Addressing Rigor in Scientific Studies
-In the context of animal studies overseen by IACUC

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Rigor in Merriam-Webster

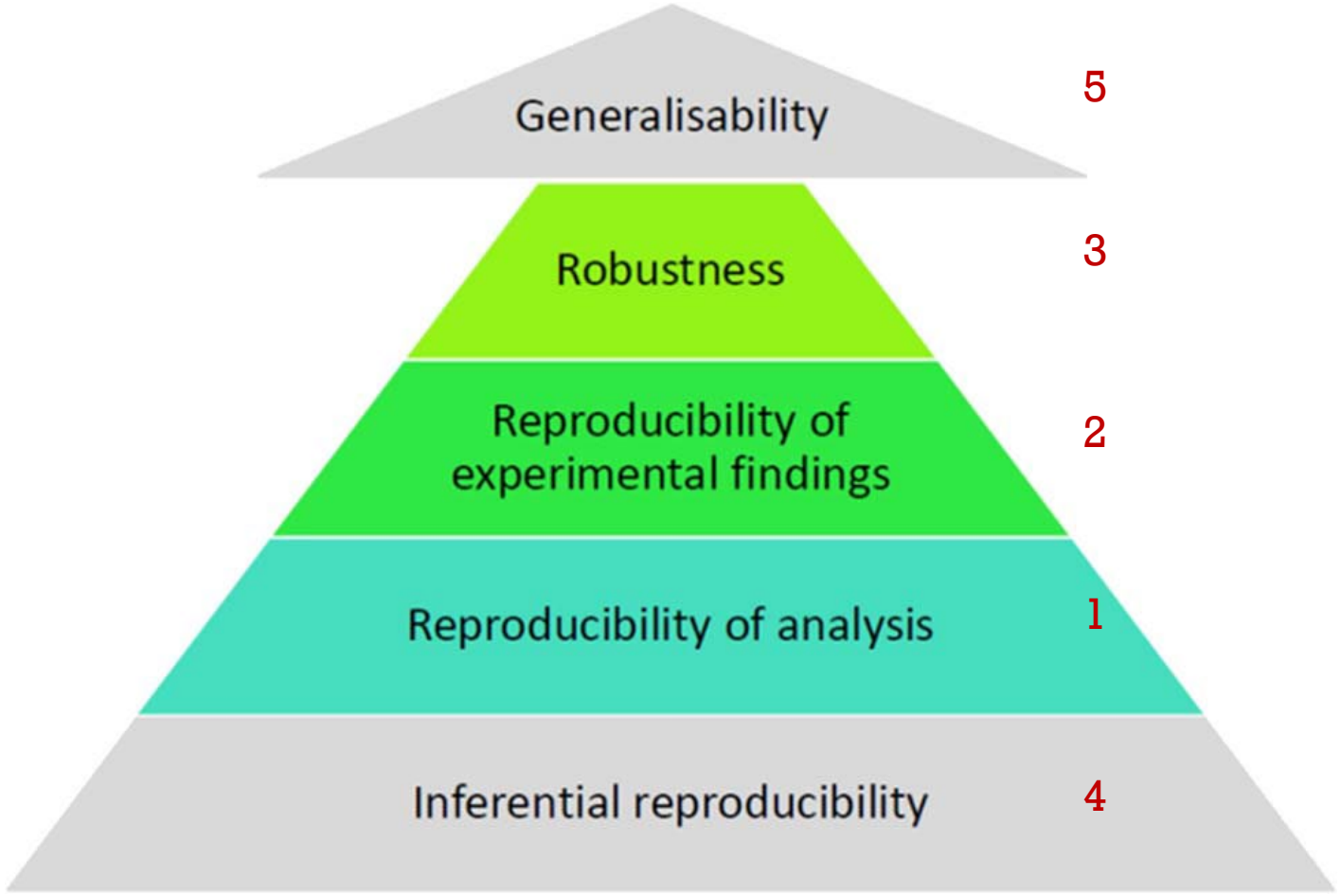
- Hardship, Severity, and Accuracy

Synonyms & Similar Words

severity	rigidity	strictness
stringency	inflexibility	sternness
rigidness	hardness	harshness
rigorousness	exactingness	implacability
callousness	obduracy	resolve
gruffness	steadfastness	pitilessness
determination	monasticism	firmness
asceticism	dourness	obstinacy
stubbornness	hard-heartedness	obdurateness
accuracy	precision	preciseness
perfection	exactness	exactitude
fidelity	definiteness	veracity
rigorousness	truth	nicety
fineness	definitiveness	accurateness
strictness	delicacy	closeness
correctness	ultraprecision	rightness
subtlety	determinacy	meticulousness
fastidiousness	carefulness	definitude
care	persnicketiness	

