NIH POLICY on Enhancing Reproducibility through Rigor and Transparency: NIH Expectations of Investigators and Revised Grant Proposal Requirements

Information Session
May 5, 2016

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What is the goal of these changes?

- Exemplify and promote the highest level of scientific integrity, public accountability, and social responsibility in the conduct of science.
- Enhance reproducibility by promoting greater scientific rigor and transparency in funding applications and publications.
- Encourage robust peer review.
- Provide adequate training.
- Emphasize overlooked areas such as the consideration of sex as a biological variable in research.
To what proposals does the policy apply?

- The rigor and transparency policy applies to most research grant and mentored career development award applications.
- **Applications submitted for due dates after January 25, 2016** must address the rigor and transparency requirements outlined in the application instructions.
- Updated application forms (FORMS-D) must be used for due dates beginning May 25, 2016.
Four elements of rigor and transparency must be addressed in proposals:

1. The **scientific premise** of the proposed research
2. Rigorous **experimental design** for robust and unbiased results
3. Consideration of relevant **biological variables**
4. **Authentication** of key biological and/or chemical resources
Element #1: The Scientific Premise

- The **scientific premise** for an application is the research used to form the basis for the proposed research questions.

- The **scientific premise** concerns the quality and strength of the body of evidence that supports the proposed research questions.

- NIH expects applicants to consider the strengths and weaknesses of published research or preliminary data being cited by the applicant as crucial to support the application.
Element #1: The Scientific Premise

- In application section 5.5.3 *Research Strategy, Significance*, applicants should describe the general strengths and weaknesses of the prior research cited in the proposal as crucial for its support.

- Consideration of strengths and weaknesses could include:
  - Attention to rigor of previous experiments
  - Methodology, analysis and interpretation
  - Relevant biological variables
  - Authentication of key resources
Element #1: The Scientific Premise

Updated Application Instructions: Scientific Premise

5.5.3 Research Strategy.... Significance

- Explain the importance of the problem or critical barrier to progress in the field that the proposed project addresses.
- **Describe the scientific premise for the proposed project**, including consideration of the strengths and weaknesses of published research or preliminary data crucial to the support of your application.
- Explain how the proposed project will improve scientific knowledge, technical capability, and/or clinical practice in one or more broad fields.
- Describe how the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field will be changed if the proposed aims are achieved.

Reviewer guidance has been updated to include a consideration for scientific premise to be scored in the Significance criterion.

The updated instructions to reviewers read:

- “Is there a strong scientific premise for the project?”
Element #1: The Scientific Premise

FAQs on Scientific Premise are available at: http://grants.nih.gov/reproducibility/faqs.htm

Scientific Premise

1. What does "scientific premise" mean for a grant application?

2. What is the difference between "scientific premise" and "significance"?

3. Should preliminary data presented within the application conform to the updated instructions for rigor and transparency? Will I be expected to discuss in my application the strengths and weaknesses of my own preliminary data as part of the scientific premise? What if a publication I cite does not include elements of rigor and transparency?

4. In exploratory/developmental grant applications and other activities for which preliminary data are not required, how should scientific premise and scientific rigor be addressed?
Element #2: Rigorous Experimental Design

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

- **Full transparency** in reporting permits others to reproduce and extend the research.

- NIH expects applicants to describe the proposed experimental design and methods and to explain how they will achieve robust and unbiased results.
Element #2: Rigorous Experimental Design

• In Application Section 5.5.3 Research Strategy, Approach, applicants should succinctly describe the aspects of rigor important to the proposed research.

Considerations might include:
• Use of standards
• Sample size estimation
• Randomization and/or Blinding
• Appropriate replicates
• Controlling for inter-operator variability
• Statistical methods planned
• Inclusion and Exclusion criteria
• Subject retention and attrition
• How missing data will be handled
Element #2: Rigorous Experimental Design

Application Instructions

Updated Application Instructions: Scientific rigor

5.5.3 Research Strategy....
Approach

- Describe the overall strategy, methodology, and analyses to be used to accomplish the specific aims of the project. Unless addressed separately in the Resource Sharing Plan, include how the data will be collected, analyzed, and interpreted as well as any resource sharing plans as appropriate.
- Describe the experimental design and methods proposed and how they will achieve robust and unbiased results.
- Discuss potential problems, alternative strategies, and benchmarks for success anticipated to achieve the aims.
- If the project is in the early stages of development, describe any strategy to establish feasibility, and address the management of any high risk aspects of the proposed work...

http://grants.nih.gov/reproducibility/index.htm
Reviewer guidance has been updated to include a consideration for rigorous experimental design to be scored in the Approach criterion.

