

Practical What & Where of Rigor and Reproducibility in the NIH Application

SIU School of Medicine

Office of Grants & Contracts with Sophia Ran, PhD

Recent Training Sessions

NIH Regional Seminar

*Rigor and Reproducibility:
Back to Basics*

Chicago, IL

Oct 27, 2016

Write Winning Grant Proposals

John D. Robertson, PhD

Grant Writers' Seminars and Workshops

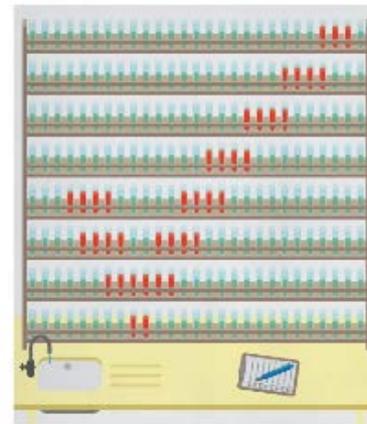
Oct 28, 2016

Why?

- ▶ NIH, researchers, educators, journals, reviewers, funding agencies, disease advocacy groups, pharmaceutical industry agree:

The inability to translate and replicate results is a **BIG** problem.

(Landis, S.C., et al., 2012)



NIH plans to enhance reproducibility

Francis S. Collins and Lawrence A. 1 initiatives that the US National Instit is exploring to restore the self-correc preclinical research.

Aggravating phenomena of concern, from scientists and laypeople, concerns the complex options for ensuring shorter terms, balance that are have been hub

enumerated by the hundreds of thousands published each year in good faith. Instead, a confluence of other factors serves to have contributed to the lack of reproducibility. Factors include poor training of researchers in experimental design, increased emphasis on making provocative statements rather than presenting technical details and publications that do not report basic elements of experimental design. Critical experimental design elements that are all too frequently ignored include blinding, randomization, replication, sample size calculation and the effect of sex differences. And some scientists reportedly take a "secret stance" to assure their experiments work—and withhold details from publications or describe them only vaguely to science colleagues. What hope is there that other scientists will be able to build on such work to further forward cell progress? Issues being discussed are the policies and methods of funding agencies, teaching centers and scientific publishers. Funding agencies often uncritically encourage the publication of novel results in high-profile journals. Some agencies contract for public information for publications in such journals, including presentation and format, and in contrast to other agencies, do not publish.

PRECLINICAL PROBLEMS Reproducibility is particularly a public health concern for the pharma. However, because cell and tissue seem to be less at risk because they



NIH to balance sex in cell and animal studies

Janine A. Clayton and Francis S. Collins unveil policies to ensure that preclinical research funded by the US National Institutes of Health considers females and males.

More than two decades ago, the US National Institutes of Health (NIH) established the Office of Research on Women's Health (ORWH). At that time, the Congressional Caucus for Women's Issues, an elected legislative group, urged NIH scientists and leaders to agree that including women in clinical research was both for women and for the science. In 1993, the NIH Revitalization Act required the inclusion of women in NIH-funded clinical research. Today, just over half of NIH-funded clinical research participants are women. This issue is not about the ratio of male and female in research, such as that seen in the general population. This issue is about the different protective effects of male and female sex hormones on the mechanisms of cell, tissue, molecular and systems level. The main challenge is to ensure that the mechanisms of cell, tissue, molecular and systems level are not only studied but also translated into clinical practice. The NIH is currently working to address this. The NIH plans to address the issue of sex and gender inclusion across biomedical research in all areas. This includes establishing policies that require the inclusion of sex and gender in all research. This issue is not about the ratio of male and female in research, such as that seen in the general population. This issue is about the different protective effects of male and female sex hormones on the mechanisms of cell, tissue, molecular and systems level. The main challenge is to ensure that the mechanisms of cell, tissue, molecular and systems level are not only studied but also translated into clinical practice. The NIH is currently working to address this. The NIH plans to address the issue of sex and gender inclusion across biomedical research in all areas. This includes establishing policies that require the inclusion of sex and gender in all research.

