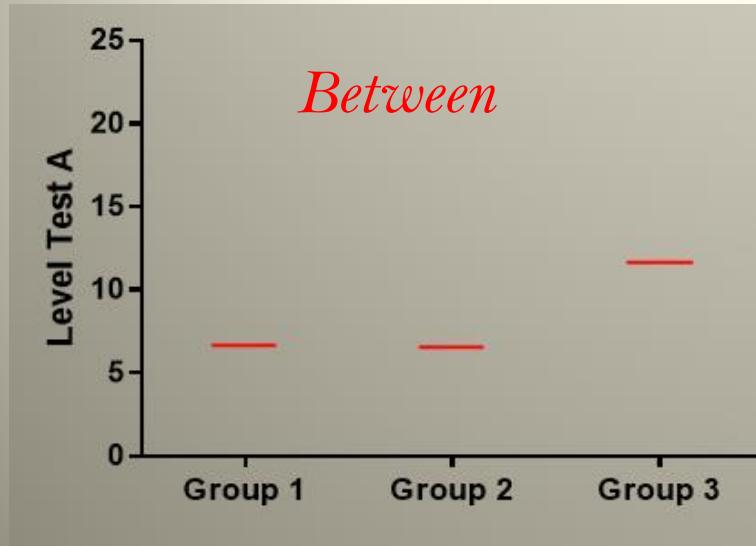
If  $p \leq 0.05$  requires post-hoc testing (AKA multiple testing, contrasts) to determine which groups differ from each other

# One-Way ANOVA: test of means for >2 groups

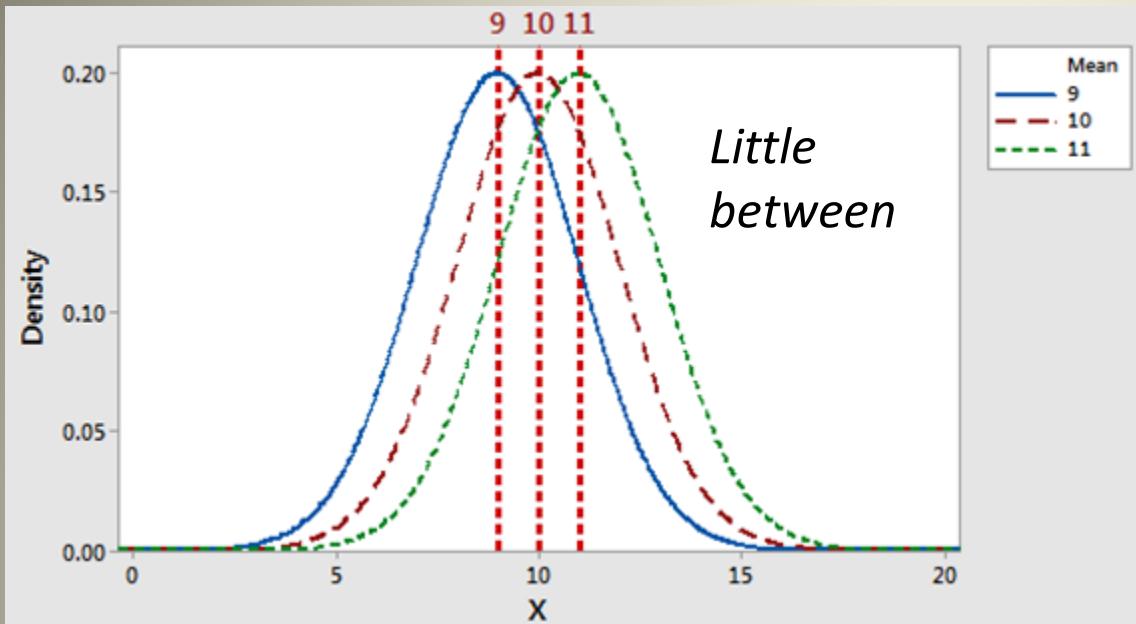
Uses the F statistic (AKA F-ratio; named after Ronald Fisher)

A ratio that compares variability *between* each group mean to variability *within* each group

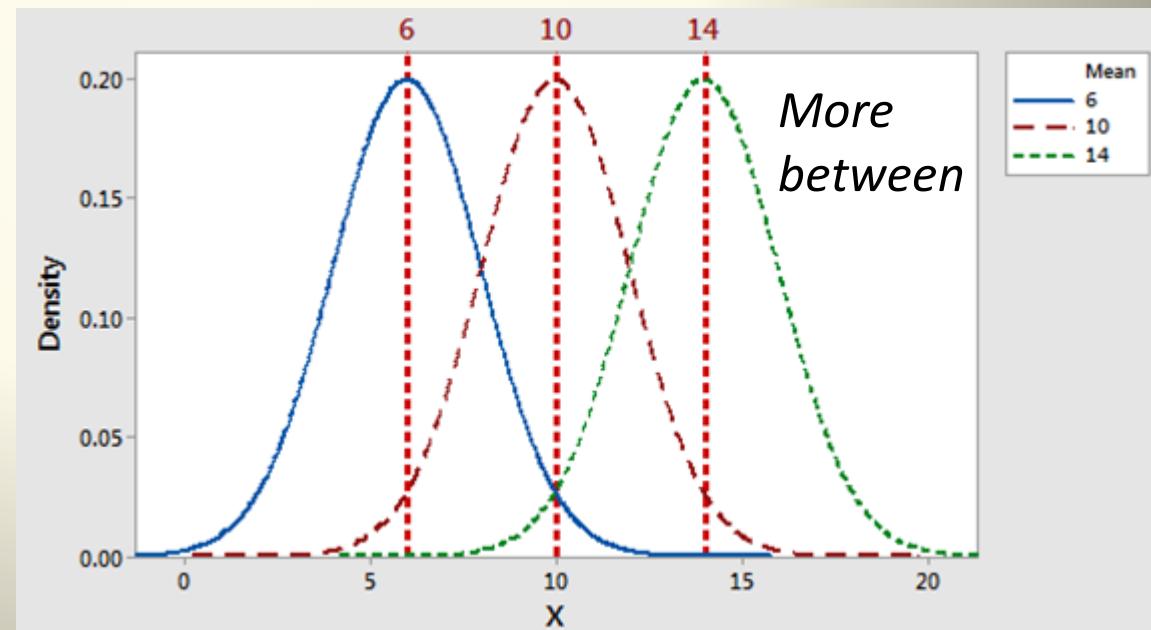
$$F = \frac{\text{Between variability}}{\text{Within variability}}$$



## ANOVA: *Between* Variability (with same *within* variability)

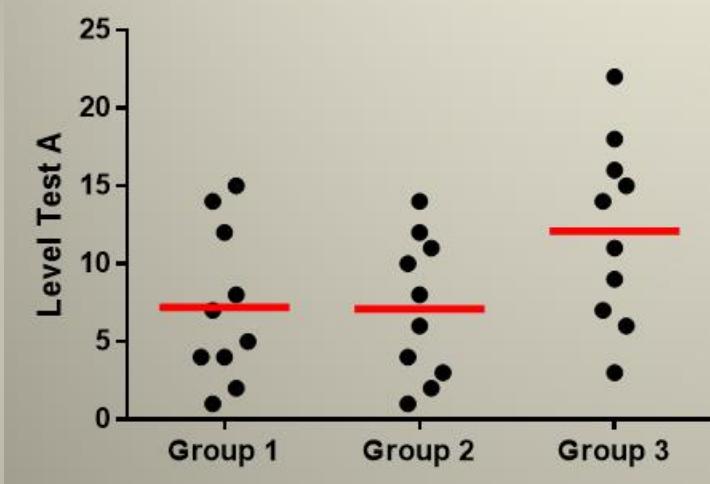


$$F = \frac{\text{Between variability}}{\text{Within variability}}$$

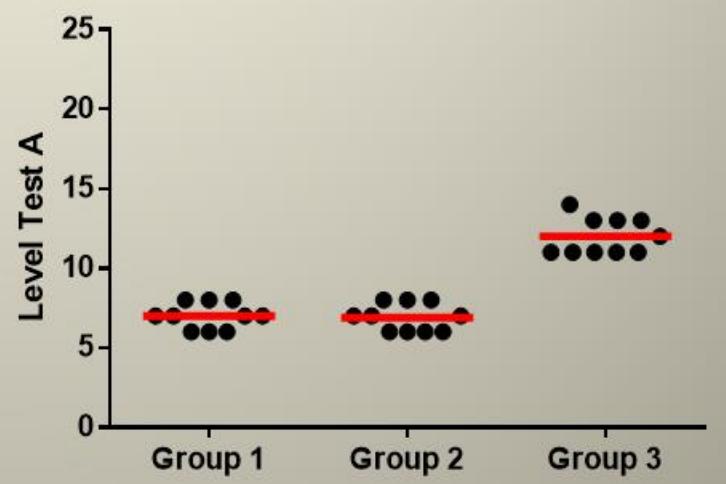


# ANOVA: Within Variability

Lots of **within** variability



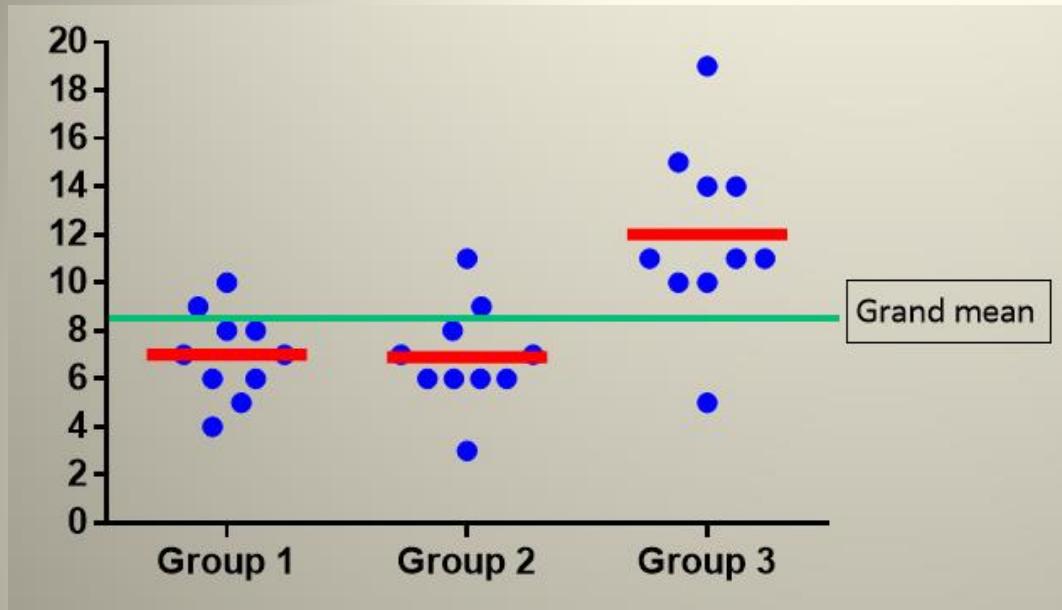
Little **within** variability



$$F = \frac{\text{Between variability}}{\text{Within variability}}$$

Something called “sum of squares” measures both types of variability

# Sum of Squares **Between** and Sum of Squares **Within** and the Grand Mean (the mean of all samples regardless of group)



SS\_**between** looks at how the means for each group vary from the grand mean

Answers your experimental question, but you need to account for variability **within** each group

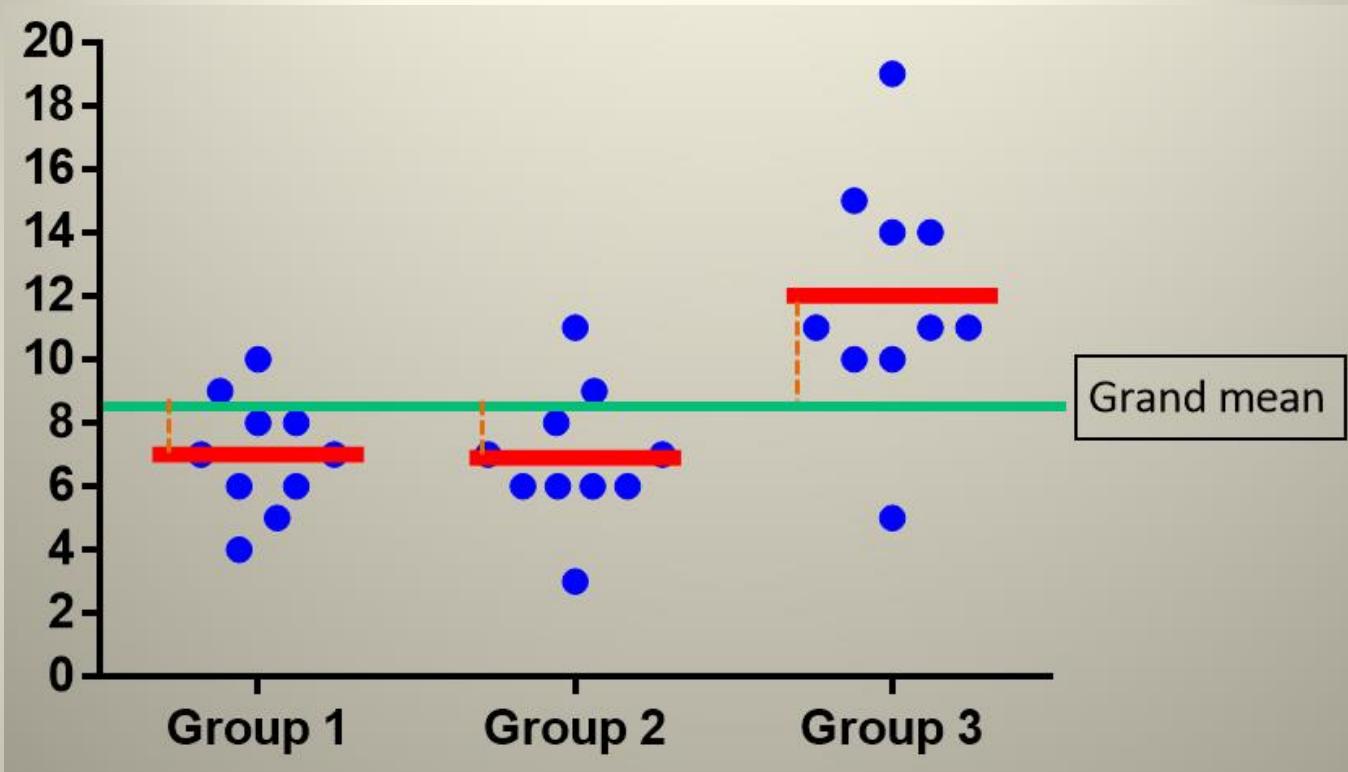
SS\_**within** looks at how individual points in each group vary from mean for that group

