

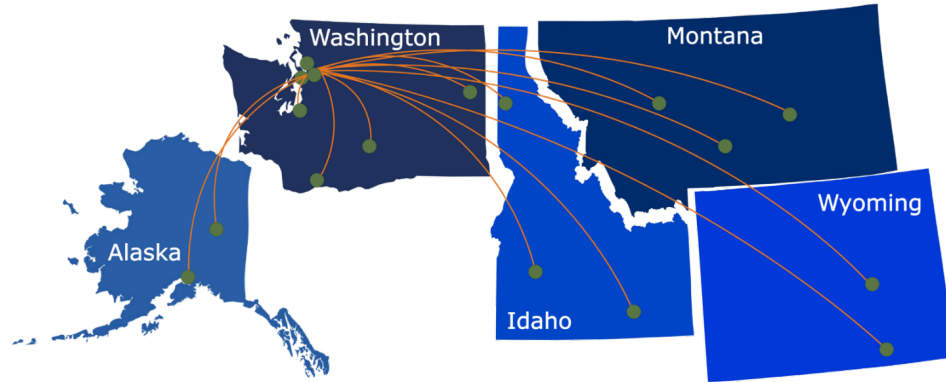
Rigor and Reproducibility

Presentation will begin at 12:00 PM (PT)



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Institute of Translational Health Sciences
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Feedback

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

Rigor and Reproducibility

Presented by:

Andrea Lazarus, PhD

**Associate Dean for Research, WSU College of
Pharmacy and Pharmaceutical Sciences**



Institute of **Translational** Health Sciences
ACCELERATING RESEARCH. IMPROVING HEALTH.

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

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

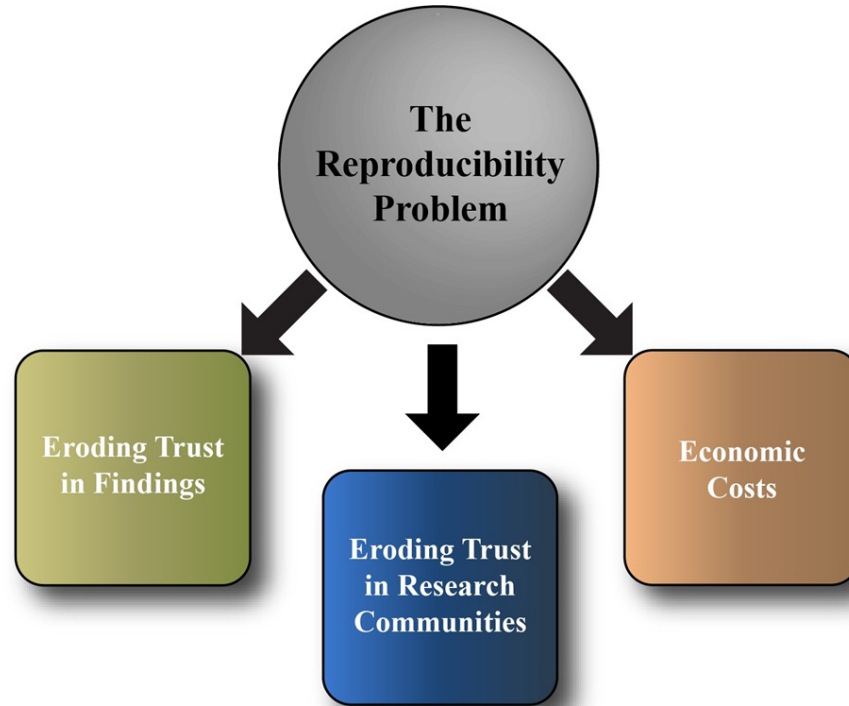
So:

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

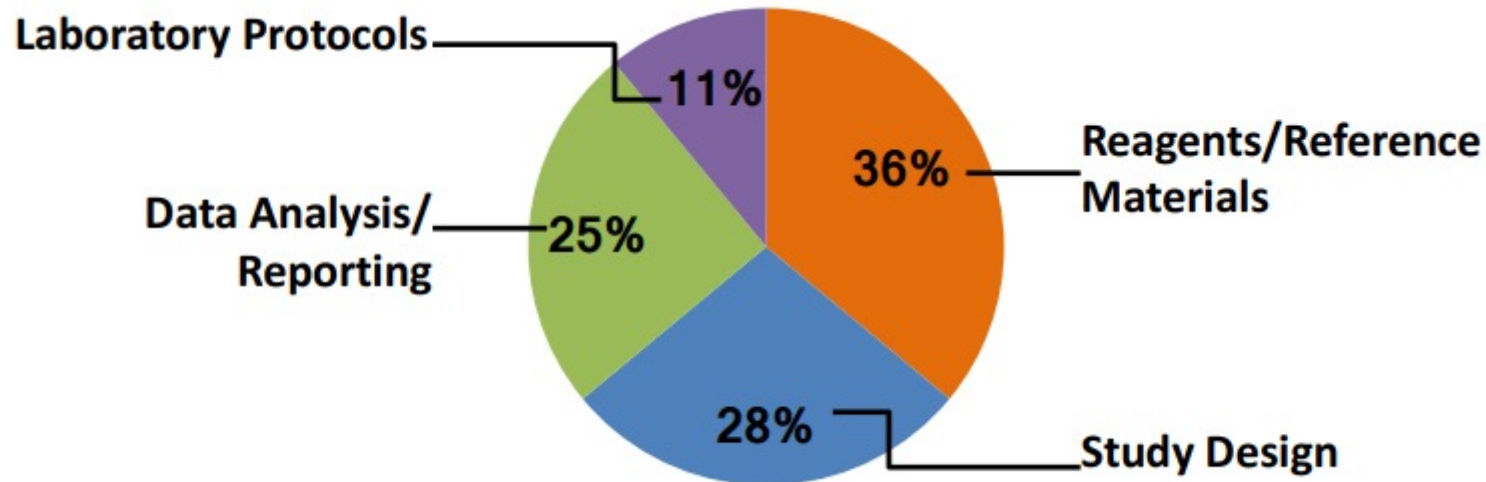
Reproducibility Problem

- 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>

HOW SCIENTISTS FOOL THEMSELVES — AND HOW THEY CAN STOP

Humans are remarkably good at self-deception. But growing concern about reproducibility is driving many researchers to seek ways to fight their own worst instincts.

COGNITIVE FALLACIES IN RESEARCH



HYPOTHESIS MYOPIA

Collecting evidence to support a hypothesis, not looking for evidence against it, and ignoring other explanations.



TEXAS SHARPSHOOTER

Seizing on random patterns in the data and mistaking them for interesting findings.



ASYMMETRIC ATTENTION

Rigorously checking unexpected results, but giving expected ones a free pass.



JUST-SO STORYTELLING

Finding stories after the fact to rationalize whatever the results turn out to be.