PERSPECTIVES

CELL BIOLOGY

Fixing problems with cell lines

Techniques and policies can improve authentication

By Dan M. Landis, Francis S. Collins, and the International Alliance for Cell Authentication

Despite the importance of cell lines in the study of biology and medicine, evidence has accumulated that cell lines are frequently misidentified or mislabeled by researchers in laboratories. This can be a substantial problem in many fields, such as cancer research, where drug and therapy development often rely on the use of cell lines. In a new study, we describe the importance of cell line authentication and the need for standardized authentication procedures. We discuss the current state of cell line authentication and the need for standardized authentication procedures. We discuss the current state of cell line authentication and the need for standardized authentication procedures.

established including publishers. This issue is not about the ratio of male and female in research, such as that seen in the general population. This issue is about the different protective effects of male and female sex hormones on the mechanisms of cell, tissue, molecular and systems level. The main challenge is to ensure that the mechanisms of cell, tissue, molecular and systems level are not only studied but also translated into clinical practice. The NIH is currently working to address this. The NIH plans to address the issue of sex and gender inclusion across biomedical research in all areas. This includes establishing policies that require the inclusion of sex and gender in all research.

Why?

- ▶ Calls for researchers to include methodological rigor in study design in order to translate results
- ▶ Ultimate goal: translate basic science into clinical studies and human intervention
 - ▶ Erroneous scientific rationale leads to unsuccessful clinical trials, exposing study patients to harm
 - ▶ Wastes resources and energy (NIH and institution)

New Policies

Applies to:

- ▶ Research
- ▶ Career Development
- ▶ Centers
- ▶ People-based
- ▶ Program Projects
- ▶ Small Business
- ▶ Resource-Related

Does Not Apply to:

- ▶ Administrative Supplements
- ▶ Conferences
- ▶ Construction
- ▶ Instrumentation
- ▶ Publication Support

Planned Policy

- ▶ Individual Fellowships
- ▶ Institutional Training
- ▶ Institutional Career Development Awards

In 2017, these will require formal training and instruction in rigorous experimental design and transparency to enhance reproducibility.

(see *NOT-OD-16-034*)

Four Areas of Clarification

1. Scientific Premise
2. Scientific Rigor
3. Relevant Biological Variables
4. Authentication of Key Biologics and/or Chemical Resources

Specific Points of Rigor & Reproducibility

- ▶ Randomization
- ▶ Blinding
- ▶ Sample Size Estimation
- ▶ Data Handling

Resources

- ▶ A CCR Statistician is available to help determine study power and statistical analysis plan:
Email: statistics@siumed.edu
- ▶ Resources such as Clayton, Collins, and Landis articles on Reference slide
- ▶ NIH website:
<https://grants.nih.gov/reproducibility/index.htm>

RPG Application and Review

Element of Rigor	Section of Application	Criterion Score	Additional Review Consideration	Contribute to Overall Impact?
Scientific Premise	Research Strategy	Significance	NA	Yes
Scientific Rigor		Approach	NA	Yes
Consideration of Relevant Biological Variables Such as Sex		Approach	NA	Yes
Authentication of Key Biological and/or Chemical Resources	New Attachment	NA	Adequate or Inadequate	No

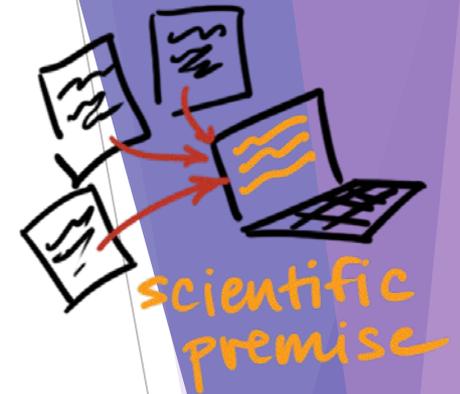
1. SCIENTIFIC PREMISE



SCIENTIFIC PREMISE

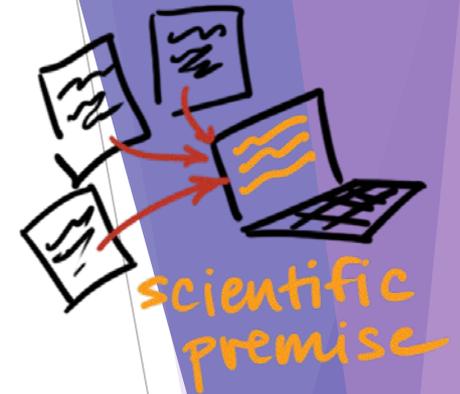
- WHAT?

