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RIGOR AND REPRODUCIBILITY IN NIH APPLICATIONS

Why is rigor and transparency important in research? Rigor and transparency in research support the highest quality of science, public accountability, and social responsibility in the conduct of science.

The goal for the National Institutes of Health (NIH) is to enhance reproducibility of research through rigor and transparency in four areas:

1. scientific premise
2. scientific rigor
3. consideration of relevant biological variables, such as sex
4. authentication of key biological and/or chemical resources

NIH Reviewer Roles are to

- assess scientific merit of applications according to review criteria, including consideration of scientific premise, rigor, and consideration of relevant biological variables, and adequacy of the authentication of key biological and/or chemical resources as an administrative issue.
- base evaluation on current best practices in the field.

A number of additional pieces of information concerning these four areas have been obtained from NIH materials, grant reviews from NIH, PI experiences, and NIH grant reviewer experiences. This information has been summarized below to assist PIs with addressing these four areas in NIH grant applications.

The NIH Rigor and Reproducibility Resource Chart http://grants.nih.gov/grants/RigorandReproducibilityChart508.pdf has been used below to define the NIH expectation in grant applications for each of the four areas.

1. **Scientific Premise** (prior research) – Include in Research Strategy (Significance)
   NIH expectation in grant application:
   When addressing scientific premise in a grant application consider the following:
   - Use the research that is published and the preliminary data in the application to address the proposed research questions
     - State the strengths and weaknesses of this prior research
     - Is there a solid basis for the work being proposed?
     - Consider rigor of previous experimental designs as well as the incorporation of relevant biological variables and authentication of key resources

   NIH Reviewer Evaluation Criteria:

"Goal is to ensure that the underlying scientific foundation of the project is sound. This would include concepts, previous work, and data.

- Pertains to the underlying evidence/data for the project
- Address under Significance (R applications) or Research Plan (Ks)
- Addition to the review criteria: "Is there a strong scientific premise?"
- Specifically, has the applicant:

- provided sufficient justification for the proposed work?
- cited appropriate work and/or preliminary data?
- appropriately identified strengths and weaknesses in prior research in the field?
- proposed to fill a significant gap in the field? OR has the applicant explained why this is not possible?

Affect overall impact score – YES"

Tips from Pitt colleagues:

- If the project is without solid evidence to support the research questions, this may be acceptable for an R21, but for an R01, the evidence needs to be solid.
- Premise was somewhat of a substitute for significance in the study section. Applicants should use the language " scientific premise", so that reviewers can find the information during the review and study section discussion.
- Top grants specifically addressed and included the word – "premise", etc. Include the specific terminology and underline or highlight it to make it easier for reviewers to find the information.
- Scientific Premise did not change the overall score because it reflects the information that would have been used for other terms such as Significance or Innovation.
- Literature review will strengthen premise. Include mechanistic insights to strengthen assumptions made.
- Reviewer comments were such as "The work did not have a strong premise."

2. **Scientific Rigor** (design/scientific method) – Include in Research Strategy (Approach)

NIH expectations in grant application:

Scientific Rigor is the application of the scientific method to ensure robust and unbiased design, methodology, analyses, interpretation, and reporting of results. Include how the experimental design and the methods proposed will achieve robust and unbiased results. Rigor and Transparency = Reproducibility

NIH Reviewer Evaluation Criteria:

"Goal is to ensure a strict application of scientific methods that supports robust and unbiased design, analysis, interpretation, and reporting of results, and sufficient information for the study to be assessed and reproduced. Give careful consideration to the methods and issues that matter in your field.

- Pertains to the proposed research
- Address under Approach (R applications) or Research Plan (Ks)
- Addition to the review criteria: Are there strategies to ensure robust and unbiased approach, as appropriate for the work proposed?
- Possible considerations, if appropriate for the scientific field and research question, include plans for
    - Determining group sizes
    - Analyzing anticipated results
    - Reducing bias

- Ensuring independent and blinded measurements
- Improving precision and reducing variability
- Including or excluding research subjects
- Managing missing data

Affect overall impact score – YES"

Tips from Pitt colleagues:

- Rigor was also a substitute for Approach in the study section.
- Applicants should use the term "rigor" in the application.
- Reviewers seems to like it when grants have a brief section on Rigor in the Approach for each Aim
- Rigor and transparency were discussed before scoring
- Reviewers liked statistical power, statistical analysis, replications, etc.
- Include enough repeats to ensure rigorous design
- Blinding, no prior knowledge of groups, statistics, and tests used to ensure a rigorous study were viewed positively. The more clearly stated, the more favorable.
- Scientific rigor is important because no longer include number of animals in application.
- Ensure study is unbiased, but pay particular attention not to exclude key investigators in the field or other relevant studies.
- Reviewers considered positively well thought-out consideration of statistical analyses of acquired data, establishing proper controls, and defining a proposed number of replicates for analyses of individual samples.
  Need to address Rigor in 10-12 lines.
- Reviewers were asked at the beginning of the study section meeting to specifically address how the applicant addressed rigor and premise. Transparency was not mentioned.