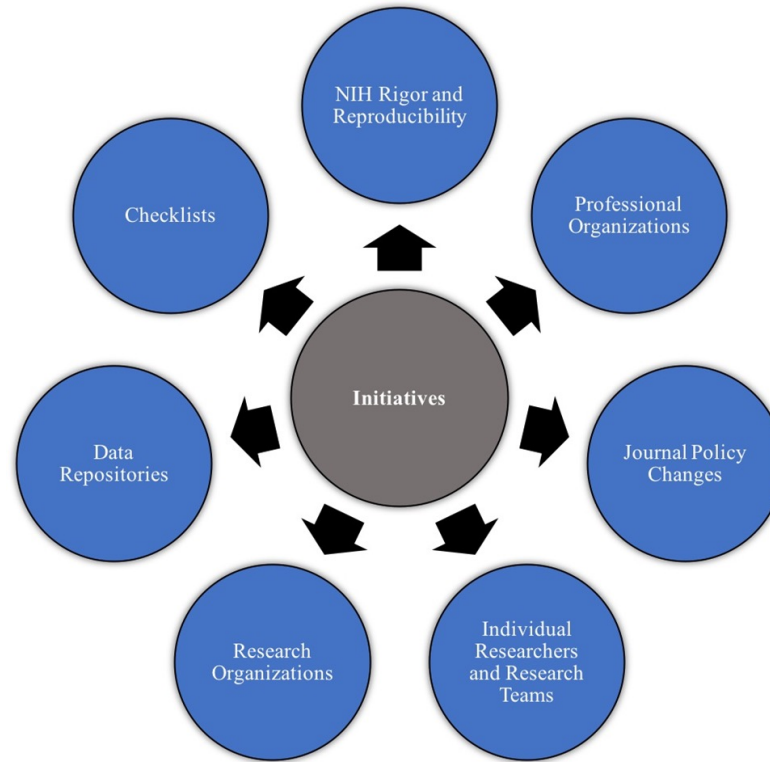
Rigor and Transparency

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

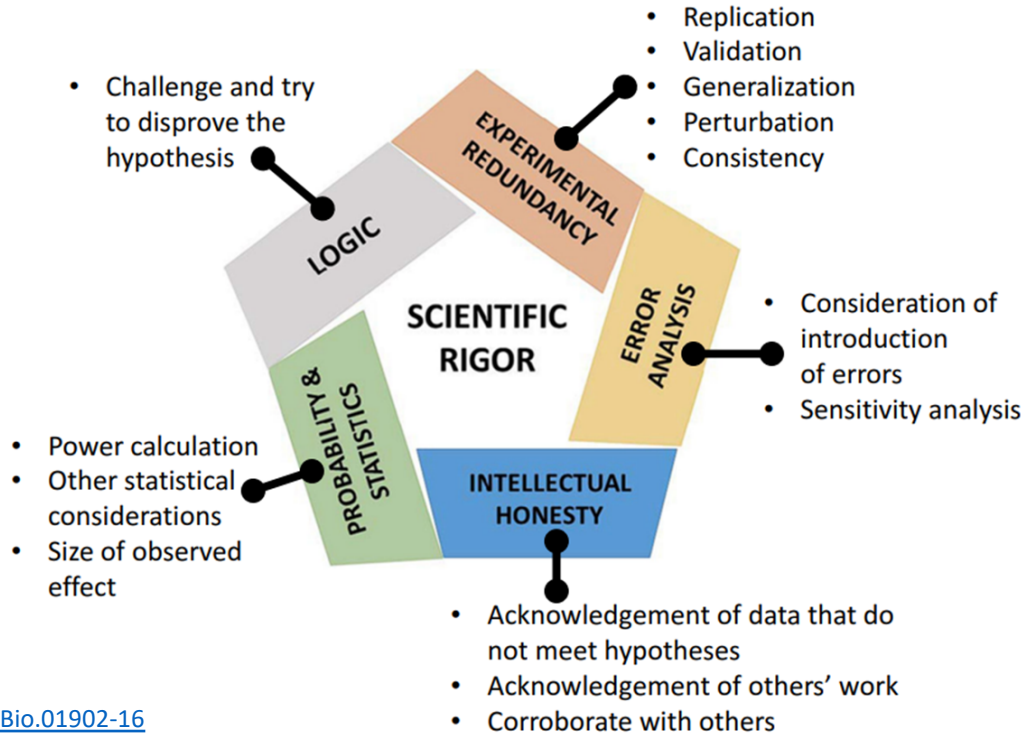
What is Being Done to Increase Rigor and Reproducibility?



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](https://doi.org/10.1128/mBio.01902-16)

Steps to Increase Rigor and Reproducibility: Researcher

DEBIASING TECHNIQUES



DEVIL'S ADVOCACY

Explicitly consider alternative hypotheses — then test them out head-to-head.