- ▶ The project's foundation of knowledge (work completed in the past)
- ▶ Critical analysis of the quality and strength of the research used to form the basis for the proposed hypothesis/ research question



SCIENTIFIC PREMISE - WORK BY OTHERS

- ▶ Cite research by others that helped spark the idea for your proposed hypothesis/ research question
 - published literature
 - PA/RFA “Purpose” and “Background”



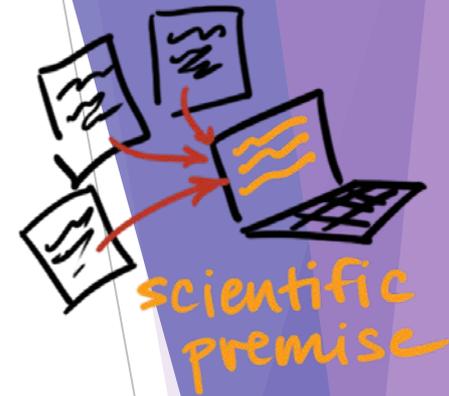
Read the instructions.

SCIENTIFIC PREMISE

- WHAT RE: PRELIM DATA?

Special considerations for preliminary data:

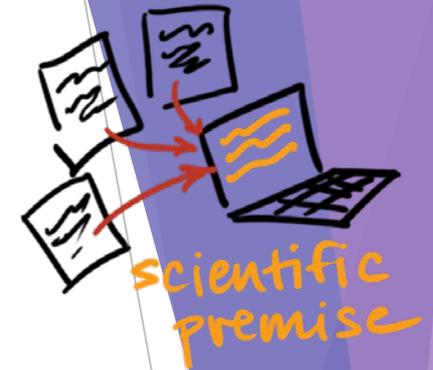
- ▶ Strong scientific rationale?
- ▶ Rigorous experimental design?
- ▶ Consideration of relevant biological variables?
- ▶ Authenticated biological and chemical resources?



SCIENTIFIC PREMISE

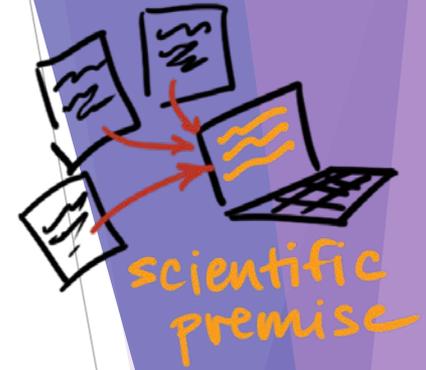
- WHERE?

- ▶ Specific Aims
- ▶ Research Strategy
 - ▶ Significance
 - ▶ Innovation
 - ▶ Approach
 - Research Design
 - Potential Pitfalls & Alternate Approaches
- ▶ Future Directions



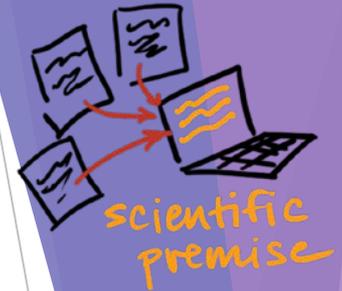
SCIENTIFIC PREMISE

SPECIFIC AIMS



SPECIFIC AIMS

- WHAT?