The updated instructions to reviewers read:

• “Have the investigators presented strategies to ensure a robust and unbiased approach, as appropriate for the work proposed?”
Element #2: Rigorous Experimental Design: Resources

Institute-specific guidelines:

• National Institute of Neurological Disorders and Stroke

• National Institute of Mental Health

• National Institute on Drug Abuse
Element #2: Rigorous Experimental Design: Resources

Training modules on:
- Lack of Transparency
- Blinding and Randomization
- Biological and Technical Replicates
- Sample Size, Outliers, and Exclusion Criteria

Available at:
Element #2: Rigorous Experimental Design: Resources

FAQs on Rigorous Experimental Design are available at: http://grants.nih.gov/reproducibility/faqs.htm

Scientific Rigor

1. What does "scientific rigor" mean?

2. What is meant by "robust" and "unbiased"?

3. Can reviewers be directed to publications where the method is described?

4. How will applicants be expected to address scientific rigor in their applications if they are proposing highly innovative research projects?

5. How can we apply scientific rigor to a pre-resource that is developing a technology, or to activities that are more resource-driven versus a research component?

6. Are guidelines available on how much detail to include in my application regarding rigor and transparency?

7. What if an application includes a power analysis that peer reviewers or program staff identify as inappropriate?
Debiassing 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**
Analyze data that look real but are not exactly what you collected – and then lift the blind.

Adapted from Nature (go.nature.com/2qyoHl)
Element #3: Consideration of Relevant Biological Variables

- NIH expects that biological variables will be factored into research designs, analyses, and reporting in *vertebrate animal and human* studies.
- Relevant variables may include *sex, age, weight/size, underlying health conditions, others*.
- Policy is intended to address any biological variable that the investigator feels is relevant, but has a *strong focus on sex as a biological variable*.
- Consideration of relevant biological variables should be addressed in Application Section: 5.5.3 *Research Strategy, Approach*. 
Sex as a Biological Variable


Source: Dr. Janine Clayton, Director, NIH/ORWH
Sex as a Biological Variable

• Researchers must examine sex as a biological variable at every stage of the research continuum, from basic to applied.

• Researchers are expected to explain how relevant biological variables, including sex, are factored into research designs and analyses.

• Experimental design should include consideration of effect size and power calculations to determine the number of samples/subjects in the study, if applicable.
Sex as a Biological Variable

• Develop a **data analysis plan prospectively** that provides for the collection of data disaggregated by sex.

• Report when sex differences **are or are not detected** in analyses that may be valuable for future meta-analysis.

• Strong justification from the scientific literature, preliminary data, or other relevant considerations **must be provided for applications proposing to study only one sex**. Justification may include:
  • acutely scarce resources
  • sex-specific conditions
  • research where study of one sex is scientifically appropriate and justified.
Element #3: Relevant Biological Variables

Application Instructions

Updated Application Instructions: Relevant Biological Variables

5.5.3 Research Strategy....

Approach

• Describe the overall strategy, methodology, and analyses to be used to accomplish the specific aims of the project. Unless addressed separately in the Resource Sharing Plan, include how the data will be collected, analyzed, and interpreted as well as any resource sharing plans as appropriate.
• Describe the experimental design and methods proposed and how they will achieve robust and unbiased results.
• Discuss potential problems, alternative strategies, and benchmarks for success anticipated to achieve the aims.
• If the project is in the early stages of development, describe any strategy to establish feasibility, and address the management of any high risk aspects of the proposed work.
• Explain how relevant biological variables, such as sex, are factored into research designs and analyses for studies in vertebrate animals and humans.
  • For example, strong justification from the scientific literature, preliminary data, or other relevant considerations, must be provided for applications proposing to study only one sex.
  • Please refer to NOT-OD-15-102 for further consideration of NIH expectations about sex as a biological variable.

http://grants.nih.gov/reproducibility/index.htm
Reviewer guidance has been updated to include a consideration of relevant biological variables to be scored in the Approach criterion.

The updated instructions to reviewers read:

• “Have the investigators presented adequate plans to address relevant biological variables, such as sex, for studies in vertebrate animals or human subjects?”
Element #3: Relevant Biological Variables

Resources

• NIH Policy: Consideration of Sex as a Biological Variable in NIH-funded Research

• NIH Guidance: Consideration of Sex as a Biological Variable in NIH-funded Research
Element #3: Relevant Biological Variables

Resources

FAQs on Rigorous Experimental Design are available at: http://grants.nih.gov/reproducibility/faqs.htm