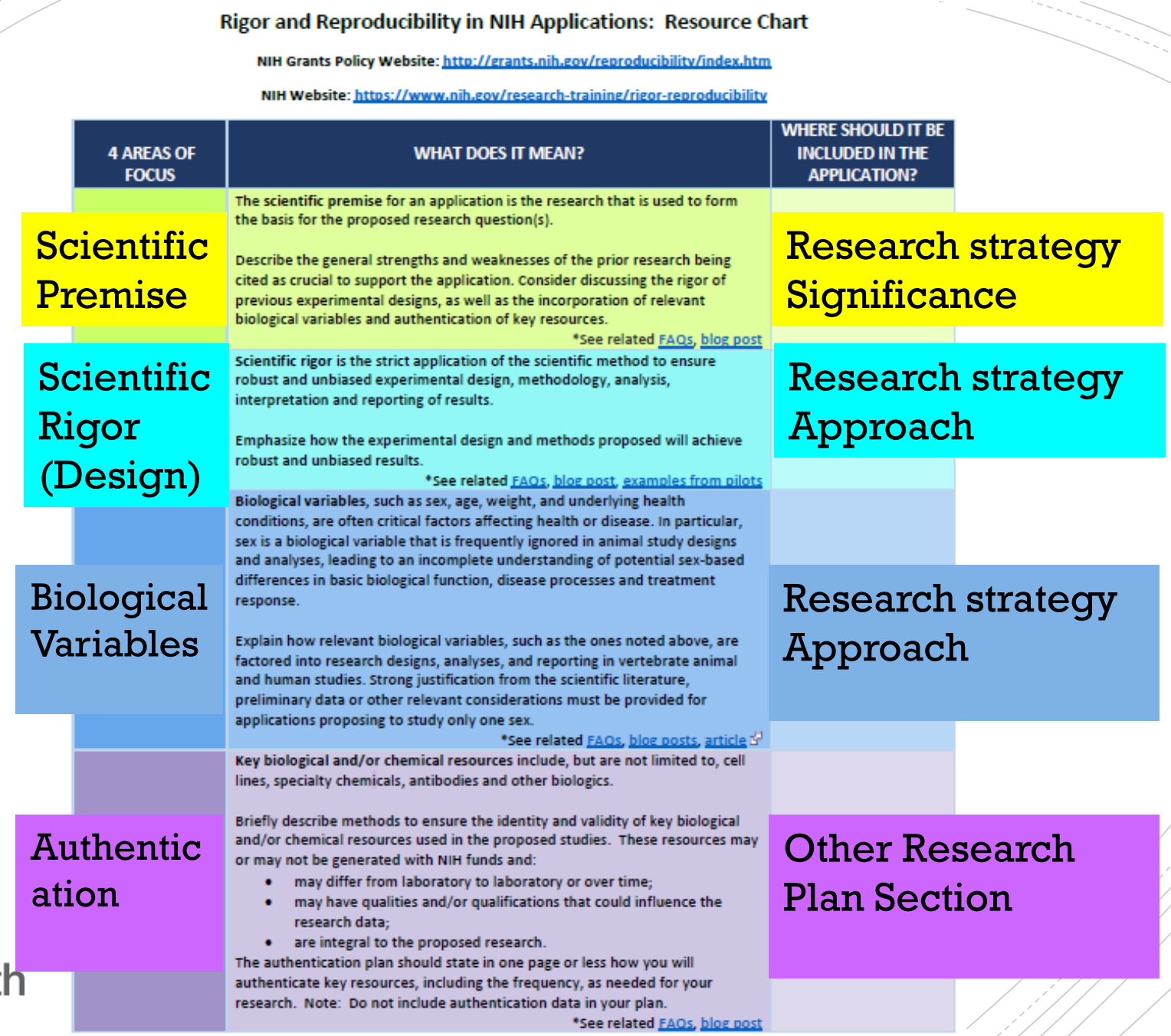
https://www.merriam-webster.com/thesaurus/rigor#thesaurus-entry-1-3

Research Rigor



Macleod M. and Mohan S. ILAR Journal, 2019, 60(1), 17–23, doi: 10.1093/ilar/ilz015;
Goodman SN *et. al*. Science Translational Medicine 2016, 8(341), 341ps312, doi: 10.1126/scitranslmed.aaf5027

