# Reporting checklist for study using laboratory animals.

Based on the ARRIVE guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the ARRIVE reporting guidelines, and cite them as:

Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research.

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|  |  | Reporting Item | Page Number |
|  | [#1](https://www.goodreports.org/arrive/info/#1) | Provide as accurate and concise a description of the content of the article as possible. | 1 |
|  | [#2](https://www.goodreports.org/arrive/info/#2) | Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study. | 3 |
| Background | [#3a](https://www.goodreports.org/arrive/info/#3a) | Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. | 4-5 |
|  | [#3b](https://www.goodreports.org/arrive/info/#3b) | Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study’s relevance to human biology | Page 5We used C57 mice as study subjects because this is an inbred strain and the individuals have an identical genetic makeup. Variables like diet, age, physiological status, and original gut microbiota were controlled for in experimental mice, except for these variables: bedding material and sanitary living conditions. This experiment could not be exerted on human directly. |
| Objectives | [#4](https://www.goodreports.org/arrive/info/#4) | Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested. | 5 |
| Ethical statement | [#5](https://www.goodreports.org/arrive/info/#5) | Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research. | 15 |
| Study design | [#6a](https://www.goodreports.org/arrive/info/#6a) | For each experiment, give brief details of the study design including the number of experimental and control groups. A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out. | 13-15 |
|  | [#6b](https://www.goodreports.org/arrive/info/#6b) | Report any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). | 13,14 |
|  | [#6c](https://www.goodreports.org/arrive/info/#6c) | Describe the experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out. | Figure s1 |
| Experimental procedures | [#7](https://www.goodreports.org/arrive/info/#7) | For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used) | 13-16 |
| Experimental animals | [#8a](https://www.goodreports.org/arrive/info/#8a) | Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). | 13, table s2 |
|  | [#8b](https://www.goodreports.org/arrive/info/#8b) | Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health / immune status, drug or test naïve, previous procedures, etc | 13,14 |
| Housing and husbandry | [#9a](https://www.goodreports.org/arrive/info/#9a) | Provide details of housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). | table s2, figure s1  |
|  | [#9b](https://www.goodreports.org/arrive/info/#9b) | Report husbandry conditions (e.g. breeding programme, light / dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment). | 13-15 |
|  | [#9c](https://www.goodreports.org/arrive/info/#9c) | Describe welfare-related assessments and interventions that were carried out prior to, during, or after the experiment | 15,16 |
| Sample size | [#10](https://www.goodreports.org/arrive/info/#10) | a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. c. Indicate the number of independent replications of each experiment, if relevant. | a. table s2, figure s1b.$ n=\frac{φ^{2}\left(\sum\_{i=1}^{k}S\_{i}^{2}/k\right)}{\sum\_{i=1}^{k}\left(\overbar{X}\_{i}-\overbar{X}\right)^{2}/\left(k-1\right)}$, where n is the average group sample size, k is the number of groups,$\overbar{X}\_{i}$ is sample mean for each group,$ S\_{i}^{2}$ is the sample variance for each group, $φ$ is obtained in $φ$ value table for multiple means compare.c. N=9 |
| Allocating animals to experimental groups | [#11](https://www.goodreports.org/arrive/info/#11) | a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed. | 13-16 |
| Experimental outcomes | [#12](https://www.goodreports.org/arrive/info/#12) | Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes). | 17-19 |
| Statistical methods | [#13](https://www.goodreports.org/arrive/info/#13) | a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach | 17-19 |
| Baseline data | [#14](https://www.goodreports.org/arrive/info/#14) | For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing (this information can often be tabulated). | 15,16 |
| Numbers analysed | [#15](https://www.goodreports.org/arrive/info/#15) | a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10 / 20, not 50%2). b. If any animals or data were not included in the analysis, explain why. | table s2, figure s1 |
| Outcomes and estimation | [#16](https://www.goodreports.org/arrive/info/#16) | Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval). | 5-10 |
| Adverse events | [#17](https://www.goodreports.org/arrive/info/#17) | a. Give details of all important adverse events in each experimental group. b. Describe any modifications to the experimental protocols made to reduce adverse events. | No, we had not met such adverse events in the research. |
| Interpretation / scientific implications | [#18a](https://www.goodreports.org/arrive/info/#18a) | Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. | 10-13 |
|  | [#18b](https://www.goodreports.org/arrive/info/#18b) | Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results | 10-13 |
|  | [#18c](https://www.goodreports.org/arrive/info/#18c) | Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research. | No. Because we used the high through put sequencing method to analyze the diversity of gut microbiota and the complicated sequencing data need enough samples to reduce the systematic errors. |
| Generalisability / translation | [#19](https://www.goodreports.org/arrive/info/#19) | Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology. | 12,13 |
| Funding | [#20](https://www.goodreports.org/arrive/info/#20) | List all funding sources (including grant number) and the role of the funder(s) in the study. | 19 |

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