1. Consideration of Relevant Biological Variables, Such as Sex
   1. Which relevant biological variables do we need to consider?
   2. What does it mean to consider sex as a biological variable?
   3. Does the Consideration of Sex as a Biological Variable policy (NOT-OD-15-102) include cell lines?
   4. Is consideration of sex as a biological variable required for all grant applications?
   5. How do I decide whether to include both males and females in my basic research?
   6. How is consideration of sex as a relevant biological variable different from NIH's inclusion requirements?
   7. Will animal costs increase as a result of the policy on Consideration of Sex as a Biological Variable?
   8. How should applicants address scientific rigor and sex as a biological variable when the research involves scarce animal resources?
   9. Must sex as a biological variable be considered by IACUCs during review of animal use protocols or is this review the purview of NIH study sections?
  10. May IACUCs approve animal use protocols that require a larger number of animals because of the new NIH requirement to include both sexes in research designs where relevant?
  11. What did NIH learn from respondents to the RFI: Consideration of Sex as a Biological Variable (SABV) in Biomedical Research (NOT-OD-14-128)?
Element #4: Authentication of Key Resources

• Researchers must ensure that the resources they are working with are genuine, have the expected properties and are valid for use in their work.

• NIH expects that applicants will authenticate key biological and/or chemical resources regularly.

• Applicants are asked to briefly describe methods to ensure the identity and validity of key biological and/or chemical resources. They should report the plan for authentication transparently.

• Applicants include this plan in the Other Research Plan section as an attachment.
Element #4: Authentication of Key Resources

NIH describes key biological or chemical resources as resources that:

• may differ from lab to lab, or over time.
• have qualities or qualifications that could influence results.
• are integral to the proposed research.
• Examples (not an inclusive list): cell lines, specialty chemicals, antibodies, other biologics.
• Standard laboratory reagents not expected to vary do not need to be included in the plan.
• If key resources have been purchased or obtained from an outside source that provided data on prior authentication, the applicant is still expected to provide their own authentication plans for these key resources.
Element #4: Authentication of Key Resources

Application Instructions

Updated Application Instructions: Resource Authentication

Other Research Plan Attachment:

Authentication of Key Biological and/or Chemical Resources
Briefly describe methods to ensure the identity and validity of key biological and/or chemical resources used in the proposed studies.
Key biological and/or chemical resources may or may not be generated with NIH funds and:
1) may differ from laboratory to laboratory or over time;
2) may have qualities and/or qualifications that could influence the research data; and
3) are integral to the proposed research.
These include, but are not limited to, cell lines, specialty chemicals, antibodies, and other biologics.
Standard laboratory reagents that are not expected to vary do not need to be included in the plan. Examples are buffers and other common biologicals or chemicals.
Reviewers will assess the information provided in this Section. Any reviewer questions associated with key biological and/or chemical resource authentication will need to be addressed prior to award.
Information in this section must focus only on authentication and/or validation of key resources to be used in the study; all other methods and preliminary data must be included within the page limits of the research strategy. Applications identified as non-compliant with this limitation will be withdrawn from the review process (see NOT-OD-15-095).

http://grants.nih.gov/reproducibility/index.htm
Element #4: Authentication of Key Resources

Instructions to Reviewers

Reviewer guidance has been updated to include Resource Authentication as an Additional Review Consideration that is not to affect the Overall Impact Score.

The updated instructions to reviewers read:

• “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.”
Element #4: Authentication of Key Resources

Additional Information

FAQs on Authentication of Key Resources are available at:  http://grants.nih.gov/reproducibility/faqs.htm

Authentication of Key Biological and/or Chemical Resources

1. What are "key biological and/or chemical resources"?

2. What is the proper way to authenticate reagents? Are there any NIH-wide guidelines about acceptable procedures for authentication?

3. What kind of information should I include in the Authentication Plan? Do I need to include an Authentication Plan?

4. If a cell line or other research resource (e.g., an antibody) is still under development, how should resource authentication be addressed in the application?

5. Do applicants have to authenticate primary cell cultures?

6. How will meritorious applications designated as inadequate for their plans for authentication of key resources be handled administratively?
# Summary of Required Elements

<table>
<thead>
<tr>
<th>Element of Rigor</th>
<th>Section of Application</th>
<th>Criterion Score</th>
<th>Additional Review Consideration</th>
<th>Contribute to Overall Impact?</th>
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<tbody>
<tr>
<td>Scientific Premise</td>
<td></td>
<td>Significance</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>Scientific Rigor</td>
<td>Research Strategy</td>
<td>Approach</td>
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<td>Yes</td>
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<tr>
<td>Consideration of Sex and Other Relevant Biological Variables</td>
<td>Approach</td>
<td>Approach</td>
<td>NA</td>
<td>Yes</td>
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<tr>
<td>Authentication of Key Biological and/or Chemical Resources</td>
<td>New Attachment</td>
<td>NA</td>
<td>Adequate or Inadequate</td>
<td>No</td>
</tr>
</tbody>
</table>

