**PRILE 2021 guidelines for reporting laboratory studies in Endodontontology: a consensus-based development**

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**Keywords:** Consensus, endodontics, guidelines, laboratory study.

**Running title:** PRILE 2021

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Abstract

Reproducible, skillfully-conducted and unbiased laboratory studies provide new knowledge, which can inform clinical research and eventually translate into better patient care. To help researchers improve the quality and reproducibility of their research prior to a publication peer-review, this paper describes the process that was followed during the development of the Preferred Reporting Items for Laboratory studies in Endodontology (PRILE) 2021 guidelines and which used a well-documented consensus-based methodology. A steering committee was created with eight individuals (PM, RO, OP, IR, JS, EP, JJ and SP), plus the project leaders (PD, VN). The steering committee then formed a PRILE Delphi Group (PDG) and PRILE Online Meeting Group (POMG) to provide expert advice and feedback on the initial draft checklist and flowchart. The members of the PDG participated in an online Delphi process to achieve consensus on the items within the PRILE 2021 checklist and the accompanying flowchart for clarity and suitability. The PRILE checklist and flowchart developed by the online Delphi process were discussed further by the POMG. This online meeting was conducted on 12th February 2021 via the Zoom platform. Following this meeting, the steering committee developed a final version of the PRILE 2021 guidelines and flowchart, which was piloted by several authors when writing-up a laboratory study for publication. Authors are encouraged to use the PRILE 2021 guidelines and flowchart to improve the clarity, completeness and quality of reports describing laboratory studies in Endodontology. The PRILE 2021 checklist and flowchart are freely available and downloadable from the Preferred Reporting
Items for study Designs in Endodontology (PRIDE) website (http://pride-endodonticguidelines.org/prile/)

**Keywords:** Consensus, endodontics, guidelines, laboratory study
Introduction
Cutting edge laboratory studies in Endodontology include a wide range of experiments conducted in well-controlled environments that allow the precise effects of variables to be measured and compared in order to detect differences between individual treatment/intervention groups and controls. Laboratory studies make up the majority of research that is undertaken in Endodontology (Krithikadatta et al. 2014), however, manuscripts reporting such studies have a very low rate of acceptance by journals, with over 85% of the manuscripts submitted to a leading Endodontic journal being rejected (Ahmad et al. 2019). The reasons for rejection have been attributed to lack of originality, lack of conformity to ethical guidelines, and major experimental design and/or methodological flaws. Laboratory studies are highly task-oriented, potentially expensive, and time-consuming and as a consequence, the rejection of manuscripts is a significant financial and professional problem (Nagendrababu et al. 2019a,b).

The transparent and accurate reporting of laboratory studies should deliver improved validity, reproducibility and translation of research findings into clinical practice (Nagendrababu et al. 2019a, b). Only a few guidelines for reporting laboratory studies in Dentistry have been proposed (Faggion 2012, Krithikadatta et al. 2014). The Consolidated Standards for Reporting Trials (CONSORT) was adapted for reporting in vitro studies on dental materials (Faggion 2012) and a Checklist for Reporting In-vitro Studies (CRIS) in Dentistry has been proposed (Krithikadatta et al. 2014). Considering the importance of laboratory studies in Endodontology, the need for well-structured and comprehensive reporting guidelines for researchers in the field of Endodontology is essential.

The Preferred Reporting Items for Laboratory Studies in Endodontology (PRILE) 2021 guidelines have been developed to address the need for reporting guidelines exclusively for Endodontology. The PRILE guidelines are intended to improve the quality, accuracy, reproducibility, completeness and transparency in reports of all types of laboratory studies within the specialty (Nagendrababu et al. 2019a,b). The items within the PRILE guidelines will help authors plan and report...
their laboratory studies more effectively as well as guide reviewers and editors of journals to evaluate the suitability of manuscripts for publication. The aim of this current project is to report the development of the PRILE guidelines for reporting laboratory studies in Endodontology through a consensus-based approach.

**Methods**

The study was approved by the Institutional Review Board on Research and Ethics of the International Medical University (IMU), Kuala Lumpur, Malaysia (No: IMU 450/2019) and University of Sharjah, Sharjah, UAE (REC-20-11-06-01). The PRILE guidelines are based on the recommendations given in the Guidance for Developers of Health Research Reporting Guidelines (Moher et al. 2010) and the development protocol has been published (Nagendrababu et al. 2019b).