Measures the “noise” from biological variability and error

The ANOVA test statistic, F, is the ratio of

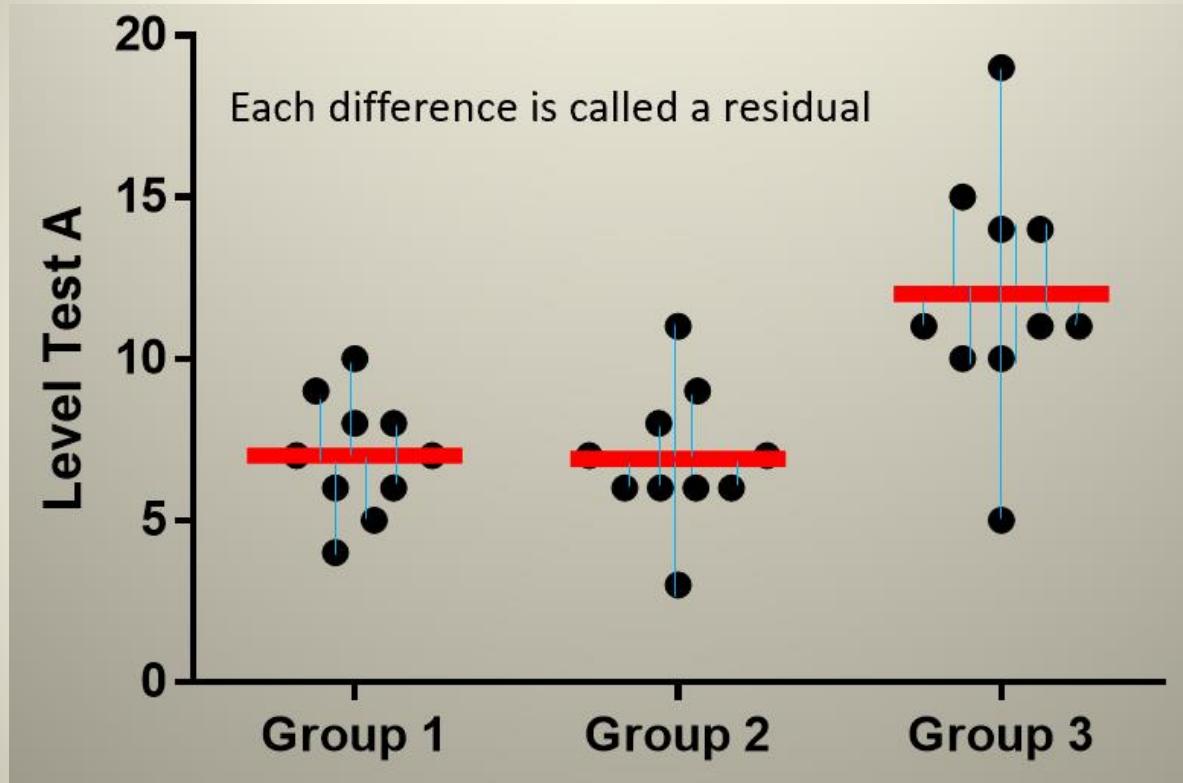
$$\frac{(\text{SS\_between}/\text{df})}{(\text{SS\_within}/\text{df})}$$

**SS\_between** looks at how the means for each group vary from the grand mean



$$SS_{\text{between}} = n_1x(\boxed{\phantom{0}})^2 + n_2x(\boxed{\phantom{0}})^2 + n_3x(\boxed{\phantom{0}})^2$$

**SS\_within** looks at how individual points in each group vary from mean for that group



$$\text{SS}_{\text{within}} = \text{sum of each } (| | )^2$$

## The F Statistic

$$F = \frac{\frac{SS_{\text{between}}}{df (\# \text{groups} - 1)}}{\frac{SS_{\text{within}}}{df (n - \# \text{groups})}} = \frac{\text{Mean of Squares}_{\text{between}}}{\text{Mean of Squares}_{\text{within}}}$$

#groups = 3 in our example

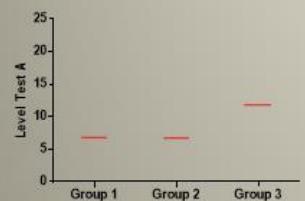
n = the total sample size, in our example n=30

An  $F > 1.0$  indicates that there is more difference between group means than within groups.

The larger the F statistic, the more likely  $p \leq 0.05$

# ANOVA: Sum of Squares to Measure Variability

## Example: 3 groups with 10 values in each group

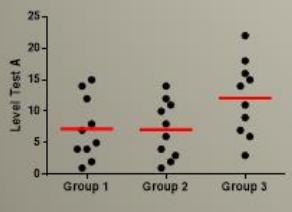


The “grand mean” is the mean for all data combined

**SS\_between** = sum of (mean for each group *minus* grand mean)<sup>2</sup>

With 3 groups, you will have 3 calculations to sum

This measures the variability of means by group



**SS\_within** = sum of (each individual value *minus* mean for their group)<sup>2</sup>

With 3 groups of 10 each, you will have 30 calculations

This measures the variability of data points within each group

**SS\_total** = sum of (each individual value *minus* grand mean)<sup>2</sup>

Grand mean is the mean of all variables (or of 3 group means)

With 3 groups of 10 each, you will have 30 calculations to sum

This measures total variability

# ANOVA Table

Summary ANOVA				
Source	Sum of Squares	Degrees of Freedom	Variance Estimate (Mean Square)	F Ratio
Between	$SS_B$	$K - 1$	$MS_B = \frac{SS_B}{K - 1}$	$\frac{MS_B}{MS_W}$
Within	$SS_W$	$N - K$	$MS_W = \frac{SS_W}{N - K}$	
Total	$SS_T = SS_B + SS_W$	$N - 1$		

ANOVA F Test Statistic

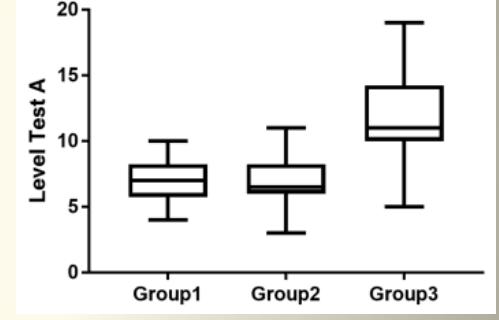
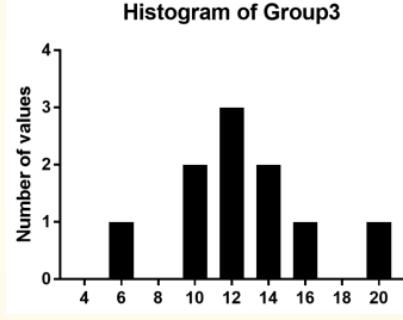
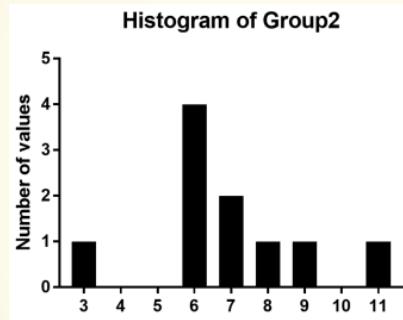
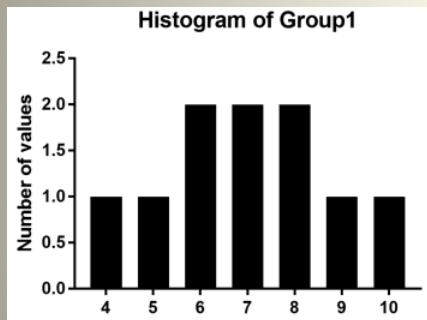
$N$  = total observations in all groups

$K$  = number of groups

# One-Way ANOVA by Hand: Assumptions

The three groups are independent (measurements done in different people)

The three groups appear to be fairly symmetrical without significant skew or outliers



Are the SD the same?

	Group 1	Group 2	Group3	Ratio largest/smallest
SD	1.83	2.13	3.74	$3.74/1.83=2.04$

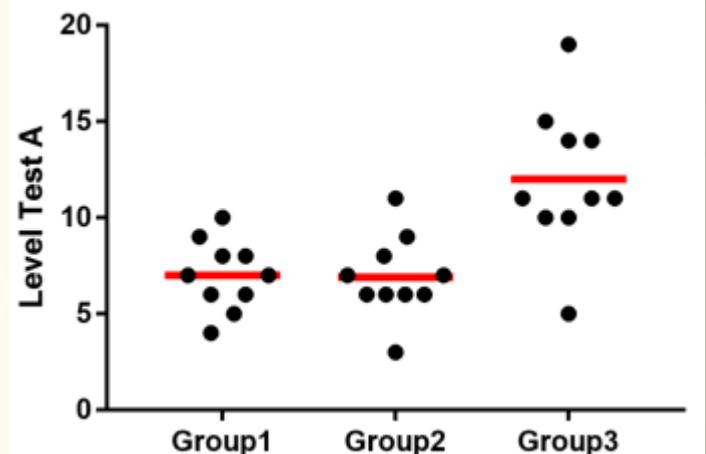
Brown-Forsythe test  $p=0.31$  (SD not different)

# One-Way ANOVA by Hand (with an assist from Excel)

## Are the three groups different?

Number	group1	group2	group3
1	9	7	10
2	8	9	19
3	6	6	14
4	8	6	5
5	10	6	10
6	4	11	11
7	6	6	14
8	5	3	15
9	7	8	11
10	7	7	11
mean	7.0	6.9	12.0
SD	1.83	2.13	3.74

Univariate Scatter Plot with Means



For the purposes of this example, we will assume test assumptions are met

$$H_0: \mu_1 = \mu_2 = \mu_3$$

$H_A$ : at least one is different from one other

$$H_A: \mu_1 = \mu_2 \neq \mu_3 \quad \text{-- or --} \quad H_A: \mu_1 \neq \mu_2 = \mu_3 \quad \text{-- or --}$$

$$H_A: \mu_1 \neq \mu_2 \neq \mu_3 \quad \text{-- or --} \quad H_A: \mu_1 = \mu_3 \neq \mu_2$$

# One-way ANOVA by Hand: Grand Mean

Number	group1	group2	group3
1	9	7	10
2	8	9	19
3	6	6	14
4	8	6	5
5	10	6	10
6	4	11	11
7	6	6	14
8	5	3	15
9	7	8	11
10	7	7	11
mean	7.0	6.9	12.0
SD	1.83	2.13	3.74

Group	All Data
1	9
1	8
1	6
1	8
1	10
1	4
1	6
1	5
1	7
1	7
2	7
2	9
2	6
2	6
2	6
2	11
2	6
2	3
2	8
2	7
3	10
3	19
3	14
3	5
3	10
3	11
3	14
3	15
3	11
3	11
grand mean	8.63

# ANOVA by Hand: Sum of Squares Total

Number	group1	group2	group3
1	9	7	10
2	8	9	19
3	6	6	14
4	8	6	5
5	10	6	10
6	4	11	11
7	6	6	14
8	5	3	15
9	7	8	11
10	7	7	11
mean	7.0	6.9	12.0
SD	1.83	2.13	3.74

SS\_total individual values

$$\begin{aligned}
 1. (x_{11} - \bar{x})^2 &= (9 - 8.63)^2 = & 0.14 \\
 2. (x_{12} - \bar{x})^2 &= (8 - 8.63)^2 = & 0.40 \\
 3. (x_{13} - \bar{x})^2 &= (6 - 8.63)^2 = & 6.92 \\
 4. (x_{14} - \bar{x})^2 &= (8 - 8.63)^2 = & 0.40 \\
 5. (x_{15} - \bar{x})^2 &= (10 - 8.63)^2 = & 1.88 \\
 \cdot & & \\
 \cdot & & \\
 \cdot & & \\
 30. (x_{310} - \bar{x})^2 &= (11 - 8.63)^2 = & 5.62
 \end{aligned}$$


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$$SS_{\text{total}} = 366.97$$

Grand mean = 8.63

# One-Way ANOVA by Hand: Sum of Squares Between

Number	group1	group2	group3
1	9	7	10
2	8	9	19
3	6	6	14
4	8	6	5
5	10	6	10
6	4	11	11
7	6	6	14
8	5	3	15
9	7	8	11
10	7	7	11
mean	7.0	6.9	12.0
SD	1.83	2.13	3.74

