W5C2 IMPROVING RESEARCH REPORTING AND SCIENTIFIC COMMUNICATION

FELASA WORKSHOP 2022-06-16, 09:00-10:30 Morgiou

Inadequate communication (reporting) of animals and methods in the scientific literature is a factor implicated in poor research, failures in reproducibility, and failures in translation. Workshop participants will work in small groups to critique selections from peer reviewed literature, and ~~(re)write a methods section~~ that aligns with the ARRIVE Guidelines and ILAR reporting guidance. This session will emphasize description of animals, relevant husbandry, environmental, and experimental conditions, and welfare concerns. Participants should gain appreciation and ~~skills~~ resources to write a concise, complete, and compliant methods section for reporting experiments with research animals. Three examples are selected to represent genetic and environmental influences, infectious agents, ~~cancer~~ and neurobehavioral research.

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Why we're here: BETTER METHODS AND METHODS REPORTING

Q 1: How often do you read a paper where you **completely understand**:

- Animals / strains, ages, sexes, n used in each test group;
- Housing/ experimental conditions including diet, bedding, water, cages, change schedule, microbial status, handling, sex of handlers, caretakers and researchers;
- AND Experimental Procedures,

TO the extent that you could confidently replicate the study ??

PLAN: 90 min total 0900-1030

- 5min Introduction
- 5min Small groups
- 30min Read excerpts, Discuss in groups, Consider Survey questions
- 5min Break

945am

- 10min Surveys
- 10min REVIEW results
- 20min Discuss outliers/opposing views <5min per article
  Consider better communication options

3 ARTICLES (excerpted methods below)

1. **Green 1941:** Genetic and Non-Genetic Factors Which Influence the Type of the Skeleton in an Inbred Strain of Mice https://pubmed.ncbi.nlm.nih.gov/17247002/ GENETICS 26: 192-222
2. **Crabbe Wahlsten Dudek. 1999**. Genetics of Mouse Behavior: Interactions with Laboratory Environment https://science.sciencemag.org/content/284/5420/1670/ SCIENCE 284: 1670-1672
3. **Beura et al 2018.** Normalizing the environment recapitulates adult human immune traits in laboratory mice. https://www.nature.com/articles/nature17655 NATURE 53 512-518

FYI Brief history of Guidance on animal care, nomenclature, reporting; laws, regs, organization etc)

1940 (1st) Report of the Committee on **mouse genetics nomenclature**. J Heredity
1950 Animal Care Panel (ACP) [→ AALAS 1967]
1952 **Standardized nomenclature** for inbred strains of mice. Cancer Res.
1953 IAR (→ ILAR 1956) 1956 ICLA (→ ICLAS 1967) 1957 ABLAM (→ ACLAM 1960)
1963 **1st Guide** for Care and Use of Animals in Research [ACP/ILAR → NIH eds 2-6; ILAR eds 7,8..]
1965 AAALAC
1966 Laboratory Animal Welfare Act - PL 89-544 → (AWA in 1970)
1971 **NIH Policy & US Gov principles** for the use of Laboratory animals
…. Institutions should have an oversight committee

1972 **AVMA Panel** on euthanasia included lab animals
1973 **1st (US) PHS Policy** on Humane Care and Use of Laboratory Animals
…. 1979 revision required oversight committee, 1984 more precise
1970's GV-SOLAS/ LASA/ Scand-LAS → FELASA 1978
1985 **(US) Health Research Extension Act** PL99-158 "Animals in Research"
…. REQUIRED 'animal care committee' etc
1985 **1st Intl Guiding principles** for Biomedical research involving animals (ICLAS CIOMS)
1996 Pubmed Online (remember GratefulMed, Medline, Index Medicus?)
2000 ECLAM (EBVS recognized 2008)
2010 **OIE terrestrial code** 1st adopted (rev 2013) Chapter 7.8 Animals in Research and Education:
https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=chapitre_aw_research_education.htm
2010 **EU directive AND Kilkenny ARRIVE guidelines;**
2011 **ILAR Guidance** on reporting animal research
2020 **ARRIVE 2.0**

1. Green EL **1941** **excepted methods** [*CB text*]
Genetic and Non-Genetic Factors Which Influence the Type of the Skeleton in an Inbred Strain of Mice

