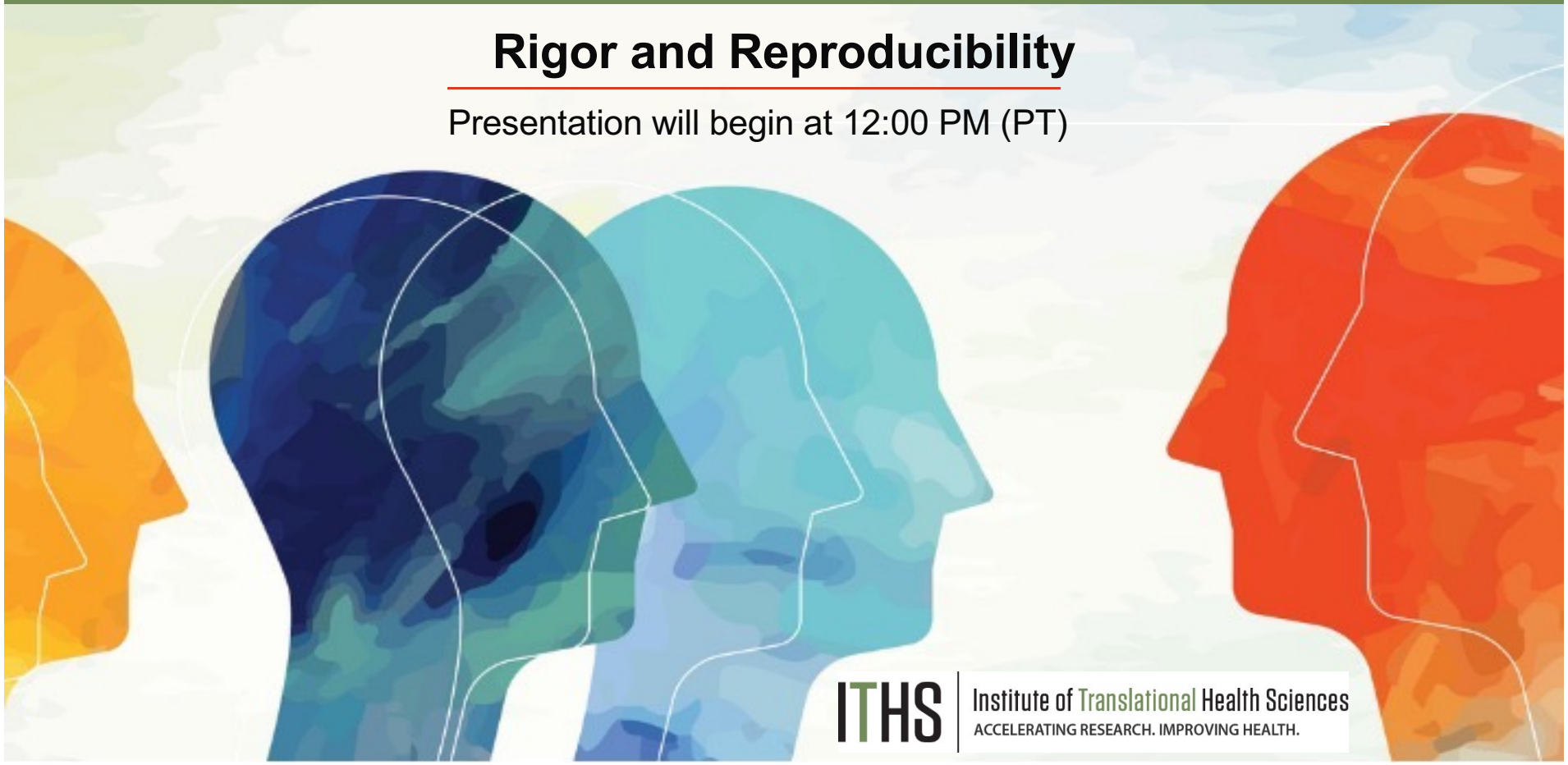
Career Development Series 2022

Rigor and Reproducibility

Presentation will begin at 12:00 PM (PT)



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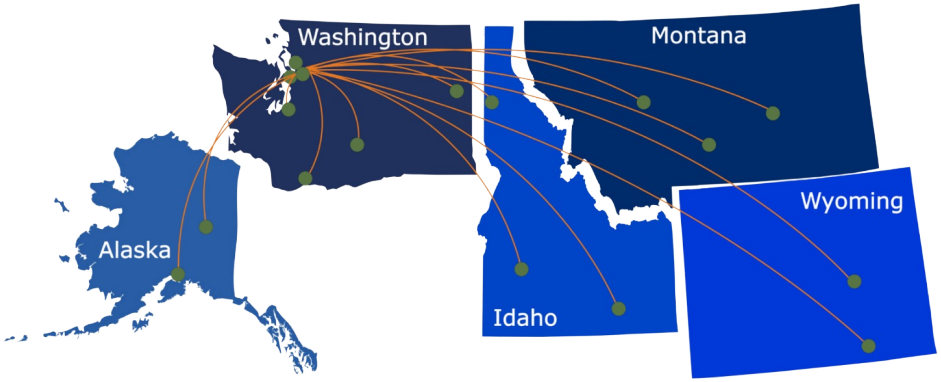

What We Offer:

1. **Research Support Services:** Members gain access to the different research services, resources, and tools offered by ITHS, including the ITHS Research Navigator.

2. **Community Engagement:** Members can connect with regional and community based practice networks

3. **Education & Training:** Members can access a variety of workforce development and mentoring programs and apply for formal training programs.

4. **Funding:** Members can apply for local and national pilot grants and other funding opportunities. ITHS also offers letters of support for grant submissions.

Contact ITHS

Director of Research Development

- Project Consultation
- Strategic Direction
- Resources and Networking

Melissa D. Vaught, Ph.D.
ithsnav@uw.edu
206.616.3875

Scientific Success Committee

- Clinical Trials Consulting
- Guidance on Study Design, Approach and Implementation
- Feedback on Design and Feasibility

https://www.iths.org/investigators/services/clinical-trials-consulting/

Feedback

At the end of the seminar, a link to the feedback survey will be sent to the email address you used to register.

Career Development Series 2022

Rigor and Reproducibility

Presented by:
Andrea Lazarus, PhD
Associate Dean for Research, WSU College of Pharmacy and Pharmaceutical Sciences

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Learning Objectives

1. Define reproducibility, replicability, rigor, and transparency
2. Understand why lack of reproducibility is a problem
3. Describe the factors that lead to a lack of reproducibility
4. Describe strategies to minimize irreproducibility and increase rigor

Expectations in Scientific Research

- If an experiment is conducted under the same conditions as another experiment, the results should be the same
- Rules should be true not only in one specific context, but should be generalizable
- Scientific results should be subject to checking by peers
- Science aims for degrees of confidence, rather than complete certainty: Uncertainty is inherent in all scientific knowledge, but researchers need to understand the uncertainty associated with research findings
- New evidence can result in revisions to current understanding

5 Requirements for “Good” Experimental Design

Cox, DR. Planning Experiments. John Wiley & Sons, New York, 1958

- Be unbiased
- Have high precision
- Have a wide range of applicability
- Be simple
- Have the ability to calculate uncertainty

Definitions

Reproducibility is obtaining consistent results using the same input data, computational steps, methods, and code; and conditions of analysis. Synonymous with "computational reproducibility"

Replicability is obtaining consistent results across studies aimed at answering the same scientific question, each of which has obtained its own data. Two studies may be considered to have replicated if they obtain consistent results given the level of uncertainty inherent in the system under study.