Research Rigor and reproducibility in NIH applications



Rigor and Reproducibility in NIH Applications: Resource Chart

NIH Grants Policy Website: http://grants.nih.gov/reproducibility/index.htm

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

4 AREAS OF FOCUS	WHAT DOES IT MEAN?	WHERE SHOULD IT BE INCLUDED IN THE APPLICATION?
Scientific Premise	The scientific premise for an application is the research that is used to form the basis for the proposed research question(s). Describe the general strengths and weaknesses of the prior research being cited as crucial to support the application. Consider discussing the rigor of previous experimental designs, as well as the incorporation of relevant biological variables and authentication of key resources. *See related FAQs, blog post	Research strategy Significance
Scientific Rigor (Design)	Scientific rigor is the strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation and reporting of results. Emphasize how the experimental design and methods proposed will achieve robust and unbiased results. *See related FAQs, blog post, examples from pilots	Research strategy Approach
Biological Variables	**Biological variables**, such as sex, age, weight, and underlying health conditions, are often critical factors affecting health or disease. In particular, sex is a biological variable that is frequently ignored in animal study designs and analyses, leading to an incomplete understanding of potential sex-based differences in basic biological function, disease processes and treatment response. Explain how relevant biological variables, such as the ones noted above, are factored into research designs, analyses, and reporting in vertebrate animal and human studies. Strong justification from the scientific literature, preliminary data or other relevant considerations must be provided for applications proposing to study only one sex. *See related FAQs, blog posts, article	Research strategy Approach
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 be generated with NIH funds and: • may differ from laboratory to laboratory or over time; • may have qualities and/or qualifications that could influence the research data; • are integral to the proposed research. The authentication plan should state in one page or less how you will authenticate key resources, including the frequency, as needed for your research. Note: Do not include authentication data in your plan. *See related FAQs, blog post	Other Research Plan Section

**This chart is based on general instructions for research grant and mentored career development applications. It should only be used as a guide. For all applications, please read the applicable Funding Opportunity Announcement (FOA) & Application Guide for specific instructions.

National Institutes of Health
Turning Discovery Into Health

https://grants.nih.gov/grants/Rigor-and-Reproducibility-Chart-508.pdf

Research Rigor in Animal Research

3Rs

- **Replacement** refers to technologies or approaches that directly replace or avoid the use of animals.
- **Reduction** involves methods that help obtain comparable levels of information from the use of fewer animals.
- **Refinement** refers to modifications of husbandry or experimental procedures that minimize or eliminate animals' pain and distress and improve their welfare.

https://www.nal.usda.gov/animal-health-and-welfare/animal-use-alternatives#:~:text=The%20%E2%80%9C3Rs%20alternatives%E2%80%9D%20refers%20to,Principles%20of%20Humane%20Experimental%20Technique%22.

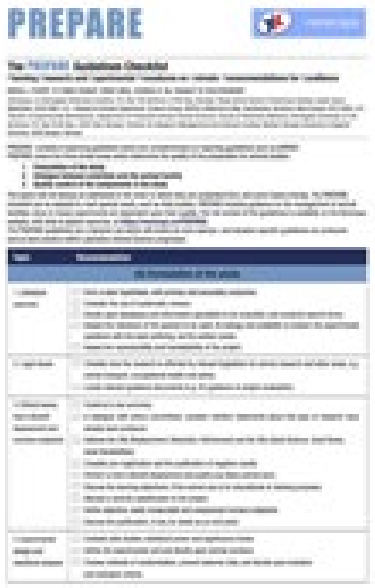
Improve Research Rigor in Animal Research

- **PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) (https://norecopa.no/prepare/) -** Covers the three broad areas which determine the quality of the preparation for animal studies.

✓ Formulation of the study

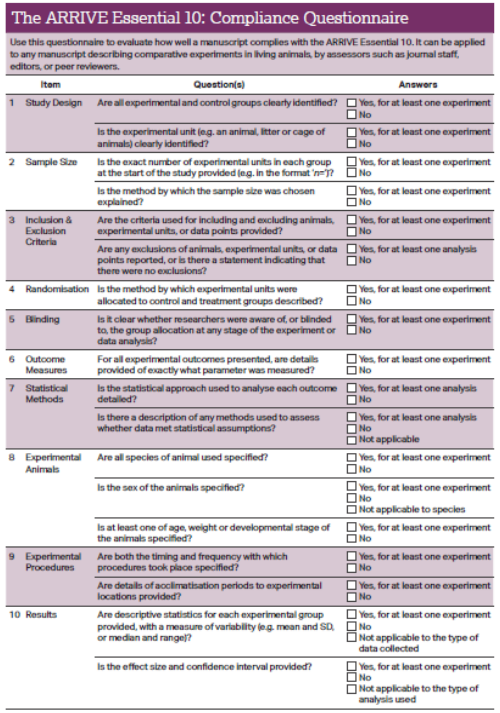
✓ Dialogue between scientists and the animal facility

