Study Design

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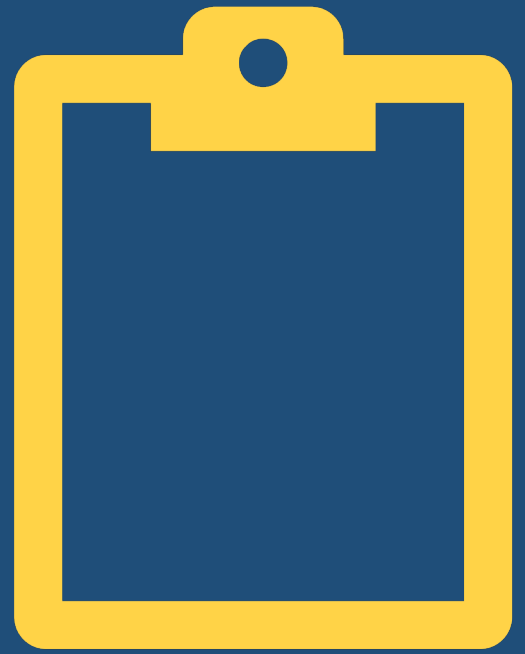
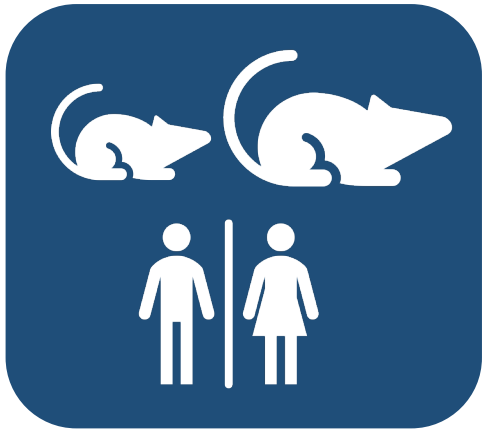
Overview

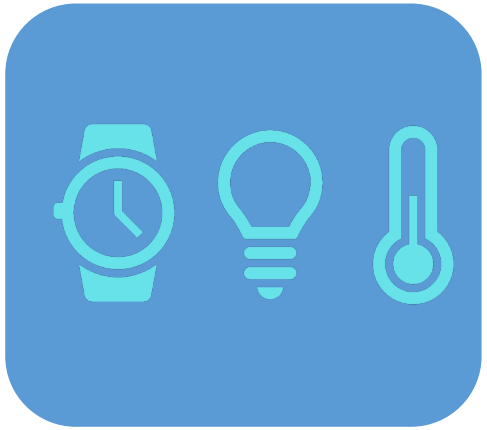
- Sources of Error and Variation
- Biological Variation
- Sample Size
- Avoiding Bias
  - Randomization
  - Blinding
- Replicates



Sources of Error & Variability

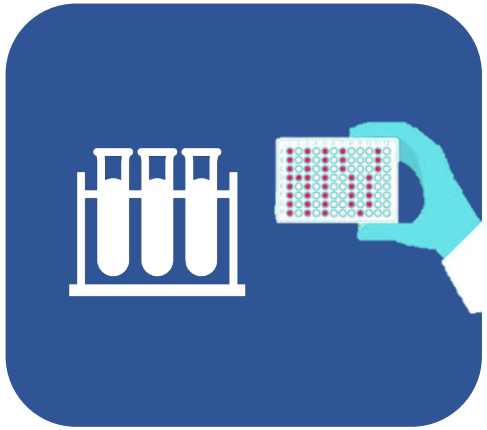


Biological Variation



Environmental

Procedural



Processing Order/
Batch Effects

Technical
/Instrumental

Human Error

Errors can be **random** or **systematic**

Impact of random errors can be reduced through **replication**

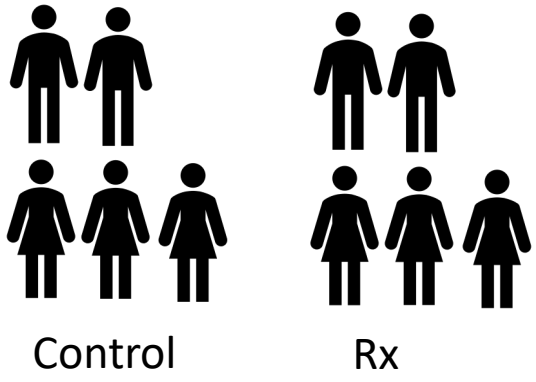
Systematic errors can sometimes be avoided through **randomization**