SS\_between individual values

$$n_1(\bar{x}_1 - \bar{x})^2 = 10 \times (7.0 - 8.63)^2 = 26.57$$

$$n_2(\bar{x}_2 - \bar{x})^2 = 10 \times (6.9 - 8.63)^2 = 29.93$$

$$n_3(\bar{x}_3 - \bar{x})^2 = 10 \times (12.0 - 8.63)^2 = 113.57$$

$$\text{SS}_{\text{between}} = 170.07$$

Grand mean = 8.63

n = 10 (for each group)

# One-Way ANOVA by Hand: Sum of Squares Within

Number	group1	group2	group3
1	9	7	10
2	8	9	19
3	6	6	14
4	8	6	5
5	10	6	10
6	4	11	11
7	6	6	14
8	5	3	15
9	7	8	11
10	7	7	11
mean	7.0	6.9	12.0
SD	1.83	2.13	3.74

SS\_Within individual values

$$1. (x_{11} - \bar{x}_1)^2 = (9 - 7.0)^2 = 4.00$$

$$2. (x_{12} - \bar{x}_1)^2 = (8 - 7.0)^2 = 1.00$$

$$3. (x_{13} - \bar{x}_1)^2 = (6 - 7.0)^2 = 1.00$$

$$4. (x_{14} - \bar{x}_1)^2 = (8 - 7.0)^2 = 1.00$$

$$5. (x_{15} - \bar{x}_1)^2 = (10 - 7.0)^2 = 9.00$$

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$$30. (x_{310} - \bar{x}_3)^2 = (11 - 12.0)^2 = 1.00$$

$$\underline{\text{SS_within}} = 196.90$$

# ANOVA by Hand: Put it all together

Summary ANOVA

Source	Sum of Squares	Degrees of Freedom	Variance Estimate (Mean Square)	F Ratio
Between	$SS_B$	$K - 1$	$MS_B = \frac{SS_B}{K - 1}$	$\frac{MS_B}{MS_W}$
Within	$SS_W$	$N - K$	$MS_W = \frac{SS_W}{N - K}$	$K = \text{number of groups} = 3$ $N = \text{total sample size} = 30$
Total	$SS_T = SS_B + SS_W$	$N - 1$		

			Variance est	
Source	Sum of squares	df	(mean square)	F ratio
Between	170.07	2	85.04	11.66
Within	196.90	27	7.29	
Total	366.97	29		

$$F = \frac{MS_{\text{Between}}}{MS_{\text{Within}}}$$

$$F(2,27) = 11.66$$

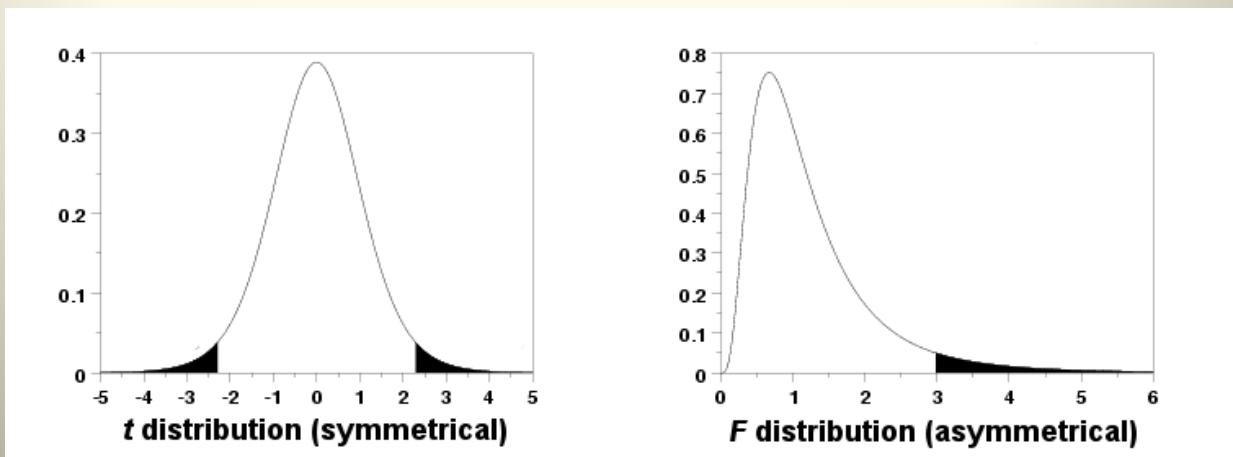
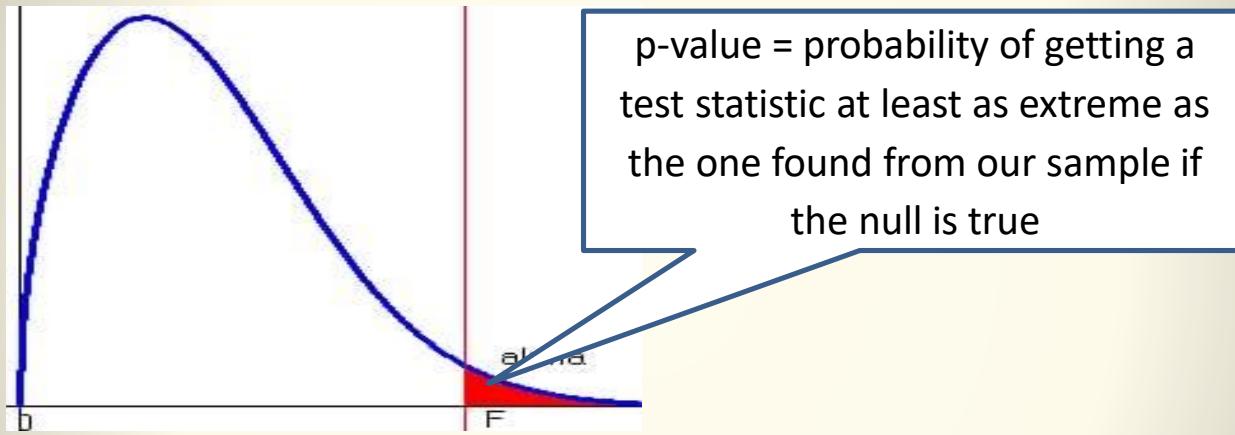
$$= 85.04/7.29 = 11.66$$

# The ANOVA F Statistic is one-sided

The p-value for ANOVA is calculated using the F-distribution

Because it squares the differences, the F statistic is always positive.

F can be  $<1.0$ , but not  $<0$ .



The ANOVA F Statistic is a one-sided but the test can be two-tailed

The ANOVA F statistic is one-sided, but it is non-directional  
a two-tailed test is non-directional, i.e., the difference can be either  
larger or smaller

As the difference between sample means grows larger (in EITHER  
direction), the F grows larger.

ANOVA is best thought of as a two-tailed test even though literally only  
one tail of the F distribution is used

# Finding the Critical Value for F(2,27)

F - Distribution ( $\alpha = 0.05$  in the Right Tail)

Denominator Degrees of Freedom <i>df<sub>2</sub></i>	Numerator Degrees of Freedom <i>df<sub>1</sub></i>	Numerator Degrees of Freedom								
		1	2	3	4	5	6	7	8	9
1	161.45	199.50	215.71	224.58	230.16	233.99	236.77	238.88	240.54	
2	18.513	19.000	19.164	19.247	19.296	19.330	19.353	19.371	19.385	
3	10.128	9.5521	9.2766	9.1172	9.0135	8.9406	8.8867	8.8452	8.8123	
4	7.7086	9.9443	6.5914	6.3882	6.2561	6.1631	6.0942	6.0410	6.9988	
5	6.6079	5.7861	5.4095	5.1922	5.0503	4.9503	4.8759	4.8183	4.7725	
6	5.9874	5.1433	4.7571	4.5337	4.3874	4.2839	4.2067	4.1468	4.0990	
7	5.5914	4.7374	4.3468	4.1203	3.9715	3.8660	3.7870	3.7257	3.6767	
8	5.3177	4.4590	4.0662	3.8379	3.6875	3.5806	3.5005	3.4381	3.3881	
9	5.1174	4.2565	3.8625	3.6331	3.4817	3.3738	3.2927	3.2296	3.1789	
10	4.9646	4.1028	3.7083	3.4780	3.3258	3.2172	3.1355	3.0717	3.0204	
11	4.8443	3.9823	3.5874	3.3567	3.2039	3.0946	3.0123	2.9480	2.8962	
12	4.7472	3.8853	3.4903	3.2592	3.1059	2.9961	2.9134	2.8486	2.7964	
13	4.6672	3.8056	3.4105	3.1791	3.0254	2.9153	2.8321	2.7669	2.7144	
14	4.6001	3.7389	3.3439	3.1122	2.9582	2.8477	2.7642	2.6987	2.6458	
15	4.5431	3.6823	3.2874	3.0556	2.9013	2.7905	2.7066	2.6408	2.5876	
16	4.4940	3.6337	3.2389	3.0069	2.8524	2.7413	2.6572	2.5911	2.5377	
17	4.4513	3.5915	3.1968	2.9647	2.8100	2.6987	2.6143	2.5480	2.4943	
18	4.4139	3.5546	3.1599	2.9277	2.7729	2.6613	2.5767	2.5102	2.4563	
19	4.3807	3.5219	3.1274	2.8951	2.7401	2.6283	2.5435	2.4768	2.4227	
20	4.3512	3.4928	3.0984	2.8661	2.7109	2.5990	2.5140	2.4471	2.3928	
21	4.3248	3.4668	3.0725	2.8401	2.6848	2.5727	2.4876	2.4205	2.3660	
22	4.3009	3.4434	3.0491	2.8167	2.6613	2.5491	2.4638	2.3965	2.3419	
23	4.2793	3.4221	3.0280	2.7955	2.6400	2.5277	2.4422	2.3748	2.3201	
24	4.2597	3.4028	3.0088	2.7763	2.6207	2.5082	2.4226	2.3551	2.3002	
25	4.2417	3.3852	2.9912	2.7587	2.6030	2.4904	2.4047	2.3371	2.2821	
26	4.2252	3.3690	2.9752	2.7426	2.5868	2.4741	2.3883	2.3205	2.2655	
27	4.2100	3.3541	2.9604	2.7278	2.5719	2.4591	2.3732	2.3053	2.2501	
28	4.1900	3.3404	2.9467	2.7141	2.5581	2.4453	2.3593	2.2913	2.2360	
29	4.1830	3.3277	2.9340	2.7014	2.5454	2.4324	2.3463	2.2783	2.2229	
30	4.1709	3.3158	2.9223	2.6896	2.5336	2.4205	2.3343	2.2662	2.2107	
40	4.0847	3.2317	2.8387	2.6060	2.4495	2.3359	2.2490	2.1802	2.1240	
60	4.0012	3.1504	2.7581	2.5252	2.3683	2.2541	2.1665	2.0970	2.0401	
120	3.9201	3.0718	2.6802	2.4472	2.2899	2.1750	2.0868	2.0164	1.9588	
$\infty$	3.8415	2.9957	2.6049	2.3719	2.2141	2.0986	2.0096	1.9384	1.8799	

$$F(2,27) = 3.3541$$

Our F statistic = 11.66

11.66 > 3.3541 so  
p<0.05

We reject the null hypothesis and conclude that at least one mean is different from one other

# Now, let's use Prism to do the one-way ANOVA

New Data Table and Graph

**New table & graph**

- XY
- Column
- Grouped
- Contingency
- Survival
- Parts of whole
- Multiple variables
- Nested

**Existing file**

Clone a graph

Prism Tips

Column tables have one grouping variable, with each group defined by a column

Data table:

	A	B
Control	Y	Treated
1		Y
2		

Learn more

Control Treated

Options:

- Enter or import data into a new table
- Start with sample data to follow a tutorial

Enter: Mean, SD, N

Cancel Create

ANOVAbyHand.xlsx

	Group A	Group B	Group C	
	Group1	Group2	Group3	
1	9	7	10	
2	8	9	19	
3	6	6	14	
4	8	6	5	
5	10	6	10	
6	4	11	11	
7	6	6	14	
8	5	3	15	
9	7	8	11	
10	7	7	11	
11				

## Analyze Data

Built-in analysis

Which analysis?

 Transform, Normalize...

- Transform
- Transform concentrations (X)
- Normalize
- Prune rows
- Remove baseline and column math
- Transpose X and Y
- Fraction of total

 XY analyses Column analyses

- t tests (and nonparametric tests)
- One-way ANOVA (and nonparametric or mixed)**

One sample t and Wilcoxon test

Descriptive statistics

Normality and Lognormality Tests

Frequency distribution

ROC Curve

Bland-Altman method comparison

Identify outliers

Analyze a stack of P values

 Grouped analyses Contingency table analyses Survival analyses Parts of whole analyses

Analyze which data sets?

- A:Group1
- B:Group2
- C:Group3

Parameters: One-Way ANOVA (and Nonparametric or Mixed)

Experimental Design    Repeated Measures    Multiple Comparisons    Options    Residuals

**Experimental design**

- No matching or pairing  
 Each row represents matched, or repeated measures, data

	Group A	Group B	Group C	Group D
	Data Set-A	Data Set-B	Data Set-C	Title
1	Y	Y	Y	Y
2				
3				

**Assume Gaussian distribution of residuals?**

- Yes. Use ANOVA.  
 No. Use nonparametric test.

**Assume equal SDs?**

- Yes. Use ordinary ANOVA test.  
 No. Use Brown-Forsythe and Welch ANOVA tests.

Based on your choices (on all tabs), Prism will perform:

- Ordinary one-way ANOVA.

