Iceman’s Run Bonus Webinar

“NIH’s new grant application rules for 2016”

Tom Hollon, PhD
Agenda

1. How to get editing help
2. To cover NIH’s new rules one more time – so you can use them to your advantage instead of having them used against you
How to get editing from me and OVPRGS

For taking this course, I guarantee you one edit from me for your Abstract, Specific Aims, Significance & Innovation sections. Let me know your grant deadline. I’ll edit as fast as I can, but remember I may be editing for more person at a time.
How to get editing from me and OVPRGS

To get me to edit the rest of your grant, ask OVPRGS. You must do this because I don’t choose who I edit for. I’m assigned. Ask to be assigned to me if I’m available. If you don’t get me, you’ll be assigned one of MSU’s outside consultants.
How to get editing from me and OVPRGS

Contact OVPRGS at: RGS.review@campusad.msu.edu

State your deadline and that you’ve been in the *Iceman’s Run* class. Jeff Croff at OVPRGS will contact you about editing arrangements.
How to get editing from me and OVPRGS

OVPRGS will ask you to commit to having your grant peer-reviewed by two colleagues before it gets submitted. This is a really, really, really good idea to help you win. Please do it.
NIH’s new rules for 2016

Most of today’s slides are copied and adapted from my webinars on *Significance and Innovation, Approach, and Odds and Ends*
Handouts on new rules for 2016

1. New grant rules for 2016 infographic
2. 4 Examples of Rigor in NIH Grant Applications.pdf
3. Reviewer Guidance on Rigor & Transparency.pdf
4. Example Authentication of Key Biological & Chemical Resources.pdf
Handouts on new rules for 2016

5. FAQs on NIH Forms Changes for 2016
6. Simplification of Vertebrate Animals
   Section NOT-OD-16-006.pdf
7. Vertebrate Animals Handout
8. Sex as a Relevant Biological Variable in
   Your NIH Grant.pdf
9. PHS Assignment Request Handout
Handouts on new rules for 2016

10. Changes to Policies, Instructions & Forms for 2016 Grant Applications
    NOT-OD-16-004.pdf

11. Enhancing Reproducibility through Rigor & Transparency
    NOT-OD-15-103.pdf
“New grant rules for 2016 infographic.pdf”

WHAT ARE THE UPDATES?

1. UPDATES TO RESEARCH STRATEGY GUIDANCE

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

- The new research strategy guidelines require that you:
  - State the strengths and weaknesses of published research or preliminary data crucial to the support of your application
  - Describe how your experimental design and methods will achieve robust and unbiased results
  - Explain how biological variables, such as sex, are factored into research design and provide justification if only one sex is used

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

From now on, 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
- SPECIALTY CHEMICALS
- ANTIBODIES
- 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.

3. NEW REVIEWER GUIDELINES

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

- Is there a strong scientific premise for the project?
- Have the investigators presented adequate plans to address relevant biological variables, such as sex, for studies in vertebrate animals or human subjects?
- Have the investigators presented strategies to ensure a robust and unbiased approach, as appropriate for the work proposed?

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

Send inquiries to reproducibility@nih.gov

See also NIH Notice NOT-OD-16-011
Also see the FAQs – Rigor & Transparency handout.
New requirements for 2016 -- you must now address:

- Scientific premise (in Significance)
- Scientific rigor and reproducibility (in Approach)
- Relevant biological variables (Approach)
- Authentication of key biological and chemical resources (in a separate attachment)
Scandal and Crackdown

Most of the new rules result from NIH’s search for a way to crack down on irreproducible data after scandalous reports of millions (maybe billions) wasted on research that couldn’t be duplicated.
NIH’s new rules change how you write about reproducibility and…

✓ the literature in your field
✓ your preliminary data
✓ why your grant is based on sound science
✓ how you’ll do your experiments
This is so new, everyone wonders...

how do you describe scientific rigor and reproducibility and sex and other relevant biological variables in a minimum of space, since NIH added these requirements without increasing the page limit?
And can the new rules be converted from a barrier to funding into an advantage for funding?
NIH’s changes make great statistics advice more important than ever

https://www.cstat.msu.edu/
Very few PIs ask CSTAT’s help until almost deadline, says CSTAT director Brian Maurer
Great stat advice early is an edge in winning grants