✓ Quality control of the components in the study



Improve Research Rigor in Animal Research

- **ARRIVE (Animal Research: Reporting of In Vivo Experiments) (https://arriveguidelines.org/)** - A checklist of recommendations to improve the reporting of research involving animals – maximizing the quality and reliability of published research, and enabling others to better scrutinize, evaluate and reproduce it.



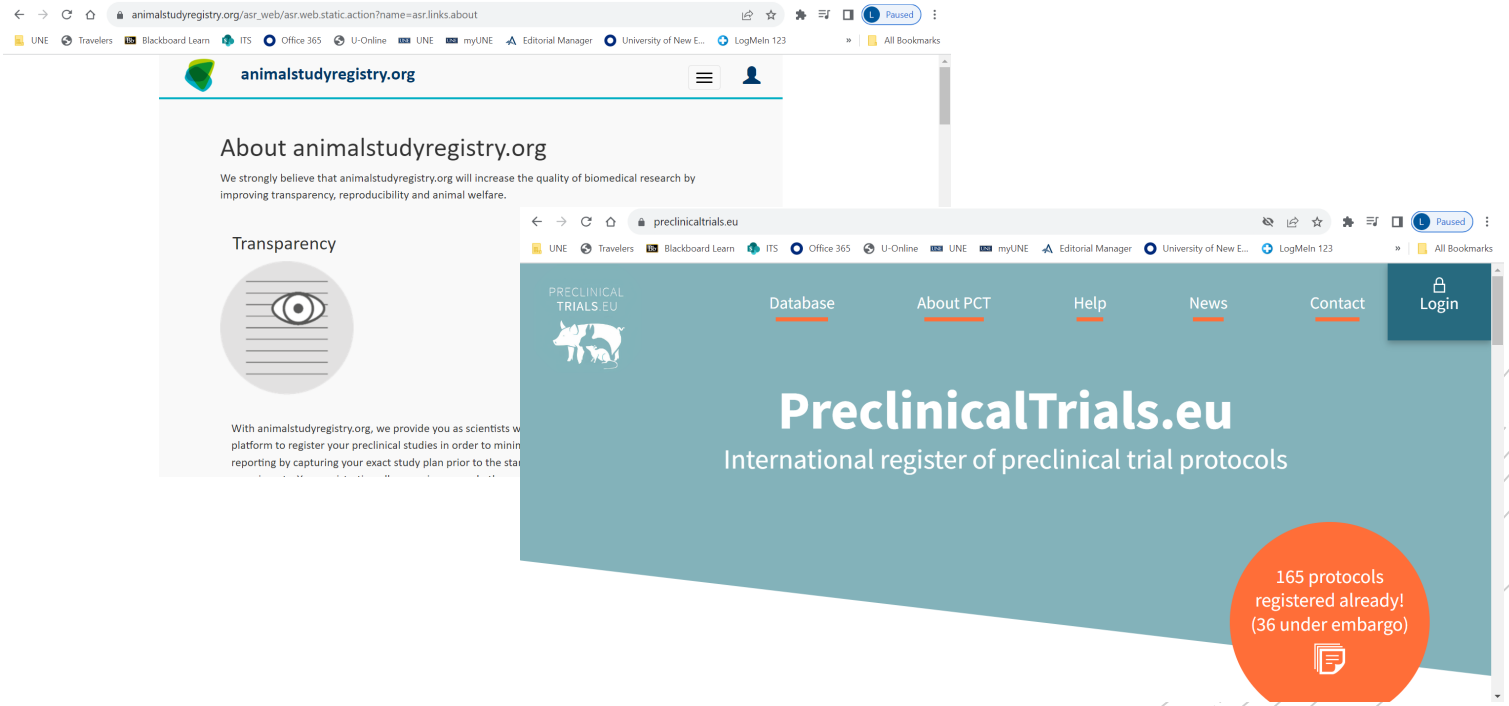
The ARRIVE Essential 10: Compliance Questionnaire

Use this questionnaire to evaluate how well a manuscript complies with the ARRIVE Essential 10. It can be applied to any manuscript describing comparative experiments in living animals, by assessors such as journal staff, editors, or peer reviewers.