Learn

Cancel

OK

## Parameters: One-Way ANOVA (and Nonparametric or Mixed)

Experimental Design   Repeated Measures   Multiple Comparisons   Options   Residuals

### Followup tests

- None.
- Compare the mean of each column with the mean of every other column.
- Compare the mean of each column with the mean of a control column.  
Control column: Column A : Group1
- Compare the means of preselected pairs of columns.  
Selected pairs:
- Test for linear trend between column mean and left-to-right column order.

### Which test?

Use choices on the Options tab to choose the test, and to set the defaults for future ANOVAs.

Learn

Cancel

## Parameters: One-Way ANOVA (and Nonparametric or Mixed)

Experimental Design   Repeated Measures   Multiple Comparisons   Options   Residuals

### Multiple comparisons test

- Correct for multiple comparisons using statistical hypothesis testing. Recommended.  
Test: Tukey (recommended)
- Correct for multiple comparisons by controlling the False Discovery Rate.  
Test: Two-stage step-up method of Benjamini, Krieger and Yekutieli (recommended)
- Don't correct for multiple comparisons. Each comparison stands alone.  
Test: Fisher's LSD test

### Multiple comparisons options

- Swap direction of comparisons (A-B) vs. (B-A).
  - Report multiplicity adjusted P value for each comparison.  
Each P value is adjusted to account for multiple comparisons.
- Family-wise significance and confidence level:

### Graphing

- Graph confidence intervals.
- Graph ranks (nonparametric).
- Graph differences (repeated measures).

### Additional results

- Descriptive statistics for each data set.
- Report comparison of models using AICc.
- Report goodness of fit.

### Output

Show this many significant digits (for everything except P values):

P value style: GP: 0.1234 (ns), 0.0332 (\*), 0.0021 (\*\*), 0.000 N =

- Make options on this tab be the default for future One-Way ANOVAs.

Learn

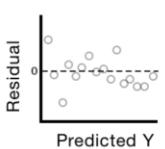
Cancel

OK

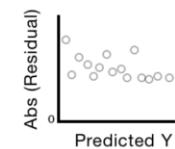
Experimental Design   Repeated Measures   Multiple Comparisons   Options   Residuals

**What graphs to create?** Residual plot

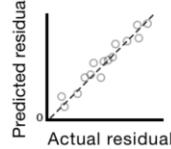
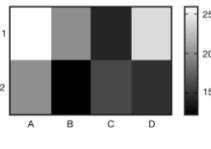
Correct model?

 Homoscedasticity plot

Equal variance?

 QQ plot

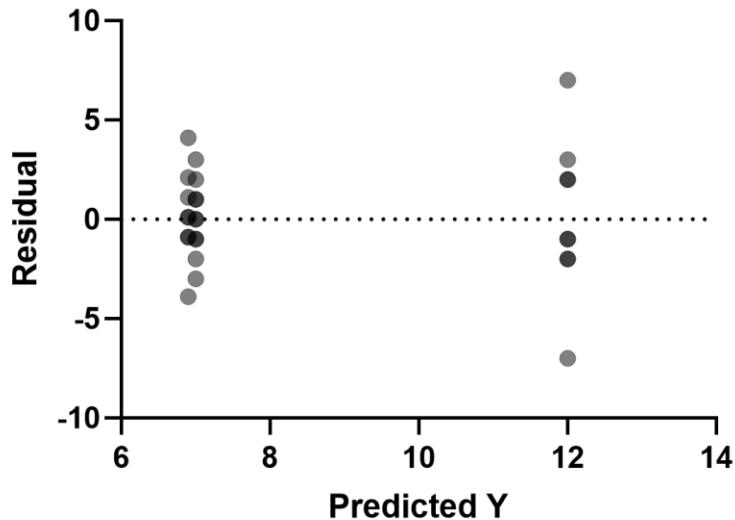
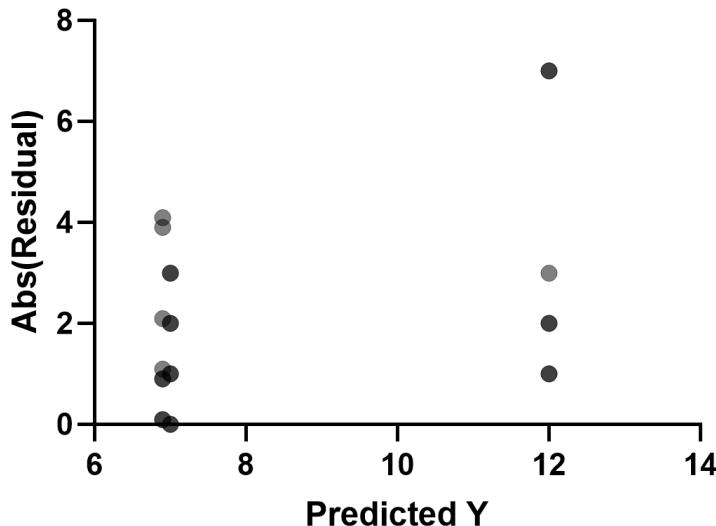
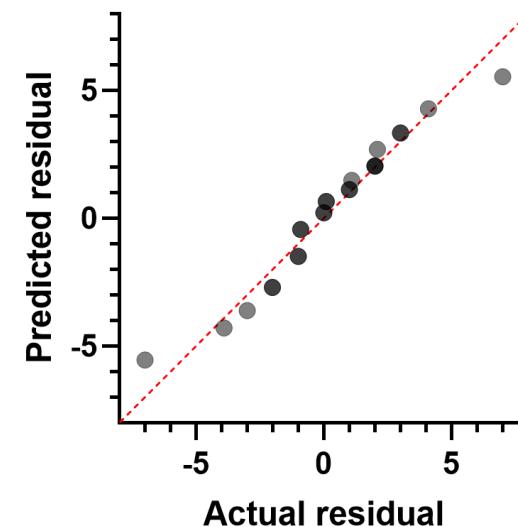
Normality?

 Heatmap plot**Diagnostics for residuals** Are residuals clustered or heteroscedastic?

Brown-Forsythe and Barlett's tests.

 Are the residuals Gaussian?

Normality tests of Anderson-Darling, D'Agostino, Shapiro-Wilk and Kolmogorov-Smirnov.

 Make options on this tab be the**Residual plot****Homoscedasticity plot****QQ plot**

ANOVA results		Multiple comparisons				
<b>Ordinary one-way ANOVA</b>						
ANOVA results						
1	Table Analyzed	One Way ANOVA				
2	Data sets analyzed	A-C				
3						
4	<b>ANOVA summary</b>					
5	F	11.66				
6	P value	0.0002				
7	P value summary	***				
8	Significant diff. among means (P < 0.05)?	Yes				
9	R square	0.4634				
10						
11	<b>Brown-Forsythe test</b>					
12	F (DFn, DFd)	1.212 (2, 27)				
13	P value	0.3134				
14	P value summary	ns				
15	Are SDs significantly different (P < 0.05)?	No				
16						
17	<b>Bartlett's test</b>					
18	Bartlett's statistic (corrected)	5.177				
19	P value	0.0751				
20	P value summary	ns				
21	Are SDs significantly different (P < 0.05)?	No				
22						
23	<b>ANOVA table</b>	SS	DF	MS	F (DFn, DFd)	P value
24	Treatment (between columns)	170.1	2	85.03	F (2, 27) = 11.66	P=0.0002
25	Residual (within columns)	196.9	27	7.293		
26	Total	367.0	29			
27						
28	<b>Normality of Residuals</b>					
29	<b>Test name</b>	Statistics	P value	Passed norm	P value summary	
30	Anderson-Darling (A2*)	0.6244	0.0943	Yes	ns	
31	D'Agostino-Pearson omnibus (K2)	3.481	0.1755	Yes	ns	
32	Shapiro-Wilk (W)	0.9538	0.2136	Yes	ns	
33	Kolmogorov-Smirnov (distance)	0.1506	0.0807	Yes	ns	
34						
35	<b>Data summary</b>					
36	Number of treatments (columns)	3				
37	Number of values (total)	30				
38						

R-Square defines the proportion of the total variance explained by the model.

How much of the variance in the variable is explained by group? 46.4%

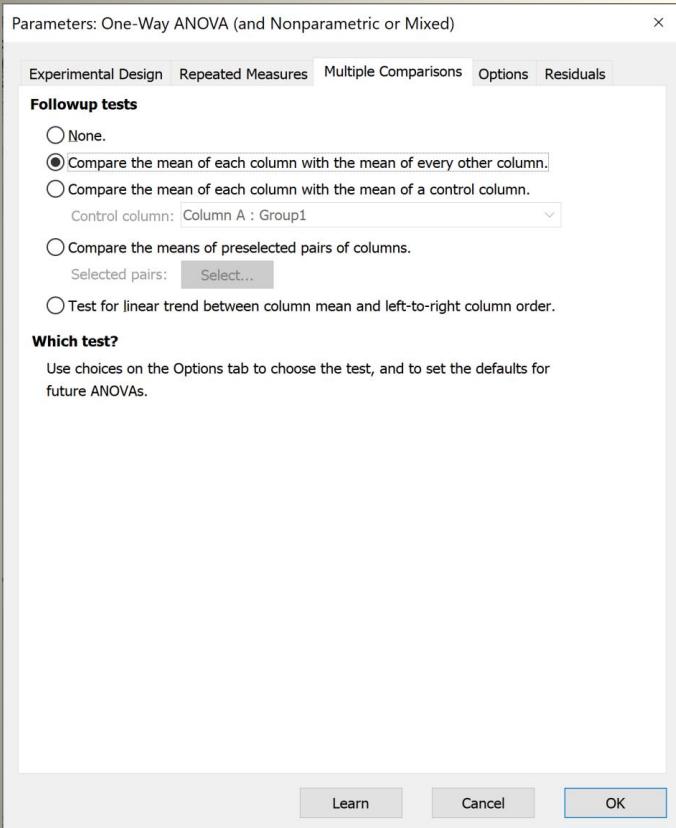
## Prism Results

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	170.1	2	85.03	$F(2, 27) = 11.66$	$P=0.0002$
Residual (within columns)	196.9	27	7.293		
Total	367.0	29			

## Our By Hand Results

			Variance est	
Source	Sum of squares	df	(mean square)	F ratio
Between	170.07	2	85.04	11.66
Within	196.90	27	7.29	
Total	366.97	29		

# Post hoc tests (AKA Multiple Comparison Tests)



If there is a significant ANOVA result, you need to run post hoc tests to tell which groups are different

Pairwise comparisons are made to determine which groups are different from the others

These post-hoc tests are t-tests with p-value adjustments to keep the type 1 error (false positives) to a minimum

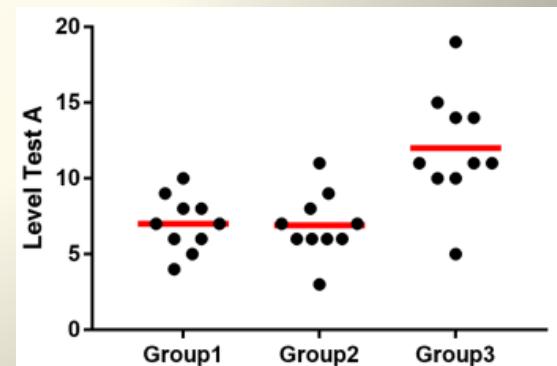
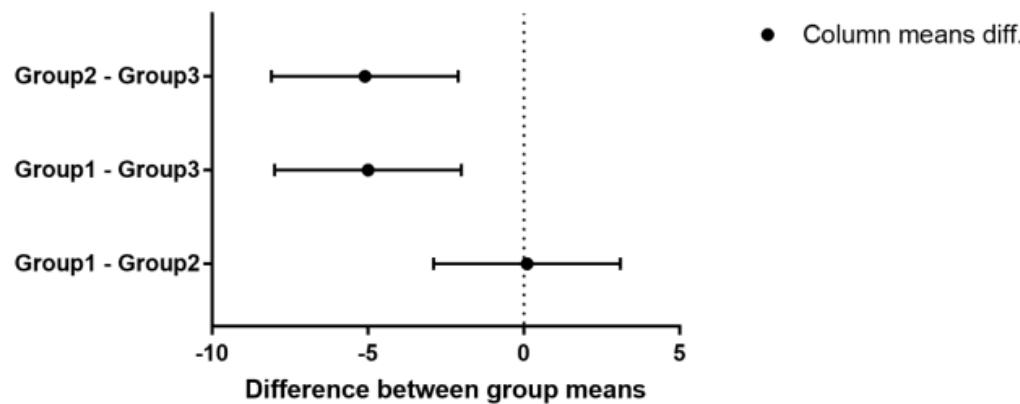
For the ANOVA, Prism recommends the commonly used Tukey's test