“Those who think about statistics early tend to be more successful.”
– Brian Maurer, CSTAT director
Upcoming CSTAT sample size analysis workshop: Tues. June 28

Title: Sample Size Analysis for Preparation of Grant Applications
Host: CSTAT
Presenter: Dr. Alla Sikorskii
Date: Tuesday June 28, 2016
Time: 09:00 AM - 04:00 PM
Location: 106 Farrall Hall
Registration Begins: Tuesday April 26, 2016
Description:

Click here for more information.
New for Significance in 2016
NIH’s 4 major changes for research plans in 2016. See today’s infographics handout.
Now your Significance section MUST HAVE a “Scientific Premise” paragraph. Or you may want to call it a “Strength of Scientific Premise” paragraph.
The Scientific Premise requirement applies to R01s, R21s, U01s, etc., regardless of whether you do lab research, social science, clinical trials, or math and statistics; and even if it’s a competing renewal grant.
A Scientific Premise paragraph justifies your grant in terms of reproducibility and considers general strengths and weaknesses of:

1. published research you are citing

or

2. your preliminary data crucial to supporting your application
Considerations of general strengths and weaknesses should include attention to:

1. the rigor of previous study designs, including lab protocols and data analysis
2. relevant biological variables
3. authentication of key resources
Examples of weaknesses to mention about the literature

• Lack of statistical power
• Studies not blinded
• Lacked detail on sex of animals
• Lacked detail about cell line authentication
• Used clinically irrelevant models
If your application includes preliminary data, your Scientific Premise paragraph must discuss its general strengths and weaknesses, regardless of whether it is published.
In discussing prelim data in Scientific Premise, NIH says you must acknowledge “any weaknesses or gaps in rigor, or reporting on rigor, with a plan to address those gaps going forward.”
The Scientific Premise requirement does *not* require you *show* your preliminary data in Significance; Only that you *discuss* its *general* strengths and weaknesses. You can still show all your prelim data in Approach as usual, and discuss its details there too.
Important!

If you admit a prelim data weakness, immediately say something good about it to prevent reviewers from using the weakness to kill your grant.
“You’d be well advised to ensure that any dark cloud you describe is paired with a silver lining tailored to that cloud.”

– Robert Cialdini
Try to turn Scientific Premise to your advantage by explaining you are avoiding your field’s past causes of irreproducibility – and therefore your high-quality proposal is all the more valuable and worthy of funding.
You don’t have any extra space to write your Scientific Paragraph, so try to get it done in one paragraph. Really important — give it a strong ending, projecting confidence you’re proposing a project that deserves funding.
A.5. Strength of scientific premise. Investigations in this field rely heavily on molecular genetic approaches, which sometimes produce oversimplifications and incorrect interpretations. For example, the idea that Cripto-1 interacts with the type I Nodal receptor ALK4 comes from co-immunoprecipitation (co-IP) experiments. In sharp contrast, our data using purified proteins do not support a Cripto-1:ALK4 interaction. A possible explanation is that in co-IPs studies ALK4 and Cripto-1 were indirectly associated by ligands in the medium.\textsuperscript{36} To avoid such problems, we use purified proteins and quantitative assays, which allow high reproducibility and quality control. We test every interaction using independent, quantitative methods, including surface plasmon resonance (SPR) and reporter gene assays. We collect data on at least 3 biological replicates for every assay and perform rigorous statistical analysis. \textit{We benchmark activity for every new protein}. For example, when we began work with BMP-4, we obtained samples from 3 different vendors. Only BMP-4 from RnD Systems was fully active, as reflected in binding affinity to 3 different receptors, and as shown using BMP-4 mediated luciferase gene expression assays. When we prepare recombinant ligands, we benchmark their binding activity against the receptor binding activities of demonstrably active ligands. This is vital in TGF-β family research, as some groups have published findings based on obviously inactive materials. For example, C-terminally tagged ligands cannot fold due to steric constraints, but expression of bioactive, C-terminally tagged ligands in bacteria has been claimed.\textsuperscript{37} For cell-based assays, we only use 3\textsuperscript{rd} passage cell line stocks that we create from cells obtained directly from ATCC. We minimize passage number and use fresh stocks once we have passaged cells 20-25 times. Thus, we assure consistency of our cell line populations. In sum, \textit{our robust, reproducible and quantitative approaches give us great confidence that our preliminary data and our working hypotheses are based on a very strong experimental foundation.}