PRE-COMMITMENT

Publicly declare a data collection and analysis plan before starting the study.




TEAM OF RIVALS

Invite your academic adversaries to collaborate with you on a study.



BLIND DATA ANALYSIS

Analyse data that look real but are not exactly what you collected — and then lift the blind.

 go.nature.com/nqyohl

© Nature

Steps to Increase Rigor and Reproducibility: NIH

NIH ENHANCING REPRODUCIBILITY GUIDELINES

what you need to know

WHERE IN THE APPLICATION?

1 RESEARCH STRATEGY

The research strategy is where you discuss the significance, innovation, and approach of your research plan. Let's look at an R01, for example:

- Introduction to Resubmission and Revision Applications
- Specific Aims
- Research Strategy
- Progress Report Publication List
- Vertebrate Animals

The **research strategy** guidelines require that you:

- Describe the strengths and weaknesses in the rigor of the prior research that serves as key support.
- Describe plans to address weaknesses in the rigor of the prior research.
- Describe how your experimental design and methods will achieve robust and unbiased results.
- Explain how relevant biological variables, such as sex, are factored into research designs and analyses.

WHAT ARE THE FOUR ELEMENTS OF RIGOR?

- 1 RIGOR OF THE PRIOR RESEARCH**
- 2 RIGOR OF THE PROPOSED RESEARCH**
- 3 BIOLOGICAL VARIABLES**
- 4 AUTHENTICATION**

Send inquiries to reproducibility@nih.gov

See also NIH Notice NOT-OD-18-228
<https://grants.nih.gov/grants/guide/notice-files/NOT-OD-18-228.html>

2 ATTACHMENT FOR AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

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

These include, but are not limited to:

CELL LINES ↔ ANTIBODIES ↔ SPECIALTY CHEMICALS ↔ 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.

- DO NOT** put experimental methods or preliminary data in this section
- DO** focus on authentication and validation of key resources

3 REVIEW GUIDELINES

Here are the additional criteria the reviewers will be asked to use:

- Is the **prior research** that serves as the key support for the proposed project **rigorous**?
- Have the investigators included plans to **address weaknesses in the rigor of prior research** that serves as the key support for the proposed project?
- Have the investigators presented **strategies to ensure a robust and unbiased approach**, as appropriate for the work proposed?
- Have the investigators presented adequate plans to address **relevant biological variables, such as sex**, for studies in vertebrate animals or human subjects?

Reviewers will also be asked to comment on that new attachment (see **Update 2!**)

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

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	<p>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”:</p> <p>*Only required if Key Biological and/or Chemical Resources are mentioned in the research strategy</p>

NIH: 2019

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

4 AREAS OF FOCUS	WHAT DOES IT MEAN?	WHERE SHOULD IT BE INCLUDED IN THE APPLICATION?
Rigor of the Prior Research	<p>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.</p> <p>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.</p> <p>Describe plans to address weaknesses in the rigor of the prior research that serves as the key support for the proposed project</p> <p><i>*See related FAQs, blog post</i></p>	<p>Research Strategy</p> <ul style="list-style-type: none"> ➤ Significance ➤ Approach
Scientific Rigor (Design)	<p>Scientific rigor is the strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation and reporting of results.</p> <p>Emphasize how the experimental design and methods proposed will achieve robust and unbiased results.</p> <p><i>*See related FAQs, blog post, examples from pilots</i></p>	<p>Research Strategy</p> <ul style="list-style-type: none"> ➤ Approach
Biological Variables	<p>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.</p> <p>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.</p> <p><i>*See related FAQs, blog posts, article</i></p>	<p>Research Strategy</p> <ul style="list-style-type: none"> ➤ Approach
Authentication	<p>Key biological and/or chemical resources include, but are not limited to, cell lines, specialty chemicals, antibodies and other biologics.</p> <p>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:</p> <ul style="list-style-type: none"> • 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. <p>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.</p> <p><i>*See related FAQs, blog post, examples</i></p>	<p>Other Research Plan Section</p> <ul style="list-style-type: none"> ➤ Include as an attachment ➤ <u>Do not include</u> in the Research Strategy.