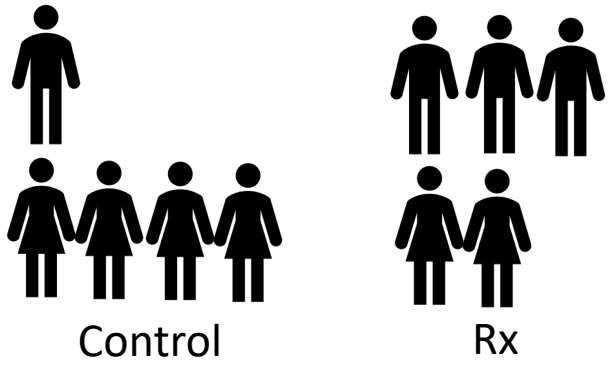
Avoiding Errors

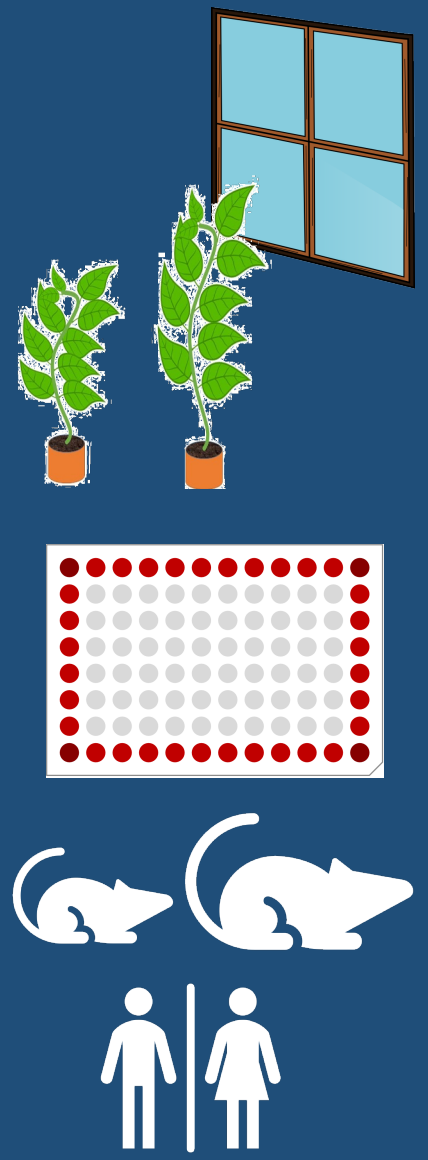
- Calibrate/maintain equipment
- Use high quality reagents/samples & store them appropriately
- Document everything
- Be skeptical of new protocols, even published ones

Biological Variation

- Can introduce bias or mask real effects
- Caused by genetic or environmental factors
- Select matched samples/subjects
  - Same age, sex, weight, environment etc
- Randomize assignment to groups
  - Block known confounding variables prior to randomization







Sample Size

- Statistical Power
- Outliers / Errors / Attrition
- Cost

Key Assumptions in Sample Size Calculations

- What is a clinically/biologically meaningful *effect size*?

$$\textit{Effect Size} = \frac{\textit{Mean}_1 - \textit{Mean}_2}{\textit{Standard Deviation}}$$

- What is the anticipated *variation* within your experimental groups?
  - Is this variation the same or different between groups?

Factors Affecting Statistical Power and Sample Size

1. Size of the effect (difference between group means) ↑
2. Variation in the outcome variable ↓
3. Bigger sample size
4. More stringent significance level desired ↓