3. **Consideration of Relevant Biological Variables, such as Sex** – Include in Research Strategy (Approach)

How are these relevant biological variables factored into the design, analyses, and reporting for animal and human studies. Use literature, preliminary data, and other relevant considerations when proposing only one sex. Cost is not a valid justification. Biological variables could also be age, disease status, weight, etc. The biological variable depends on the nature of the research.

Sex as a Biological Variable:

Consideration of sex, included under the umbrella of "Relevant Biological Variables" is required in all studies involving human subjects or vertebrate animals.

NIH expectations in grant applications:

- If little is known about sex differences, the application should include both sexes.
  - Sufficient numbers should be provided to inform the presence or absence of sex differences. Statistically powered comparisons between sexes may not be warranted.
  - Specific hypotheses about sex differences may not be possible.
  - Findings should be reported separately by sex in progress reports and publications.

- If sex differences are not known to exist, a strong justification should be provided if the application proposes to study one sex.
- If sex differences are known, experiments should be designed with appropriate group sizes to detect sex differences."

NIH Reviewer Evaluation Criteria:

"Goal is to ensure that the research accounts for sex and other relevant biological variables in developing research questions and study designs. The ways in which sex and other biological variables need to be accounted for will differ across research questions and fields of study:

- Pertains to the proposed research
- Applies to studies in vertebrate animals and/or human subjects
- Address in Approach (R applications) or Research Plans (K)
- Addition to review criteria: Are there "Adequate plans to address relevant biological variables for studies in vertebrate animals or human subjects"?
- Consideration of sex is required in all studies involving human subjects or vertebrate animals
- Special considerations to assess include:
    - Applies broadly to all biological variables relevant to the research such as sex, age, source, weight, or genetic strain.
    - Has the applicant considered biological variables, such as sex, that are relevant to the experimental design?
    - Will relevant biological variables be controlled or factored into the study design appropriately?

Affect overall impact score – YES"

Tips from Pitt Colleagues:

- Addressing sex is definitely important.
- If all females and no justification as to why, then application will be dinged.
- In some study sections age and instrumentation were not discussed.

4. **Authentication of Key Biological and/or Chemical Resources** – Include in New Attachment

NIH expectations in grant applications:

Briefly describe methods to ensure the identity and validity of key biological and/or chemical resources used in the proposed studies. The resources may or may not be generated with NIH funds and may differ from laboratory to laboratory over time, may have qualities and/or qualifications that could influence the research data, and are integral to the proposed project.

The authentication plan should be one page or less and include how you will authenticate key resources, including frequency as needed for the research. The authentication data should NOT be included in this authentication plan.

- Ensure cell lines are not misidentified, contaminated, etc., if applicable.
- Include how you will independently verify rather than just accept other source information. For example for an antibody, how will you evaluate it to ensure that it targets what you are

expecting it to target and to function as you are expecting? How will you ensure it is doing what you think it is doing?
    - For example, cells obtained from ATCC are within 5-10 passages. These cells are without continual passaging as a result of having a master stock of cells, a grand master stock of cells, and minimal feeder passages of cells.
    - For example, you will genotype high passage cell lines and conduct testing for mycoplasma.
- Describe where you will obtain the materials, and if received from a specific person, discuss, for example, the maintenance of a seed stock for propagation and sequencing of the construct.

NIH Reviewer Evaluation Criteria

"Goal is to ensure processes are in place to identify and regularly validate key resources used in the research and avoid unreliable research as a result of misidentified or contaminated resources.

- Researchers are expected to authenticate key biological and/or chemical resources used in their research, to ensure that the resources are genuine.
- New Additional Review Consideration
    - 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.
- Rate as acceptable/unacceptable (provide brief explanation if unacceptable)

Affect overall impact score – NO"

Tips from Pitt Colleagues:

- For reviewers, this seems to be the most problematic section.
- This has been a section that has caused great frustration to the reviewers, because it's unclear what this actually is about and thus has resulted in the review sessions becoming bogged down.
- Discussed at study section after scoring

Related Issues to consider:

- Different research fields may have different best practices for and reach different conclusions about scientific premise and rigor. Assess based on best practices in the field.
- Page limits have not changed. Page limits, cost and time are not valid reasons to disregard attention to these issues.
- Rigor and transparency considerations apply to R03 (Small grant) and R21 (exploratory/developmental) applications. However, preliminary data are not required and the extent to which approach details can be provided may differ.
- Reviewers are asked to evaluate the scientific merit of these applications, including rigor and transparency, in light of the goals and reviewer guidelines for these activities.