\textit{--- Erik Martinez-Hackert}
Erik’s Scientific Premise has 4 parts

1. **Examples** of reproducibility problems in his field, **without assigning blame**

2. Possible explanations for the causes
Erik’s Scientific Premise has 4 parts

3. How he is avoiding the problems in his prelim data and proposed project, including testing commercial reagents

4. A summary expressing confidence his project has a firm foundation
Noting irreproducible literature without assigning blame by citing papers

“For example, the idea that Crypto-1 interacts with the type I Nodal receptor ALK4 comes from co-immunoprecipitation experiments. In sharp contrast, our data....”

-- Erik Martinez-Hackert
Erik’s upbeat summary at the end of Scientific Premise:

“In sum, our robust, reproducible and quantitative approaches give us great confidence that our preliminary data and our working hypotheses are based on a very strong experimental foundation.”
Should you show prelim data in Significance?

If you have prelim data to show you’re avoiding reproducibility problems in your field, you may want to show some or all of it in Significance. Or tell reviewers you’ll show the data in Approach.
New for Approach & Prelim Data in 2016
NIH’s 4 major changes for research plans in 2016. See today’s infographics handout.

1. PREMISE
   The scientific premise forming the basis of the proposed research

2. DESIGN
   Rigorous experimental design for robust and unbiased results

3. VARIABLES
   Consideration of relevant biological variables

4. AUTHENTICATION
   Authentication of key biological and/or chemical resources
Preliminary Data in Significance vs. Approach

• In Significance, **discuss** your prelim data’s general strengths and weaknesses in your Scientific Premise to show your project has a sound scientific basis.

• In Approach, **show** your prelim data as usual. Note its reproducibility.
If you are preparing a competing renewal R01, you must critique the strengths and weaknesses of your Progress Report data in the same manner expected for your Scientific Premise paragraph.
You must convince NIH your Approach will have scientific rigor. But what is rigor?

“The strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation and reporting of results” (including experimental details sufficient for others to reproduce and extend the finding).”

– NIH FAQs on Rigor and Transparency
“’Robust and unbiased’ are goals, not absolute standards to be met, and may vary across scientific disciplines” – NIH FAQs on Rigor and Transparency
In a nutshell, scientific rigor means convincing NIH your work will be reproducible.
NIH examples on writing about reproducibility in Approach in a minimum of words
This handout contains 4 examples chosen by NIH to illustrate how to explain in Approach why your research will be reproducible.
Rigor & reproducibility example

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, α 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].

In red: power & significance level; blinded investigators; extensive prior experience; others replicated our earlier results
Rigor & reproducibility example

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 Dunnott or Bonferroni, as appropriate.

Aim 2: Each set of compounds will include a blinded negative control compound that has been determined to be inactive and that is solubilized in the same manner as test compounds. Mice will be randomly assigned within a litter, and data will be collected and submitted to the PI. For compounds that demonstrate extended survival, the PI will be sure to have these tested in {the collaborators’} labs, and data will be merged and evaluated. To calculate the number of the experimental mice, we will perform an SSD sample size power analysis to ensure that the appropriately minimal number of mice is used in each experimental context. Typically for each compound in life span studies, we will need ~20 SMA animals in the treated group; ~20 SMA animals in the vehicle treated group; ~20 SMA animals in the untreated group. If we can administer the compound in aqueous solution without expedient, the vehicle and untreated groups might be combined, as these should have identical survival. Therefore, no more than 80 SMA animals will be needed per compound.

In red: points in triplicate; repeat tests 3X; controls; sample size of vertebrate animals
Rigor & reproducibility example

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 bispecific 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.

Vertebrate Animal Section: 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.

In red: number of repeated measurements; power & significance level; number of animals is mentioned
Note the mention of numbers of animals in examples 2 and 3

As a new rule for 2016, you must now justify the number of animals you’ll use in Approach. This justification used to go in the Vertebrate Animals section.
2016 rule change about justifying the number of vertebrate animals

“Justification for the number of animals has been eliminated from the Vertebrate Animals Section attachment and is now addressed in the Research Strategy as part of experimental design.”