Item		Question(s)	Answers
1	Study Design	Are all experimental and control groups clearly identified?	☐ Yes, for at least one experiment ☐ No
		Is the experimental unit (e.g. an animal, litter or cage of animals) clearly identified?	☐ Yes, for at least one experiment ☐ No
2	Sample Size	Is the exact number of experimental units in each group at the start of the study provided (e.g. in the format "n="?)	☐ Yes, for at least one experiment ☐ No
		Is the method by which the sample size was chosen explained?	☐ Yes, for at least one experiment ☐ No
3	Inclusion & Exclusion Criteria	Are the criteria used for including and excluding animals, experimental units, or data points provided?	☐ Yes, for at least one experiment ☐ No
		Are any exclusions of animals, experimental units, or data points reported, or is there a statement indicating that there were no exclusions?	☐ Yes, for at least one analysis ☐ No
4	Randomisation	Is the method by which experimental units were allocated to control and treatment groups described?	☐ Yes, for at least one experiment ☐ No
5	Blinding	Is it clear whether researchers were aware of, or blinded to, the group allocation at any stage of the experiment or data analysis?	☐ Yes, for at least one experiment ☐ No
6	Outcome Measures	For all experimental outcomes presented, are details provided of exactly what parameter was measured?	☐ Yes, for at least one experiment ☐ No
7	Statistical Methods	Is the statistical approach used to analyse each outcome detailed?	☐ Yes, for at least one analysis ☐ No
		Is there a description of any methods used to assess whether data met statistical assumptions?	☐ Yes, for at least one analysis ☐ No ☐ Not applicable
8	Experimental Animals	Are all species of animal used specified?	☐ Yes, for at least one experiment ☐ No
		Is the sex of the animals specified?	☐ Yes, for at least one experiment ☐ No ☐ Not applicable to species
		Is at least one of age, weight or developmental stage of the animals specified?	☐ Yes, for at least one experiment ☐ No
9	Experimental Procedures	Are both the timing and frequency with which procedures took place specified?	☐ Yes, for at least one experiment ☐ No
		Are details of acclimatisation periods to experimental locations provided?	☐ Yes, for at least one experiment ☐ No
10	Results	Are descriptive statistics for each experimental group provided, with a measure of variability (e.g. mean and SD, or median and range)?	☐ Yes, for at least one experiment ☐ No ☐ Not applicable to the type of data collected
		Is the effect size and confidence interval provided?	☐ Yes, for at least one experiment ☐ No ☐ Not applicable to the type of analysis used

Improve Research Rigor in Animal Research

- **Adoption of animal study registries to improve transparency and prevent selective reporting** – Examples: https://preclinicaltrials.eu/ (International register of preclinical trial protocols) https://animalstudyregistry.org/ (an online registry for scientific studies involving animals conducted around the world).