Generalizability refers to whether the results of a study apply in other contexts or populations that differ from the original one

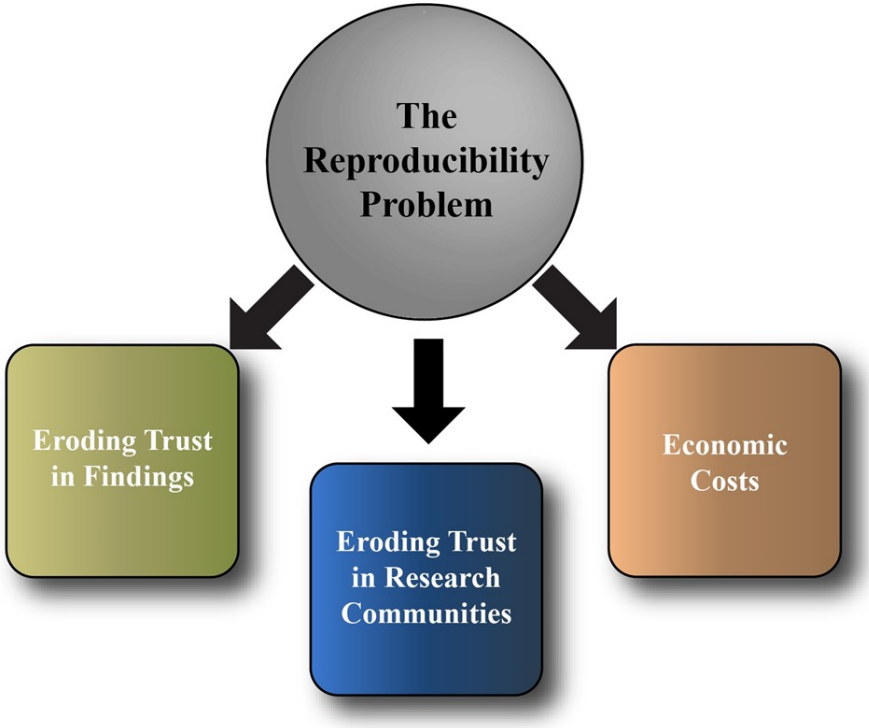
So:

Reproducibility involves the original data, while **replicability** involves new data to test for consistency with previous results.

Data should be reproducible if the researcher has been transparent in reporting the study and any underlying artifacts

However, even if a study is rigorously conducted according to best practices, analyzed correctly, and reported with transparency, it may fail to be replicated.

Why is NIH interested in rigor and reproducibility?



Reproducibility Problem

Results are considered statistically significant if the probability they occurred by chance is at the level of 0.05 or lower.

So, about 5% of the time it is expected that results are a statistical fluke, and correspondingly, one would expect a 5% failure-to-replicate rate.

However, efforts to reproduce and replicate results fail at a much higher rate

Reproducibility Problem

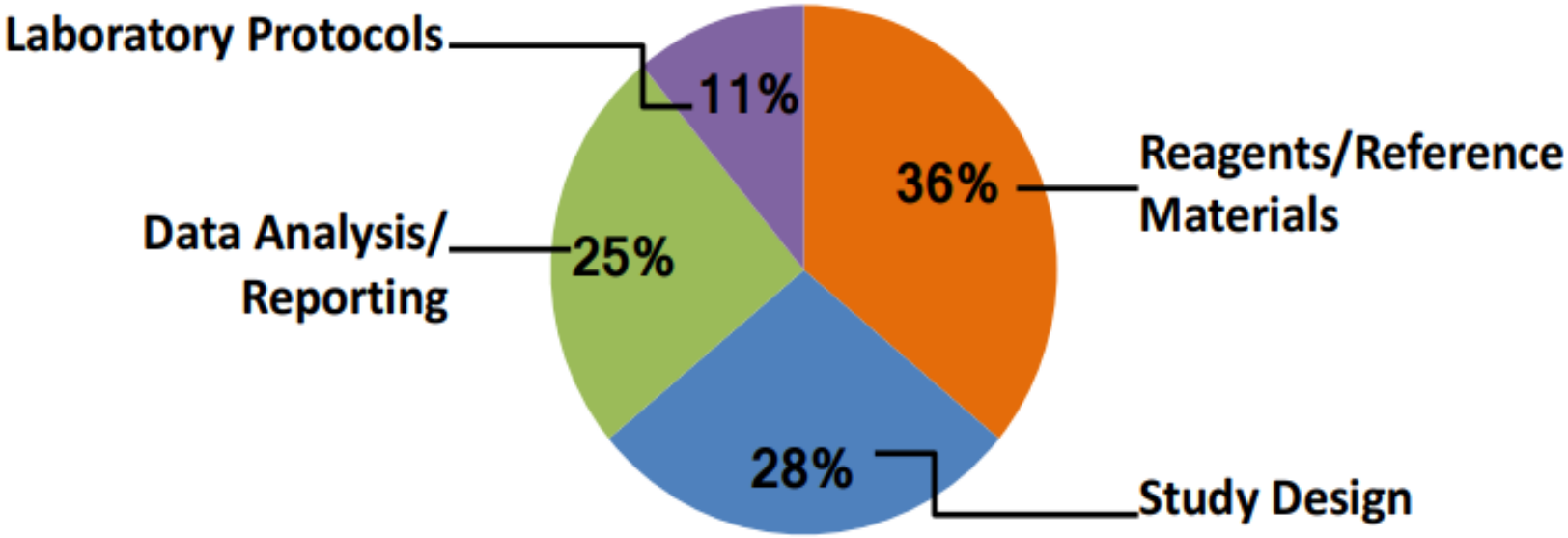
- Bayer tried to replicate some of its preclinical studies, but could only completely validate 20-25% of published studies (Prinz et al, 2011)
- Amgen selected 53 “landmark” clinical studies and tried to replicate them

  Only 6/53 of the studies (11%) were able to be replicated (Begley & Ellis, 2012)
- An attempt was made to independently replicate 100 psychological science publications, 97 of which reported statistically significant findings (Open Science Collaboration, 2015)
  - They contacted the original authors to get them to reproduce the findings, but only 35% still showed statistical significance

Reproducibility Problem

- A *Nature* study found that the potential economic cost of irreproducibility is $10-50 billion/yr (Freedman, 2015)
    - In that study, the rate of irreproducibility was 53%
- More than 70% of researchers say that they have not been able to reproduce another researcher's findings
- More than 50% of researchers report that they have been unable to replicate their own findings.

Four Categories of Irreproducibility



Freedman, *Nature*, 2015

Why Is There A Problem?

Method creep

- Over time, small changes in procedures, equipment, materials and techniques can occur, which add up and can affect results

Poor methodology

- Poor study design and lack of experimental controls create threats to internal and external validity, and this variation can make it difficult to replicate results

Data management errors

- Accidental changes to data sets introduce errors
- Eg, errors inputting data, when creating a database, formatting, using software that perpetuates a mistake

Inadequate recordkeeping

- Researchers do not transparently report, provide open access to, or archive the relevant data

Why Is There A Problem?

Investigator bias

- If not blinded, researchers are susceptible to bias in classifying data, or altering hypothesis to fit the data (HARKing—hypothesizing after results are known)

Research misconduct

- Purposeful research misconduct (eg, omission of outlier data, fabrication of data) will make it impossible to replicate a study

Low statistical power

- Statistical power is related to sample size and the precision of measurements
- Stopping testing after finding significance rather than using the *n* from power calculations can create false positives (P-hacking)

Science viewed as "self-correcting" and immune from reproducibility problems

- True over the long term, but not short-term

Why Is There A Problem?