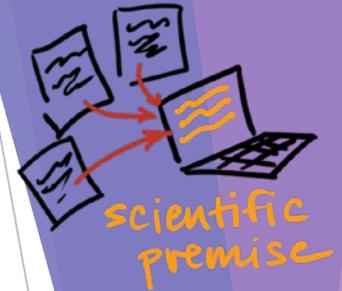


- ▶ Few citations in Specific Aims section
- ▶ Essential references to justify project need
 - Most important, seminal, 'linchpin' references
 - Not review articles

Stay within page limits.

SPECIFIC AIMS

- WHERE?



- ▶ Introductory paragraph to introduce need (gap)
- ▶ Long term goal paragraph with central hypothesis and scientific rationale

Example, Russell & Morrison 2016, pg 77

SCIENTIFIC PREMISE

RESEARCH STRATEGY

Significance

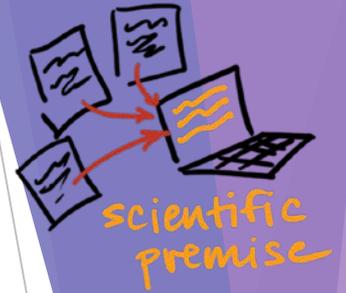
Innovation

Approach

SIGNIFICANCE

- WHAT?

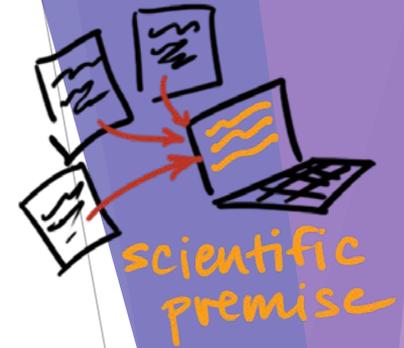
Describe the scientific premise for the proposed project, including GENERAL consideration of the strengths and weaknesses of published research or preliminary data crucial to the support of your application.



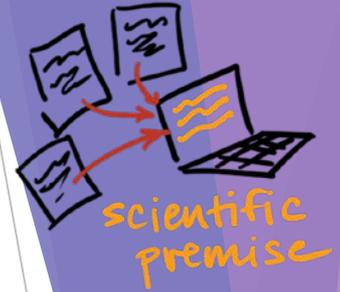
SIGNIFICANCE

- WHAT?

- ▶ Scientific rigor of cited/prelim work
- ▶ Justify the need for the proposed research by assessing the foundation of knowledge supporting the proposal
- ▶ PA/RFA “Purpose” and “Background”



SIGNIFICANCE - WHERE?



Significance:

Scientific Premise

Overall Scientific Premise

Scientific Premise for Aim #1

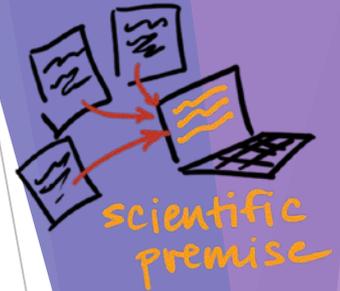
Scientific Premise for Aim #2

(add for addl aims if needed)

Sentence that frames the problem as relevant to FOIA or I/C.

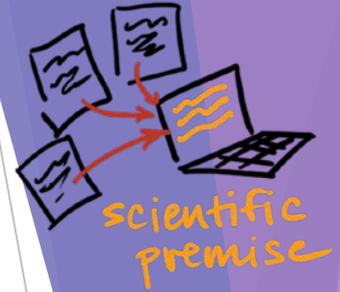
INNOVATION

- WHAT?



- ▶ Tactfully discuss the status quo and the Innovative aspects of your project that depart from it
- ▶ Establish the firm foundation supporting your claim to enable new funding agency goals that would be unattainable without the proposed work

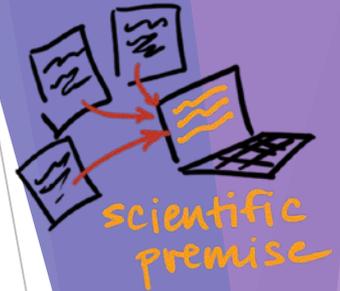
INNOVATION - WHAT NOT?



- ▶ Do not repeat assessment of strengths and weakness (should be in Significance section)

Read the instructions.