- .. eleven laboratory stocks of the house mouse, six long inbred and five recently inbred (brother x sister), were examined for their skeletal compositions. All animals were prepared …by a routine potash clearing and alizarin staining technique, such as that described by Cumley et al. (1939).
- [*For further study*]… Bagg albino stock was chosen because it has variations in number of ribs and position of sacrum occurring with such frequency that analysis is reasonably practicable.
- The **3026** individuals of this strain examined for skeletal type were descended from a single pair of the 36th generation of inbreeding. The stock was continued by matings of brother and sister, usually litter-mates, and may be divided arbitrarily into five sublines, each subline having descended from single matings in the 38th or 39th generation. Only individuals produced after the 38th or 39th generation and up to the 43rd generation have been included in this summary.
- For the purpose of estimating the effect of genetic factors on the variation in the number of presacral vertebrae, the population … [*data were analyzed*] (I) by sex, (2) by subline, and (3) by phenotypes of mated parents.
- To evaluate possible non-genetic agents acting upon the position of the sacrum, the population may be considered as being composed of (I) sibships, those individuals produced by single pairs of parents, (2) litters, and (3) individuals. Since individuals are frequently (13.5 percent) asymmetrical, opposite sides may be considered independently.
- [*and data were analyzed*] by age of mother and parity; litter size; season (time of year)….

How do you score the following:	☺ 😐 ☹ + Comments
A. Description /selection of Animals	
B. Description of Procedures	
C. … Housing/husbandry	
D. … Microbial status	
E. … Ethics statement? Welfare concerns?	

Did we learn anything useful/important?

2. Crabbe et al 1999 (excerpted methods)

Genetics of Mouse Behavior: Interactions with Laboratory Environment

- We tested males and females from the inbred strains: A/J, BALB/cByJ, C57BL/6J, DBA/2J, 129/Sv-ter, and 129/SvEvTac; the F2 hybrid cross of C57BL/6J and DBA/2J (B6D2F2); and the serotonin receptor subtype null mutant, 5-HT1B –/–, which is maintained on the 129/Sv-ter background.
- Mice were obtained from the Jackson Laboratory (Bar Harbor, ME), Taconic Farms (Germantown, NY), or the colonies of R. Hen (Columbia University, New York, NY). Because many targeted deletions are placed on the 129/SvEvTac background, we included this close relative of 129/Sv-ter….
- …testing six mouse behaviors simultaneously in three laboratories (Albany, New York; Edmonton, Alberta, Canada; and Portland, Oregon) using exactly the same inbred strains and one null mutant strain (3). We went to extraordinary lengths to equate test apparatus, testing protocols, and all possible features of animal husbandry (4). One potentially important feature was varied systematically. Because many believe that mice tested after shipping from a supplier behave differently from those reared in-house, we compared mice either shipped or bred locally at the same age (77 days) starting at the same time (0830 to 0900 hours local time on 20 April 1998) in all three labs (5). Each mouse was given the same order of tests [Day 1: locomotor activity in an open field; Day 2: an anxiety test, exploration of two enclosed and two open arms of an elevated plus maze; Day 3: walking and balancing on a rotating rod; Day 4: learning to swim to a visible platform; Day 5: locomotor activation after cocaine injection; Days 6 to 11: preference for drinking ethanol versus tap water (6)].
- Reference (4). Variables explicitly equated across laboratories included apparatus, exact testing protocols, age of shipped and laboratory-reared mice, method and time of marking before testing, food (Purina 5001; Purina 5000 for breeders), bedding (Bed-o-cob, 1/4 inch; Animal Specialties, Inc., Hubbard, OR), stainless steel cage tops, four to five mice per cage, light/dark cycle, cage changing frequency and specific days, male left in cage after births, culling only of obvious runts, postpartum pregnancy allowed, weaned at 21 days, specific days of body weight recording, and gloved handling without use of forceps. Unmatched variables included local tap water, requirement of filters over cage tops in Portland only, variation of physical arrangement of colonies and testing rooms across sites, different air handling and humidity, and different sources of batches of cocaine and alcohol
- 'Further details at (www.albany.edu/psy/obssr) 2022: https://www.albany.edu/psychology/obssr3/

How do you score the following:	☺ 😐 ☹ + Comments
F. Description /selection of animals	
G. Description /selection of Procedures	
H. … Housing/husbandry	
I. … Microbial status	
J. … Ethics statement? Welfare concerns?	

Did we learn anything useful/important?