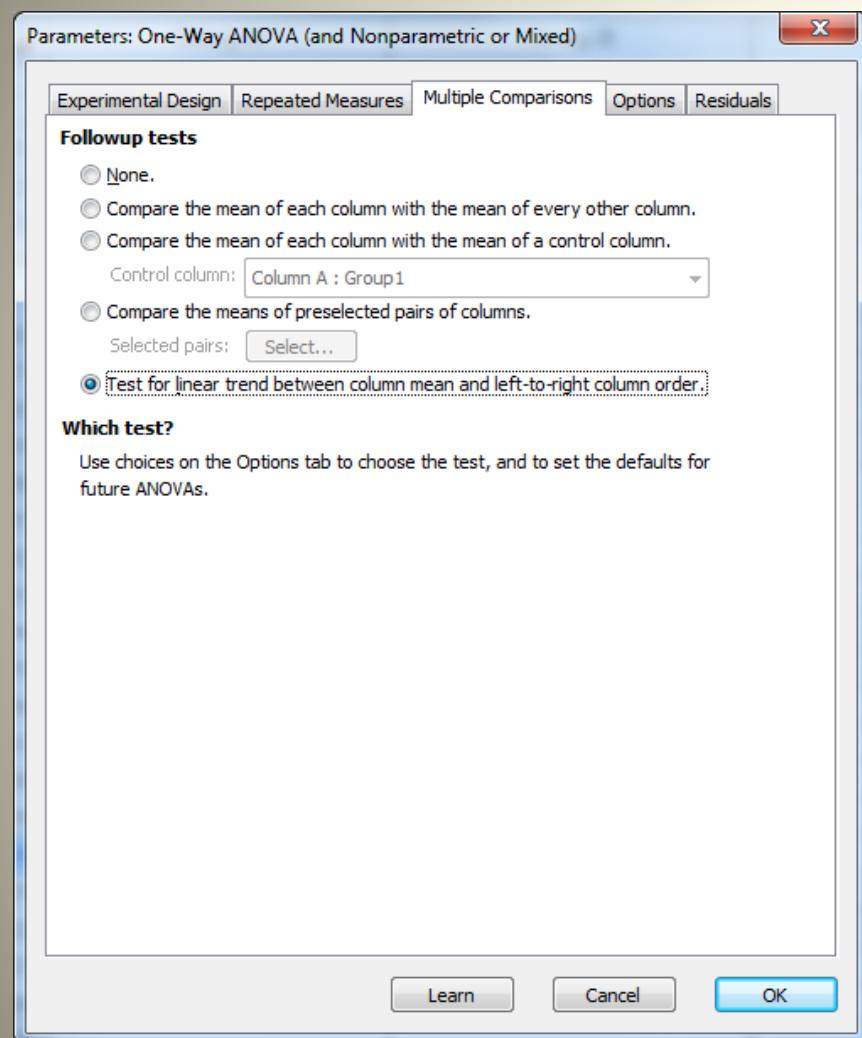
ANOVA results

Multiple comparisons

### Ordinary one-way ANOVA Multiple comparisons

1	Number of families							
2	Number of comparisons per family							
3	Alpha							
4								
5	Tukey's multiple comparisons test	95.00% CI of diff.	Significant?	Summary	Adjusted P Value			
6	Group1 vs. Group2	-2.894 to 3.094	No	ns	0.9962	A-B		
7	Group1 vs. Group3	-7.994 to -2.006	Yes	***	0.0009	A-C		
8	Group2 vs. Group3	-8.094 to -2.106	Yes	***	0.0007	B-C		
9								
10	Test details	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
11	Group1 vs. Group2	6.900	0.1000	1.208	10	10	0.1171	27
12	Group1 vs. Group3	12.00	-5.000	1.208	10	10	5.855	27
13	Group2 vs. Group3	12.00	-5.100	1.208	10	10	5.972	27
14								

**95% Confidence Intervals (Tukey)**

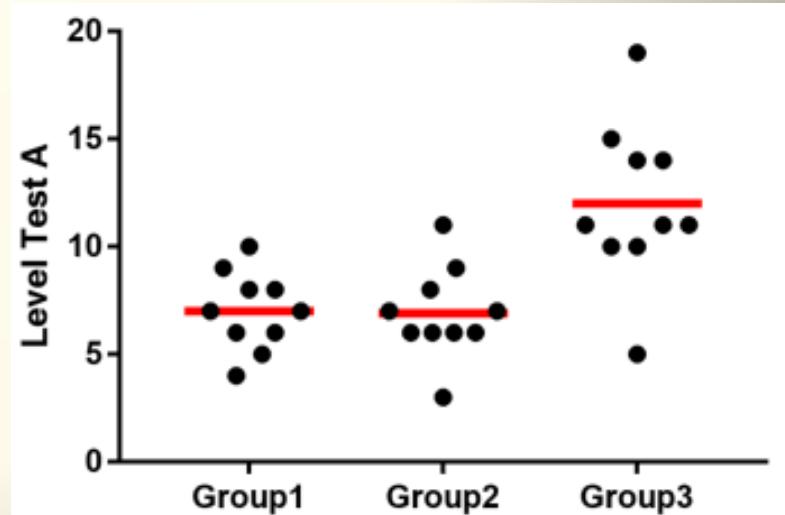


ANOVA results | Test for trend

### Ordinary one-way ANOVA

Test for trend

1	Test for linear trend	
2	Slope	2.500
3	SE of slope	0.6038
4	95% CI of slope	1.261 to 3.739
5	P value	0.0003
6	P value summary	***
7	Is linear trend significant ( $P < 0.05$ )? Yes	
o		



## Write up results

### For this class:

The data passed the assumptions for an one-way ANOVA (independence, normality/symmetry, homoscedasticity). With  $p=0.0002$  [two-tailed hypothesis,  $F(2,27)=11.66$ ,  $\alpha=0.05$ ], we reject the null hypothesis of no difference in test population means by group and conclude there is at least one group different from one other. Tukey post-hoc tests showed that Group3 (mean=12.0) was significantly different from Group1 (7.0) and Group2 (6.9). There was no difference between Groups 1 and 2.

### How you may see it:

We found there was a significant differences between the groups ( $p=0.0002$ ). Post-hoc tests found the differences were between Group3 (mean=12.0) and Group1 (7.0) and Group2 (6.9).

## The Kruskal-Wallis Test: The non-parametric alternative to the one-way ANOVA

Is the Mann-Whitney test extended to >2 groups

Use when the assumptions of one-way ANOVA are not met

Still assumes that observations are independently sampled and the groups are independent

Null hypothesis is similar to the Mann-Whitney test which can be simplified to hypothesis about the medians with additional assumption (same distribution)

## Kruskal Wallis test

Is a global test like the ANOVA  
tells us if at least one of the groups is different, but not which  
group is different

Can follow up a significant Kruskal-Wallis test with pairwise Wilcoxon  
Rank Sum tests to determine which pairs are different from each other.

Prism uses Dunn's method to adjust p-values for multiple  
comparisons

# Kruskal-Wallis Example

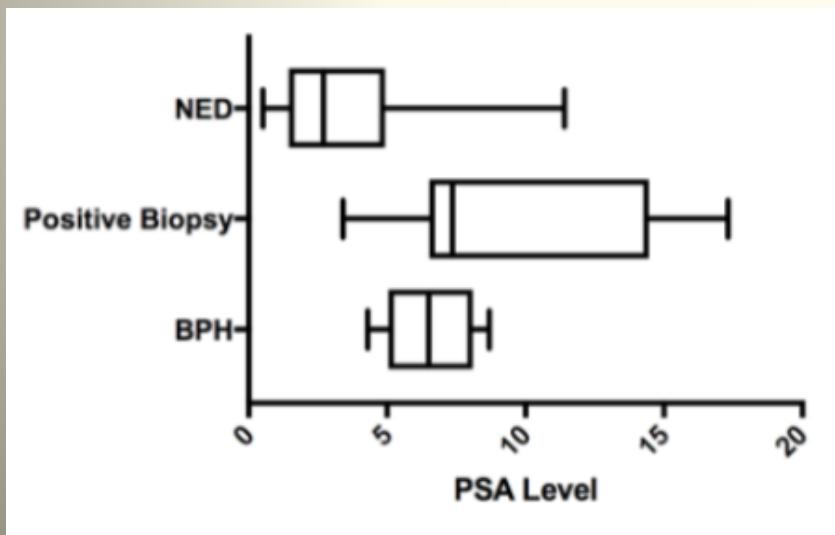
We want to compare PSA levels between 3 groups of patients:

6 with benign prostatic heterotrophy (BPH),

8 with positive biopsy for prostate cancer, and

8 with negative biopsies and no evidence of disease (NED)

Data are unbalanced, sample sizes are small, and data appear skewed,  
>2 magnitude difference between SD



	Col. stats	A	B	C
		BPH	Positive Biopsy	NED
1	Number of values	6	8	8
2				
3	Minimum	4.300	3.400	0.5000
4	25% Percentile	5.050	6.525	1.450
5	Median	6.500	7.350	2.750
6	75% Percentile	8.100	14.45	4.925
7	Maximum	8.700	17.30	11.40
8				
9	Mean	6.533	9.588	3.738
10	Std. Deviation	1.618	4.900	3.462
11	Std. Error of Mean	0.6606	1.732	1.224
12				
13	Skewness	-0.02078	0.5498	1.805
14	Kurtosis	-0.8685	-1.226	3.734
15				

# Column Table

	Group A	Group B	Group C
	BPH	Positive Biopsy	NED
1	5.3	7.1	11.4
2	7.9	6.6	0.5
3	8.7	6.5	1.6
4	4.3	14.8	2.3
5	6.6	17.3	3.2
6	6.4	3.4	1.4
7		13.4	4.4
8		7.6	5.1

Analyze Data

Built-in analysis

Which analysis?

- Transform, Normalize...
  - Transform
  - Transform concentrations (X)
  - Normalize
  - Prune rows
  - Remove baseline and column math
  - Transpose X and Y
  - Fraction of total
- XY analyses
- Column analyses
  - t tests (and nonparametric tests)
  - One-way ANOVA (and nonparametric or mixed)**
  - One sample t and Wilcoxon test
  - Descriptive statistics
  - Normality and Lognormality Tests
  - Frequency distribution
  - ROC Curve
  - Bland-Altman method comparison
  - Identify outliers
  - Analyze a stack of P values
- Grouped analyses

Analyze which data sets?

A:BPH  
 B:Positive Biopsy  
 C:NED

Select All   Deselect All   Help   Cancel   OK

Parameters: One-Way ANOVA (and Nonparametric or Mixed)

Experimental Design   Repeated Measures   Multiple Comparisons   Options   Residuals

**Experimental design**

No matching or pairing  
 Each row represents matched, or repeated measures, data

	Group A	Group B	Group C	Group D
Data Set-A	Y	Y	Y	Title
1				
2				

**Assume Gaussian distribution of residuals?**

Yes, Use ANOVA.  
 No, Use nonparametric test.

Based on your choices (on all tabs), Prism will perform:  
 - Kruskal-Wallis test.

Learn   Cancel   OK

## Parameters: One-Way ANOVA (and Nonparametric or Mixed)

X

Experimental Design

Repeated Measures

Multiple Comparisons

Options

Residuals

**Followup tests**

- None.
- Compare the mean rank of each column with the mean rank of every other column.
- Compare the mean rank of each column with the mean rank of a control column.
 

Control column:
- Compare the mean ranks of preselected pairs of columns.
 

Selected pairs:
- Test for linear trend between column mean and left-to-right column order.

**Which test?**

Use choices on the Options tab to choose the test, and to set the defaults for future ANOVAs.

ANOVA results

Multiple comparisons

Kruskal-Wallis test Multiple comparisons		Mean rank diff.	Significant?	Summary	Adjusted P Value		
					n1	n2	Z
1	Number of families	1					
2	Number of comparisons per family	3					
3	Alpha	0.05					
4							
5	Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value		
6	BPH vs. Positive Biopsy	-2.938	No	ns	>0.9999	A-B	
7	BPH vs. NED	6.375	No	ns	0.2070	A-C	
8	Positive Biopsy vs. NED	9.313	Yes	*	0.0124	B-C	
9							
10	Test details	Mean rank 1	Mean rank 2	Mean rank diff.	n1	n2	Z
11	BPH vs. Positive Biopsy	12.75	15.69	-2.938	6	8	0.8379
12	BPH vs. NED	12.75	6.375	6.375	6	8	1.818
13	Positive Biopsy vs. NED	15.69	6.375	9.313	8	8	2.869
14							

## Parameters: One-Way ANOVA (and Nonparametric or Mixed)

X

Experimental Design | Repeated Measures | Multiple Comparisons | Options | Residuals

**Multiple comparisons test**

- Correct for multiple comparisons using statistical hypothesis testing. Recommended.  
Test: Dunn's
- Correct for multiple comparisons by controlling the False Discovery Rate.  
Test: Two-stage step-up method of Benjamini, Krieger and Yekutieli (recommend)
- Don't correct for multiple comparisons. Each comparison stands alone.  
Test: Uncorrected Dunn's test

**Multiple comparisons options**

- Swap direction of comparisons (A-B) vs. (B-A).
  - Report multiplicity adjusted P value for each comparison.  
Each P value is adjusted to account for multiple comparisons.
- Family-wise significance and confidence level:

**Graphing**

- Graph confidence intervals.
- Graph ranks (nonparametric).
- Graph differences (repeated measures).

**Additional results**

- Descriptive statistics for each data set.
- Report comparison of models using AICc.
- Report goodness of fit.