INNOVATION - WHERE?



- ▶ Paragraph 1: frame the status quo *diplomatically*
 - ▶ “The current standard/status quo relating to _____ is _____.”

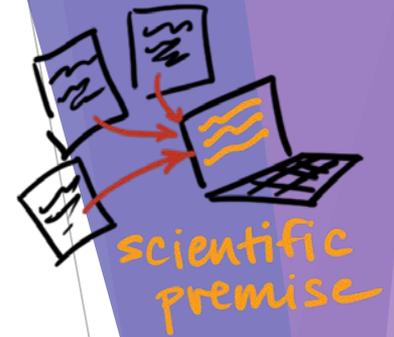
APPROACH

- WHAT?

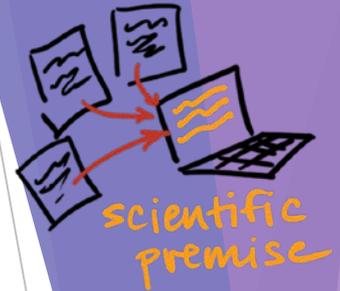
Enough detail that experiments can be replicated

- WHAT NOT?

- Manufacturer instructions
- Standard scientific procedures
- Etc.



APPROACH - WHERE?



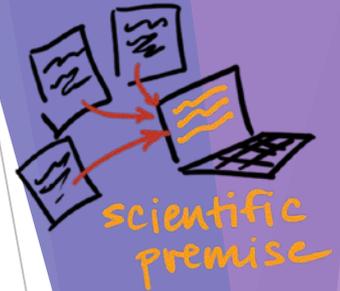
▶ Significance

- Methodologic feasibility (cited work/prelim data)
- Technical ability (prelim data)

▶ Potential Pitfalls/Alternate Strategies

- Unvalidated biologic or chemical resources
- Consideration of biological variables
- Retrospective endpoint selection
- Inadequate blinding

APPROACH - WHERE?



Research Strategy: Future Directions

- ▶ Ability to translate and replicate results
- ▶ NIH requirements are designed to improve:
 - Transparency
 - Adequate reporting on the design, conduct, and analysis of experiments

2. SCIENTIFIC RIGOR

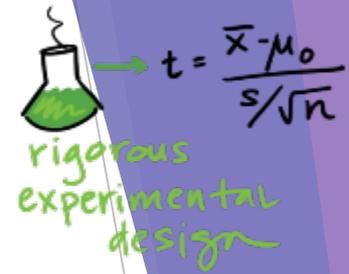

$$t = \frac{\bar{x} - \mu_0}{s/\sqrt{n}}$$

rigorous
experimental
design

Read the instructions.

SCIENTIFIC RIGOR

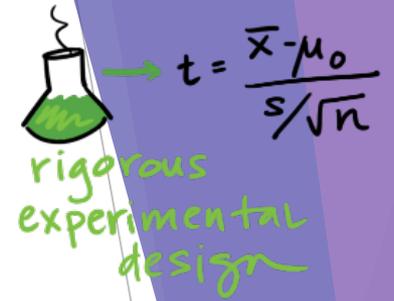
- WHAT?



Describe the experimental design and methods proposed and how they will achieve robust and unbiased results.

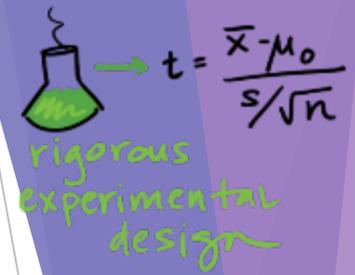
- ▶ Robust and unbiased results are:
 - Obtained with solid, well-controlled experiments and
 - Capable of being reproduced under well-controlled conditions, using reported experimental details.

SCIENTIFIC RIGOR - WHAT?



- ▶ Methods to reduce bias (examples)
 - Use independent, blinded assessors
 - Select primary endpoint prospectively
 - Randomize to treatment groups
 - Define inclusion/exclusion criteria in advance
 - Predetermine handling outliers
 - Conduct interim data analysis (statistics@siumed.edu)

SCIENTIFIC RIGOR - WHERE?

