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Recommendations for Minimum Information for Publication of Experimental Pathology Data:
MINPEPA Guidelines

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Abstract

Animal models are essential research tools in modern biomedical research, but there are concerns about their lack of reproducibility and the failure of animal data to translate into advances in human medical therapy. A major factor in improving experimental reproducibility is thorough communication of research methodologies. The recently published ARRIVE guidelines outline basic information that should be provided when reporting animal studies. This paper builds on ARRIVE by providing the minimum information needed in reports to allow proper assessment of pathology data gathered from animal tissues. This guidance covers aspects of experimental design, technical procedures, data gathering, analysis and presentation that are potential sources of variation when creating morphological, immunohistochemical (IHC) or in situ hybridisation (ISH) datasets. This reporting framework will maximise the likelihood that pathology data derived from animal experiments can be reproduced by ensuring that sufficient information is available to allow for replication of the methods and facilitate inter-study comparison by identifying potential interpretative confounders.

Introduction

Animal models are integral to understanding the pathogenesis and therapy of human disease [1,2,3]. However, the lack of reproducibility of preclinical animal studies has become an increasing cause for concern [4,5] due to an inability to replicate drug target discovery studies [6,7] and the divergent results obtained with widely accepted animal models of human disease [8'9].

Many factors cause lack of robustness in the animal data. Some are inevitable, such as interspecies biological differences [10,11,12]. Many others are avoidable, including poor study design; inappropriate data analysis; peer review by individuals who have insufficient expertise in the species, model system or methods applied; failure to follow existing guidelines and career pressure to publish fast and often [5]. A lack of transparency in reporting, presenting as either incomplete disclosure of methods (sometimes due to journal word count restrictions) and/or a reluctance to report negative results often exacerbate these avoidable factors.

Pathology data are a crucial endpoint in animal studies, the importance of which is often overlooked by research teams [13]. Morphological data can often explain other phenotypic data and are highly translatable, allowing direct comparison of pathological processes and/or histologically identifiable changes in animals with the human disease that is being modelled. The reporting of pathology data is often limited to a figure (often restricted in size by journal formats so that diagnostic features are not, or only poorly, visible) and a brief description in the text (sometimes found only in the figure legend or the supplementary data section). Specific technical information is often not included or is incomplete in published reports, perhaps in part because many non-pathologists consider that basic pathology techniques are completely standardised in comparison with modern molecular, biochemical, genetic and "omics" platforms, or are arcane. In reality, many variables can affect the outcome of even "routine" pathology techniques, and failure

to define and account for these factors in a publication makes it impossible to fully review or critically evaluate the results.

Improving reproducibility of animal models will require all aspects of studies to be transparent and open to scrutiny by journals, peer reviewers and readers. Guidelines are available and are being adopted to a variable degree by journals [14] covering general aspects of experimental design in animal studies (Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines, [15,16,17]; technique-specific details (MIQE guidelines for rtPCR, [18,19]); the application of protein and nucleic acid biomarkers [20] and preclinical imaging techniques [21]. In human diagnostic medical pathology in the UK, guidance is provided for reporting pathology findings from many organ systems and conditions in the minimum datasets published by the Royal College of Pathology [22]. In contrast there is limited guidance on the reporting of pathological findings from human tissues in research [23] and no guidance to date covering the reporting of pathology data from animal tissues.

The aim of this paper is to recommend the minimum information for publication of experimental pathology data (MINPEPA) (in addition to that suggested by the ARRIVE guidelines) that should be included when presenting pathology datasets from experimental animal studies. The guidance has been developed as a collaboration between expert pathologists, pathology journal editors and statisticians following consultation with the NC3Rs who sponsored development of the ARRIVE guidelines in the UK and should provide other researchers with the ability to reproduce and validate novel findings. The information presented here may also serve as a primer of factors that can affect pathology data, and its interpretation, for researchers unfamiliar with histochemical, immunohistochemical (IHC), in situ hybridisation (ISH), and other routine techniques in morphological pathology. *Critical Pathology Data to Be Reported*

1. ARRIVE guidelines

The ARRIVE guidelines provide clear guidance on the minimum information for reporting all experiments involving animals [15]. They include information that allows an experiment to be reproduced, and the internal and external validity of the results to be assessed, where *internal validity* is the extent to which the effects detected in an experiment are truly caused by the treatment and *external validity* is the extent to which the results can be generalised to a broader population. However the ARRIVE guidelines are generic, and additional pathology-specific criteria are needed to complement them to ensure that animal-based experiments can be interpreted. For example, features unique to pathology (e.g., image preparation) generally need detailed consideration to permit their critical analysis by readers. The critical features recommended for reporting pathology data are summarised in Table 1.