**Initial steps**

The project leaders (VN and PD) identified the need for developing guidelines for reporting laboratory studies in Endodontology. At first, a checklist of items to be included in the PRILE guidelines was drafted by a steering committee consisting of ten members, including the project leaders (PD, VN, PM, RO, OP, IR, JS, EP, JJ, SP). The initial draft checklist was based on the modified CONSORT checklist of items for reporting *in vitro* studies of dental materials (Faggion 2012) and the Clinical and Laboratory Images in Publications (CLIP) principles (Lang et al. 2012) to fit the specialty of Endodontology. Following this, the draft checklist and a flowchart were subjected to an online Delphi process to build consensus on the contents of the checklist and the design of the flowchart.

**Online Delphi process**

The Delphi consensus phase of the study involved creating a PRILE Delphi Group (PDG). The PDG included 30 members including 22 academics or researchers, four Endodontists, two general dentists and two representatives of the public. The PDG members with a professional background fulfilled at least one of the following...
eligibility criteria to be included: 1) had published at least two laboratory studies in Endodontology; 2) published guidelines for reporting research; 3) a minimum of 15 years academic or clinical experience in Dentistry. All the eligible PDG members were invited via e-mail to participate in the online Delphi process; the invitation introduced the aims and rationale for developing the PRILE guidelines, described the Delphi process and set out the role of the PDG members.

The members who confirmed their participation were provided with a Delphi document that gave detailed information on the anonymous consensus building process and included the draft PRILE checklist with 40 items and a flowchart. The PDG members were informed about the criteria and scoring scheme for inclusion or exclusion of items in the draft checklist, which were assessed for their suitability and clarity. The clarity of an item was assessed using ‘yes’ or ‘no’, whilst the suitability of an item was evaluated using a 9-point Likert scale (1 = ‘definitely not include’ to 9 = ‘definitely include’). PDG members were encouraged to add comments on each item to help the steering committee understand why they had awarded the score as well as provide an additional perspective to improve the quality of the checklist and the flowchart.

The steering committee analysed the scores of the items based on the previously determined set of inclusion and exclusion criteria. Items that achieved a score between 7 and 9 by at least 70% of PDG members and items with a score of 1-3 by less than 30% of members were included whereas, items were excluded from the checklist if they received a score between 1 and 3 by more than 70% of members or a score of 7 to 9 by less than 30% of members. Subsequent Delphi rounds continued until the pre-set standard of consensus was achieved and a final set of items was approved (Agha et al. 2017). Thereafter, the revised PRILE checklist and flowchart was discussed in detail during a PRILE online meeting.

*Online meeting*
A PRILE Online Meeting group (POMG) was formed that included 24 individuals. The eligibility criteria for POMG members were the same as those of the PDG with several individuals being members of both groups. During the online meeting, the results of the two online Delphi rounds, the revised PRILE checklist and flowchart, agenda of the meeting as well as the details of the meeting (date, time, zoom link) were shared with the POMG. The online meeting was conducted on 12\textsuperscript{th} February 2021 using the Zoom online platform.

Post-meeting activities

Based on the comments received during the meeting, the steering committee revised the checklist and flowchart. Several experts were then asked to pilot the PRILE guidelines by drafting a manuscript using the PRILE 2021 checklist and flowchart.

Results

Online Delphi process

The online Delphi process was conducted over two rounds and included feedback from 30 individuals with a 100\% response rate each time. Round 1 consisted of a PRILE checklist with 40 items and a flowchart. Among the 40 items, 39 received sufficient scores to allow them to be included in the PRILE checklist whereas there was disagreement over one item. Based on the feedback provided by PDG members, the steering committee revised that one item. In addition, even though Item 6a within the Results domain - \textit{The estimated effect size and its precision for all the outcomes (primary and secondary) for each group including controls must be provided} - was scored between 7 and 9 by ≥70\% of members, the large number of comments received on this item convinced the steering committee to include this item once again in round 2 to confirm its “inclusion/exclusion” in the PRILE checklist. Thus, round 2 included just two items (Item 6a and 11b). Finally, both these items were included in the version of the PRILE checklist that was discussed at the online meeting. The flowchart was approved in round 1.
**Online meeting**

An online virtual Zoom meeting was conducted *in lieu* of the anticipated face to face meeting that was cancelled due to the COVID-19 pandemic. The meeting was attended by 24 individuals including two postgraduate students and three steering committee members (PD, VN, RO). The online session was chaired by two steering committee members (PD, VN). The attendees discussed the suitability of the items for inclusion in the PRILE checklist and the design of the flowchart.