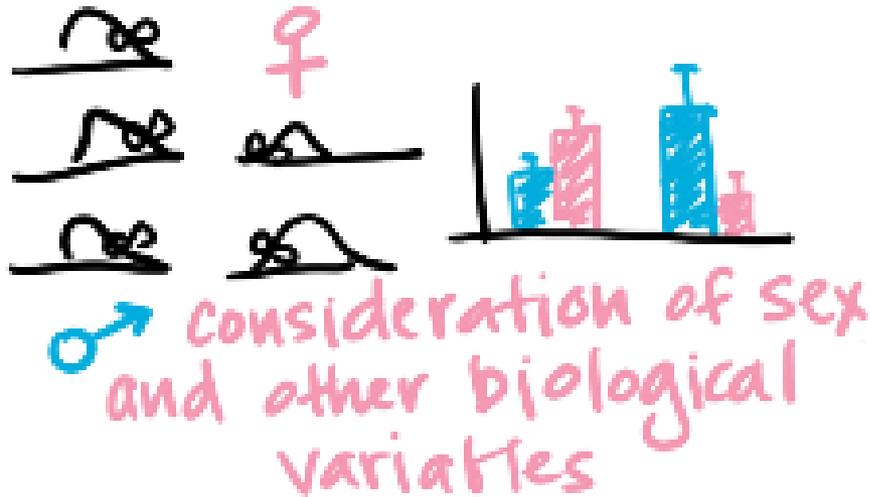


Research Strategy: Approach

- ▶ Succinctly state what is planned
 - Include information on sample numbers, blinding, statistical power and analyses
 - Describe experimental animal numbers here (power); VAS no longer requires justification of animal numbers.
- ▶ Be transparent about your plans for analysis

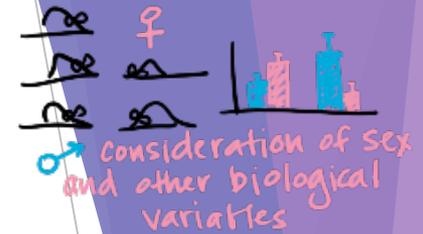
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3. RELEVANT BIOLOGICAL VARIABLES



RELEVANT BIOLOGICAL VARIABLES

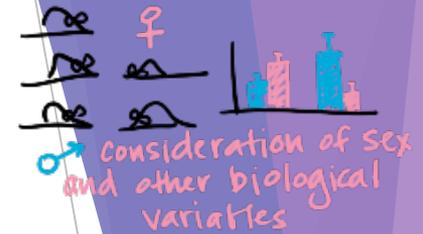
- WHAT?



- ▶ Biological variables: sex, age, weight, underlying health conditions, types of strains, vendor source, suppliers, housing conditions (room temp., light/dark cycles)
- ▶ Types of studies: tissues, primary cells, samples from vertebrate animals and humans
 - If cells, tissues, or other samples are being implanted into a host, sex of both sample & host should be considered

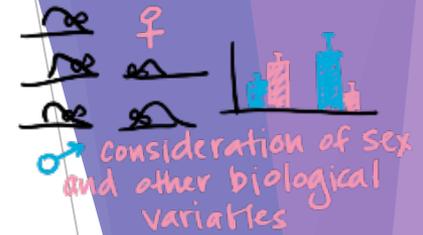
RELEVANT BIOLOGICAL VARIABLES

- WHERE?



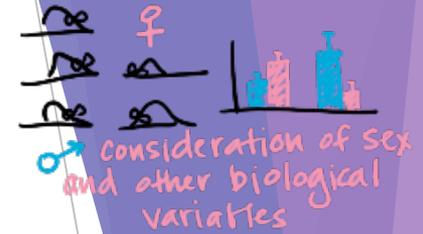
- ▶ Research Strategy (design, analyses), Vertebrate Animals, Human Subjects
- ▶ Propose to study one sex ONLY with strong justification from scientific literature, preliminary data, or other relevant considerations
 - ▶ Single-sex studies: pregnancy, ovarian & prostate cancer
 - ▶ Clinical Studies: Inclusion of Women and Minorities and Inclusion of Children

RELEVANT BIOLOGICAL VARIABLE DATA



- ▶ Data should be disaggregated, whether study was statistically powered to detect sex differences or not
- ▶ Reporting descriptive statistics for males and females provides usual information for further study and understanding of differences in biology
- ▶ Studies that control for sex in multivariate analyses should also report sex-specific results

FAQ: Will I have to double my sample numbers?