2. Experimental design - managing variation and generalisability

An experiment is designed to manage sources of variation and isolate the effect of interest such that there is confidence that any differences observed are due to the effect of interest and not arising from any other source. This approach requires researchers to identify potential sources of variation in studies in advance so that they can be managed. Most sources of variation are managed by standardisation (e.g., use of the same reagent lot or staining technique). At times it is not possible to standardise a possible source of variation (e.g., batch-to-batch variation in staining), and in this case the study design needs to account for this (e.g., randomising the slides for staining between batches so that any variation is equal for both control and treatment groups). It is therefore important to report how sources of variation are managed.

Generalisability is the ability to apply the conclusion from an experiment to a broader scenario. For pathology-related experiments, it is important that tissues examined are described in detail (including whether these were harvested and handled consistently, e.g., sampling the

same liver lobe in every animal) so that it is clear that the evaluation was limited to a subset of tissues or alternatively that the whole animal was examined systematically. Where only a limited number of tissues are examined to provide an illustration, this fact should be clearly stated. Targeted examination of limited tissue sets reflecting a particular research interest may help address a specific hypothesis, but will generally not allow full characterisation of an animal model. Methodological standardisation, for example focusing only on a single time point or gender, limits variation and increases sensitivity. What is important in reporting a pathology dataset is to fully explain the study design and objective(s) while stating in detail the methodological considerations that impose possible limitations on the conclusions.

3. Tissue harvesting and sampling at necropsy

The methods section of research reports should give a detailed account of the techniques used to acquire the pathology data at necropsy including method of euthanasia, measurements taken (e.g., body weight), samples gathered (e.g., fluids, tissues) and whether animals were necropsied in a specific order. A detailed discussion of methods used to analyse all samples, such as clinical biochemistry and haematology for fluids or histopathologic procedures and morphometry techniques for tissues, should also be provided.

Tissues for pathology evaluation are generally harvested after death. The speed with which samples are collected and fixed impacts cell and tissue preservation. Death does not result in immediate cessation of metabolism, and continuing biochemical changes can result in ultrastructural alterations and, ultimately, autolytic degradation of cells and tissues. These changes alter not only the structural appearance of tissue and cells, but also the antigenicity of tissue proteins [24]. All biochemical processes are heat-dependent, so the rate of change can be altered by the ambient temperature. Important factors to be reported are the time from

death until tissue harvest and initial fixation and whether the carcass was kept refrigerated (4°C) or held at another temperature.

4. Fixation and post-fixation techniques

The type of fixative (chemical or freezing); method of its application (immersion or perfusion); length of time in fixative; and temperature of fixation can affect both tissue morphology and the subsequent uses of tissue samples. Neutral buffered 10% formalin (approximately 3.7% formaldehyde) is the most common fixative in routine use for research studies, providing good preservation of morphological detail by cross-linking molecules to stabilize cell and tissue structures. This fixative results in a tissue cellular appearance that is well recognised by pathologists and most non-pathologists who perform animal-based research. Formalin has its limitations, including tissue shrinkage [25] and the potential to form molecular cross-links that mask antigens and nucleic acids from IHC and ISH detection [26]. Paraformaldehyde is preferred as a fixative in some laboratories because it lacks additives (such as methanol) that are used to stabilise formaldehyde for long-term storage. Alcohol (ethanol or methanol) and acetone are sometimes used as fixatives since they coagulate tissues by removing water rather than cross-linking molecules. These coagulating agents generally result in poorer morphology and mRNA preservation but may lead to better antigen preservation relative to cross-linking fixatives. Freezing is often preferred for molecular pathology techniques since it does not damage molecular structure, but the absence of chemical preservation generally results in comparatively poor morphology. Clear reporting of the fixation method, including the fixative used, volume of tissue to fixative ratio (ideally >1:10) and the length of time spent in fixative, is essential when evaluating and seeking to reproduce pathology data. Specific details should be given regarding the fixative procedure, in particular the fixative composition—agent(s) and

its/their percentage strength(s)—as well as the sequence in which multiple fixatives are applied.