Publication bias

- Until a finding has been replicated, the study has limited credibility—however, journals often will not publish studies that replicate prior work, and replication studies are less likely to be cited by others

Pressures from promotion and tenure

- Promotion and tenure rewards new studies, not replication of existing studies

Large labs

- The larger the size of a lab, the less individual attention and individual lab member might receive, which leads to cutting corners

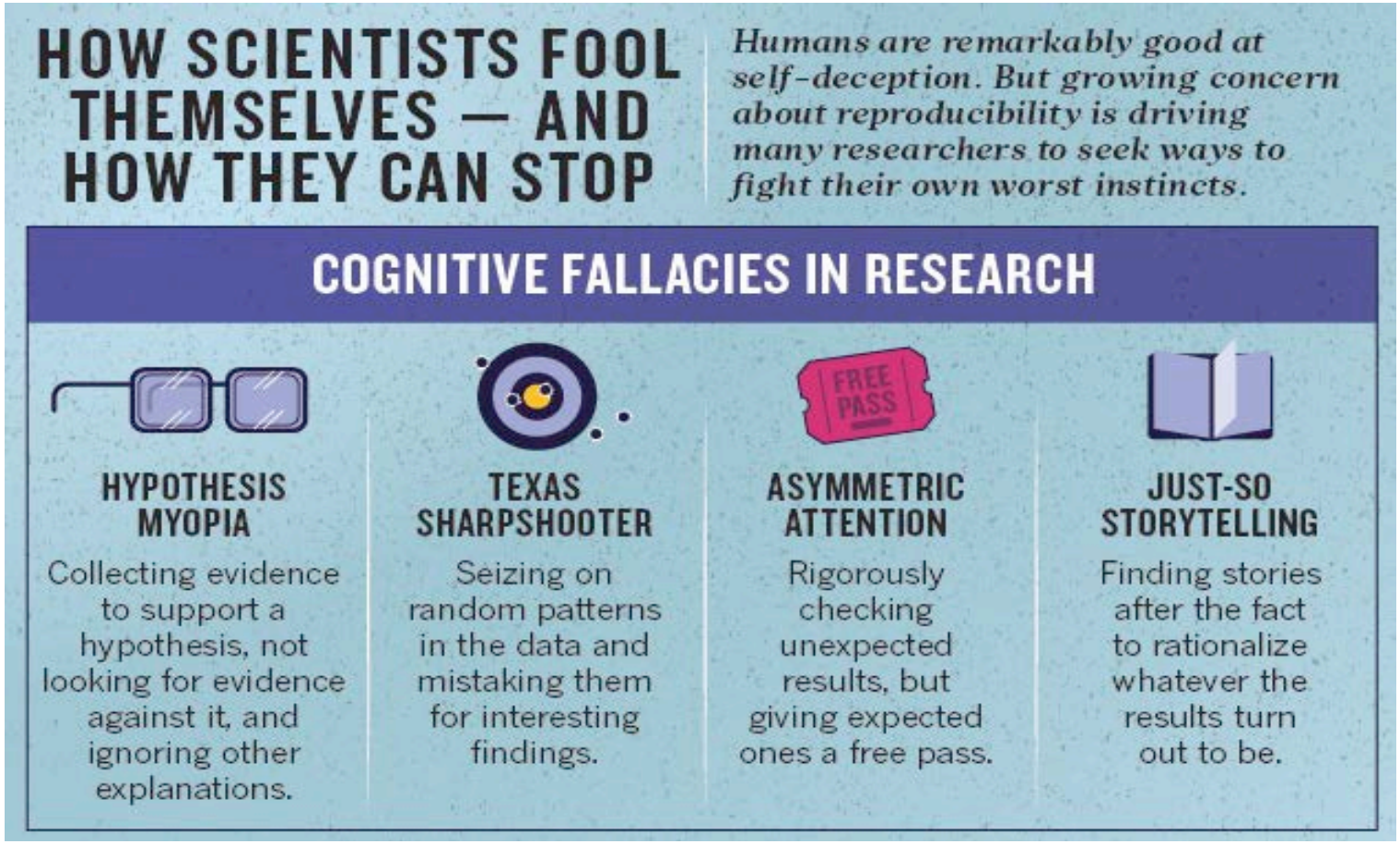
Inadequate reporting of methods

- Journals encourage shorter articles to reduce printing and mailing costs, so descriptions of methodology may leave out important details
- Online publication has relieved some of this pressure

What is Meant by Inadequate Reporting of Research?

- Omissions of crucial aspects of study methods, such as inclusion and exclusion criteria, precise details of interventions, measurement of outcomes, and statistical methods
- Statistical errors
- Selective reporting of results for only some of the assessed outcomes
- Selective reporting of statistical analyses (eg, subgroup analyses)
- Inadequate reporting of harms
- Confusing or misleading presentation of data and graphs
- Incomplete numerical presentation of data precluding inclusion in a later meta-analysis
- Selective presentation of results in abstracts or inconsistency with the main text
- Selective or inappropriate citation of other studies
- Misinterpretation of study findings in the main article and abstract ('Spin')

Guidelines for Reporting Health Research: A User's Manual. EQUATOR https://www.equator-network.org/wp-content/uploads/2016/10/AltmanMoher-Chapter-1-Guidelines-for-Reporting-Health-Research-A-Users-Manual.pdf



Nuzzo, *Nature*, 2015

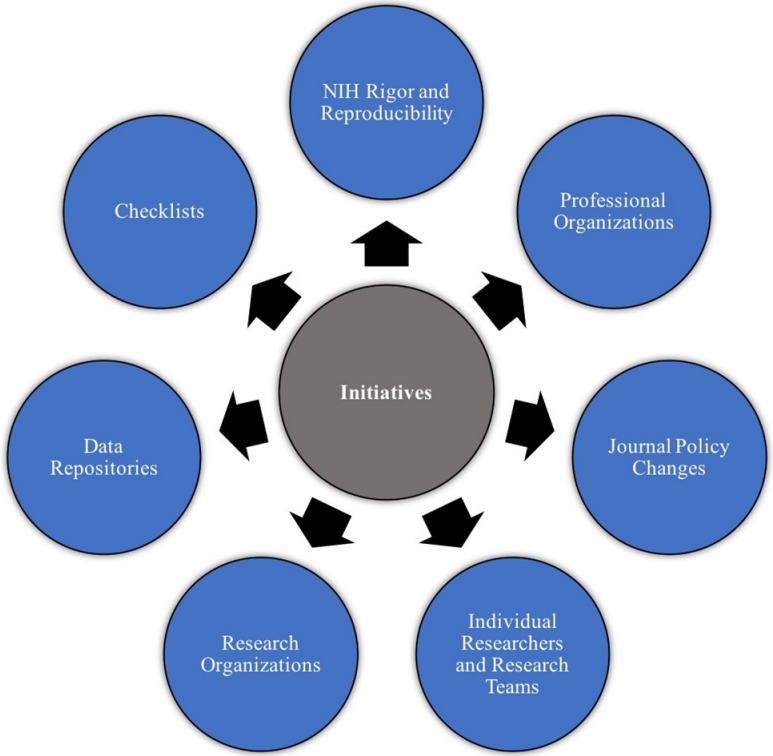
Rigor and Transparency

Rigor: the strict application of the scientific method to ensure robust and unbiased experimental design

Rigor does not guarantee that a study will be replicated, but conducting a study with rigor—with a well-thought- out plan and strict adherence to best practices—will make it more likely

Transparency: sharing the details about research, including study design, materials used, details of the system under study, operationalization of variables, measurement techniques, uncertainties in measurement, and how data were collected and analyzed