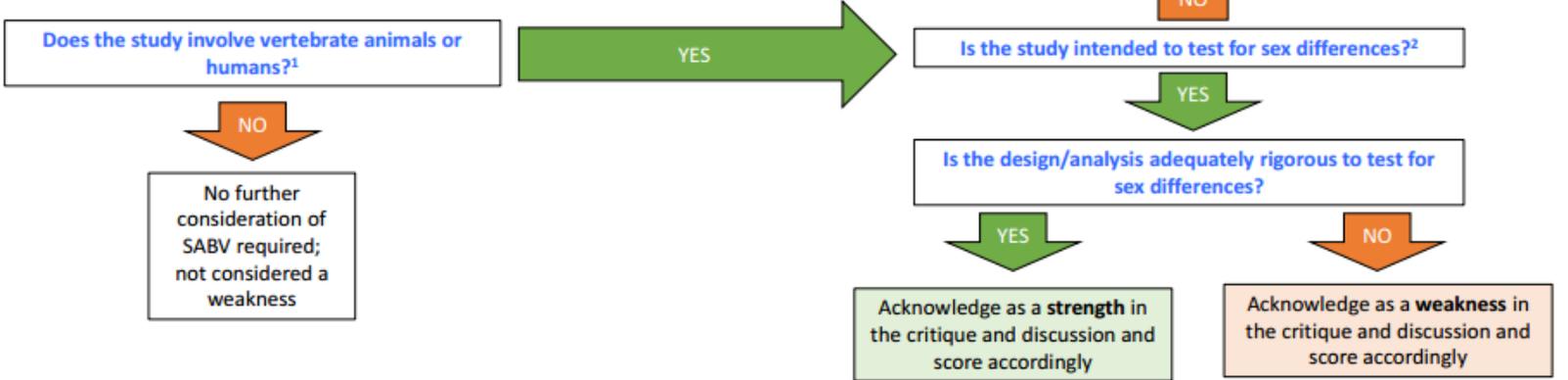


- ▶ Statistical analysis will determine the sample size needed for statistically significant data
- ▶ May not need to double, but may need to use more

Reviewer Guidance to Evaluate Sex as a Biological Variable (SABV)

Main points

- NIH expects that sex as a biological variable will be factored into research designs, analyses, and reporting in vertebrate animal and human studies.
- Strong justification from the scientific literature, preliminary data, or other relevant considerations must be provided for applications proposing to study only one sex.
- This decision tree is meant to be used as a guide, but does not encompass the entire policy. See [NOT-OD-15-102](#) for more information.



Notes

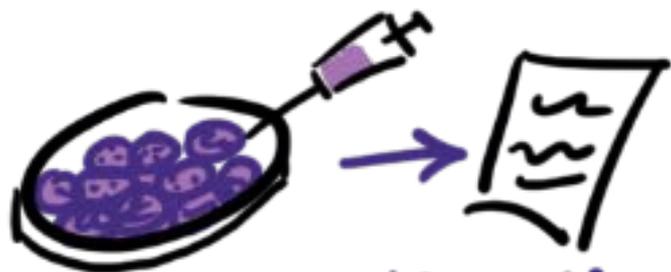
¹ See FAQs on [inclusion, primary cells and tissues](#), and [established cell lines](#).

² See FAQs on [considering sex as a biological variable](#) and [use of males and females in basic research](#).

³ See FAQ on [justification of single sex studies](#).