Post-fixation techniques may be required to allow examination of specific tissue types. The most common example of this is the need to decalcify tissues containing bone to allow them to be sectioned by routine techniques following embedding in wax. Decalcification generally relies on the use of calcium chelators or strong acids which can significantly affect tissue morphology and protein and nucleic acid preservation. Exact details of post-fixation processing should be referenced [27].

1. Tissue processing

Tissue processing procedures generally use aqueous alcohol gradients and a transitional clearing agent (miscible with water and wax) to permit embedding of water-rich tissues in a wax or resin. Processing timings and schedules vary among laboratories, and there is little information on the effect that these variations have on tissue morphology. Health and safety concerns around historical clearing agents (e.g. benzene, toluene, chloroform and xylene) mean that there has been considerable interest in finding safer alternatives [28], but objective comparison of these newer agents (e.g. D-limonene based substitutes and isopropanol/mineral oil mixtures) with current practice has been limited. In practice, variations in chemicals or methods can produce subtle differences in tissue morphology and staining. In particular, xylene-free processing has been associated with increased staining variability (C. Scudamore, MRC Harwell, and K Smith, Royal Veterinary College, unpublished observations). Therefore, tissue processing schedules, processing equipment and reagents should be referenced or included in supplementary data. Following processing, tissue sections are cut

from blocks of embedded tissue. The thickness of tissue sections should be indicated as it can significantly alter the appearance of cells and tissues.

2. Conventional “tinctorial” staining.

A variety of stains are commonly used for light microscopy with the most common being the “standard” combination of haematoxylin and eosin (H&E). Other more specific stains may be used for demonstrating structural components including carbohydrates (periodic acid–Schiff); connective tissue (trichrome, picosirius red, Martius scarlet blue); fats (oil red O, Sudan black) and iron (Perl’s Prussian blue reaction). It is important for researchers to be aware of what stains actually demonstrate, the correct staining procedure, possible artefacts, the effect of fixation and processing on the stains, and their limitations. Stains are all dyes whose tinctorial characteristics can vary depending on their source and use. Even common stains like haematoxylin come in multiple chemical forms (often with differing stabilisers and impurities). The stain reference number, source, technique and randomisation method implemented should all be reported. Variations in staining among batches of slides stained with a particular dye can significantly affect the appearance of tissues and may be sufficiently extreme to confound interpretation [29]. One of the major sources of staining variability is the use of manual rather than automated procedures; if possible, manual staining should be avoided. For all staining techniques, precautions must be taken so that any tinctorial variability does not lead to an erroneous conclusion. This can be accomplished by including a mix of control and treated sections in each batch stained.

3. Molecular pathology techniques (immunolabelling and in situ hybridisation)

Immunolabelling is common in pathological analysis of human tissue and is becoming increasingly common for studies involving animal (especially mouse and, in diagnostic settings,

dog and cat) tissues, allowing detection of specific molecules. Immunolabelling includes the techniques of immunohistochemistry (IHC, detection via an enzyme substrate reaction) and immunofluorescence (IF, detection via a UV-activated fluorochrome). Many primary antibodies can be detected with either method, but optimal results are obtained with IF for some techniques and IF is often preferred for multicolour labelling of multiple antigens. IHC developments [30] are beginning to allow detection of the protein phosphorylation state (e.g. phospho-Akt, phospho-Mek, phospho-Erk) and other post-translational modifications as well as identifying specific alleles/single nucleotide polymorphisms (SNPs) of genes (for example [31]).

To ensure reproducibility, reports of immunolabelling should define the exact details of the labelling procedure. The batch of antibody should be described (supplier, monoclonal [including clone identification and immunoglobulin subclass] or polyclonal, and species of origin), including whether it has been affinity purified and whether the specificity of the antibody has been validated by Western blotting. Labelling conditions should be defined precisely (antigen retrieval methodology, antibody concentration or dilution, duration and temperature of incubation, detection methodology with full details of secondary and tertiary antisera, use of an automated or manual system). Controls for both reagents and tissues should be given [32,33]. Immunofluorescence studies should include details of the detecting reagents and the conjugated fluorochromes, their order of application in multicolour studies, the fluorescence microscope, and the image capture software used.