**Post-meeting activities**

The comments from the POMG meeting were considered by the steering committee and revisions made as necessary. The PRILE checklist and flowchart were then piloted by three authors when writing manuscripts describing laboratory studies. The final PRILE 2021 checklist consists of 11 sections with 40 individual items (Table 1). The PRILE 2021 flowchart (Figure 1) that includes 11 domains summarizes the key steps in the reporting of a laboratory study.

**Discussion**

Cutting edge endodontic research encompasses a wide range of laboratory-based studies that overlaps all of the scientific disciplines. Although, the multi-year task was convoluted and involved multiple revisions, guidelines were developed for endodontic researchers to avoid the most common pitfalls which can make their laboratory research fail during the publication peer-review process, (Nagendrababu *et al.* 2019b). This present report describes the process that was followed during the development of these reporting guidelines.

The PRILE 2021 guidelines provide guidance for the development of more reproducible, effective, accurate, skilfully-conducted and unbiased manuscripts reporting laboratory studies in Endodontontology. The implementation of the PRILE 2021 guidelines will assure greater standardization in the design, conduct and reporting of laboratory studies using a logical and comprehensive template.
Statistical tests are almost always a necessary element of laboratory studies, but because of the bewildering array of statistical tests and ad hoc tests for researchers to choose from, and due to the complexity of statistical software, it can be easy to obtain inaccurate probability values. Ideally, to detect and prevent statistical mistakes and to ensure probability reproducibility, the statistical analysis of datasets should never rely upon only one person for data collection or its analysis, or upon only one statistical test type, or upon only one software package. It is essential that a research team can replicate their own results prior to reporting them in a publication. The replication of data gives an assurance that the results are accurate and reliable, and also detect problems, such as equipment malfunctions, assay mistakes, or cross-contamination, which can help to prevent embarrassing article retractions or corrections.

The importance of an a priori sample size calculation for quantitative data has been highlighted in the PRILE 2021 guidelines in order that true differences between two or more interventions / assessed parameters in a study can be identified. Underpowered studies with small sample sizes tend to produce imprecise estimates with wider confidence intervals (Montori et al. 2004, Faggion 2012). Thus, sample size calculation plays a critical role during the planning phase of laboratory-based research and its detailed reporting in the methodology section is mandatory. In the absence of pilot data to estimate a priori sample sizes, sample sizes in prior publications can serve as a useful guide.

In Endodontology, the method of randomization and concealment of samples until the moment of assignment is often not implemented nor reported in the majority of published laboratory-based research. Randomization, by flipping a coin or card shuffling could be utilized prior to allocating the sample to a specific group. Similarly, extracted teeth can be stored in sequentially numbered, opaque sealed containers to follow allocation concealment (Faggion 2012). Planning and reporting these two parameters produces more dependable results and this has been emphasized in the PRILE 2021 guidelines. However, the randomisation of samples
may not be necessary in experiments where the samples are homogeneous, such as for the physico-chemical tests of materials such as radiopacity, setting time, solubility, cytotoxicity, or cyclic fatigue test on endodontic instruments.