**Output**

Show this many significant digits (for everything except P values):

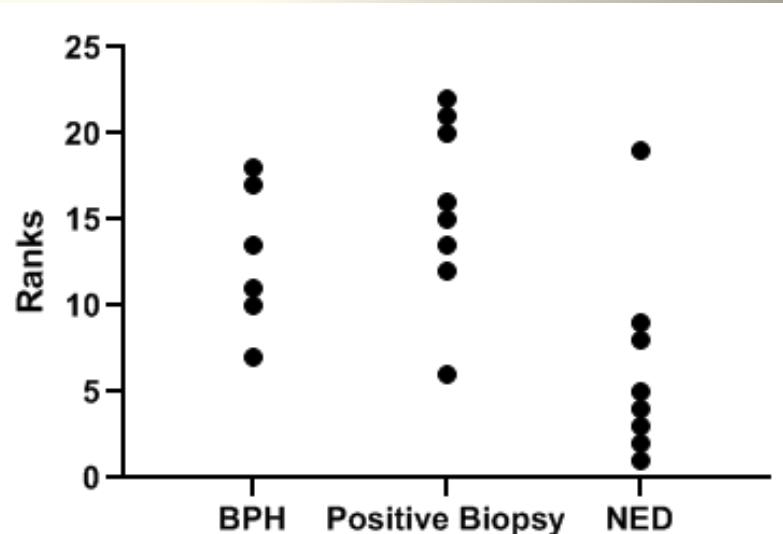
P value style: GP: 0.1234 (ns), 0.0332 (\*), 0.0021 (\*\*), 0.1 N =

Make options on this tab be the default for future One-Way ANOVAs.

Learn

Cancel

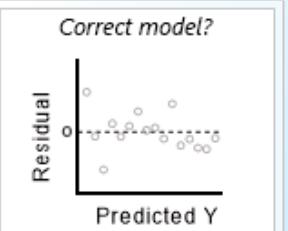
OK

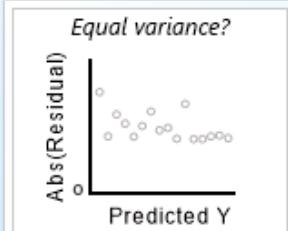


Parameters: One-Way ANOVA (and Nonparametric or Mixed)

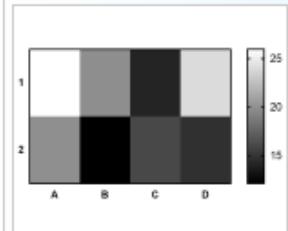
Experimental Design   Repeated Measures   Multiple Comparisons   Options   Residuals

**What graphs to create?**

Residual plot  
*Correct model?*  
  
 Residual   Predicted Y

Homoscedasticity plot  
*Equal variance?*  
  
 Abs(Residual)   Predicted Y

QQ plot  
*Normality?*  
  
 Standardized residual   Actual residual

Heatmap plot  
  
 A   B   C   D

**Diagnostics for residuals**

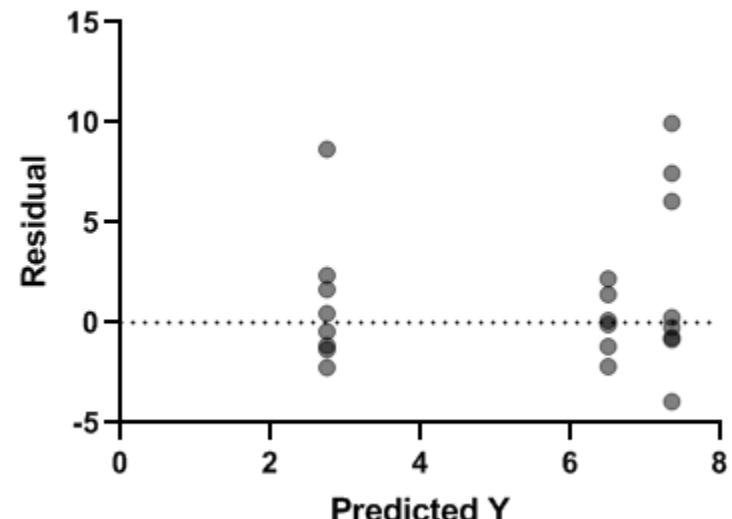
Are residuals clustered or heteroscedastic?  
 Brown-Forsythe and Barlett's tests.

Are the residuals Gaussian?  
 Normality tests of Anderson-Darling, D'Agostino, Shapiro-Wilk and Kolmogorov-Smirnov.

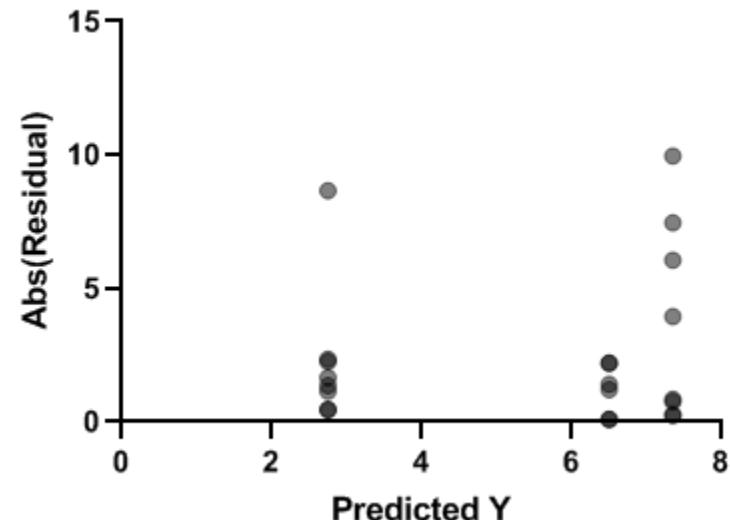
Make options on this tab be the default for future One-Way ANOVAs.

Learn   Cancel   OK

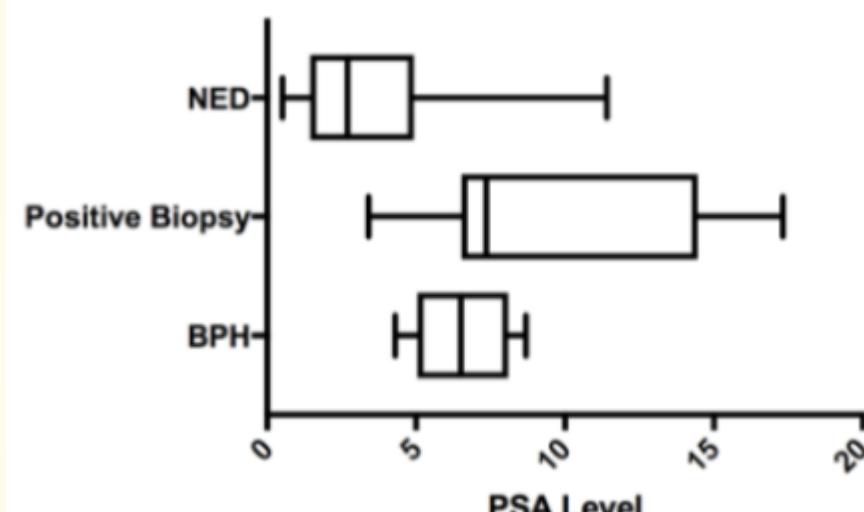
Residual plot



Homoscedasticity plot



ANOVA results	
	Kruskal-Wallis test
	ANOVA results
1	Table Analyzed
2	
3	Kruskal-Wallis test
4	P value
5	Exact or approximate P value?
6	P value summary
7	Do the medians vary signif. ( $P < 0.05$ )? Yes
8	Number of groups
9	Kruskal-Wallis statistic
10	
11	Data summary
12	Number of treatments (columns)
13	Number of values (total)



5	Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted P Value	
6						
7	BPH vs. Positive Biopsy	-2.938	No	ns	>0.9999	A-B
8	BPH vs. NED	6.375	No	ns	0.2070	A-C
9	Positive Biopsy vs. NED	9.313	Yes	*	0.0124	B-C
10						

Kruskal-Wallis test		
ANOVA results		
1	Table Analyzed	PSA Values
2		
3	Kruskal-Wallis test	
4	P value	0.0089
5	Exact or approximate P value?	Exact
6	P value summary	**
7	Do the medians vary signif. ( $P < 0.05$ )?	Yes
8	Number of groups	3
9	Kruskal-Wallis statistic	8.537
10		
11	Data summary	
12	Number of treatments (columns)	3
13	Number of values (total)	22

Ordinary one-way ANOVA		
ANOVA results		
1	Table Analyzed	PSA Values
2	Data sets analyzed	A-C
3		
4	ANOVA summary	
5	F	4.909
6	P value	0.0191
7	P value summary	*
8	Significant diff. among means ( $P < 0.05$ )?	Yes
9	R square	0.3407
10		

## Write up results

### For this class:

The data did not pass the assumptions for an one-way ANOVA (independence, normality/symmetry, homoscedasticity) as the data were unbalanced, group distributions were skewed, and there was evidence of heteroscedasticity. Using the Kruskal-Wallis test, with  $p=0.001$  (two-tailed hypothesis,  $\alpha=0.05$ ), we reject the null hypothesis of no difference in PSA medians by group and conclude there is at least one group different from one other. Dunn's post-hoc tests showed that Positive Biopsy (median PSA=7.35) was significantly different from NED (2.75). BPH PSA (6.5) was not different from either of the other groups.

# Two-Way ANOVA

## AKA Two Factor ANOVA, Factorial ANOVA

A two-way ANOVA has 2 categorical (independent) factors

Gender and diet type are both factors (a total of 4 groups)

Is there was a gender effect on weight lost as well as type of diet effect and do they combine to increase (or decrease) weight loss?

Similar results as separate one-way ANOVAs (or in this case t-tests) on each factor, but **interaction** between factors can be tested with a two way ANOVA

The categorical variables Diet and Gender are factors (independent) variables

Between groups factor

Between groups factor

Continuous (Dependent) variable

	WeightLOST	Diet	gender
16	1.1	1	Male
17	1.5	1	Female
18	1.7	2	Female
19	1.7	2	Male
20	1.9	1	Female
21	2.0	2	Female
22	2.0	2	Female
23	2.0	1	Female
24	2.0	1	Female

## Two-way ANOVA

Continuous (Dependent) variable: Weight Loss

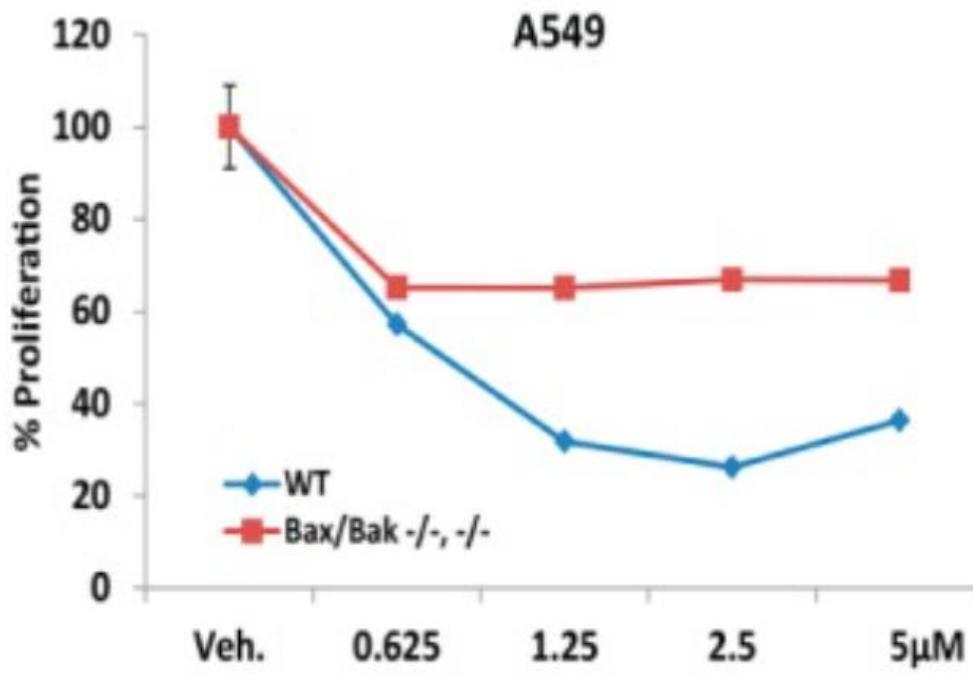
Categorical (Independent) variables (factors): Diet type and Gender

A two-way ANOVA tests three hypotheses, the null's are:

1.  $H_0$ : Mean weight loss in the population does not differ by diet type  
Test of *main* effects for one categorical variable (factor)
2.  $H_0$ : Mean weight loss in the population does not differ by gender  
Test of *main* effects for the other categorical variable (factor)
3.  $H_0$ : There is no interaction between diet type and gender  
Test of interaction between diet and gender

## Example of a Two-Way ANOVA plot

D.