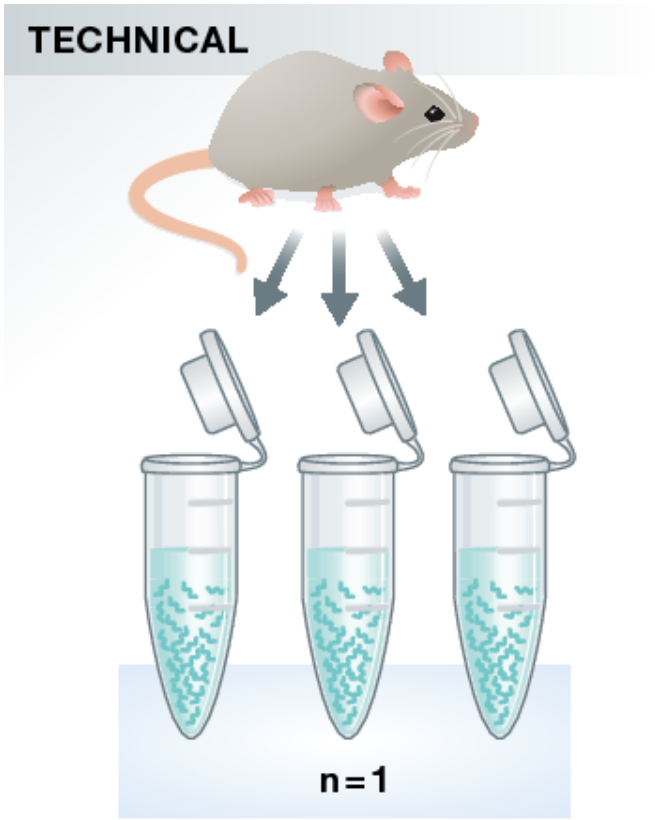
Bias

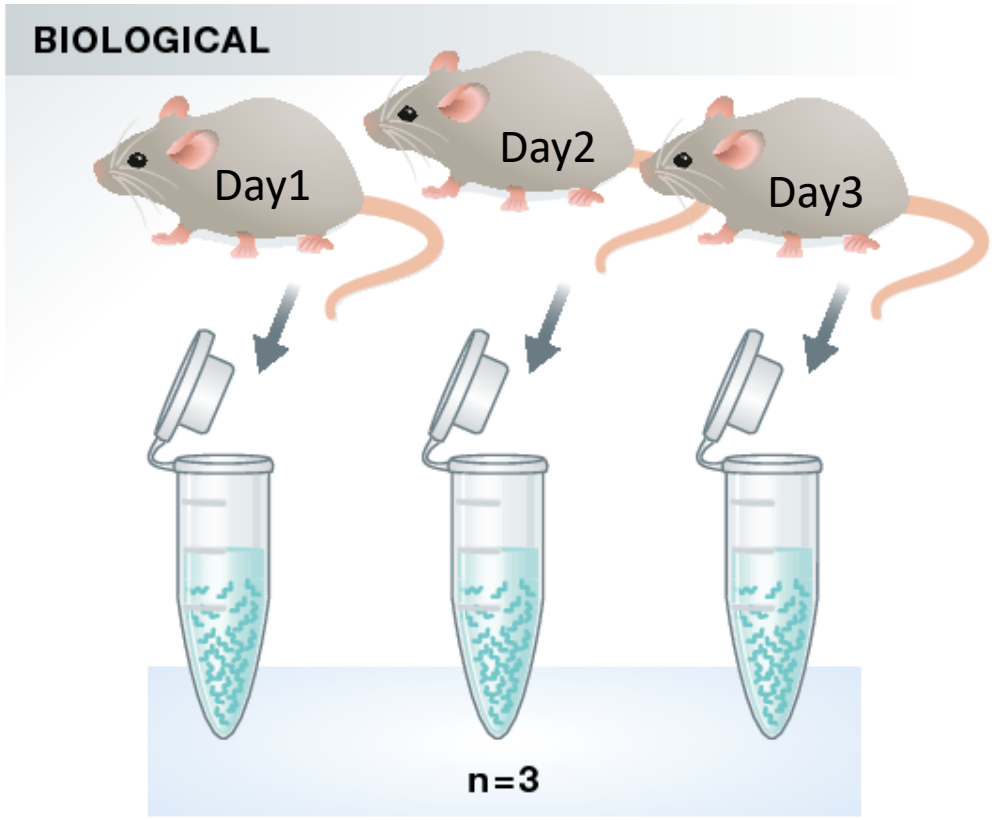
- Confirmation bias
  - Looking for data to support the hypothesis
- Detection bias
  - Increased screening for treated population
- Observer bias
  - Different standards/protocols used by different researchers
- Procedural bias
  - Order of processing / batch effects

Avoiding Bias

- Randomization
- Blinding of researcher (and patient)
- Standardization of procedures