Source:
http://grants.nih.gov/grants/forms_updates_faq.htm
Last revised: April 6, 2016
Rigor & reproducibility example

Aim 1: Statistical considerations: In our preliminary studies consisting of this same cohort of DFUs (n=100) and utilizing 16S rRNA sequencing, we were able to detect dimensions of DFU microbiome, including microbial diversity, that were significantly associated with DFU outcomes. We therefore anticipate that the sample size will provide sufficient power to detect significant differences using metagenomic sequencing, as this is a more sensitive and less-biased assay of microbial identification and diversity.

Aim 3: Random Forests, a machine learning approach for classification, will be used to determine which metagenome features differentiate groups (e.g., antibiotics vs. no antibiotics; pre- vs. post-debridement). Random Forest uses a bootstrap method to assess test error, ideal in our situation of small sample size (n=18). For diversity and load measures, significance between groups will be assessed using non-parametric Wilcoxon rank-sum tests.

*In red: sample size; statistical methods are appropriate*
NIH’s rigor & reproducibility examples emphasize:

1. Sample sizes, randomization, statistical power, and differences detectable based on power
2. Blinded investigators
3. Experience with randomization techniques
NIH’s rigor & reproducibility examples emphasize:

4. Number of times experiments are repeated and data points replicated

5. Names of the statistical tests being used and their appropriateness
Things not in NIH’s examples that you may want to mention

• Standards used for experiments
• Inclusion and exclusion criteria
• Methods to reduce bias; e.g., having multiple individuals record assessments; defining terminology in advance; using independent blinded assessors.
There are probably too many potential reproducibility problems to say how you’ll avoid them all. Mention a few your reviewers will consider key.
Another way to discuss rigor and reproducibility in Approach is to use a list based on NIH's definition of scientific rigor.
NIH says scientific rigor is…

“The strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation and reporting of results” (including experimental details sufficient for others to reproduce and extend the finding).” – NIH FAQs on Rigor and Transparency
Rigor, reproducibility and biological variables discussed as a list

Scientific Rigor, Reproducibility, and Relevant Biological Variables

1. **Experimental design:** 10 male and 10 female adult C57BL6 mice will be randomized to blinded treatment and control groups, giving 80% power to detect a treatment effect size of 65% compared to a baseline response of 5% at a significance level of 0.05.

2. **Methodology:** (1) ELIZAs in Aims 1 and 2 will use a protocol we used previously [41-43] and will be repeated 3 times to assure replicability; (2) We tested anti-7UP monoclonal antibodies from 3 different vendors and selected Supralabs’ product for superior ELIZA replicability; see our “Authentication of Key Resources” appended as part of this application (3) XYZ apparatus tests in Aim 3 will use multiple operators to reduce bias.

3. **Analysis and interpretation:** Our consulting statistician Dr. Severus Snape of MSU will compare Treatment vs Controls by the Montauk Tier Test and assess reproducibility between experiments. See Letter of Support and statistical analysis plan below.

4. **Reporting of results:** We will follow the guidelines developed by NIH, the Nature Publishing Group and Science in 2014 [22] and publish with full details needed to repeat and extend our work.

5. **Biological variables:** Major biological variables in this glaucoma study are adulthood, gender and seb genotype. Although our study is not powered for male vs female differences, we will report these differences in our future publications for their potential to generate hypotheses to deepen understanding of gender effects on seb biology.
Rigor, reproducibility and biological variables listed in a table

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<th>Table 1. Scientific Rigor, Reproducibility, and Relevant Biological Variables</th>
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<td><strong>Experimental design</strong></td>
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<td><strong>Reporting of results</strong></td>
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<td><strong>Biological variables</strong></td>
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Here’s the citation for the NIH, Nature, Science publishing guidelines in my list:

A rigor and reproducibility figure near the beginning of Approach

Figure 2. Scientific Rigor and Reproducibility: Overcoming one of the biggest hurdles in TGF-β family biochemistry: expressing and purifying ligands and receptors without losing biological activity. (A) Ecto domains of receptors and Cripto-1/Cryptic are fused to human IgG1 Fc via a linker containing a TEV site. (B) Fc-fusion proteins are captured from conditioned medium using a protein A affinity column. Proteins can be used directly for SPR and further purified by SEC. The Fc tag is removed by TEV cleavage. Isolated domains can be used in inhibition assays or are deglycosylated and further purified with SEC for structural studies. (C) SDS PAGE of protein A captured proteins. Yields are dependable and range between 30 - 150 mg/L. Residual amounts of free Fc shows degradation is negligible. (D) Deglycosylation of Fc-free ectodomains using Sialidase (SIA), PNGase F (PNGF) and Endo-F3 (ENDO). Deglycosylated proteins are active and mono-disperse by SEC. Ligands are synthesized as precursors and processed into pro- and mature domains. Pro-domains exist in TGF-β-like (left) and BMP-9-like (right) forms. Mature domains (shown as ovals) lose activity when tagged at the C-terminus. Purification tags are therefore introduced at the N-terminus of the pro-domain. TGF-β-like ligands (Activins) have Fc tags; BMP-9-like ligands (Nodal, BMP-4) only have an 8XHis tag. Gaussia luciferase (Luci) helps secretion and tracing. (F) Ligands are captured using protein A or Metal affinity chromatography. Mature domains are purified by acidification and RPC. (G) Intermolecular disulfides (3 CC or 4 CC) determine the orientation of the mature domain N-terminus and thus pro-domain type. (H) Non-reducing gels show disulfide linked dimeric (active) ligands obtained from CHO cells. Reduced Nodal detected by Western. Methods to verify biological activity are described in the text.

Courtesy, Erik Martinez-Hackert
Erik’s figure’s subheading emphasizes reproducibility:

“Overcoming one of the biggest hurdles in TGF-β biochemistry: expressing and purifying ligands and receptors without losing biological activity.”
Sex as a biological variable

In addition, in 2016 Approach must consider sex as a biological variable. And you must report data by gender.
The requirement to consider sex as a biological variable applies to animals and humans. Reviewers will expect you to consider this in designing, analyzing and reporting your experiments.
Considering sex as a biological variable is not a demand for sex differences research, NIH says

Source: “What Does It Mean to Consider Sex as a Relevant Biological Variable in Your NIH Grant Application?”. See D2L folder.
Janine Clayton, Director of NIH’s Office of Research on Women’s Health, says…

“In its efforts to enhance reproducibility and transparency by expecting investigators to consider SABV, NIH will not require any specific research design or method for accomplishing this goal.”
“Rather, the existing state of knowledge in a particular scientific area and the specific research question under study will both affect how an investigator considers sex and other basic biological Variables.” -- Janine Clayton
“NIH policy changes to ensure consideration of basic biological variables like sex do not imply the necessary doubling of research animals in every experiment. “

– Janine Clayton
“However, to look explicitly for sex differences may require larger numbers of animals, or equal numbers of animals of both sexes, for adequate power to detect statistically significant effects. Typically, such projects grow from preliminary data that hints of sex-based influences that generate a testable hypothesis in larger sample sizes.”

-- Janine Clayton
In other words, NIH expects you to consider sex as a biological variable and then justify in Approach whatever you decide to do.
Considering sex as a biological variable may include:

✔ Relevant review of available 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
Considering sex as a biological variable may include:

- 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
Considering sex as a biological variable may include:

✓ 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
Reporting data by gender

“When studies incorporate both males and females, … disaggregate this data, whether the study was statistically powered to detect sex differences or not.” This “enables its use for further study… Studies controlling for sex in multivariate analyses should also report sex-specific results.” – NIH FAQs on Rigor and Transparency
If you study only one sex...

you must justify your decision based on the literature, your preliminary data, or other considerations. Also make it clear which biological variables in your study are tested and controlled.

-- NIH FAQs on Rigor and Transparency
Authentication of sex of established cell lines is not required at this time because NIH recognizes it can be hard to do. But report sex of cell lines, when known. Reporting sex of primary cells will be expected.
Authentication of Key Biological &/or Chemical Resources
NIH’s 4 major changes for research plans in 2016. See today’s infographics handout.
“Authentication of Key Biological and Chemical Resources” is new for 2016

It is *not* part of your research plan – It’s a new attachment. Don’t make it more than one page.
NIH now expects you to *regularly* authenticate key biological and/or chemical resources to ensure their identity and validity in your project.
Examples of key resources you are expected to regularly authenticate include, but are not limited to:

- cell lines
- specialty chemicals
- antibodies
- other biologics
Tip: Do your suppliers affect your project’s reproducibility?