3. Beura et al 2018 (excerpted methods)

Normalizing the environment recapitulates adult human immune traits in laboratory mice

- Pet store mice were purchased from various Twin Cities area pet stores. Feral mice were trapped on a horse farm or rural outdoor petting zoo in Minnesota or Georgia, USA. Male or female pet store mice were introduced into the cages of 6–8-week-old C57BL/6 mice of the same sex purchased from the National Cancer Institute. Co-housing occurred within a BSL-3 facility.
- Age-matched C57BL/6 laboratory mice maintained in SPF facilities served as controls.
- The number of animals needed to reach statistical significance was determined on the basis of previous experience. All animals that survived the experimental treatment were included in the final analysis. No method of randomization was used to allocate animals to experimental groups. Investigators were not blinded to the group allocation during experiments. *L. monocytogenes* was grown in tryptic soy broth containing streptomycin to log phase growth. The indicated groups of mice were infected intravenously (i.v.) with 8.5×10^4 c.f.u. of wild-type *L. monocytogenes* (provided by J. Harty). Bacterial load in the spleen and liver was determined 3 days post-challenge as previously described[31,32]. *L. monocytogenes* immune mice were generated by primary infection with recombinant *L. monocytogenes* expressing OVA (LM-OVA) (provided by H. Shen)[33] 5 months before secondary challenge with wild-type *L. monocytogenes*. *P. berghei* ANKA (provided by S. K. Pierce) was propagated by passage in mice and blood collection. One-million parasitized RBCs were injected intraperitoneally (i.p.) into the indicated mice. Parasitaemia was measured by flow cytometry of peripheral blood[34].
- All mice were used in accordance with the guidelines of the Institutional Animal Care and Use Committees at the University of Minnesota.

How do you score the following:	☺ 😐 ☹ + Comments/questions
K. Description /selection of animals	
L. Description /selection of Procedures	
M. … Housing/husbandry	
N. … Microbial status	
O. … Ethics statement? Welfare concerns?	

Did we learn anything useful/important?

CONCLUSIONS regarding these examples:

- Accurate reporting, with sufficient detail, IS possible even with page limits.
- Differences/variations may be informative.
- GENETICs matter, Strain matters, Substrain matters…
- NON genetic factors (Extrinsic X factors) matter…
- Beware of lies, damn lies and statistics…
- Consider Pathology
- Reproducibility: Is it A new crisis?

Additional resources

1959 Russell & Burch: Principles of humane experimental technique
https://caat.jhsph.edu/principles/the-principles-of-humane-experimental-technique NOT just animal welfare and 3R's - its about good science and worth reading.

2010 ARRIVE guidelines – Kilkenny & al 2010 – NC3R's https://www.ncbi.nlm.nih.gov/pubmed/20613859

2010 OIE terrestrial code Chapter 7.8 Animals in Research and Education 1st adopted (rev 2013): https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=chapitre_aw_research_education.htm

2011 ILAR GUIDANCE (NRC 2011) Guidance for the Description of Animal Research in Scientific Publications. Washington, DC: National Academies (**NOT 'THE Guide' 2010 8th ed)**
http://www.nap.edu/catalog.php?record_id=13241 /https://www.ncbi.nlm.nih.gov/pubmed/22379656

2014 NIH.gov Principles and Guidelines for Reporting Preclinical Research
https://www.nih.gov/research-training/rigor-reproducibility/principles-guidelines-reporting-preclinical-research https://grants.nih.gov/policy/reproducibility/index.htm

2016 FASEB Recommendations
https://www.faseb.org/Portals/2/PDFs/opa/2016/FASEB_Enhancing%20Research%20Reproducibility.pdf