Continuous variable:  
% Proliferation

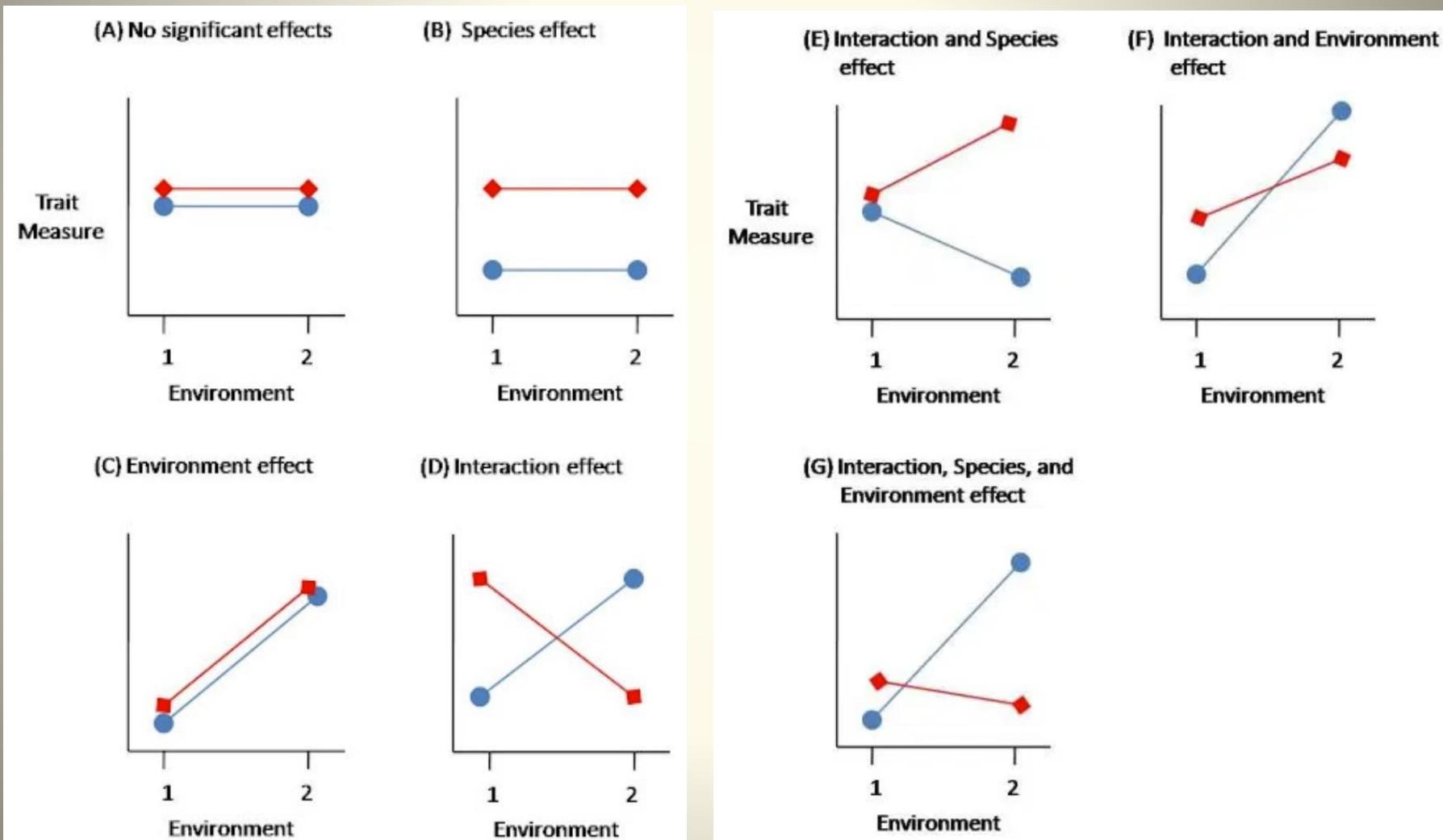
Factor1: Borrelinidin dose  
(5 groups)

Factor2: cell type  
(2 groups)

(D) Proliferation assay with  $\text{BAX}^{(-/-/-/-)}$   $\text{BAK}^{(-/-)}$  A549 cells at 16 h  
two-way ANOVA, dose  $p < 0.0001$ , between wildtype and knockout  $p < 0.0001$ ,  
and interaction  $p = 0.0016$

# Interpreting Effects from a Two-way ANOVA

measuring a biological trait from two species raised in two environments



# Assumptions

Means	Male	Female
Diet1	10.2	9.8
Diet2	12.5	8.7

All cells (groups) of the study design should be normal

Less important for large sample sizes

The samples must be independent

Observations or participants must be independent

Participants in one group cannot influence participants in another

Homoscedasticity in every cell.

The cells should have the same sample size

## The Limitations of Prism

Prism does not test for violations of assumption of normality for a two-way ANOVA

Prism advises that if you don't think your data are sampled from a normal distribution (and no transformation will make the distribution normal), you should consider performing nonparametric two-way ANOVA.

But Prism does not offer this test

Prism also cannot test the assumption of equal variances

But you can eyeball the data as we have done before

## Two-Way ANOVA Example

	ABILITY	METHOD	X
1	none	blue-book	23
2	none	blue-book	32
3	none	blue-book	25
4	some	blue-book	29
5	some	blue-book	30
6	some	blue-book	34
7	lots	blue-book	31
8	lots	blue-book	36
9	lots	blue-book	33
10	none	computer	32
11	none	computer	26
12	none	computer	26
13	some	computer	34
14	some	computer	41
15	some	computer	35
16	lots	computer	23
17	lots	computer	26
18	lots	computer	32
18	18	18	18

A statistics teacher is concerned that allowing some students to use a computer for the final exam might give them an unfair advantage if their data entry (aka typing) skill are good (a person can usually type faster than they can write). She is interested in the effect the of type of medium (bluebook or computer) and typing ability on test results.

Her main question is about how typing affects test scores for people using computers or bluebooks

For a practice exam, she randomly divided the classes in half: 50% wrote in a bluebook, 50% used computers. In addition the students were partitioned into three groups, no typing ability, some typing ability, and lots of typing ability.

The continuous (dependent) variable is the final exam score (X).

## Assumptions for this example

Assumption of independence seems to be OK

However, Prism can't test the assumptions of normality and homoscedasticity by cells (it assumes these to be met)

Cell # = 3 (levels of typing ability) x 2 (exam methods) = 6

We could restructure the data and test the 6 cells in a column (ANOVA) analysis

But each cell only has an n of 3

Too small for statistical tests and what would graphing tell us?

What do we do?

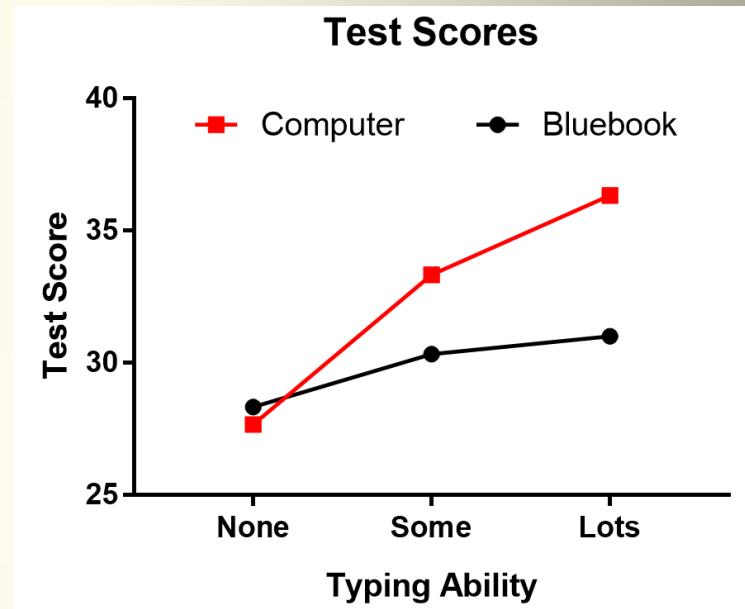
Run the example as it is for now.

If n's are small, seek professional statistical help before you publish

## A first look at the data

If lines are not parallel, there could be interaction

If scores for medium factor (computer vs. Bluebook) have different levels for different categories of the other factor (typing ability), there could be interaction



	Computer			Bluebook		
	n	mean	SD	n	mean	SD
None	3	27.7	2.08	3	28.3	1.53
Some	3	33.3	1.15	3	30.3	1.53
Lots	3	36.3	1.53	3	31.0	2.00

## New Data Table and Graph

## New table &amp; graph

XY

Column

Grouped

Contingency

Survival

Parts of whole

Multiple variables

Nested

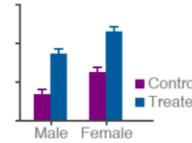
## Existing file

Clone a graph

**Grouped tables have two grouping variables, one defined by columns and the other defined by rows**

Table format  
Grouped

	A			B		
	Control			Treated		
	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Male					
2	Femal					


[Learn more](#)

## Data table:

- Enter or import data into a new table
- Start with sample data to follow a tutorial

## Options:

- Enter and plot a single Y value for each point
- Enter  replicate values in side-by-side subcolumns
- Enter and plot error values already calculated elsewhere

Enter: Mean, SD, N

Prism Tips

Table format:  
Grouped

		X	A:1	A:2	A:3	B:1	B:2	B:3	C
1	None		30	28	27	30	26	27	
2	Some		29	30	32	34	32	34	
3	Lots		31	33	29	38	36	35	

## Graphing the data differently by different data structures

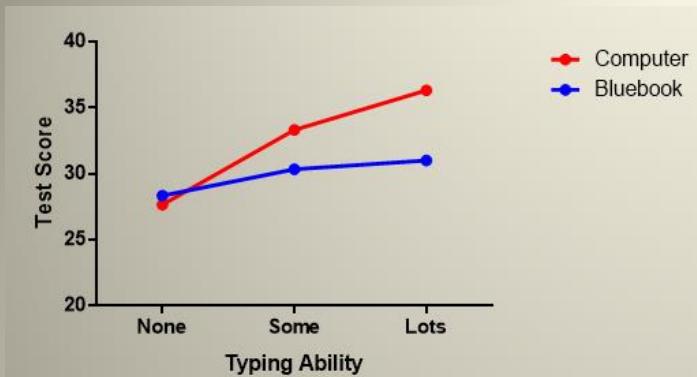


Table format: <b>Grouped</b>		Group A			Group B			C
		Bluebook			Computer			
1	None	30	28	27	30	26	27	C1
2	Some	29	30	32	34	32	34	C2
3	Lots	31	33	29	38	36	35	C3
4	Title							C4
5	Total							C5

This set-up better answers her main question

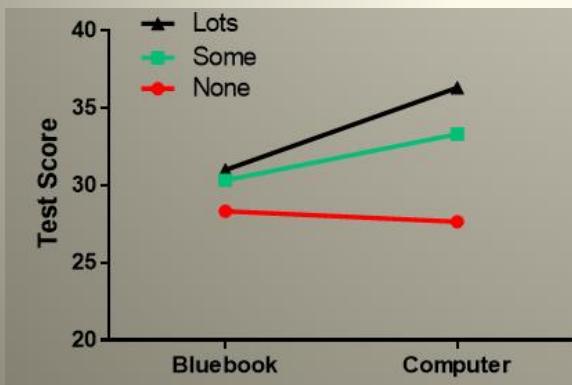


Table format: Grouped		Group A Bluebook			Group B Computer			
		A:1	A:2	A:3	B:1	B:2	B:3	C
1	None	30	28	27	30	26	27	
2	Some	29	30	32	34	32	34	
3	Lots	31	33	29	38	36	35	

Analyze Data

Built-in analysis

Which analysis?

- Transform, Normalize...**
  - Transform
  - Transform concentrations (X)
  - Normalize
  - Prune rows
  - Remove baseline and column math
  - Transpose X and Y
  - Fraction of total
- XY analyses**
- Column analyses**
- Grouped analyses**
  - Two-way ANOVA (or mixed model)
  - Three-way ANOVA (or mixed model)
  - Row means with SD or SEM
  - Multiple t tests - one per row
- Contingency table analyses**
- Survival analyses**
- Parts of whole analyses**
- Multiple variable analyses**
- Nested analyses**
- Generate curve**
- Simulate data**
- Recently used**

Analyze which data sets?

A:Bluebook  
 B:Computer

Select All   Deselect All

Help   Cancel   OK

Parameters: Two-Way ANOVA (or Mixed Model)

RM Design   RM Analysis   Factor names   Multiple Comparisons   Options   Residuals

Data arrangement

Table format: Grouped	Group A		Group B		Group C	
	Title		Title		Title	
	A:Y1	A:Y2	B:Y1	B:Y2	C:Y1	C:Y2
1	Title					
2	Title					
3	Title					
4	Title					

Matching by which factor(s)?

Each column represents a different time point, so matched values are spread across a row.  
 Each row represents a different time point, so matched values are stacked into a subcolumn.

Assume sphericity (equal variability of differences)?

No. Use the Geisser-Greenhouse correction. Recommended.  
 Yes. No correction.

Based on your choices (on all tabs), Prism will perform:  
- Ordinary two-way ANOVA

Learn   Cancel   OK

Parameters: Two-Way ANOVA (or Mixed Model)

RM Design RM Analysis Factor names Multiple Comparisons Options Residuals

Analyses of repeated measures data can be reported in two ways.

- ANOVA (partition sum-of-squares). This is the same as the general linear model (GLM).
- Mixed-effects model. This uses the restricted maximum likelihood method.

If there are no missing values, the two approaches give the same main results (F and P values). But the methods are very different, so the other reported results differ.

Analyze using which method

- Repeated measures ANOVA (based on GLM).  
Same as Prism 7 and earlier.  
Requires balanced data (no missing values).
- Mixed-effects model.  
Results are presented in a format different than ANOVA.  
Works fine with missing values.
- It depends.  
Use ANOVA if there are no missing values.  
Use mixed-effects model if there are missing values.

What to do if a random effect is zero (or negative)?

- Remove term(s) from model and fit a simpler model (recommended).
- Fit the full model anyway (corresponds to NOBOUND parameter in SAS).

These choices are available only when you entered replicate data into subcolumns, and choose a repeated measures design on the first (RM Design) tab.

Make these choices the default for future ANOVAs (One-, Two- and Three-way).