Ideally, each immunolabelling run should include for each primary antibody a known positive control (either a suitably characterised tissue or cell block composed of cells expressing the antigen either naturally or as a result of transfection) and a negative control. The latter can be achieved by using a species- and isotype-matched primary antibody with specificity for an

irrelevant epitope, at the same protein concentration as the primary antibody of interest.

Simply substituting the primary antibody with diluent (e.g., PBS) is not regarded as an adequate control on its own but may provide additional information on binding of secondary antibodies [33,34]. The use of mouse primary antibodies on mouse tissue (“mouse on mouse”) requires special consideration because of the potential for artefact introduced by the use of anti-mouse secondary antibodies that non-specifically detect residual serum proteins within mouse tissue sections.

In situ hybridisation (ISH) can detect mRNA and DNA on formalin-fixed, paraffin-embedded material and is often performed on an automated platform. As for IHC, a detailed description of the probe characteristics (i.e., sequences, lengths), probe cocktail, tissue pre-treatment and probe detection method should be reported. Appropriate controls must also be described, including (i) positive and negative controls for the probe set being used (ideally blocks made from cell lines known to express and not to express the target RNA/DNA sequence) and (ii) RNA/DNA preservation in the tissue of interest by means of probing a serial section for expression of a reference target such as Actb (Actin-beta) when detecting mRNAs as this offers consistent levels and patterns of expression in different cells and tissues. Ideally, a further negative control should be performed on the tissue of interest, using an irrelevant probe set (e.g., directed against a bacterial target) or sense probes (for RNA-ISH), to detect any non-specific labelling. When ISH for DNA is performed to look for translocations, other rearrangements and copy number changes, similar principles and considerations apply to those relevant for investigations of human tissues [35,36].

Similar considerations apply when performing and reporting other molecular pathology applications used on animal tissues. Where laser capture microdissection is used, “before” and “after” pictures are useful to confirm that the correct cells have been sampled. Other common

research procedures include the use of enzyme histochemistry (to detect functional proteins) and lectin histochemistry (to demonstrate carbohydrates added during post-translational modification of proteins). In general, full details regarding reagents, methods, and control tissues should be given in all reports.

4. Lesion terminology and scoring

The pharmaceutical industry and governmental organisations have performed pathological analysis on laboratory animals in a systematic way for many years and have produced many publications giving terminology for describing and naming background pathology [37]. An ongoing global effort to harmonise terminology via the InHAND (International Harmonization of Nomenclature and Diagnostic Criteria) initiative [38] provides a comprehensive resource to allow pathologists and researchers to identify and interpret common pathological changes. Such nomenclature may not be directly translatable to descriptions of human disease entities for non-pathologists. For this reason, an ontological approach to recording pathology data is also advocated by some parties [39].

Lesion scoring systems depend on the nature of the finding. Neoplastic lesions generally are not scored. For non-neoplastic proliferative and non-proliferative lesions, semi-quantitative scoring of the severity of lesions has proven a robust technique for high-throughput toxicological pathology analysis [40,41] that can be applied to other experimental pathology studies. In some instances, established scoring techniques are available in the literature; such as those used for colitis, experimental autoimmune encephalitis, lupus nephritis and collagen-induced arthritis [42]. For certain organ systems, detailed classification schemes have been defined for animal models with the aim of relating animal scoring criteria as closely as possible to the WHO (World Health Organisation) equivalents for human diseases (e.g., the murine classification of haematopoietic neoplasms; [43,44]). To allow comparison with other animal

studies and human disease, if utilised, the basis of any terminology used, the scoring system employed, and any modifications to published methods should be referenced or described. If a previously published scheme is lacking, text descriptions and/or illustrations of the criteria used for each score in the system must be provided.

Semi-quantitative lesion scoring techniques result in the production of numerical data. The resulting lesion scores should be displayed in some form (e.g., bar graphs, incidence tables) in the body of the paper or supplementary data [45].

5. Data processing and statistical analysis

As with all experiments, pathology data should only be discarded if there are clear technical reasons to do so, rather than because a result appears to be an outlier. Ideally, reasons for discarding data should be defined in advance of the experiment and should be applied equally to control and treatment groups. These reasons include technical concerns (e.g., tissue fixation / decalcification problems, inappropriate orientation of material at embedding, insufficient sample for analysis); inappropriate results for control samples; and pathological changes more likely to be related to the use of the animal strain or species. If data are discarded, the reasons for doing so should be reported.

