Enhancing Rigor and Reproducibility in the Biological and Biomedical Sciences at Yale University

What is rigorous research?
According to the NIH, scientific rigor is the strict application of the scientific method to ensure unbiased experimental design, methodology, analysis, interpretation and reporting of results. This includes full transparency in reporting experimental details so that others may reproduce and extend the findings. Robust results are obtained with solid, well-controlled experiments capable of being reproduced under well-controlled conditions by reporting illustrative experimental details. The aim is to reduce bias within research design and execution by promoting best practices at each stage of an experiment.

NIH Policy from 2016 has four elements to facilitate the interpretation and replication of results:

1. *Prior research*. New research should be built on a solid foundation, understanding the strengths and weaknesses of the studies that came before.
2. *Elements of Experimental Design*. Define terminology in advance, and when possible use blinding and randomization. Also, identify sample size and build an analysis plan before you begin.
    a. Use comprehensive reporting methods (Landis et.al. 2012) adopted by Nature Publishing Group and 30 other top journals in 2014:
        - Use community standards and nomenclature; distinguish between biological data points and technical replicates; note whether experiments were randomized by which method; provide the statistical model for power analyses and computed sample size; report all excluded cases with rationale beyond 'outlier.' Share your data and materials to encourage dataset transparency for future analyses.
3. *Consider relevant biological variables*. Sex, weight, age, and underlying health conditions can influence your results. Sex-based differences in basic genetics, cellular and biochemical organizations, and the exclusion of females from preclinical studies has led to treatments with adverse effects more common or severe in women than in men.
4. *Authenticate and validate key biological or chemical resources*. What methods will you employ to authenticate your resources prior to use and at regular intervals as needed?
    a. Since the 1960s, more than 400 cell lines widely-used worldwide have been shown to have been misidentified.
    b. Use best practices for biological material with enough information to uniquely identify reagents. Report the source, characteristics, dilutions and validation method for antibodies. Report the source, authentication and mycoplasma contamination status of cell lines. Report the source, strain, species, sex, age, and husbandry of animals.

References and Resources:

- https://nexus.od.nih.gov/all/2018/12/13/resources-for-rigorous-research/
- https://www.nigms.nih.gov/training/pages/clearinghouse-for-training-modules-to-enhance-data-reproducibility.aspx
- https://grants.nih.gov/policy/reproducibility/index.htm
- https://www.nig.gov/research-training/rigor-reproducibility
- https://ctsi.ucla.edu/funding/files/view/docs/rigorandreproducibility_Rochester.pdf
- https://research.medicine.umich.edu/our_units/grant-services-analysis/research-development/proposal-development/rigor_reproducibility
- www.Rwjms.umdnj.edu/gsbs/current/RigorandReproducibilityTraining.html
- https://as.nyu.edu/biology/programs/phd/phd_resources/phd-best-practices-for-research-rigor-and-reproducibility.html
- https://grants.nih.gov/reproducibility/faqs.htm#4846
- https://www.brown.edu/research/resourcesandtrainings
- Rogers, W.A., Ballantyne, A.J. Australian gender equity in health research group 2008. Exclusion of women from clinical research: myth or reality*? Mayo Clin. Proc.,* 83 (2008), pp. 536–542.

For Graduate Students to Consider in their Research:

1. Learn the standard operating procedures of your lab and ask questions about how and why things are done a certain way.
    a. Ask a senior lab member or your adviser if you are ever unsure of a next step in design or execution of experiments.
    b. Learn how to maintain your lab notebook for accurate record keeping and transparency in reporting - also important if you need to share data or materials.
2. During your literature review of prior research:
    a. Think about inherent biases and how you can transparently report your research process.
    b. Note conflicting results in previous studies, and consider how your work would seek to clarify misconceptions or complications.
    c. Understand that rigor of the studies on which you base your hypothesis is a vital component of the robust design of your research study.
3. Consider these features of experimental design as you plan your study:
    a. Familiarize yourself with publications standards of journals in your field.
    b. Estimate your sample size and plan your statistical methods and analyses in advance.
    c. Use appropriate methods to randomize your sample.
    d. Use blinding for experimental groups and outcomes analyses.
    e. Control for inter-operator variability.
    f. Consider appropriate biological (# of different samples) and technical replicates (# of times you measure any one sample).
    g. Discuss how you will handle missing data.
    h. Define your inclusion and exclusion criteria for your sample.
    i. Reflect upon possible impacts of subject retention and attrition.
4. Consider sex as a biological variable:
    a. Conduct a literature review on whether there is an influence of biological sex in your field.
    b. Form research questions and study the design and results from the perspective of biological sex.
    c. Use both sexes in your research or provide strong justification for only using one sex (from literature review, preliminary data or other relevant considerations).
    d. Characterize your study results by sex: examine treatment or toxicity effects separately for males and females; have a large enough sample size to explore the possible impact of sex on results.
5. Key biological resources are materials that may differ from lab to lab, have qualities or qualifications that could influence the results, and are integral to the proposed research.
    a. Independently verify the identity and activity of the product you obtained to ensure the identity and validity of key resources. Some examples:
        - Authenticate a cell line by using short tandem repeat (STR) profiling and mycoplasma testing.
        - Authenticate chemical resources using liquid or gas chromatography or mass spectrometry.
        - To confirm genome modifications in an animal or cell, conduct PCR amplification or Southern blot testing.
6. For further discussion:
    a. What potential problems might you encounter during the various stages of your research plan? What limitations are inherent in your research plan? What alternative strategies or explanations might explain your results? How will you proceed if your results are not what you expected?

References and Resources:

- Benson, M.B., & Scott, S.F. (2018). *NIH & AHRQ: Rigor and Reproducibility Policy*, [powerpoint slides].
- Carroll, A. Indiana University School of Medicine, R25 GM116146 (NIH training modules) https://www.youtube.com/watch?v=EvoVb_QLRK8
- Casadevall A, Fang FC. 2016. Rigorous science: a how-to guide. *mBio* 7(6): e01902-16. doi:10.1128/mBio.01902-16.
- Clayton, J.A. Studying both sexes: a guiding principle for biomedicine, *FASEB J February 2016* 30:519-524.
- Landis, et.al. (2012). A call for transparent reporting to optimize the predictive value of preclinical research, *Nature, 490(7419),* 187-91. Doi:10.1038/nature11556.
- Prendergast, B.J., Onishi, K.G., & Zucker, I. Female mice liberated for inclusion in neuroscience and biomedical research, *Neuroscience & Biobehavioral Reviews, Volume 40*, March 2014, Pages 1-5.