Scientific Rigor

Reduce bias

- Different/multiple individuals recording assessments
- Define terminology in advance
- Use independent and blinded assessors
- Etc.

Robust results

- Well-controlled experiments
- Reproducible results when repeated using the details reported in experimental design under well-controlled conditions

Area 1: Scientific premise

Area 2: Rigorous experimental design

Area 3: Relevant biological samples

Area 4: Authentication

Rigor of Prior Research

Enhancing Reproducibility in NIH Applications: Resource Chart

NIH Grants Policy Website: <https://grants.nih.gov/ResearchPolicy/index.htm>
 NIH Website: <https://www.nih.gov/research-training/igor-reproducibility>

4 AREAS OF FOCUS	WHAT SHOULD IT ADDRESS?	WHERE SHOULD IT BE INCLUDED IN THE APPLICATION?
Rigor of the Prior Research	<p>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.</p> <p>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.</p> <p>Describe the plans to address weaknesses in the rigor of the prior research that serves as the key support for the proposed project. <small>*See related FAQs, blog post</small></p>	<p>Research Strategy</p> <ul style="list-style-type: none"> > Significance > Approach
Scientific Rigor (Design)	<p>Scientific rigor is the strict application of the scientific method to ensure <u>fair and unbiased experimental design, methodology, analysis, interpretation, and reporting of results</u>.</p> <p>Emphasize how the experimental design and methods proposed will achieve robust and unbiased results. <small>*See related FAQs, blog post, examples from pilots</small></p>	<p>Research Strategy</p> <ul style="list-style-type: none"> > Approach
Biological Variables	<p>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.</p> <p>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. <small>*See related FAQs, blog posts, article ¹⁷</small></p>	<p>Research Strategy</p> <ul style="list-style-type: none"> > Approach
Authentication	<p>Key biological and/or chemical resources include, but are not limited to, cell lines, specialty chemicals, antibodies and other biologics.</p> <p>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:</p> <ul style="list-style-type: none"> • 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. <p>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. <small>*See related FAQs, blog posts, article ¹⁸</small></p>	<p>Other Research Plan Section</p> <ul style="list-style-type: none"> > 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, 2023 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.

*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.*

- *In **Significance** section: 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.*
- *In **Approach** section: Describe plans to address weaknesses in the rigor of the prior research that serves as the key support for the proposed project.*

Rigor of the Prior Research

Enhancing Reproducibility in NIH Applications: Resource Chart

NIH Grants Policy Website: <https://www.nih.gov/grants-policy/index.html>
 NIH Website: <https://www.nih.gov/research-training/reproducibility>

4 AREAS OF FOCUS	WHERE FOCUS IS APPLIED	WHERE SHOULD IT BE INCLUDED IN THE APPLICATION?
Rigor of the Prior Research	<p>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.</p> <p>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.</p> <p>Describe plans to address weaknesses in the rigor of the prior research that serves as the key support for the proposed project.</p>	<p>Research Strategy</p> <ul style="list-style-type: none"> > Significance > Approach
Scientific Rigor (Design)	<p>Scientific rigor is the strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation, and reporting of results.</p> <p>Emphasize how the experimental design and methods proposed will achieve robust and unbiased results.</p>	<p>Research Strategy</p> <ul style="list-style-type: none"> > Approach
Biological Variables	<p>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 design and analysis, leading to an incomplete understanding of potential sex-based differences in basic biological function, disease processes and treatment response.</p> <p>Explain how relevant biological variables, such as the ones noted above, are factored into research design, analysis, and reporting in nonvertebrate 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.</p>	<p>Research Strategy</p> <ul style="list-style-type: none"> > Approach
Authentication	<p>Key biological and/or chemical resources include, but are not limited to, cell lines, specialty chemicals, antibodies and other biologics.</p> <p>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:</p> <ul style="list-style-type: none"> 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. <p>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.</p>	<p>Other Research Plan Sections</p> <ul style="list-style-type: none"> > 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, 2023 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.