Role of IACUC

Improve Research Rigor in Animal Research

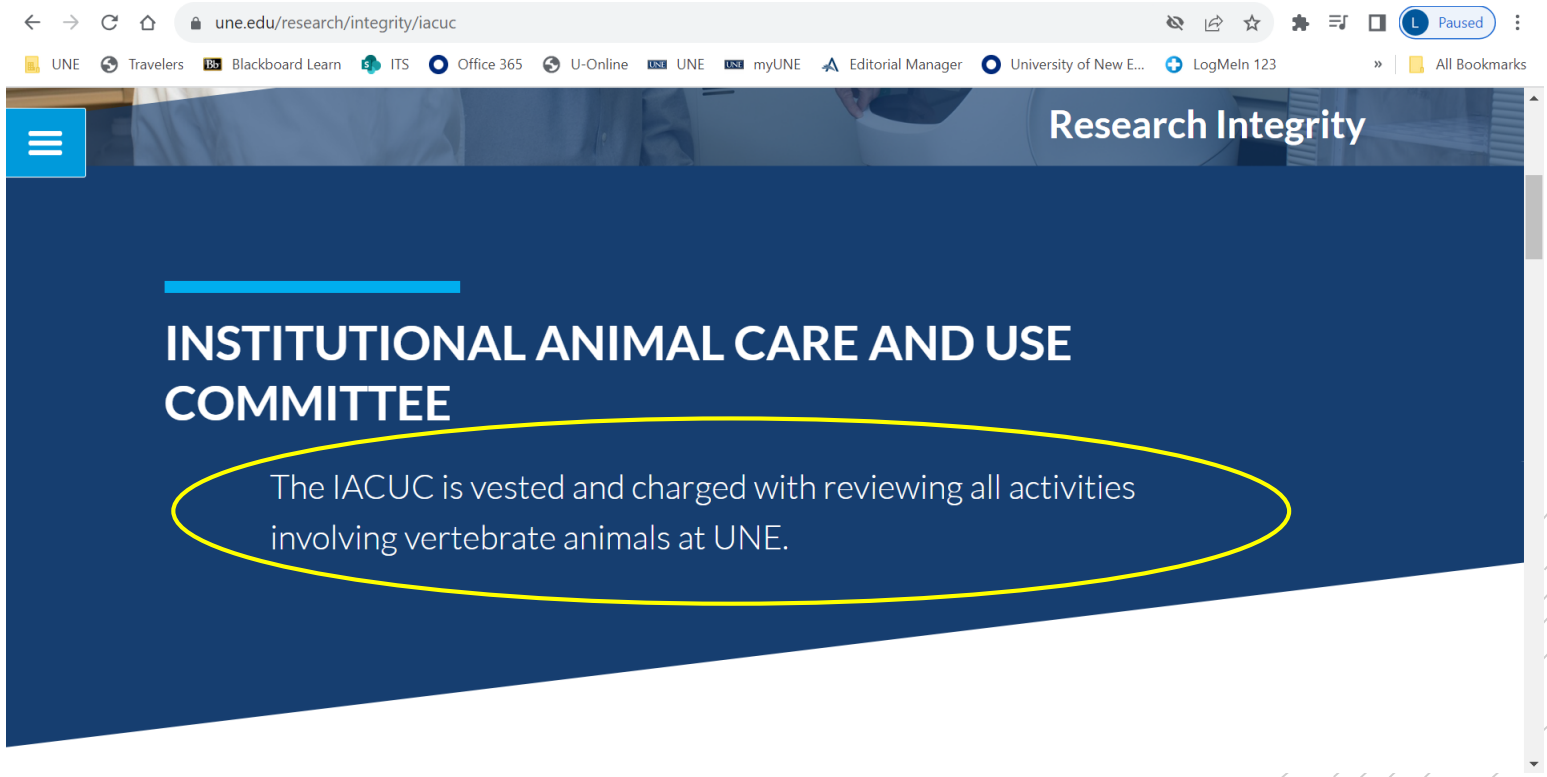
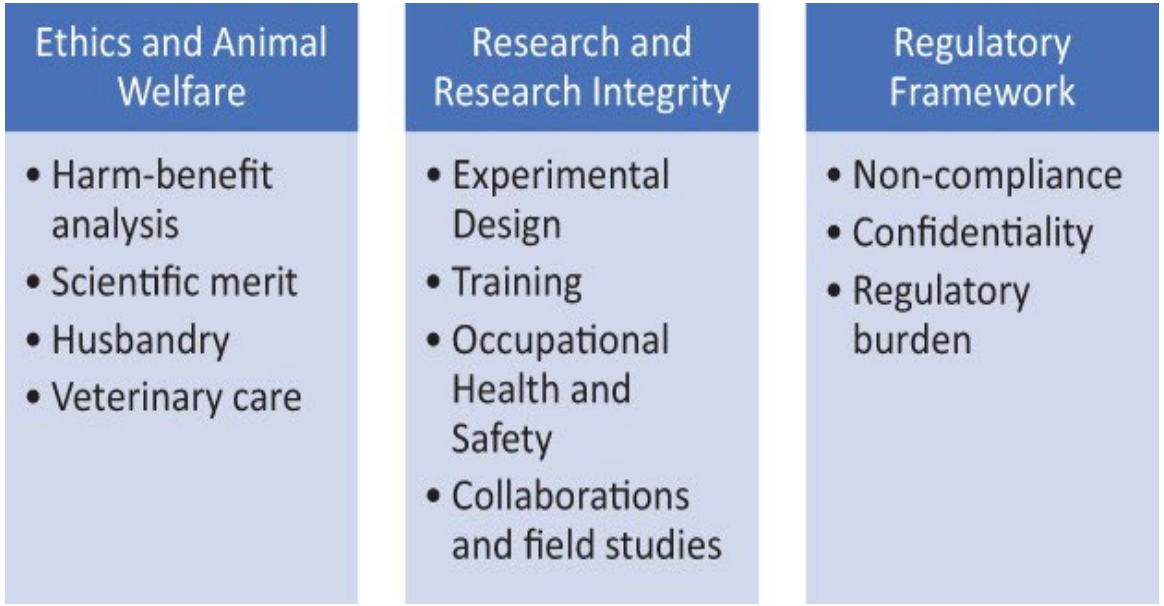