Statistical analysis is used to assess whether a difference is likely a sampling effect or a real population difference. Common pathology data types are semi-quantitative (ordinal [e.g., obtained from scoring of lesions or IHC labelling]) or quantitative (continuous [e.g., measurements like length, weight and cell number]). Selecting the appropriate statistical test is a function of the biological goal, the data characteristics, and the experimental design (e.g. the structure in the data [45]). Structure in data can arise from how experimental variation is managed (e.g., if multiple samples were stained in a batch and batch variation is high, then

samples from the same batch will be more similar) or from the design (e.g., depending on the experimental unit). The experimental unit (for statistical analysis) is the physical entity which can be assigned, at random, to a treatment. The experimental unit (whether this is an animal, tissue or fluid sample, or an entire litter of offspring) in pathology studies needs to be defined and treated as the unit in advance. If the statistics used are inappropriate, then the control of false positives (erroneous calls) will be poor. If in doubt regarding the appropriate tests, it is critical that a statistician be consulted when designing the study. For reporting purposes, it is necessary to report the experimental design, the experimental unit, the statistical analysis applied, the confidence level (α), and the calculations to ensure appropriate statistical power.

6. “Blind” review

There is much discussion regarding whether the concept of blind (also known as coded or masked) review is applicable to pathology data, and under what circumstances it should be used [46]. Blind review involves the evaluation of samples (usually stained tissue sections on slides) without knowledge of the experimental group from which the tissue is derived. Blinding to one pathology sample type (e.g., tissue sections) does not necessarily mean that the observer is also blind to other data gained during the course of a study. Blind evaluation is considered important to avoid unintentional experimenter bias affecting the study outcome. It has been shown for studies involving animals or cell lines that unblinded (decoded) analysis is three times more likely to yield a positive result [47].

Blinding is recommended in pathology studies where well-defined criteria for specific histopathological findings can be defined prior to the study [48]. In toxicological pathology studies, blinding *a priori* is not considered best practice because it may reduce sensitivity, particularly when changes represent part of a continuum or variation from “normal”

background findings, and reduces the value to be obtained from having concurrent controls [49,50]. Blind analysis also does not help the “inductive reasoning” process that is required to detect changes in novel animal models and which allows best use of an experienced comparative pathologist in interpreting the relevance of lesions. In practice, obvious tissue changes may effectively and rapidly unblind the examination. To address this unique issue in toxicological and experimental pathology, a common approach is to do an initial analysis of all tissues with full knowledge of the experimental details to ensure that all findings are detected and then use a subsequent blind review to confirm subtle or borderline differences in selected tissues [41,50,51]. The decision to evaluate slides in a blinded or non-blinded fashion or alternatively as non-blinded initial evaluation followed by blinded review needs to be considered on a case-by-case basis, and the rationale for the decision reported in the manuscript.

7. Data interpretation

Histopathology data may be produced by a subjective (descriptive or semi-quantitative) visual interpretation of tissue sections or by objective quantitative image analysis. Both approaches have considerable merit, and their relative usefulness depends on the circumstances and the pathophysiological questions to be answered [46,52,53]. The quality of the data and its interpretation will generally depend on the experience of the analyst looking at the sections. There are a number of examples where interpretation by inexperienced or unqualified researchers (‘do-it-yourself pathology’) has resulted in inaccurate interpretation of findings in publications [54], and there are many more undocumented examples where the lesion images in papers do not illustrate claims made in the text. Morphological pathology is a subjective skill, and there is no substitute for specific training and experience [55]. Fully trained experimental and comparative pathologists are usually best placed to report and interpret

pathology data, but there is a severe and chronic shortage of such professionals available to researchers [56]. While researchers should ideally collaborate with pathologists to ensure the accuracy of their data, where this is not possible investigators should ensure that they have appropriate understanding of the techniques involved and sufficient experience to identify and interpret lesions accurately. The quality of the pathology data is perhaps uniquely dependent on the skill and experience of the observer, and for this reason the person(s) responsible for acquiring, interpreting, and reporting these data should be clearly identified in the authors list or acknowledgements section. In addition, journal editors should ensure that suitably qualified pathologists review submitted publications where morphological data is critical to understanding.