If you can buy a key resource from more than one supplier, you may want to test which supplier’s product gives the most reproducible results.
A key biological or chemical resource:

• may or may not be generated with NIH funds
• may differ from lab to lab or over time
• may have qualities or qualifications that could influence the research data
NIH recognizes that standard authentication methods may not exist for many key resources.

Where standard methods are absent, your obligation to NIH is to clearly describe how you’ve chosen to conduct authentication. Justify any unusual choices.
Do not put experimental methods or preliminary data in this attachment
Example Authentication of Key Biological & Chemical Resources.pdf is in your D2L folder

-- Jim Pestka

This Authentication form example included in a 2016 R01 grant application is courtesy of Dr. Jim Pestka of MSU.

Authentication of Key Biological and/or Chemical Resources

The biological resource used in this study will be saliva from the parents of children with ASD. To ensure the identity and validity of saliva specimen collected for accurate results, we will follow the procedures described below. Each participant will be assigned with a specific study ID used in all data collections and analyses in the study. When collecting saliva specimen from each participant, the tube will be labeled with the unique study ID by the research staff immediately upon receiving the sample.

We will collect saliva samples at baseline and at the end of the intervention (or the equivalent time for the control group). Samples will be collected immediately before the initiation of the training session (or the equivalent time of the day for the control group). We plan on collecting saliva samples between 8:00 and 9:00 am to control for circadian variation, and keep the intra-personal time difference between pre and post training to 15 minutes or less. We will quantify levels of cortisol as a biologically relevant marker of physiological stress responses (1, 97-100 REFERENCES). This marker is reliably measured in saliva by enzyme-linked immunosorbent assays (ELISAs) and have been shown to increase in response to various stress paradigms (98, 99, 101 REFERENCES). Specifically, cortisol is a mediator of HPA axis adaptation to acute stress exposure. Cortisol is a well-established marker for catabolic stress responses (98, 102, 103 REFERENCES).

The steps of collecting saliva samples are summarized below.

A) We will conduct a brief interview over the phone to inquire the following information and schedule an appointment.
   1) For women with menstrual cycles: we will schedule saliva collection appointment during one of her luteal days.
   2) For women who are post menopausal or have had a hysterectomy, we will schedule the appointment at any day of her convenience.
   3) For women and men who are taking hormone-related medications or cortisol supplements, we will obtain her/his permission to contact their physician for information of the medication and consult medical professionals before scheduling an appointment.

B) Once an appointment is scheduled, we will call the parents one day prior to the appointment and give them the following instruction.
   1) Do not apply lotions or cream during night and in the morning before coming to the appointment.
   2) Do not eat one hour prior to the appointment.
   3) Do not brush or floss teeth 30 minutes prior to the appointment.

C) During collections, our research staff will assist each parent to collect saliva with appropriate equipment and fill up the tubes to the sufficient amount for lab testing. The staff will immediately label each tube with the participant’s unique study ID and also enter all necessary information in the test form and specimen bag.

D) After collections, the staff will double check the tubes are sealed securely and placed in a zip-top bag. The bag with saliva specimen will be placed together with the participant’s form in a sealed box. The samples will be kept in the freezer in the autism center and be carried to the laboratory by the research staff. The laboratory is about one hour away by driving from the autism research center.
Cover Letter
& PHS Assignment Request Form
Cover letters are optional – and in 2016 their use changes

Until now, people have used cover letters to steer their grants to the right study sections and away from competitors. Cover letters are read by NIH staff and are not shown to reviewers. A cover letter is not a form. It’s written on your office stationary.
In 2016 there’s a new way to request a study section

While the cover letter hasn’t gone away, as of May 25 it will no longer be used to request study section assignments. For that, use the new *PHS Assignment Request Form*. 
If you don’t want to request a study section for your grant, you don’t have to. You can let NIH decide.