Figure 1 Broad categorization of IACUC functions that contribute to responsible conduct of animal research. Some of these domains may overlap however, the cumulative effect of these roles serves to promote animal welfare, facilitate good research practices, satisfy regulatory requirements, and ensure research quality.



"…healthy animals housed in optimal conditions yield the most reliable data, whereas compromised welfare negatively impacts physiology, immunology, and behavior of animals leading to skewed and misrepresented results."

Mohan S and Huneke R ILAR J, Volume 60, Issue 1, 2019, Pages 43–49, https://doi.org/10.1093/ilar/ilz016

Role of IACUC

Scientific Rigor (Design)

Biological Variables

Authentic ation

PREPARE

(A) Formulation of the study	
1. Literature searches	☐ Form a clear hypothesis, with primary and secondary outcomes. ☐ Consider the use of systematic reviews. ☐ Decide upon databases and information specialists to be consulted, and construct search terms. ☐ Assess the relevance of the species to be used, its biology and suitability to answer the experimental questions with the least suffering, and its welfare needs. ☐ Assess the reproducibility and translatability of the project.

UNE IACUC application

UNE UNIVERSITY OF NEW ENGLAND INNOVATION FOR A HEALTHIER PLANET	Application for Initial & *De Novo* IACUC Review	Page 1 of 8

https://www.une.edu/research/integrity/iacuc

- ✓ Lay summary (`300 words) - Research for the good of society
- ✓ Rational for animal use
- ✓ Species & strain justification
- ✓ Justification of animal numbers
- ✓ Description of experimental design & animal procedures + Supplemental forms C &D
- ✓ Special concerns or requirements of the study

Scientific Premise

Role of IACUC

PREPARE

2. Legal issues	☐ Consider how the research is affected by relevant legislation for animal research and other areas, e.g. animal transport, occupational health and safety. ☐ Locate relevant guidance documents (e.g. EU guidance on project evaluation).

UNE IACUC application

- ✓ Funding type
- ✓ Specify the specific source(s) of the animal(s)
- ✓ Wild animals
- ✓ Supplemental Form D: Use of Biological Materials, Chemicals, Drugs, Hazardous Agents, or Other Substances in Animal Studies

Role of IACUC

Scientific Rigor (Design)

Biological Variables

Authentic ation

PI may be invited to the IACUC meeting

PREPARE

3. Ethical issues, harm-benefit assessment and humane endpoints	☐ Construct a lay summary. ☐ In dialogue with ethics committees, consider whether statements about this type of research have already been produced. ☐ Address the 3Rs (replacement, reduction, refinement) and the 3Ss (good science, good sense, good sensibilities). ☐ Consider pre-registration and the publication of negative results. ☐ Perform a harm-benefit assessment and justify any likely animal harm. ☐ Discuss the learning objectives, if the animal use is for educational or training purposes. ☐ Allocate a severity classification to the project. ☐ Define objective, easily measurable and unequivocal humane endpoints. ☐ Discuss the justification, if any, for death as an end-point.

UNE IACUC application

- ✓ Lay summary (`300 words)
- ✓ Rational for animal use
- ✓ Species & strain justification
- ✓ Justification of animal numbers
- ✓ Description of experimental design & animal procedures (for each animal, from the starting to the end of the study in chronological order)
- ✓ Pain & distress classification & consideration of alternative procedures –list numbers in each pain category
- ✓ Methods of euthanasia or disposition of animals at the end of study (AVMA recommendation)
- ✓ Special concerns or requirements of the study (housing, special location, food/water, experimental drugs, etc.)
- ✓ Supplemental Form C: Animal Surgical Procedures
- ✓ Supplemental Form D: Use of Biological Materials, Chemicals, Drugs, Hazardous Agents, or Other Substances in Animal Studies
- ✓ Supplemental Form A: Study Team Training & Qualification Summary
- ✓ Occupational health and safety program (OHSP) – including animal care staff

Role of IACUC

Scientific Rigor (Design)

Biological Variables

Authentic ation

PREPARE

4. Experimental design and statistical analysis	☐ Consider pilot studies, statistical power and significance levels. ☐ Define the experimental unit and decide upon animal numbers. ☐ Choose methods of randomisation, prevent observer bias, and decide upon inclusion and exclusion criteria.