The uniqueness of root canal anatomy and physiology between different teeth and the possible confounding impact of complex anatomical variations on the outcome of laboratory studies, must be recognized while planning, designing and reporting research to minimize any potential bias. It is commonly understood that obliterated root canals are naturally more difficult to instrument, and the apical regions of root canals are generally more difficult to disinfect; these difficulties must be considered to ensure comparative studies are dependable and robust (Babb et al. 2009, De-Deus 2012). As a consequence, anatomical matching of tooth specimens by pre-experimental analysis of root canal anatomy will create experimental/control groups with similar baseline features, which ultimately allows the investigator to answer the research question with minimal bias (De-Deus et al. 2020). The method used to ensure the similarity of the samples must be reported in the methodology section. On the other hand, it is important that authors acknowledge to what extent the new findings can be generalized to other anatomical groups or conditions. It is also necessary to discuss the external validity of laboratory experiments. Strict inclusion criteria come with another limitation: the findings may not be applicable to tooth types or canal shapes that differ from the study population and therefore the results cannot be generalized to all teeth or canal shapes (low external validity). Studies in single-rooted teeth that exclude the common complex anatomy of posterior teeth will inevitably limit the results to cases in which a treatment failure is less common. External validity can be improved by using broad inclusion criteria and a sample that can be generalized to the clinical context. However, this may increase the variability of the results and require a larger sample size in order to detect true differences.
The biological testing of disinfection, demineralization, cell and molecular activity requires both negative and positive assay controls. A positive control is any well-characterized material and/or substance that, when evaluated by a specific test method, demonstrates the suitability of the test system to yield a reproducible, appropriately positive or reactive response. Whereas, a negative control is a well-characterized material and/or substance that, when evaluated by a specific test method, demonstrates the suitability of the test system to yield a reproducible, appropriately negative, non-reactive or minimal response. The negative control can also be important to define background or baseline values (Camilleri et al. 2020).

Internal controls are also necessary for molecular assays to ensure that the assays are functioning with a high degree of specificity. Conformance with ISO 7405 and 10993 and other international and national standards is necessary for evaluating the safety of dental devices. However, one should take into consideration the conflicting properties of antimicrobial activity and the cytotoxicity, and any potential differences between the in vitro testing of devices and their clinical use, such as inflammatory responses.

In Endodontology, the use of sterilization procedures and aseptic techniques are important in certain type of studies related to microbiology and cell biology. Inadequately sterilized specimens or infection during handling will lead to inaccurate testing with false positives/negatives. The conditions used during the testing are important. Some sterilization procedures have been shown to be ineffective on specific substrates (White & Hays 19995) and may also induce chemical changes on the substrate (Farrugia et al. 2015, André et al. 2018). The sterilization of biological samples and tissues is important to prevent cross-contamination, and to ensure the safety of the personnel handling the specimens (such as to prevent the potential spread of infections from saliva, blood, tissues, plaque, or extracted teeth). In some laboratory-based studies, sterilization may be irrelevant (such as for the mechanical testing of materials).
The performance of research which adheres to biomaterials and device testing standards developed by the International Standards Organization (ISO) and other professional standardization agencies (ADA, ANSI, FDA etc.) are important to ensure patient safety and to preserve the reproducibility and continuity of the scientific literature. However, care is needed to ensure that the standardized methods are not used to improperly obtain pass or fail compliance criteria. Due to patient safety concerns, the ad hoc modification of ISO or other professional standards without a valid justification is not recommended (Camilleri 2020, Darvell 2020, Schmalz et al. 2021).

The presentation of methods and results should include relevant bar charts, figures, images, radiographs, photographs, flow charts and illustrations, which each contain a text legend to succinctly describe the image. The use of clear illustrations also helps researchers to support their results, communicate new discoveries and generate new hypotheses (Kotz & Cals 2013, Polepalli Ramesh et al. 2015). Due to the high frequency of quality-control problems with images submitted for peer-review, the PRILE 2021 checklist includes eight “quality of image” checklist items, to provide guidance to authors.

Flowcharts within the Consolidated Standards of Reporting Trials (CONSORT) and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines have been reported to enhance the quality of the reporting of randomized clinical trials and systematic reviews (Egger et al. 2001, Vu-Ngoc et al. 2018) as they help readers to understand the flow of a trial or a review process. As a consequence, a flowchart has also been included in the PRILE 2021 guidelines in order to provide a pictorial representation of the major steps involved in the research.

Future plans
1. Explanation and elaboration document: The purpose and relevance of each item in the checklist and flowchart will be described further in an explanation and elaboration document, which will be prepared by the steering committee and include
suitable examples from the literature or hypothetical examples to support the understanding of each item in the checklist and the flowchart.

2. **Translation**: Translation of the PRILE 2021 guidelines into various languages will be done for the benefit of non-English authors and readers across the world.

3. **Dedicated website**: The PRILE 2021 checklist and flowchart will be available and freely downloadable on the Preferred Reporting Items for study Designs in Endodontology (PRIDE) website (http://pride-endodonticguidelines.org/prile/).