Scientific Rigor–Design

Enhancing Reproducibility in NIH Applications: Resource Chart

NIH Grants Policy Website: <https://www.nih.gov/grants-policy/index.html>
 NIH Website: <https://www.nih.gov/research-training/scientific-rigor-reproducibility>

4 AREAS OF FOCUS	WHAT DOES IT MEAN?	WHICH 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.	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. <i>*See related FAQs, link post, examples from pilots</i>	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 design, analysis, 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. <i>*See related FAQs, link post, articles</i>	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: <ul style="list-style-type: none"> 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. <i>*See related FAQs, link post, examples</i>	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 26, 2019 Ann 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.

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

- *In Approach section:
Emphasize how the experimental design and methods proposed will achieve robust and unbiased results.*

Scientific Rigor–Design

Enhancing Reproducibility in NIH Applications: Resource Chart

NIH Grants Policy Website: <https://www.nih.gov/grantspolicy/index.html>
 NIH Website: <https://www.nih.gov/research-training/scientific-reproducibility>

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.	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. <i>*See related FOOs, line items, attachments from allobs</i>	Research Strategy > Approach
Biological Variables	Biological variables , such as age, sex, weight, and metabolic health, under certain 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 consider items must be provided for applications proposing to study only one sex. <i>*See related FOOs, line items, attachments</i>	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. <i>*See related FOOs, line items, attachments</i>	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, 2023 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.

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.

Approach

- 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.

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

Enhancing Reproducibility in NIH Applications: Resource Chart

NIH Grants Policy Website: <https://grants.nih.gov/grants/reproducibility/index.htm>
 NIH Website: <https://www.nih.gov/research-training/reproducibility>

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. <small>*See related FAQs, NIH news</small>	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. <small>*See related FAQs, NIH news, NIH press releases</small>	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 design, analyses, and reporting in vertebrate animal and human studies. Strong justifications from the scientific literature, preliminary data or other relevant considerations must be provided for applications proposing to study only one sex. <small>*See related FAQs, NIH news, NIH press releases</small>	Research Strategy ➢ Approach
Authentication	All chemical and/or chemical resources include, but are not limited to, lines, specially characterized cell lines, and reagents. 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: <ul style="list-style-type: none"> • 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. <small>*See related FAQs, NIH news, NIH press releases</small>	Other Research Plan Sections ➢ 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, 2023 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.

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.

- **In Approach section:**
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.*

How to Consider Sex as a Biological Variable

Sex is a biological variable that has been 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 responses.

- 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:
 - review of literature on the influence of biological sex
 - formulation of 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
 - appropriate generalization of research findings

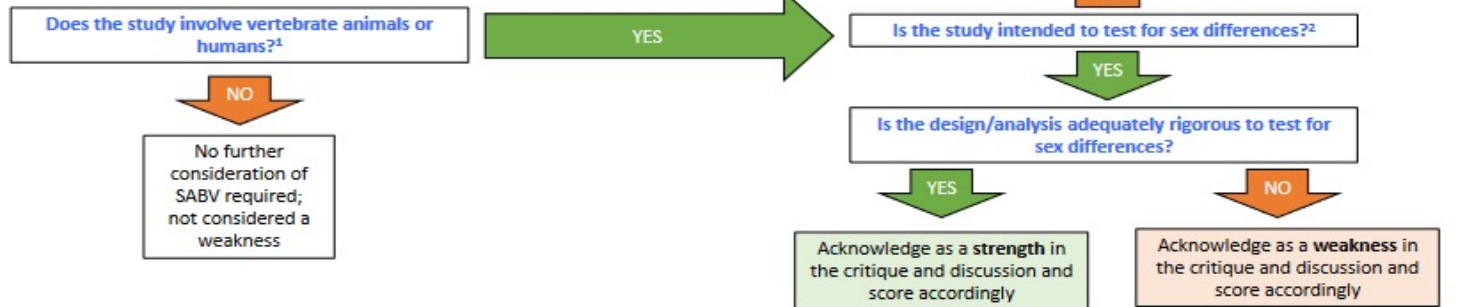
Animal Studies

- Need to provide a justification for the species that are appropriate for the proposed research in the vertebrate animals 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

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.