UNE IACUC application

- ✓ Justification of animal numbers
  - Animal calculation process (pilot-validation criteria if applicable, attrition rate, litter size, unwanted genotypes, inclusion/exclusion, etc.)
  - Statistical methods, power analysis
  - Ensure the total numbers matches the numbers listed in each sub set of studies.

- ✓ Description of experimental design & animal procedures (for each animal, from the starting to the end of the study in chronological order) – Randomization and blinding
  - Flow charts – time sequence
  - Individual procedures – USDA pain category
  - Standard operating procedures (SOPs) – reproducibility, preparation of protocol, review process
  - Humane endpoints – special monitoring

Role of IACUC

IACUC: iacuc@une.edu

Attending Veterinarian: Dr. Art Lage
(artlage123@gmail.com; 617-699-2256)

PREPARE

(B) Dialogue between scientists and the animal facility	
5. Objectives and timescale, funding and division of labour	☐ Arrange meetings with all relevant staff when early plans for the project exist. ☐ Construct an approximate timescale for the project, indicating the need for assistance with preparation, animal care, procedures and waste disposal/decontamination. ☐ Discuss and disclose all expected and potential costs. ☐ Construct a detailed plan for division of labour and expenses at all stages of the study.
6. Facility evaluation	☐ Conduct a physical inspection of the facilities, to evaluate building and equipment standards and needs. ☐ Discuss staffing levels at times of extra risk.
7. Education and training	☐ Assess the current competence of staff members and the need for further education or training prior to the study.
8. Health risks, waste disposal and decontamination	☐ Perform a risk assessment, in collaboration with the animal facility, for all persons and animals affected directly or indirectly by the study. ☐ Assess, and if necessary produce, specific guidance for all stages of the project. ☐ Discuss means for containment, decontamination, and disposal of all items in the study.

UNE IACUC application

- ✓ Funding type – Approval time line
- ✓ Supplemental Form A: Study Team Training & Qualification Summary
- ✓ Animal requirements (Contact facility managers, special housing/locations, specific types of animals, etc.)
- ✓ Transportation of animals
- ✓ Pain & distress classification & consideration of alternative (consultation with Dr. Lage)
- ✓ Special concerns or requirements of the study (UNE Behavior Core)
- ✓ Supplemental Form D: Use of Biological Materials, Chemicals, Drugs, Hazardous Agents, or Other Substances in Animal Studies

Role of IACUC

Scientific Rigor (Design)

Biological Variables

Authentic ation

PREPARE

	(C) Quality control of the components in the study
9. Test substances and procedures	☐ Provide as much information as possible about test substances. ☐ Consider the feasibility and validity of test procedures and the skills needed to perform them.
10. Experimental animals	☐ Decide upon the characteristics of the animals that are essential for the study and for reporting. ☐ Avoid generation of surplus animals.
11. Quarantine and health monitoring	☐ Discuss the animals' likely health status, any needs for transport, quarantine and isolation, health monitoring and consequences for the personnel.
12. Housing and husbandry	☐ Attend to the animals' specific instincts and needs, in collaboration with expert staff. ☐ Discuss acclimatization, optimal housing conditions and procedures, environmental factors and any experimental limitations on these (e.g. food deprivation, solitary housing).
13. Experimental procedures	☐ Develop refined procedures for capture, immobilisation, marking, and release or rehoming. ☐ Develop refined procedures for substance administration, sampling, sedation and anaesthesia, surgery and other techniques.
14. Humane killing, release, reuse or rehoming	☐ Consult relevant legislation and guidelines well in advance of the study. ☐ Define primary and emergency methods for humane killing. ☐ Assess the competence of those who may have to perform these tasks.
15. Necropsy	☐ Construct a systematic plan for all stages of necropsy, including location, and identification of all animals and samples.

UNE IACUC application

- ✓ Proper controls, randomization, blinding, biological variables (age, sex, strains, etc.), experimenter skills and proper training

IACUC and Research Rigor

- Resource, partner, but not a barrier to animal research
- "Safeguard responsible animal research by ensuring ethical, scientifically sound, standardized practices in animal research". (*Mohan S and Huneke R ILAR J, Volume 60, Issue 1, 2019, Pages 43–49*)
- Improve reproducibility and the rigor of animal research

THAK YOU!