4. **Endorsement**: The Editors of relevant dental journals will be contacted to seek their support in the adoption of the PRILE 2021 guidelines.

5. **Update of the PRILE guidelines**: The steering committee will periodically revise and update the PRILE guidelines based on feedback received from stakeholders.

6. **Workshop/webinar**: The steering committee will actively promote the PRILE 2021 guidelines by conducting workshops/seminars at various conferences as well as producing educational videos and webinars.

**Conclusion**

A well-documented and validated consensus process was used in the development and validation of the PRILE 2021 guidelines. The guidelines consist of a checklist of 40 items under 11 sections. The items within the PRILE 2021 guidelines will help authors plan and report their laboratory studies more effectively as well as guide reviewers and editors of journals to evaluate the suitability of manuscripts for publication.
Acknowledgements

The first author (V Nagendrababu) was associated previously with the School of Dentistry, International Medical University, Kuala Lumpur, Malaysia where the ethical clearance was obtained (No: IMU 450/2019) and where the study was conducted. The first author (V Nagendrababu) is now associated with the University of Sharjah, Sharjah, United Arab Emirates, where a further ethical clearance was obtained (REC-20-11-06-01). The project leaders (PD, VN) would like to thank the Tehran University of Medical Sciences (TUMS), Tehran, Iran for managing the Zoom platform during the online meeting.

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Legends

Figure 1: PRILE 2021 Flowchart

Table 1: PRILE 2021 checklist of items to be included when reporting laboratory studies in Endodontology
References


De-Deus G, Simões-Carvalho M, Belladonna FG *et al.* (2020) Creation of well-balanced experimental groups for comparative endodontic laboratory studies: a


Figure 1: PRILE 2021 Flowchart.

1. **RATIONALE/JUSTIFICATION** (what the investigation contributes to the literature)
2. **AIM/HYPOTHESIS**
3. **ETHICAL APPROVAL (IF APPLICABLE)**
   (e.g. Institutional review board approval to collect and research human teeth)
4. **SAMPLES** (e.g. Instruments, materials, extracted human mandibular teeth, histology slides, cells, or proteins)
5. **EXPERIMENTAL AND CONTROL GROUPS, INCLUDE INDEPENDENT VARIABLES**
   (e.g. Group 1 – Microbiology samples from infected retreated root canals (n = 10),
   Group 2 - Microbiology samples from disinfected root canals (n = 10),
   Group 3 – Controls for comparison, pure microbiology samples (n = 10))
6. **OUTCOME(S) ASSESSED, INCLUDE DEPENDENT VARIABLES AND TYPE**
   (e.g. Microbiology changes in infected root canals)
7. **METHOD USED TO ASSESS THE OUTCOME(S) AND WHO ASSESSED THE OUTCOME(S)**
   (e.g. ELISA by laboratory staff)
8. **RESULTS**
   (e.g. Report success and failures)
9. **CONCLUSIONS**
10. **FUNDING DETAILS**
11. **CONFLICT OF INTEREST**
Table 1: PRILE 2021 checklist of items to be included when reporting laboratory studies in Endodontontology