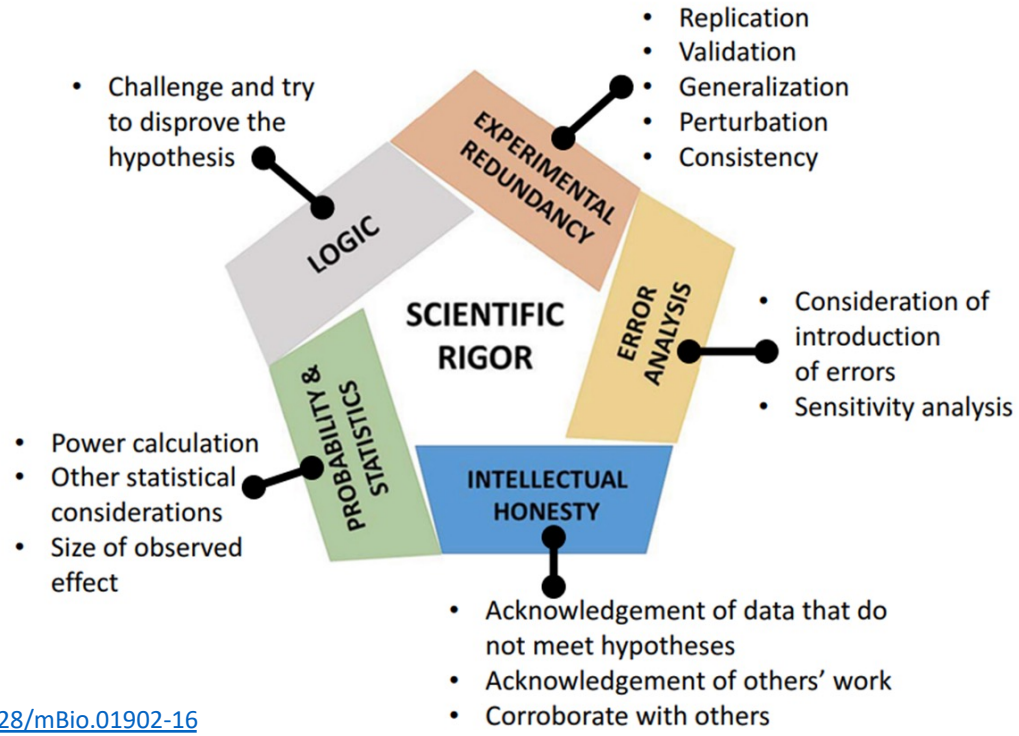
What is Being Done to Increase Rigor and Reproducibility?



Steps to Increase Rigor and Reproducibility: Journals

- Online publication means more room to show additional data and expanded methodology
- Accepting more articles with negative results
- Greater emphasis on checking statistical accuracy
- Requirement to provide study details, such as method of randomization, blinding, sample-size estimates, inclusion and exclusion criteria, and standards
- Stipulation that datasets be made available upon request during review of the manuscript, and be placed in public databases where appropriate

Steps to Increase Rigor and Reproducibility: Researcher



Casadevall & Fang, DOI: 10.1128/mBio.01902-16

Steps to Increase Rigor and Reproducibility: NIH

NIH interests:

- Ensuring that applicants describe their work adequately and provide sufficient information for reviewers
- Demonstrate to public stakeholders that NIH is taking their concerns seriously
- Protecting their investment by funding the best research
- Maximizing public confidence in scientific research

Which Grants are Affected by the NIH Policy on Rigor?

Any grant that funds research, training, or career development (R series, F, K, T awards)

- Training grants are expected to include a formal plan for training in rigor and transparency
- The Program Plan section of training grant applications needs to include a description of how the program and faculty will provide training in rigorous research design, relevant data science, and quantitative approaches

Steps to Increase Rigor and Reproducibility: NIH (2016)

Key Area	Application Instructions
Scientific Premise	Research Strategy: Significance (scored)
Scientific Rigor	Research Strategy: Approach (scored)
Consideration of Relevant Biological Variables, such as sex	Research Strategy: Approach (scored)
Authentication of Key Biological and/or Chemical Resources	Separate Attachment (not scored): to be saved as a single file named "Authentication of Key Resources Plan" attached in FORMS-D, and FORMS-E, "Other Research Plan Sections": *Only required if Key Biological and/or Chemical Resources are mentioned in the research strategy

Steps to Increase Rigor and Reproducibility: NIH

https://grants.nih.gov/policy/reproducibility/guidance.htm

NIH: 2019

4 AREAS OF FOCUS	WHAT DOES IT MEAN?	WHERE SHOULD IT BE INCLUDED IN THE APPLICATION?
Rigor of the Prior Research	A careful assessment of the **rigor of the prior research** that serves as the key support for a proposed project will help applicants identify any weaknesses or gaps in the line of research. Describe the strengths and weaknesses in the rigor of the prior research (both published and unpublished) that serves as the key support for the proposed project. Describe plans to address weaknesses in the rigor of the prior research that serves as the key support for the proposed project *See related FAQs, blog post	**Research Strategy** ➢ Significance ➢ Approach
Scientific Rigor (Design)	**Scientific rigor** is the strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation and reporting of results. Emphasize how the experimental design and methods proposed will achieve robust and unbiased results. *See related FAQs, blog post, examples from pilots	**Research Strategy** ➢ Approach
Biological Variables	**Biological variables**, such as sex, age, weight, and underlying health conditions, are often critical factors affecting health or disease. In particular, sex is a biological variable that is frequently ignored in animal study designs and analyses, leading to an incomplete understanding of potential sex-based differences in basic biological function, disease processes and treatment response. Explain how relevant biological variables, such as the ones noted above, are 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. *See related FAQs, blog posts, article	**Research Strategy** ➢ Approach
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https://grants.nih.gov/policy/reproducibility/guidance.htm

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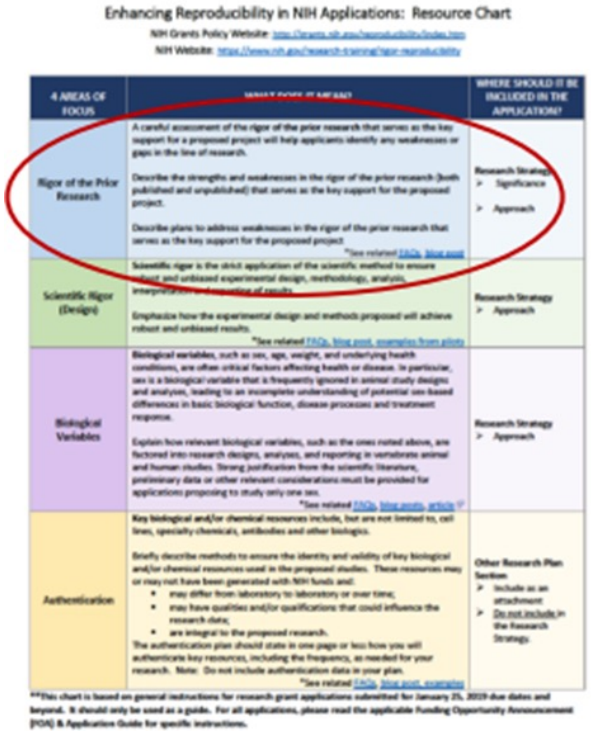
SIGNIFICANCE

APPROACH

https://grants.nih.gov/policy/reproducibility/guidance.htm

Rigor of the Prior Research



Enhancing Reproducibility in NIH Applications: Resource Chart

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NIH Website: https://www.nih.gov/research-training/rigor-reproducibility

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**This chart is based on general instructions for research grant applications submitted for January 25, 2019 due dates and beyond. It should only be used as a guide. For all applications, please read the applicable Funding Opportunity Announcement (FOA) & Application Guide for specific instructions.