Authentication

Enhancing Reproducibility in NIH Applications: Resource Chart

NIH Grants Policy Website: <https://grants.nih.gov/nihsupport/ohrt/index.html>
NIH Website: <https://www.nih.gov/research-3/ensure/repro-reproducibility>

4 AREAS OF FOCUS	WHAT DOES IT MEAN?	WHERE SHOULD IT BE INCLUDED IN THE APPLICATION?
Rigor of the Prior Research	<p>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.</p> <p>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.</p> <p>Describe the plans to address weaknesses in the rigor of the prior research that serves as the key support for the proposed project.</p> <p><small>**See related FAQs, Blog posts, Examples from pilots</small></p>	Research Strategy ➢ Significance ➢ Approach
Scientific Rigor (Design)	<p>Scientific rigor is the strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation and reporting of results.</p> <p>Emphasize how the experimental design and methods proposed will achieve robust and unbiased results.</p> <p><small>**See related FAQs, Blog posts, Examples from pilots</small></p>	Research Strategy ➢ Approach
Biological Variables	<p>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 responses.</p> <p>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 applicants proposing to study sex-based differences.</p> <p><small>**See related FAQs, Blog posts, Examples from pilots</small></p>	Research Strategy ➢ Approach
Authentication	<p>Key biological and/or chemical resources include, but are not limited to, cell lines, specialty chemicals, antibodies and other biologics.</p> <p>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:</p> <ul style="list-style-type: none">• 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. <p>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.</p> <p><small>**See related FAQs, Blog posts, Examples from pilots</small></p>	Other Research Plan Section ➢ Include as an attachment ➢ Do not include in the Research Strategy.

**This chart is based on proposed submissions for research grant applications submitted for January 25, 2025. It should only be used as a guide. For more information, please read the [NIH Grants Policy System \(GPS\) & 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 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.

Authentication

Enhancing Reproducibility in NIH Applications: Resource Chart
 NIH Grants Policy Website: <https://grants.nih.gov/monoclonal-antibodies/index.htm>
 NIH Website: <https://www.nih.gov/research-training/reproducibility>

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 the plans to address weaknesses in the rigor of the prior research that serves as the key support for the proposed project. <i>**See related FAQs, Blog post, Blog post.</i>	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. <i>**See related FAQs, Blog post, Examples from pilot.</i>	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 responses. Explain how relevant biological variables, such as the ones noted above, are factored into research design, analysis, and reporting in vertebrate animal and human studies. Strong justification from the scientific literature, preliminary data or other relevant considerations must be provided for applicants proposing to study sex-specific differences . <i>**See related FAQs, Blog posts, Blog posts.</i>	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. <i>**See related FAQs, Blog posts, Blog posts.</i>	Other Research Plan Section > Include as an attachment > Do not include in the Research Strategy.

**This chart is based on the proposed guidelines for research grant applications submitted for January 25, 2023 and beyond. It should only be used as a guide. For more information, please read the [Research Training Community Announcement \(PCA\)](#) & 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.

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 μ g 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
- Scientific premise and rigor of the prior research (organize overall or by aim)
 - Numerous studies have...
 - However, studies X and Y have important limitations...
 - In addition, the rigor of 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 are used as 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-checked to ensure that there is no or little variation between control strains.
- **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 data; 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

Thank You!

Open for Questions

ITHS

Institute of **Translational** Health Sciences
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.