<table>
<thead>
<tr>
<th>Section/Topic</th>
<th>Item Number</th>
<th>Checklist Items</th>
<th>Reported on page number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title</td>
<td>1a</td>
<td>The Title must identify the study as being laboratory-based, e.g. “laboratory investigation” or “in vitro,” or “ex vivo” or another appropriate term</td>
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<td></td>
<td>1b</td>
<td>The area/field of interest must be provided (briefly) in the Title</td>
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<td>Keywords</td>
<td>2a</td>
<td>At least two keywords related to the subject and content of the investigation must be provided</td>
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<td>Abstract</td>
<td>3a</td>
<td>The rationale/justification of what the investigation contributes to the literature and/or addresses a gap in knowledge must be provided</td>
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<td></td>
<td>3b</td>
<td>The aim/objectives of the investigation must be provided</td>
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<td></td>
<td>3c</td>
<td>The body of the Abstract must describe the materials and methods used in the investigation and include information on data management and statistical analysis</td>
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<td></td>
<td>3d</td>
<td>The body of the Abstract must describe the most significant scientific results for all experimental and control groups</td>
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<td></td>
<td>3e</td>
<td>The main conclusion(s) of the study must be provided</td>
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<tr>
<td>Introduction</td>
<td>4a</td>
<td>A background summary of the scientific investigation with relevant information must be provided</td>
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<td></td>
<td>4b</td>
<td>The aim(s), purpose(s) or hypothesis(es) of an investigation must be provided ensuring they align with the methods and results</td>
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<td>Materials and Methods</td>
<td>5a</td>
<td>A clear ethics statement and the ethical approval granted by an ethics board, such as an Institutional Review Board or Institutional Animal Care and Use Committee, must be described</td>
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<td>5b</td>
<td>When harvesting cells and tissues for research, all the legal, ethical, and welfare rights of human subjects and animal donors must be respected and applicable procedures described</td>
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<td>5c</td>
<td>The use of reference samples must be included, as well as negative and positive control samples, and the adequacy of the sample size justified</td>
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<td>5d</td>
<td>Sufficient information about the methods/materials/supplies/samples/specimens/instruments used in the study must be provided to enable it to be replicated</td>
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<td>5e</td>
<td>The use of categories must be defined, reliable and be described in detail</td>
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<td>5f</td>
<td>The numbers of replicated identical samples must be described within each test group. The number of times each test was repeated must be described</td>
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<td>5g</td>
<td>The details of all the sterilization, disinfection, and handling conditions must be provided, if relevant</td>
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<td>5h</td>
<td>The process of randomization and allocation concealment, including who generated the random allocation sequence, who decided on which specimens to be included and who assigned specimens to the intervention must be provided, if relevant</td>
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<td>5i</td>
<td>The process of blinding the operator who is conducting the experiment (if applicable) and the examiners when assessing the results must be provided</td>
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<td>5j</td>
<td>Information on data management and analysis including the statistical tests and software used must be provided</td>
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<td>Results</td>
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<td>6a</td>
<td>The estimated effect size and its precision for all the objective (primary and secondary) for each group including controls must be provided</td>
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<td>6b</td>
<td>Information on the loss of samples during experimentation and the reasons must be provided, if relevant</td>
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<td>6c</td>
<td>All the statistical results, including all comparisons between groups must be provided</td>
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<td>Discussion</td>
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<td>7a</td>
<td>The relevant literature and status of the hypothesis must be described</td>
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<td>7b</td>
<td>The true significance of the investigation must be described</td>
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<td>7c</td>
<td>The strength(s) of the study must be described</td>
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<td>7d</td>
<td>The limitations of the study must be described</td>
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<td>7e</td>
<td>The implications for future research must be described</td>
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<tr>
<td><strong>Conclusion(s)</strong></td>
<td>8a</td>
<td>The rationale for the conclusion(s) must be provided</td>
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<tr>
<td></td>
<td>8b</td>
<td>Explicit conclusion(s) must be provided, i.e. the main “take-away” lessons</td>
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<tr>
<td><strong>Funding and support</strong></td>
<td>9a</td>
<td>Sources of funding and other support (such as supply of drugs, equipment) as well as the role of funders must be acknowledged and described</td>
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<tr>
<td><strong>Conflicts of interest</strong></td>
<td>10a</td>
<td>An explicit statement on conflicts of interest must be provided</td>
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<tr>
<td><strong>Quality of images</strong></td>
<td>11a</td>
<td>Details of the relevant equipment, software and settings used to acquire the image(s) must be described in the text or legend</td>
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<td>11b</td>
<td>If an image(s) is included in the manuscript, the reason why the image(s) was acquired and why it is included must be provided in the text</td>
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<td>11c</td>
<td>The circumstances (conditions) under which the image(s) were viewed and evaluated must be provided in the text</td>
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<td>11d</td>
<td>The resolution and any magnification of the image(s) or any modifications/ enhancements (e.g. brightness, image smoothing, staining etc.) that were carried out must be described in the text or legend</td>
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<td>11e</td>
<td>An interpretation of the findings (meaning and implications) from the image(s) must be provided in the text</td>
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<td>11f</td>
<td>The legend associated with each image must describe clearly what the subject is and what specific feature(s) it illustrates</td>
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<td>11g</td>
<td>Markers/labels must be used to identify the key information in the image(s) and defined in the legend</td>
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<td>11h</td>
<td>If relevant, the legend of each image must include an explanation whether it is pre-experiment, intra-experiment or post-experiment and, if relevant, how images over time were standardised</td>
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