In this sense, the PHS Assignment Request Form is optional, just like the cover letter. You don’t have to use either.
But if you do want to request a study section – and I think you should – then the *PHS Assignment Request Form* is the form NIH requires for this purpose.
Searching the NIH Reporter and talking to your Program Officer should give you a really good idea of which study section is best for you, and which ones to stay away from.
PHS Assignment Request Form

Funding Opportunity Number:

Funding Opportunity Title:

Awarding Component Assignment Request (optional)

If you have a preference for an Awarding Component (e.g., NIH Institute/Center) assignment, please use the link below to identify the most appropriate assignment and then enter the short abbreviation (e.g., NCI for National Cancer Institute) in “Assign to/Do Not Assign To Awarding Component” sections below. Your first choice should be in column 1. All requests will be considered; however, locus of review is predetermined for some applications and assignment requests cannot always be honored.

Information about Awarding Components can be found here: https://grants.nih.gov/grants/phs_assignment_information.html#Awarding Components

Assign to Awarding Component:

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Study Section Assignment Request (optional)

If you have a preference for a study section assignment, please use the link below to identify the most appropriate study section and then enter the short abbreviation for that study section in “Assign to/Do Not Assign to Study Section” sections below. Your first choice should be in column 1. All requests will be considered; however, locus of review is predetermined for some applications and assignment requests cannot always be honored.

For example, you would enter “CAMPS” if you wish to request assignment to the Cancer Molecular Pathobiology study section or enter “ZRG1 HDM-R” if you wish to request assignment to the Healthcare Delivery and Methodologies SBIR/STTR panel for information. Be careful to accurately capture all formatting (e.g., spaces, hyphens) when you type in the request.

Information about Study Sections can be found here: https://grants.nih.gov/grants/phs_assignment_information.html#Study Section

Assign to Study Section:

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
</table>

Only 20 characters allowed

Do Not Assign to Study Section:

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
</table>

Only 20 characters allowed
Page 2 is for avoiding unwanted reviewers and requesting expertise.
If you have a competitor who might give you a bad review

You can request that competitors not be allowed to review your grant. Do this even for competitors not on your study section’s roster, in case they become ad hoc reviewers.
Example of avoiding an unwanted reviewer

“Because of a long standing scientific disagreement that has been publically discussed at several scientific meetings, we request that Dr. ABC not be involved in reviewing this application.”
You cannot request reviewers by name

But you can request that reviewers have specialties aligned with the science in your proposal. Use the *PHS Assignment Request Form* for this.
If the cover letter is no longer for requesting study sections, what’s it good for?
Use the cover letter to explain…

• why you’re submitting late (and it can’t be a run-of-the-mill excuse)
• that you’re an Early Stage Investigator
• that you have written permission to apply for more than $500,000/yr
• etc.
New font guidelines for 2016
Allowable fonts

✓ Arial, Helvetica, Palatino Linotype, or Georgia
✓ Font size: 11 points or larger
✓ No more than 6 lines/inch
✓ No more than 15 characters/inch
When color text is OK

• “Color text in figures, graphs, diagrams, charts, tables, footnotes and headings is acceptable”. -- NIH

• Otherwise text must be black.

Source:
http://grants.nih.gov/forms_updates_faq.htm
Using colored text

**SIGNIFICANCE**

**Bacterial vaginosis, sexually transmitted infections, and preterm birth**  Bacterial vaginosis (BV) is an exceedingly common and poorly understood disorder associated with significant adverse sequelae. Nationwidé point-prevalence estimates of BV among reproductive aged women are roughly 30%, corresponding to 21 million women with BV [2]. Rates are higher in pregnant women and in African-American populations [2]. Although the symptoms associated with BV are not life threatening, BV substantially increases the risk of a number of significant health outcomes. Chief among these is preterm birth (PTB). The nationwide rate of PTB (parturition at less than 37 weeks gestation) is 12.7% (March of Dimes Peristats). BV causes 90,000 excess preterm births per year (at an overall cost in excess of $1 billion) and accounts for at least 30% of the racial difference in PTB rates [3]. In pregnancy, women with BV are at increased risk for chorioamnionitis, post-operative wound infections, and post-partum endometritis. BV increases both acquisition and shedding of a number of sexually transmitted infections, including HIV [4]. BV increases the risk of heterosexual acquisition of HIV at least 2-fold [5,6], and exposure of HIV-infected cells to vaginal secretions from women with BV [7] or to pure cultures of *Gardnerella vaginalis* [8,9] increases production of HIV transcripts and viral shedding. Synergistic interactions between BV and other sexually transmitted infections including herpes simplex, gonorrhea, and chlamydia have been detailed [10,11].