- *NIH expects applicants to **describe the general strengths and weaknesses** in the rigor of the prior research (both published and unpublished) that serves as the key support for the proposed project.*

Significance

- *It is expected that this consideration includes attention to:*
  - *the rigor of the previous experimental designs*
  - *the incorporation of relevant biological variables and authentication of key resources*

Approach

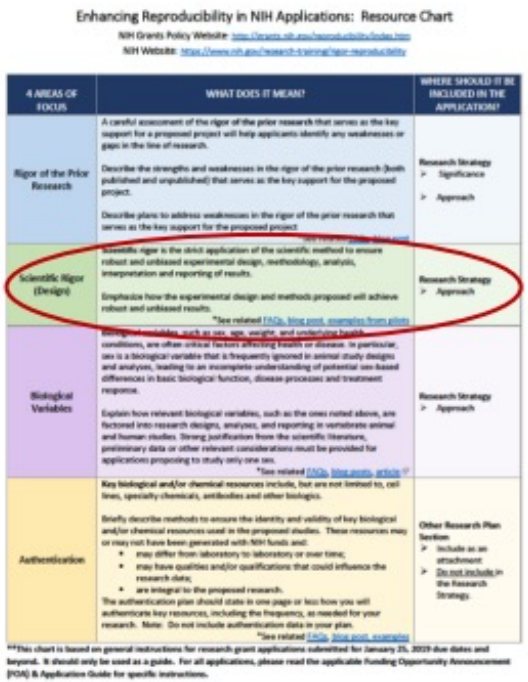
- *Applicants are expected to include plans to address any weaknesses or gaps identified.*

https://medicine.uiowa.edu/internalmedicine/sites/medicine.uiowa.edu.internalmedicine/files/wysiwyg_uploads/Blaumueller_2-19-19.pdf

Scientific Rigor (Design)	**Scientific rigor** is the strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation and reporting of results. Emphasize how the experimental design and methods proposed will achieve robust and unbiased results. *See related FAQs, blog post, examples from pilots

APPROACH

Scientific Rigor–Design



Enhancing Reproducibility in NIH Applications: Resource Chart

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Rigorous experimental design for robust and unbiased results

Scientific rigor is the strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation and reporting of results.

- ***NIH expects full transparency in proposing and reporting experimental details so that reviewers may assess the proposed research and others may reproduce and extend the findings.***

Approach

https://medicine.uiowa.edu/internalmedicine/sites/medicine.uiowa.edu.internalmedicine/files/wysiwyg_uploads/Blaumueller_2-19-19.pdf

In the Approach Section

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

- Pre-experiment power calculations (endpoint sensitivity, variability, effect
- size, desired level of confidence, definition and rationale for n).
- Controls to reduce un-recognized bias in data collection
- Random assignment to groups
- Procedures to achieve blinding
- Description of data handling and analyses
- Positive and negative controls
- Blinding, recoding, and systematic random sampling to minimize bias

Steward and Balice-Gordon, (2014) *Neuron*, 84, 572-581.

Biological Variables	**Biological variables**, such as sex, age, weight, and underlying health conditions, are often critical factors affecting health or disease. In particular, sex is a biological variable that is frequently ignored in animal study designs and analyses, leading to an incomplete understanding of potential sex-based differences in basic biological function, disease processes and treatment response. Explain how relevant biological variables, such as the ones noted above, are 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. *See related FAQs, blog posts, article

APPROACH

How to Consider Sex as a Biological Variable

Sex as a biological variable (SABV) needs to be factored into research designs and when reporting in vertebrate animal and human studies. There is an expectation that studies use both sexes, unless there is a strong justification.

- Studies that control for sex in multivariate analyses should report sex-specific results

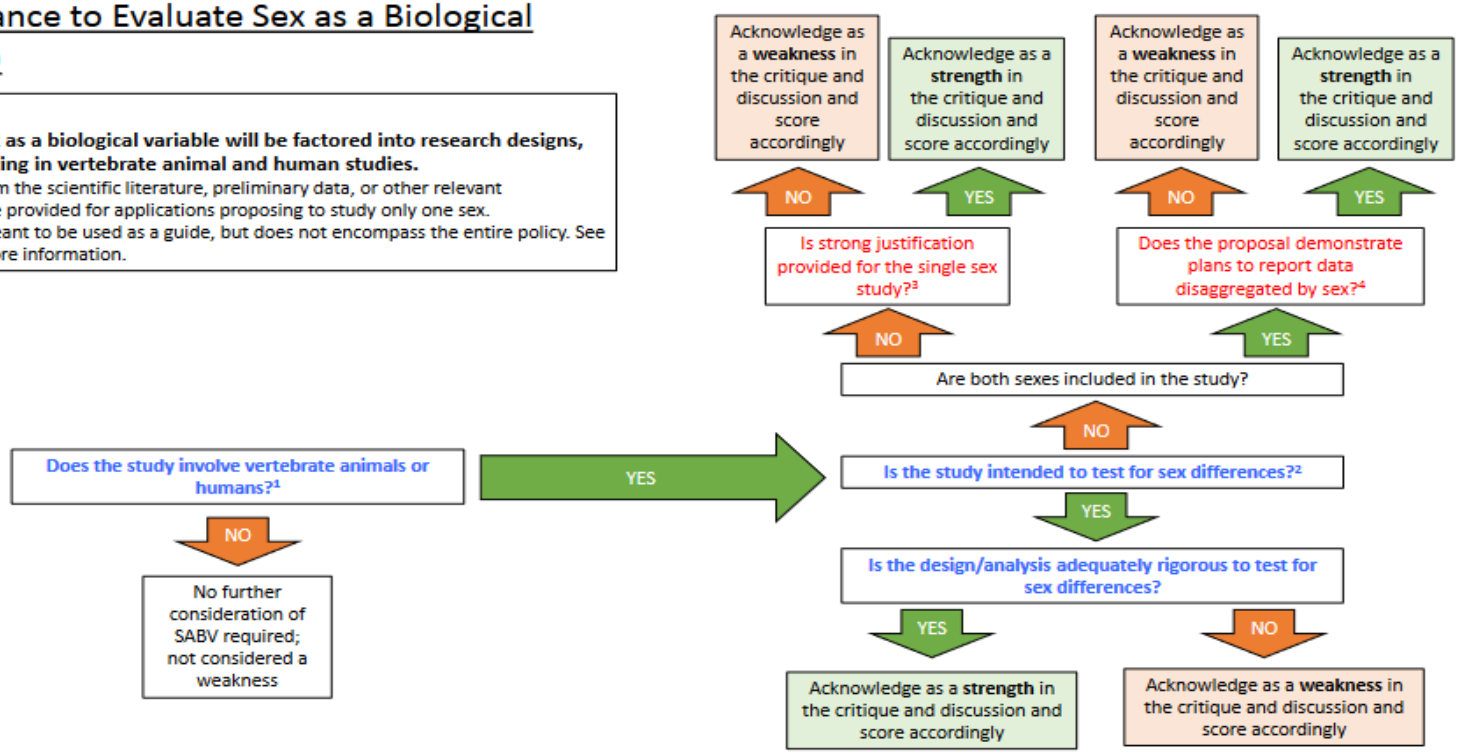
Need to consider SABV in:

- Reviewing the literature on the influence of biological sex
- Formulating research questions
- Incorporating both males and females into studies
- Articulating strong justification for a single-sex study
- Consideration of the influence of sex in study design
- Stratified randomization of males and females into experimental conditions
- Characterization of study results for males and females
- Examination of treatment or toxicity effects for each sex separately
- Consideration of the influence of sex in the interpretation of study results
- Discussing the generalization of research findings

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.

https://grants.nih.gov/grants/peer/guidelines_general/SABV_Decision_Tree_for_Reviewers.pdf

Animal Studies

- Need to provide a justification for the species that are appropriate for the proposed research in the vertebrate animal section
- Need to provide strong justification if using just one sex
  - Justification can include the study of sex-specific conditions, or where the study of just one sex is appropriate
  - Absence of evidence of sex differences does not constitute justification to study just one sex
  - Cost should not be used when justifying why just one sex is being studied
- No longer need to describe veterinary care
- No longer need to justify number of animals here—this would go in the Rigor section of the Approach

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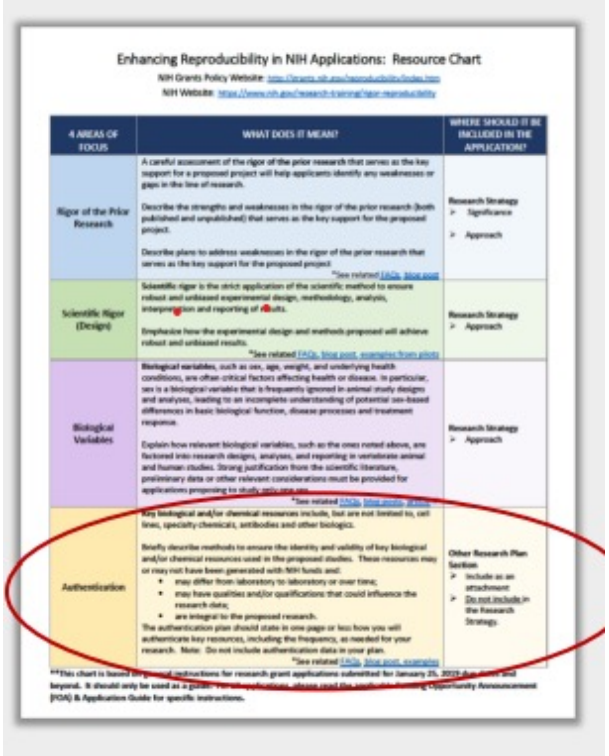
SEPARATE ATTACHMENT

Authentication



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Scientific Rigor (Design)	Scientific rigor is the strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation and reporting of results. Emphasize how the experimental design and methods proposed will achieve robust and unbiased results. *See related FAQs, blog post, examples from plots	Research Strategy ➢ Approach
Biological Variables	Biological variables, such as sex, age, weight, and underlying health conditions, are other critical factors affecting health or disease. In particular, sex is a biological variable that is frequently ignored in animal study designs and analyses, leading to an incomplete understanding of potential sex-based differences in basic biological function, disease processes and treatment response. Explain how relevant biological variables, such as the ones noted above, are 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. *See related FAQs, blog post, examples	Research Strategy ➢ Approach
Authentication	Key biological and/or chemical resources include, but are not limited to, cell lines, specialty chemicals, antibodies and other biologics. Briefly describe methods to ensure the identity and validity of key biological and/or chemical resources used in the proposed studies. These resources may or may not have been generated with NIH funds and: • may differ from laboratory to laboratory or over time; • may have qualities and/or qualifications that could influence the research data; • are integral to the proposed research. The authentication plan should state in one page or less how you will authenticate key resources, including the frequency, as needed for your research. Note: Do not include authentication data in your plan. *See related FAQs, blog post, examples	Other Research Plan Section ➢ Include as an attachment ➢ Do not include in the Research Strategy

**This chart is based on general instructions for research grant applications submitted for January 25, 2019 due dates and beyond. It should only be used as a guide. For all applications, please read the applicable Funding Opportunity Announcement (FOA) & Application Guide for specific instructions.

Key biological and/or chemical resources include, *but are not limited to, cell lines, specialty chemicals, antibodies and other biologics.*

Briefly describe methods to ensure the identity and validity of key biological and/or chemical resources used in the proposed studies.

These resources may or may not have been generated with NIH funds and:

- *may differ from laboratory to laboratory or over time;*
- *may have qualities and/or qualifications that could influence the research data;*
- *are integral to the proposed research*

The quality of resources used to conduct research is critical to the ability to reproduce the results.

Each investigator will have to determine which resources used in their research fit these criteria and are therefore key to the proposed research.

https://medicine.uiowa.edu/internalmedicine/sites/medicine.uiowa.edu.internalmedicine/files/wysiwyg_uploads/Blaumueller_2-19-19.pdf

PHS 398 Research Plan

OMB Number: 0925-0001

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		Add Attachment	Delete Attachment	View Attachment
6. Data Safety Monitoring Plan		Add Attachment	Delete Attachment	View Attachment
7. Inclusion of Women and Minorities		Add Attachment	Delete Attachment	View Attachment
8. Inclusion of Children		Add Attachment	Delete Attachment	View Attachment
Other Research Plan Section				
9. Vertebrate Animals		Add Attachment	Delete Attachment	View Attachment
10. Select Agent Research		Add Attachment	Delete Attachment	View Attachment
11. Multiple PD/PI Leadership Plan		Add Attachment	Delete Attachment	View Attachment
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	☐

Authentication of Key Biological and Chemical Resources

Why is there a need for authentication?

- Since the 1960's, >400 widely-used cell lines have been shown to be misidentified
- A 2011 study of 122 different head and neck cancer cell lines showed that 30% were misidentified
- Studies using just two misidentified cell lines were included in 3 NIH-funded grants, 2 clinical trials, 11 patents, and >100 papers (Lorsch et al, *Science*, 2014)

What resources need authentication?

- Those that differ from lab-to-lab or over time
- Those that have qualities that can influence research data and are integral to the proposed research
- Eg: cell lines, specialty chemicals, antibodies, and other biologics

Key Biological and Chemical Resources

Chemicals

Chemicals purchased commercially come with an authentication sheet identifying the purity and contaminants

Cell lines

Human cell lines from ATCC and other commercial cell line providers assess all of their lots of their cell lines by short tandem repeats, which allow them to identify specific cell lines. Non-human cell lines have interspecies analysis performed.

Primary cell lines

Primary cell lines isolated in individual labs or in commercial facilities need to be identified by surface markers unique to each cell line. These markers are identified by flow cytometry or immunohistochemistry.

Antibody specificity

Western blot analysis can be used to confirm antibody specificity

How to Authenticate Key Resources: Antibodies

Provide complete information

- Find and report resource identifiers for antibodies that will be used in the proposed research: https://scicrunch.org/resources/Antibodies/search?q=*
- Raw data: antibodyregistry.org
- Identification: What was the source of the antibody? If another lab has donated the antibody, give the antiserum code number, and if possible, the bleed. If it was obtained from commercial sources, give the catalog, and if possible, the lot number.
- Preparation of the antibody: What was the antibody actually raised against? Give the precise structure of the immunizing antigen, not just vague information about the part of the molecule that was used. What species was the antiserum raised in? Was it a polyclonal or monoclonal preparation?

Ex: "This rabbit antiserum (Company XYZ #30248) was prepared against a synthetic peptide representing amino acids 121-142 from tyrosine hydroxylase."
"This mouse monoclonal antibody, kindly donated by Dr. John Smith, University of Alabama, was raised against human placental choline acetyltransferase."