Replicates



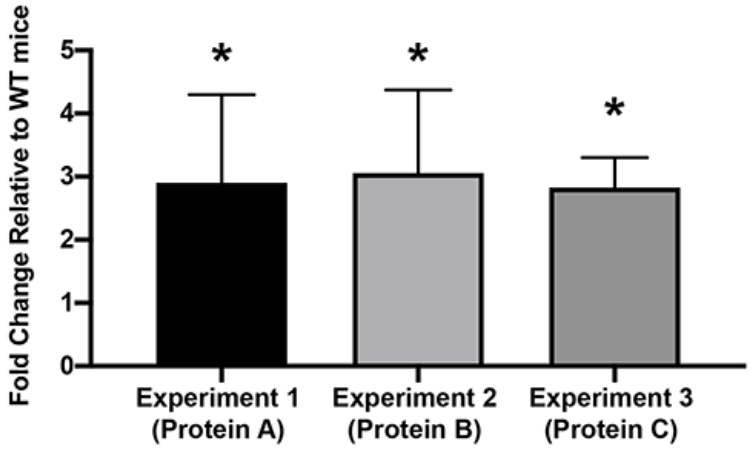


use the mean, mode or median value

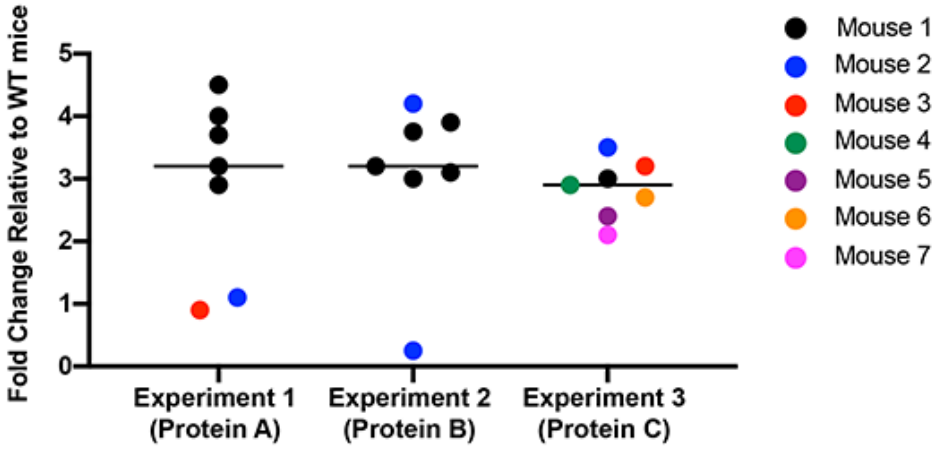
INDEPENDENT EXPERIMENTS – performed at different times!

Replicates

Figure 1



Authors report n = 7 in each experiment and conclude statistical significance



Same exact data as shown in Fig. 1, n = 7?

What do replicates tell you?

- Technical Replicates
  - Assess variation in the *assay*
  - Increase confidence in the measured value
- Biological Replicates
  - Confirm effects are reproducible across biologically distinct samples
  - Detection of outliers
- Independent Experiments
  - Account for effects caused by human error

Report whether replicates are technical or biological, how many were performed (**n!**) and whether they were performed independently!

Definitions (controversial)

- Replicability
  - Different method, different team
- Reproducibility
  - Same method, different team
- Repeatability
  - Same method, same team

Study Design Exercises

1) Biological and Technical Replicates:

- Using the faculty provided description of an experiment from a recent grant application, uploaded to the "Study Design" module in Canvas, *map out the samples that will need to be collected and analyzed for this experiment, indicating which are biological vs technical replicates*.

2) Sample Size and Power Calculations:

- Independently complete a sample size calculation for a mouse tumor growth study using the equation and desired study components outlined in the "Study Design" module in Canvas

Useful Resources

- NIH Guidance on Rigor and Reproducibility in Grant Applications
- NIH Policy on Sex as a Biological Variable
- NIH eLearning resources for Sex as a Biological Variable
- Experimental Design Assistant - National Centre for the Replacement, Refinement, & Reduction of Animals in Research
- NIH Scientific Rigor in Study Design - Examples from funded proposals
- **Empowering statistical methods for cellular and molecular biologists** https://www.molbiolcell.org/doi/full/10.1091/mbc.E15-02-0076
- **Criteria for biological reproducibility: What does "*n*" mean?** https://stke.sciencemag.org/content/8/371/fs7?rss=1
- **Rigor and Reproducibility in Experimental Design** https://smcclatchy.github.io/exp-design/

Useful Resources

Software for sample size calculations			
Software	Platform	URL[a]	Freely available?
Stand-alone programs			
G*Power	Windows and macOS	http://www.gpower.hhu.de	Yes
PS	Windows	http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize	Yes
PASS	Windows	https://www.ncss.com/software/pass	No
nQuery	Windows	https://www.statsols.com/nquery-sample-size-and-power-calculation-for-successful-clinical-trials	No
JAVA applets	Windows and macOS	https://homepage.stat.uiowa.edu/~rlenth/Power/oldversion.html	Yes
R[b] packages			
pwr	Windows, macOS and Linux	https://cran.r-project.org/web/packages/pwr	Yes
TrialSize	Windows, macOS and Linux	https://cran.r-project.org/web/packages/TrialSize	Yes
powerSurvEpi	Windows, macOS and Linux	https://CRAN.R-project.org/package=powerSurvEpi	Yes
SAS			
PROC POWER	Windows and Linux	https://support.sas.com/documentation/cdl/en/statug/63033/HTML/default/viewer.htm#power_toc.htm	No
SPSS			
SamplePower	Windows	https://www-01.ibm.com/marketing/iwm/iwmdocs/tnd/data/web/en_US/trialprograms/U741655I36057W80.html	No
Stata			
power	Windows, macOS and Linux	https://www.stata.com/features/power-and-sample-size/	No
Microsoft Excel			
PowerUp		http://www.causalevaluation.org/power-analysis.html	Yes[c]
[a] URLs are correct as of 11 April 2018.			
[b] R also has several base functions that enable power calculations to be made; e.g. power.t.test(), power.prop.test() and power.anova.test().			
[c] Requires Microsoft Excel to be installed.			
Adapted from G.L. Hickey et al. / European Journal of Cardio-Thoracic Surgery			