⁴ Based on the research question and availability of relevant data, statistically powered comparisons between the sexes may not be required. Analyzing and publishing sex-based data, even in the absence of powered sex differences analyses, would permit the consideration of the influence of sex in the interpretation of study results and the appropriate generalization of research findings.

4. AUTHENTICATION OF BIOLOGICS AND CHEMICALS



authentication
of key
resources

AUTHENTICATION OF BIOLOGICS AND CHEMICALS - WHAT?

- ▶ Quality of resources is critical to the ability to reproduce results
- ▶ Key biological and/or chemical resources should be regularly authenticated to ensure identity and validity



AUTHENTICATION OF BIOLOGICS AND CHEMICALS

- WHAT?

If applicable to the proposed science, briefly describe methods to ensure the identity and validity of key biological and/or chemical resources used in the proposed studies. No more than one page is suggested.

- ▶ Key biological and/or chemical resources include, but are not limited to, cell lines, specialty chemicals, antibodies, and other biologics.
- ▶ Standard laboratory reagents that are not expected to vary do not need to be included in the plan. Examples are buffers and other common biologicals or chemicals.
- ▶ Reviewers will assess the information provided in this Section. Any reviewer questions associated with key biological and/or chemical resource authentication will need to be addressed prior to award.



authentication
of key
resources

AUTHENTICATION PLAN - WHERE?



[View Burden Statement](#)

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction

1. Introduction to Application
(Resubmission and Revision)

 [Add Attachment](#) [Delete Attachment](#) [View Attachment](#)

Research Plan Section

2. Specific Aims

 [Add Attachment](#) [Delete Attachment](#) [View Attachment](#)

3. *Research Strategy

 [Add Attachment](#) [Delete Attachment](#) [View Attachment](#)

4. Progress Report Publication List

 [Add Attachment](#) [Delete Attachment](#) [View Attachment](#)

Human Subjects Section

5. Protection of Human Subjects

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6. Data Safety Monitoring Plan

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7. Inclusion of Women and Minorities

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8. Inclusion of Children

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Other Research Plan Section

9. Vertebrate Animals

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10. Select Agent Research

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11. Multiple PD/PI Leadership Plan

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12. Consortium/Contractual Arrangements

 [Add Attachment](#) [Delete Attachment](#) [View Attachment](#)

13. Letters of Support

 [Add Attachment](#) [Delete Attachment](#) [View Attachment](#)

14. Resource Sharing Plan(s)

 [Add Attachment](#) [Delete Attachment](#) [View Attachment](#)

15. Authentication of Key Biological and/or
Chemical Resources

 [Add Attachment](#) [Delete Attachment](#) [View Attachment](#)

Appendix

16. Appendix

[Add Attachments](#) [Delete Attachments](#) [View Attachments](#)

Read the instructions.



Contact Info

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statistics@siumed.edu

References

Clayton JA & Collins FS. **NIH to balance sex in cell and animal studies.**

Nature. 2014 May 15; 509(7500): 282-283. <http://www.nature.com/news/policy-nih-to-balance-sex-in-cell-and-animal-studies-1.15195>

Collins FS and Tabak LA. **NIH plans to enhance reproducibility.** *Nature*. 2014 Jan 30; 505(7485): 612–613.

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Lorsch JR, Collins FS, Lippincott-Schwartz J. **Fixing problems with cell lines.** *Science*. 2014 Dec 19; 346(6216): 1452-1453.

<http://science.sciencemag.org/content/346/6216/1452>

NIH Notice NOT-OD-15-102, **Consideration of Sex as a Biological Variable in NIH-funded Research.** <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-102.html>

NIH Rigor and Reproducibility. <https://www.nih.gov/research-training/rigor-reproducibility>; <http://grants.nih.gov/reproducibility/index.htm#guidance>; <http://grants.nih.gov/reproducibility/faqs.htm#4827>:

Infographic <http://grants.nih.gov/reproducibility/documents/grant-guideline.pdf>

Russell SW and Morrison DC. **The Grant Application Writer's Workbook – Forms D Edition.** April 2016. Grant Writers' Seminars and Workshops, LLC: Buellton, CA.