How to Authenticate Key Resources: Antibodies

How has the specificity of the antibody been characterized?

- If the antiserum is against a large protein, it is important to know what it stains on a gel from the tissue and species you are using. This information is often included by the manufacturer in the technical information, and can be cited, as can previous studies that provided this information.

Ex: "The antiserum stains a single band of 55 kD molecular weight on Western blot (manufacturer's technical information)."

"This antiserum stains the 150kD but not the 130kD or 110kD forms of the molecule on Western blot (Fig. 1)."

What controls are necessary for immunostaining?

- Does the antibody stain the tissue of interest from which the molecule of interest has been removed?

Ex: "No staining was seen when the antibody was used to stain tissue from an orexin knockout mouse."

"All staining was abolished when 1 ml of the diluted primary antibody was preincubated with 50 µg of the immunizing peptide."

"Staining with this antiserum was colocalized with in situ hybridization for the mRNA for the same protein"

How to Authenticate Key Resources: Cell Lines

Authentication guidelines (International Cell Line Authentication Committee):
http://iclac.org/resources/human-cell-line-authentication

Cell line checklist to determine if proposed cell lines meet quality requirements:
https://iclac.org/resources/cell-line-checklist/

- Actual data demonstrating that authenticated resources are available for the proposed research are not needed in the grant application, just a description of the methods you plan to use to authenticate the cell lines.
- If collecting primary cells for culture, plans for validating the cell lines should be included in the research strategy section.
- If primary cells or cultures from primary cells will be obtained from another laboratory, an authentication plan should be provided in the authentication section

Authentication of Key Resources Plan

- Researchers should transparently report on what they have done to authenticate key resources, so that NIH can develop understanding of consensus approaches.
- You can use one description for multiple different resources in the same category (example: authenticating cell lines)
- Actual data demonstrating that authenticated resources exist is not necessary
- If a key resource is being made as part of the project or is under development, that should be in your research strategy, not the authentication plan.
- Save this information in a single PDF file named “Authentication of Key Resources Plan,” and attach it on the Other Project Information page of the application package

Updated Review Criteria

Scored Review Criteria

Significance

Is the prior research that serves as the key support for the proposed project rigorous? (2018)

Approach

Have the investigators presented strategies to ensure a robust and unbiased approach, as appropriate for the work proposed? (2016)

Have the investigators included plans to address weaknesses in the rigor of prior research that serves as key support for the proposed project? (2018)

Have the investigators presented adequate plans to address relevant biological variables, such as sex, for studies in vertebrate animals or human subjects? (2016)

Additional Review Considerations

Authentication of Key Biological and/or Chemical Resources

For projects involving key biological and/or chemical resources, reviewers will comment on the brief plans proposed for identifying and ensuring the validity of those resources.

Grant-Writing Tips

SIGNIFICANCE (originally 0.5-0.75 pages; now up to 4-5 pages)

- *Rigor of the previous research: strengths and weaknesses*
  - *Was the study design rigorous?*
  - *Were relevant biological variables controlled for?*
- *Your preliminary data that contribute to the scientific foundation of the proposal*
- *Repeat this for each of the proposed aims*
- Significance of the expected research contribution

Grant-Writing Tips

SIGNIFICANCE

- Importance of the problem and/or critical barriers to progress that the study addresses
- Explicitly state the **scientific premise** and rigor for the proposed project
  - The scientific premise is the prior research that formed the basis for the proposal research questions, whether observations, preliminary data, or published literature
  - For example:
    - Numerous studies have shown…
    - However, studies X and Y have important limitations…
    - In addition, the rigor or study Z is not sufficient because…
    - To overcome these gaps in rigors we will…
    - Thus, our proposed studies will circumvent the limitations of…by…
- Significance of the expected research contribution

Grant-Writing Tips

APPROACH (originally 10.5-11 pages; now can be as little as 6.5-7.5 pages)

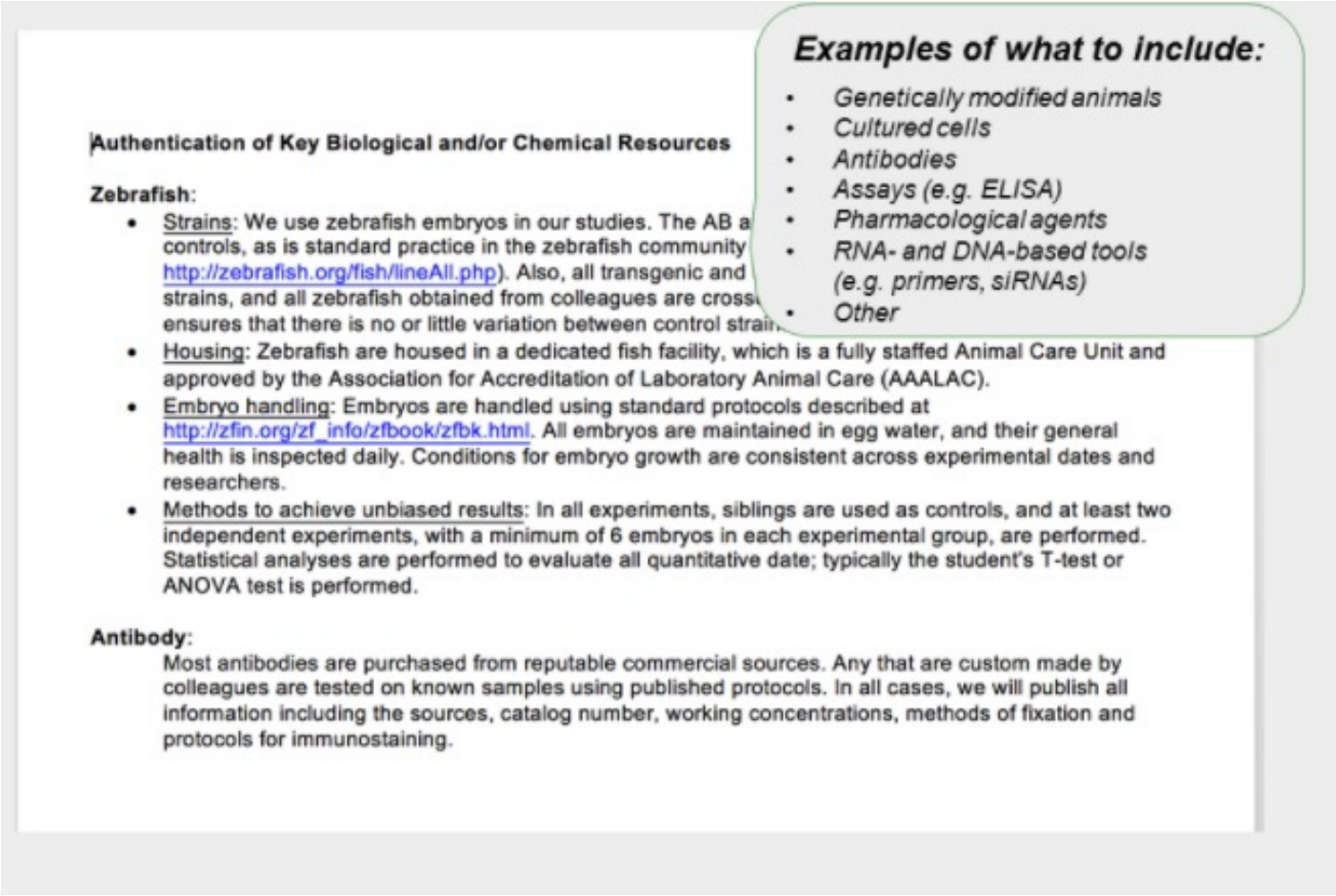
- *Issues related to rigor and reproducibility*
  - *Strategies to ensure rigor of proposed research*
  - *Consideration of biological variables* **OR**
- For each aim:
  - Title of specific aim
  - Introduction/rationale paragraph
  - Research design paragraphs
    - *Strategies to ensure rigor of proposed research*
    - *Consideration of biological variables*
  - Expected outcomes
  - Potential problems
  - Timeline and benchmarks for success
  - Future directions