**RESEARCH STRATEGY**

![Fig. 1. Changes in vaginal microflora during bacterial vaginosis. (A) Normal vaginal microflora is dominated by *Lactobacillus* species (gram-positive rods). (B) During bacterial vaginosis, there is an abundance of gram-variable coccus adherent to vaginal epithelial cells (clue cells). Figure adapted from [1].](image)

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[--- Adam Ratner---]
b) Innovation
We have assembled a multi-disciplinary team of established clinical malaria investigators, vascular biologists, and pathologists. Whereas many malaria pathology studies might use animal models, our work will be based on human patients and human cells. We are ideally placed to identify pediatric CM patients with the clinical phenotype most strongly associated with death, and to use clinically annotated parasite isolates and plasma in vitro human cell biological models and molecular genetic analyses. Linking clinical phenotypes established in Malawi with endothelial cell phenotypes characterized in the Center for Excellence in Vascular Biology, Brigham and Women’s Hospital and Harvard Medical School represents an innovative combination of the research methods developed and refined by our two teams over the past 25 years. Potential contributions to understanding malaria pathogenesis are immense because: 1) for the first time we know which process is crucial for death (brain swelling); 2) we have an unbiased approach (genome-wide transcriptional profiling); and 3) we can use parasites and plasma collected directly from well-characterized patients to provoke and characterize responses from the very endothelial cells that interact directly with parasitized red cells and are capable of contributing to processes which could generate increased brain volume.

c) Approach
Preliminary Studies
Studies of malaria pathogenesis continue to be warranted because, despite scaled-up efforts prevention and
Vertebrate Animals for 2016
(Thank you, Sue Ewart!)
“Write as if to a veterinarian”
-- Sue Ewart

1. Description of Procedures
2. Justification for use of animals
3. Minimization of pain and distress
4. Euthanasia
1. Description of Procedures

Concisely describe the proposed procedures that involve vertebrate animals in the application. Identify the species, strains, ages, sex and total number of animals by species to be used in the proposed work. If dogs or cats are proposed, provide the source of the animals.
2. Justifications

Provide justification that the species are appropriate for the proposed research. Explain why the research goals cannot be accomplished using an alternative model (e.g., computational, human, invertebrate, in vitro).
3. Minimization of pain & distress

Describe the interventions including analgesia, anesthesia, sedation, palliative care and humane endpoints to minimize discomfort, distress, pain and injury.
4. Euthanasia

For applications after May 25, 2016 the euthanasia method is eliminated from the Vertebrate Animal Section and is addressed in the FORMS-D Cover Page Supplement. If the euthanasia method does not comply with AVMA guidelines, describe the method in the Supplement.
NIH has tried to simplify this section for 2016, removing some previous requirements
2016’s simplifications to the Vertebrate Animal Section

• A description of veterinary care is no longer required;
• A description and justification of the method of euthanasia is required only if the method is not consistent with the AVMA Guidelines for the Euthanasia of Animals.
2016 rule change about justifying the number of vertebrate animals

“Justification for the number of animals has been eliminated from the Vertebrate Animals Section attachment and is now addressed in the Research Strategy as part of experimental design.”

Source:
http://grants.nih.gov/grants/forms_updates_faq.htm
Last revised: April 6, 2016
Recap.
I’ll close with NIH’s reviewer guidelines for 2016.
New reviewer guidelines, 2016

1. Is there a strong scientific premise for the project? (Cover this in Significance)

2. Has the PI presented adequate plans to address relevant biological variables, such as sex, for studies in vertebrate animals or human subjects? (Cover in Approach)
New reviewer guidelines, 2016

3. Has the PI presented strategies to ensure a robust and unbiased approach, as appropriate for the work proposed? (Cover this in Approach)
Thank you, and may you always score above payline!