*Source: Dr. Janine Clayton, Director, NIH/ORWH*
Examples from awarded applications

Source: http://grants.nih.gov/reproducibility/index.htm
Example #1

Aim 3: Male and female mice will be randomly allocated to experimental groups at age 3 months. At this age the accumulation of CUG repeat RNA, sequestration of MBNL1, splicing defects, and myotonia are fully developed. The compound will be administered at 3 doses (25%, 50%, and 100% of the MTD) for 4 weeks, compared to vehicle-treated controls. IP administration will be used unless biodistribution studies indicate a clear preference for the IV route. A group size of n = 10 (5 males, 5 females) will provide 90% power to detect a 22% reduction of the CUG repeat RNA in quadriceps muscle by qRT-PCR (ANOVA, \( \alpha \) set at 0.05). The treatment assignment will be blinded to investigators who participate in drug administration and endpoint analyses. This laboratory has previous experience with randomized allocation and blinded analysis using this mouse model [refs]. Their results showed good reproducibility when replicated by investigators in the pharmaceutical industry [ref].
Example #2

Aim 1: Primary screen: In this high throughput screening assay, we combined the SMN promoter with exons 1-6 and an exon 7 splicing cassette in a single construct that should respond to compounds that increase SMN transcription, exon 7 inclusion, or potentially stabilize the SMN RNA or protein [refs]. The details of the assay and the SMN2-luciferase reporter HEK393 cell line have been extensively validated [refs]. Each point is run in triplicate, the compounds are tested on three separate occasions, and the results are averaged to give an EC50 with standard deviation. Secondary screen: ... We analyze SMN protein levels by dose response in quantitative immunoblots with statistical analysis by one-way ANOVA with post-hoc analysis using Dunnett or Bonferroni, as appropriate.
Example #3

Aim 2: Intensity signal data will be transformed into log values and then modeled by longitudinal methods (reference cited). Specifically, the composite difference in mean intensity signals over time between the bi-specific T cells vs. control groups is assumed to be 2.8 logs with a composite standard deviation of 2.2 logs. Furthermore, we will assume at least five repeated measurements per mouse after T cell infusion and a within-mouse intra-correlation coefficient equal to 0.50. Thus, a sample size of 10 mice per group will provide at least 80% power to detect the above difference between treated versus control group with a 5% significance level. Log-rank test will be used to compare the survival distribution between groups.

VAS: Animal numbers are based on the requirement to perform each experiment (power and sample size calculations are described in the Research Strategy), which includes an independent experimental repeat.
Rigor, Reproducibility & Transparency (R2T)

The Requirement

ALL NIH & AHRQ Grant applications AND progress reports MUST focus:
1. the scientific premise of the proposed research,
2. authentication of key biological and/or chemical resources,
3. consideration of relevant biological variables, and
4. rigorous experimental design for robust and unbiased results.

The Challenge

1. New Scored Criteria
2. New Required Appendix

The Opportunity

1. Expert BERD Consultations
2. Web Resources through CCTS Research Commons
3. CCTS Project Panels
4. Rigor in Training
5. Key Approaches in Version Control, Independent Verification and Data Archiving

Sharpen your science... and R2T, too!
Access To Services Through Partnership With UAB CCTS

BERD Representatives and Project Panels can be accessed through CCTS Research Commons

**ccts@uab.edu**

**205.934.7442**

[http://www.uab.edu/ccts/researchcommons](http://www.uab.edu/ccts/researchcommons)

Faculty are encouraged to use these resources as they refine their research strategies and manuscripts in the context of rigor and transparency.
Information Resources for the Investigator

• Training Module on R&T for NIH staff: https://grants.nih.gov/reproducibility/module_1/presentation.html*

Excellent resources at NIH website to assist researchers:
• http://grants.nih.gov/reproducibility/index.htm
• https://www.nih.gov/research-training/rigor-reproducibility
• http://grants.nih.gov/reproducibility/faqs.htm
• http://orwh.od.nih.gov/sexinscience/overview/index.asp
• NOT-OD-15-095
• NOT-OD-15-102
• NOT-OD-15-103
• NOT-OD-16-011

*Highly recommended
Information Resources for the Investigator

• Article: *Nature* Commentary: "Policy: NIH plans to enhance reproducibility"  Collins & Tabak, 01/27/2014

• Article: *Nature* Commentary: "Policy: NIH to balance sex in cell and animal studies"  Clayton & Collins, 05/14/2014


• Article: *Science* Perspectives: "Fixing problems with cell lines"  Lorsch, Collins & Lippincott-Schwartz, 12/19/2014
Thank you for your attention

For more information or assistance, contact:

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