Grant-Writing Tips

APPROACH

- Research design paragraphs
  - First subaim
  - Approach to be used (include how bias will be minimized)
  - Overview of methods used
  - *Sources of biologic variation*
  - *Essential reagents and their authentication*
  - Essential minor/major equipment
  - *Number of human/animal subjects needed and justification*
  - *Statistical analyses to be used*
  - *Controls and replicates needed*
  - How results will be interpreted

Example structure for Authentication Page



Authentication of Key Biological and/or Chemical Resources

Zebrafish:

- Strains: We use zebrafish embryos in our studies. The AB a
  controls, as is standard practice in the zebrafish community
  http://zebrafish.org/fish/lineAll.php). Also, all transgenic and
  strains, and all zebrafish obtained from colleagues are cross
  ensures that there is no or little variation between control strai
- Housing: Zebrafish are housed in a dedicated fish facility, which is a fully staffed Animal Care Unit and approved by the Association for Accreditation of Laboratory Animal Care (AAALAC).
- Embryo handling: Embryos are handled using standard protocols described at http://zfin.org/zf_info/zfbook/zfbk.html. All embryos are maintained in egg water, and their general health is inspected daily. Conditions for embryo growth are consistent across experimental dates and researchers.
- Methods to achieve unbiased results: In all experiments, siblings are used as controls, and at least two independent experiments, with a minimum of 6 embryos in each experimental group, are performed. Statistical analyses are performed to evaluate all quantitative date; typically the student's T-test or ANOVA test is performed.

Antibody:

Most antibodies are purchased from reputable commercial sources. Any that are custom made by colleagues are tested on known samples using published protocols. In all cases, we will publish all information including the sources, catalog number, working concentrations, methods of fixation and protocols for immunostaining.

Examples of what to include:

- *Genetically modified animals*
- *Cultured cells*
- *Antibodies*
- *Assays (e.g. ELISA)*
- *Pharmacological agents*
- *RNA- and DNA-based tools (e.g. primers, siRNAs)*
- *Other*

Resources

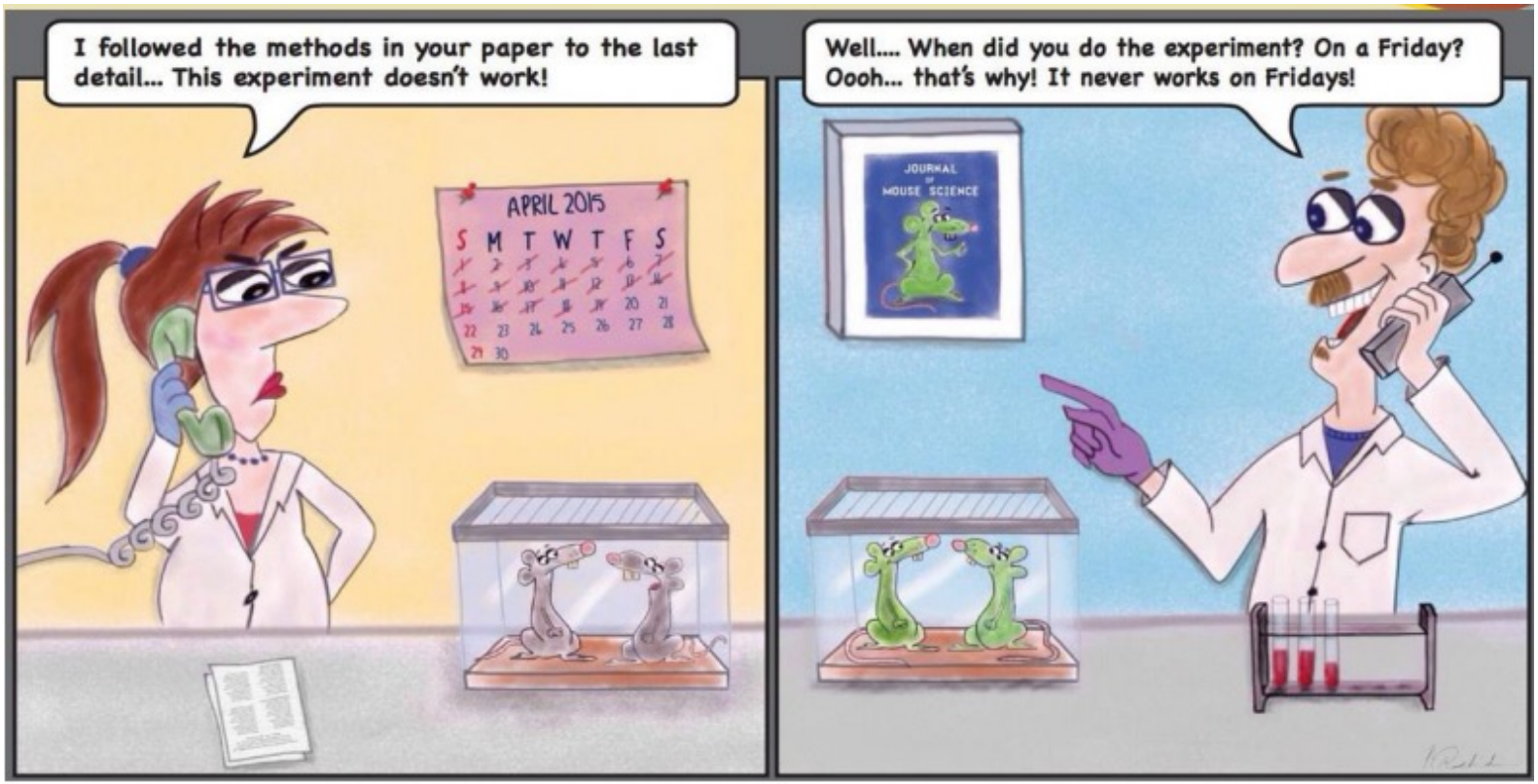
On the reproducibility problem:
Nature special issue: Challenges in irreproducible research
https://www.nature.com/collections/prbfkwmwvz/

Reproducibility and Replicability in Science, the National Academies Press (2019)
https://www.nap.edu/catalog/25303/reproducibility-and-replicability-in-science

On meeting NIH guidelines:
NIH policy on rigor and reproducibility (with examples):
https://grants.nih.gov/policy/reproducibility/index.htm

Guidance: Rigor and reproducibility in grant applications:
https://grants.nih.gov/policy/reproducibility/guidance.htm

Reviewer guidance on rigor and transparency:
https://grants.nih.gov/grants/peer/guidelines_general/Reviewer_Guidance_on_Rigor_and_Transparency.pdf


I followed the methods in your paper to the last detail... This experiment doesn't work!
APRIL 2015
S M T W T F S
Well.... When did you do the experiment? On a Friday? Oooh... that's why! It never works on Fridays!
JOURNAL OF MOUSE SCIENCE

Thank You!

Open for Questions

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ACCELERATING RESEARCH. IMPROVING HEALTH.

Feedback Survey

A link to the feedback survey has been sent to the email address you used to register.

Please get out your device, find that email, and spend a few moments completing that survey before you leave today.

Tip: If on a mobile device, shift view to landscape view (sideways) for better user experience.