Learn Cancel OK

Parameters: Two-Way ANOVA (or Mixed Model)

RM Design RM Analysis Factor names Multiple Comparisons Options Residuals

Data arrangement

Table format:  
**Grouped**

		Group A		Group B		Group C	
		Title		Title		Title	
		A:Y1	A:Y2	B:Y1	B:Y2	C:Y1	C:Y2
1	Title						
2	Title						
3	Title						
4	Title						

Factor names

Name the factor that defines the columns:  
Medium

Name the factor that defines the rows:  
Typing

Name of matched set (i.e. person or block):  
Subject

Learn Cancel OK

## Parameters: Two-Way ANOVA (or Mixed Model)

RM Design RM Analysis Factor names Multiple Comparisons Options Residuals

**What kind of comparison?**

Compare each cell mean with the other cell mean in that row

Group A		Group B	
Data Set-A		Data Set-B	
A:Y1	A:Y2	B:Y1	B:Y2
1	Mean	Mean	Mean
2	Mean	Mean	Mean
3	Mean	Mean	Mean

**How many comparisons?**

- Compare each column mean with every other column mean.  
 Compare each column mean with the control column mean.

Control column: Group A : Bluebook

**Which test?**

Use choices on the Options tab to choose the test, and to set the defaults for future ANOVAs.

Learn

Cancel

OK

## Parameters: Two-Way ANOVA (or Mixed Model)

RM Design RM Analysis Factor names Multiple Comparisons Options Residuals

**Multiple comparisons test**

- Correct for multiple comparisons using statistical hypothesis testing. Recommended.

Test: Sidak (more power, recommended)

- Correct for multiple comparisons by controlling the False Discovery Rate.

Test: Two-stage step-up method of Benjamini, Krieger and Yekutieli (recommended)

- Don't correct for multiple comparisons. Each comparison stands alone.

Test: Fisher's LSD test

**Multiple comparisons options** Swap direction of comparisons (A-B) vs. (B-A). Report multiplicity adjusted P value for each comparison.

Each P value is adjusted to account for multiple comparisons.

Family-wise significance and confidence level: 0.05 (95% confidence interval)

**Graphing options** Graph confidence intervals.**Additional results** Narrative results. Show cell/row/column/grand means. Report goodness of fit.**Output**

Show this many significant digits (for everything except P values): 4

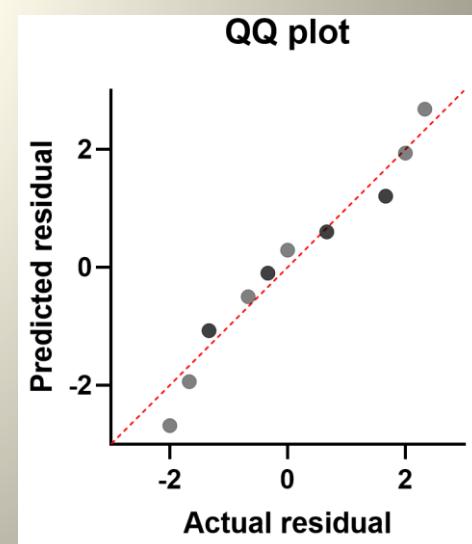
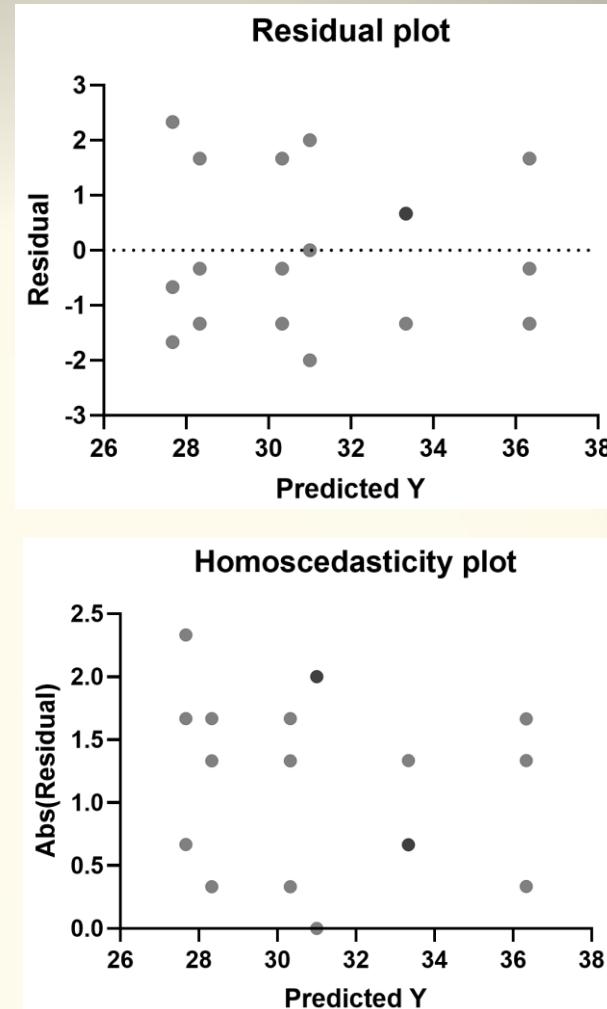
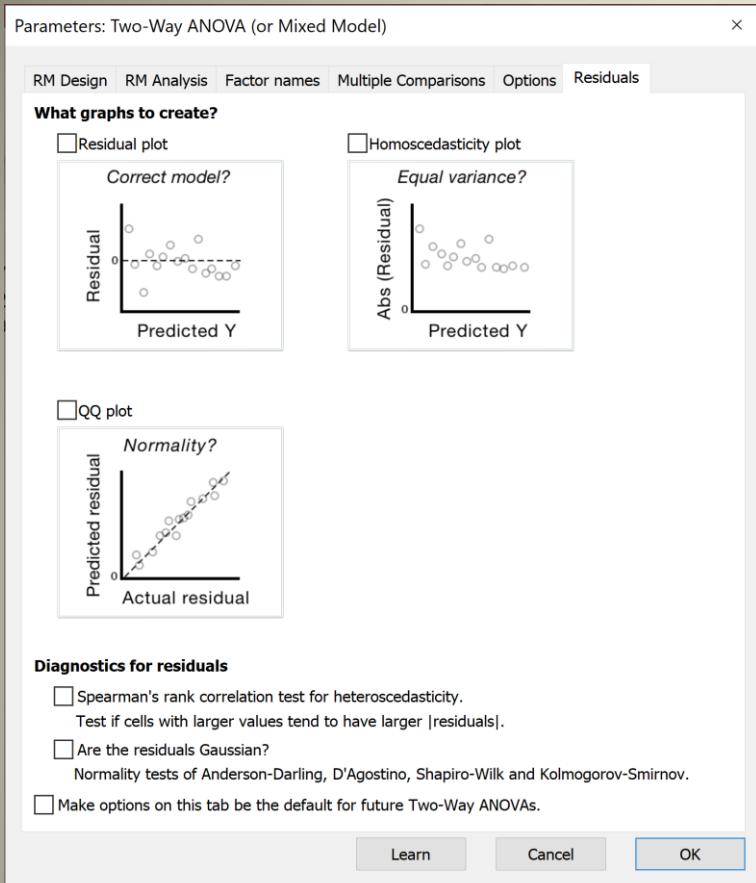
P value style: GP: 0.1234 (ns), 0.0332 (\*), 0.0021 (\*\*), 0.000 N = 6

 Make options on this tab be the default for future Two-Way ANOVAs.

Learn

Cancel

OK



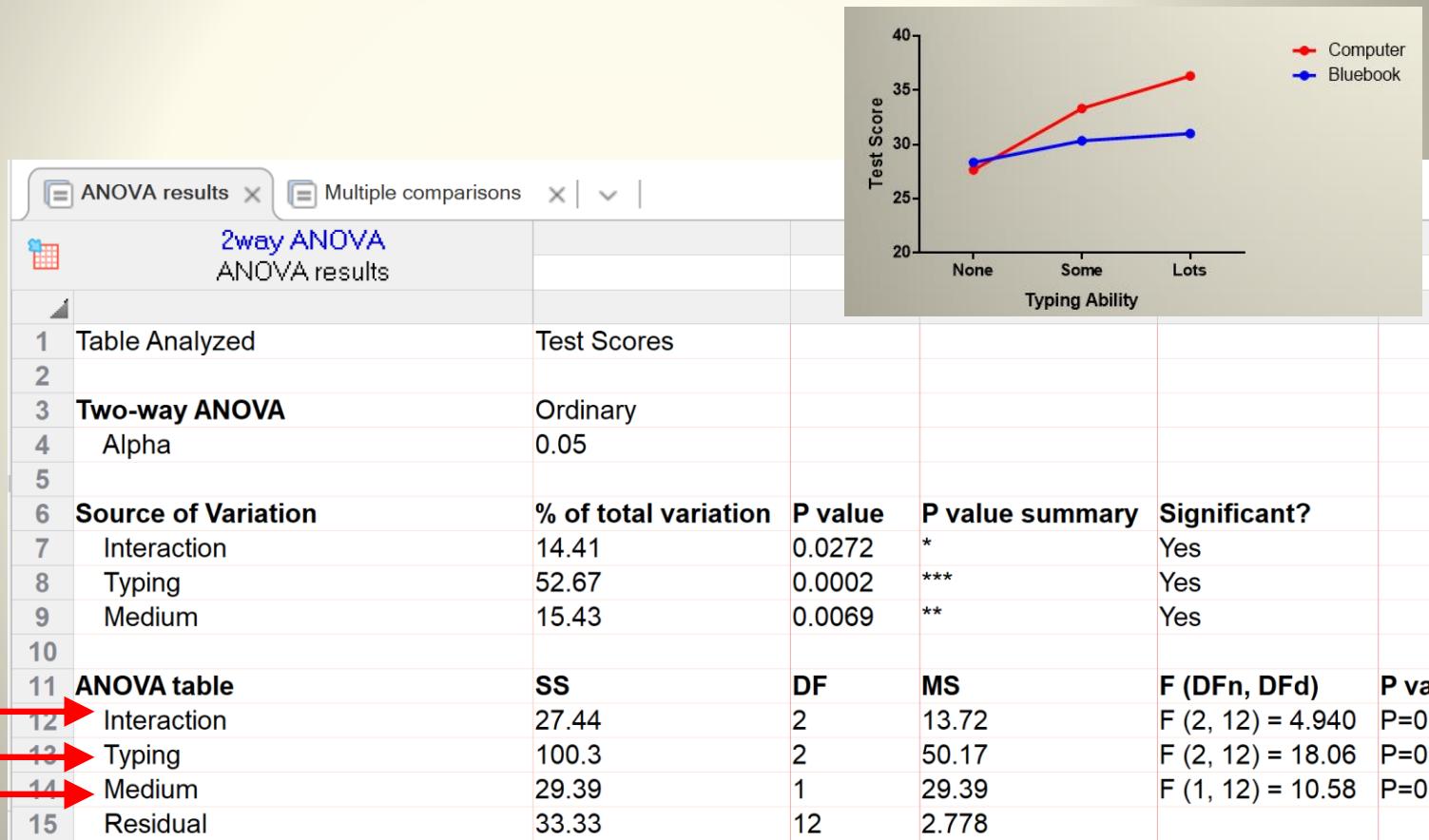


## Two-Way ANOVA: Summary Table

(Don't worry, I won't ask you to describe this one)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
A (Row)	$a - 1$	$SS(A)$	$MS(A)$	<u><math>MS(A)</math></u> MSE
B (Column)	$b - 1$	$SS(B)$	$MS(B)$	<u><math>MS(B)</math></u> MSE
AB (Interaction)	$(a-1)(b-1)$	$SS(AB)$	$MS(AB)$	<u><math>MS(AB)</math></u> MSE
Error	$n - ab$	$SSE$	$MSE$	
Total	$n - 1$	$SS(\text{Total})$		← <i>Same as Other Designs</i>

Remember the 3 null hypotheses about  
 Interaction  
 Main effect for factor1  
 Main effect for factor2



	ANOVA results	Multiple comparisons					
	2way ANOVA						
	Multiple comparisons						
1	Compare each cell mean with the other cell mean in that row						
2							
3	Number of families						
4	Number of comparisons per family						
5	Alpha						
6							
7	<b>Sidak's multiple comparisons test</b>	<b>Significant?</b>	<b>Summary</b>	<b>Adjusted P Value</b>			
8							
9	Bluebook - Computer						
10	None	No	ns	0.9506			
11	Some	No	ns	0.1365			
12	Lots	Yes	**	0.0061			
13							
14							
15	<b>Test details</b>	<b>Mean Diff.</b>	<b>SE of diff.</b>	<b>N1</b>	<b>N2</b>	<b>t</b>	<b>DF</b>
16							
17	Bluebook - Computer						
18	None	0.6667	1.361	3	3	0.4899	12.00
19	Some	-3.000	1.361	3	3	2.205	12.00
20	Lots	-5.333	1.361	3	3	3.919	12.00
21							

## Write up results

For this class:

The data passed the assumptions for an two-way ANOVA (independence, homoscedasticity for each cell by both factors). With only n of three in each cell, it was difficult to assess normality. Given that the ANOVA is robust to some deviation from normality, we will proceed with the two-way anov. Both main effects were significant so we reject the null hypotheses of no difference in score by medium used (computer vs. bluebook) or typing ability in the population. People using the computer scored higher [ $F(1,12)=10.58$ ,  $p=0.01$ ,  $\alpha=0.05$ ] as did those with higher typing ability [ $F(2,12)=18.06$ ,  $p=0.002$ ; see table 1 for mean values]. The interaction term had a p-value of 0.03 [ $F(2,12)=4.94$ ] meaning that we reject the null hypothesis of no interaction and conclude that people who used the computer and had better typing skills had the highest test scores (see Figure 1 and Table 1).

Table 1.

	Computer			Bluebook		
	n	mean	SD	n	mean	SD
None	3	27.7	2.08	3	28.3	1.53
Some	3	33.3	1.15	3	30.3	1.53
Lots	3	36.3	1.53	3	31.0	2.00