- If the reviewer in the study section indicates that the criteria were met, follow-through in written critiques may lack information about these specific points/areas.
- At the beginning of a study section meeting, all reviewers were asked to specifically address how the applicant expressed two things, rigor and premise. Transparency did not come up in the discussion.

Collection of Specific Reviewer Comments concerning Rigor and Transparency Collected from Pitt PIs:

- “The premise that X does Y is not very strong; it was from an investigator’s clinical study with limited patient numbers. A thorough literature review may help strengthen the premise. Also there is a lack of mechanistic insight why X does Y.”
- “Need a more rigorous assessment of key assays”
- "For all Aims, it is not stated whether behavioral and neuropathological analyses will be conducted by investigators blind to genotype. This would be important to avoid potential bias and to ensure rigor and reproducibility." Note – Considered a minor weakness
- "The approach was not sufficiently rigorous in the following areas:"
- “There is no indication of how the investigator will mitigate bias in data collection, analysis, and reporting”.
- “Does not include new NIH rigor and reproducibility policy”
- “Does not include NIH rigor and reproducibility policy”.
- “A more rigorous quantitative assessment of inter assay performance is needed for a key assay that will drive the next cycle of hypotheses generation and testing (Major)”
- “Specifically addressed within both of the Aims are well detailed plans for assuring Scientific Rigor by thoughtfully considering statistically analyses of acquired data, establishing proper controls, and defining the proposed number of replicates for analyses of individual samples (Major).”
- “The Rigor of the project is also concerning – there is no mention of biophysical studies to investigate the effects of mutagenesis on the protein structure and stability. Change in amino acid from charged to uncharged can destabilize a protein significantly, but there is no discussion on how the mutations will be analyzed. Finally, it seems that reliance on computation to guide the mutations may be a severe limiting factor.”
- “Sex As A Biological Variable – Unacceptable/not addressed. 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. NIH expects that sex as a biological variable will be factored into research designs, analyses, and reporting in vertebrate animal and human. Strong justification from the scientific literature, preliminary data or other relevant considerations must be provided for applications proposing to study only one sex.”
- “Vertebrate animals: Does not include new NIH rigor and reproducibility policy”
- “Sex as a biological variable is not adequately considered; mention is only made of using male animals, without explanation or justification”.
- “No concerns, male and female subjects included.
- “Authentication of Key Biological and/or Chemical Resources: Unacceptable. No detail info included.”

- "Missing Authentication Plan: …Reviewers were asked to consider information provided in this attachment as part of their evaluation of your application. The attachment was missing from your application and could not be assessed." Note – this attachment was actually in the grant application.
- "Authentication Plan needs to be well described and contain detail."
- "Human MSCs will be used, but as a biological variable is not considered…."
- "No documentation is provided for plans to authenticate key biological and chemical resources."

Attached Templates

- The attached Rigor and Transparency statements used by Project and Core Leaders for the Progress Report of a SPORE were provided by John Kirkwood, MD, UPCI.
- Authentication of Key Biological and/or Chemical Resources: Attached example, provided by Jesus Tejero, PhD, Vascular Medicine Institute, was evaluated as acceptable by all 3 grant reviewers. Highlights of the template include four references to published work, and the commercial sources were identified for the lipids which were the only chemicals requiring comment.
- Authentication of Key Biological and/or Chemical Resources: Attached example, provided by Chris Bakkenist, PhD, Radiation Oncology, was evaluated as acceptable by all three grant reviewers.

Additional Resources:

- NIH Grants Policy Website: https://grants.nih.gov/reproducibility/index.htm
- NIH Website: https://www.nih.gov/research-training/rigor-reproducibility
- Rigor and Reproducibility Resource Chart http://grants.nih.gov/grants/RigorandReproducibilityChart508.pdf
- Rigor and Reproducibility in grant applications (OER site): http://grants.nih.gov/reproducibility/index.htm
- NIH presentation of background and goals of Rigor and Transparency (video): https://grants.nih.gov/reproducibility/module_1/presentation.html
- Reviewer Guidance on Rigor and Transparency: http://grants.nih.gov/grants/peer/guidelines_general/Reviewer_Guidance_on_Rigor_and_Trans parency.pdf
- Updated Application Instructions to Enhance Rigor and Reproducibility https://www.nih.gov/research-training/rigor-reproducibility/updated-application-instructions-enhance-rigor-reproducibility
- Consideration of Sex as a biological Variable in NIH-funded Research: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-102.html
- Rigor and transparency do not apply to all applications. See List of eligibility codes: https://grants.nih.gov/sites/default/files/RigorActivityCodes-20151006.pdf
- Pitt Office of Sponsored Programs Guidance on CONSIDERATION OF SEX AS A BIOLOGICAL VARIABLE IN NIH FUNDED RESEARCH http://osp.pitt.edu/sites/default/files/OSPFiles/considerationofsexasabiologicalvariableguidance_09_01_15.pdf

Questions about the NIH policy should be directed to reproducibility@nih.gov