2018 ICLAS **HARRP** harmonized animal research reporting principles
https://pubmed.ncbi.nlm.nih.gov/29669797/

2020 ARRIVE 2.0 https://arriveguidelines.org/arrive-guidelines

- **Protocols.io**: Virtual Communities for Protocol Development and Discussion. Teytelman et al. 2016. https://www.ncbi.nlm.nih.gov/pubmed/27547938
  Find, discuss, implement, report, revise protocols, 'publish' with DOI https://www.protocols.io/
- **PREPARE**: Guidelines for planning animal research and testing. Smith et al. 2017. https://www.ncbi.nlm.nih.gov/pubmed/28771074 https://norecopa.no/prepare
- **EDA** The Experimental Design Assistant https://eda.nc3rs.org.uk/
- **Specialty group guidance:** Minimum information etc recommendations:
  e.g. MinPDX, MinPEPa (pathology), MinSC (stem cells)…
- **CAMARADES** http://www.dcn.ed.ac.uk/camarades/default.htm
- **SYRCLE** https://www.radboudumc.nl/en/research/departments/health-evidence/systematic-review-center-for-laboratory-animal-experimentation
- **PRISMA** https://www-ncbi-nlm-nih-gov.proxy1.library.jhu.edu/pubmed/19621072
- https://www.**nature**.com/nature-research/editorial-policies/ethics-and-biosecurity
- STAR METHODS https://www.**cell.com/star**-authors-guide → template
- https://www.**sciencemag**.org/authors/science-journals-editorial-policies → animal studies

Glossary of relevant terms (ARRIVE 2.0) excerpted (https://arriveguidelines.org/arrive-guidelines)

Bias: Introduction of a systematic error …caused by inadequacies in the design, conduct, or analysis of an experiment.

Effect size: Quantitative measure that estimates the magnitude of differences between groups…

Experimental unit: Biological entity subjected to an intervention independently of all other units...

External validity: Extent to which the results of an animal experiment provide a correct basis for generalisations to other …and/or other environmental conditions.

False positive: Statistically significant result obtained …when the effect ...does not exist.

False negative: Non-statistically significant result obtained when the effect …genuinely exists.

Independent variable of interest: Factor that a researcher manipulates within a controlled environment in order to test its impact on the outcome ... AKA predictor variable, ...

Internal validity: Refers to the rigour of the study design and statistical analysis to isolate cause and effect, and attribute the effect observed to manipulation of the independent variable of interest… with high

internal validity, sources of bias and chance observations are minimized... with low internal validity, the effect may be caused by bias, chance and other nuisance variables rather than the independent variable(s) of interest.

Null and alternative hypotheses: The null hypothesis (H0) … postulate that response being measured is UNaffected by the experimental manipulation… alternative hypothesis (H1) refers to the postulate that manipulating the independent variable of interest has an effect on the response measured.

Nuisance variable: Sources of variability or conditions that could potentially bias results. Also known as: confounding factor, confounding variable

Outcome measure: Any variable recorded during a study to assess the effects of a treatment or experimental intervention. AKA dependent variable, response variable

Power: Probability that a test of significance will detect an effect (i.e. a deviation from the null hypothesis), if an effect exists (i.e. true positive result).

Sample size: Number of experimental units per group, also referred to as N number.

ARRIVE 2.0 ESSENTIAL 10 items	**Recommended Set (11)**
1. **Study design (Methods)**	1. Abstract
2. Sample size	2. Background
3. Inclusion and exclusion criteria	3. Objectives
4. Randomisation	4. Ethical statement
5. Blinding	5. **Housing and husbandry**
6. Outcome measures	6. **Animal care and monitoring**
7. Statistical methods	7. Interpretation /scientific implications
8. **Experimental animals (emphasis here)**	8. Generalisability /translation
9. **Experimental procedures (emphasis here)**	9. Protocol registration
	10. Data access
10. Results	11. Declaration of interests

Validities https://pubmed.ncbi.nlm.nih.gov/25541540/

Validity Definitions adapted from van der Worp et al. (2010) and from the Cochrane Collaboration.

Construct validity: The degree to which inferences are warranted from the sampling properties of an experiment (e.g., units, settings, treatments and outcomes) to the entities these samples are intended to represent.

External validity: The extent to which the results of an animal experiment provide a correct basis for generalizations to other populations of animals (including humans) and/or other environmental conditions.

Internal validity: The extent to which the design, conduct, and analysis of the experiment eliminate the possibility of bias so that the inference of a causal relationship between an experimental treatment and variation in an outcome measure is warranted.

Standardization vs heterogenization Which is better? **Voelkl et al 2020 PMID/32488205/ etc**

Standardization (reductionist approach)	**Heterogenization**
♦ Simple system	*(NOT referring to randomization in design)*
♦ Fewer variables	♦ **More heterogeneity** / complexity in research animals and research conditions?
♦ Better defined	♦ Messier = More like real life
♦ Understand effects of interventions	♦ ***Defined sufficiently to reach valid conclusions? Vs can you achieve Internal validity in poorly defined (messy) situations?***
♦ ***BUT relevant to real life? External Validity?***	