8. Preparation and presentation of figures

The representation of pathology data in manuscripts and supplementary material is often unclear, inconsistent and/or limited to a “representative” illustrative figure. Most journals now offer colour publication of images (within text or supplementary material); in most instances, colour images are preferable. Any images presented in a figure should be representative, ethically prepared, and of sufficient size and quality to allow relevant features to be seen. An accompanying figure legend should describe specifically and clearly the essential features that readers including non-experts need to understand the lesion without reference to the text. A working knowledge of colour contrast and the effect of grayscale may enhance lesion visibility [57]. If good quality photomicrographs cannot be placed within the main manuscript due to the journal’s formatting requirements, then higher quality low- and high-power images (and potentially virtual slides) should be supplied in supplementary data. It is likely that the increasing use of digital pathology and associated computer analytical technologies will

facilitate image publication. Where data are summarised (e.g., in boxplot comparing scores), then it is critical to include information such as the numbers of animals / tissues analysed to allow the plots to be appropriately evaluated.

Unethical image presentation and manipulation is a significant factor in scientific fraud [58]. The minimum degree of image manipulation should be used to ensure that a clear, representative and reproducible image that represents the original data is shown in the final publication [59]. Ethical manipulations may include global corrections of white balance, uneven background and colour balance; conversion from colour to grayscale or among colour modes (e.g., RGB to CMYK); and image sharpening. Many journals actively encourage submission of RGB images as these will allow the best representation of the captured image when viewed on screen, leaving conversion into the more restricted CMYK colour space in the hands of their printers. Cropping and straightening an image may be required to present an image correctly but should not be used to create a false impression of the lesion or avoid showing flaws. Resizing by upscaling will increase the number of pixels, but will create data that wasn't originally present. Certain file types can result in "lossy compression" (loss of image data) as occurs with JPEG formats; TIFF files with LZW compression are "lossless" yet may reduce file sizes significantly. Certain manipulations may be unavoidable when producing an image to journal specifications for publication but should definitely be avoided when making measurements from images. A copy of the original image prior to manipulation should be retained in the pathologist's records along with the software log (for example, from a given version of Adobe Photoshop or Image J) recording the manipulations used to create the published image. A statement regarding the adjustments made should be included in the methods or in the figure legend (for example, "Images were individually adjusted in Adobe Photoshop CS5, including white balance and contrast adjustment"). Manipulation of focal

areas of an image [58], the use of some software filters, and cloning tools are examples of unacceptable image manipulations [59].

Care should be taken when specifying the magnification of images. Mentioning the original magnification provided by the microscope when the image was taken does not take into account the effect of splitters or camera attributes, or any further image manipulation or resizing [59], and generally should be avoided. As it is usually not possible to predict the final size of the image when it is published, it is best to include a scale bar in the original image that will be scaled as the image is manipulated.

The principles for documenting clinical and laboratory images in publications (the CLIP principles, [60]) are endorsed by the *Journal of Pathology* and provide clear guidance that covers the points discussed above. The summary principles are given in Table 2.

Conclusion

Morphological pathology data are extremely valuable in defining the tissue responses in animal models and allowing for translation of animal-derived data to predict biological responses in human and animal disease conditions. The approaches used in traditional pathology are well established, but the numerous reagents and methods mean that there is not a universal standard for even the most “routine” techniques. Analysis of tissue sections mounted on glass slides or as virtual slide images is still largely dependent on a human observer, and the quality of the results and their interpretation is therefore uniquely dependent on that individual’s training and experience as a morphologist. Here we extend the ARRIVE guidelines by suggesting a recommended 12-point checklist of critical pathology data to be reported in publications. This framework for reporting pathology data will maximise the likelihood that pathology data derived from experiments in animal models can be reproduced and understood.

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Author contributions

CLS, MJA and ES wrote the initial draft. NK provided expert input on experimental design and statistics. CSH and RP provided input on image presentation in line with *Journal of Pathology* guidelines. BB edited the major drafts. MJD, KS, ESW, BW, JE and CB provided additional expert comments and edits.

Table 1: Minimum set of critical pathology data to be reported in addition to the ARRIVE guidelines

		Recommendations
1	ARRIVE	<ul style="list-style-type: none"> • Generic requirements for all in vivo experiments
2	Experimental design	<ul style="list-style-type: none"> • Detail the experimental design • Describe efforts to minimise variation and bias (e.g., standardisation of reagents, randomisation of groups) • Provide details of tissues examined, numbers of animals and time points • Controls (negative and positive)
3	Tissue harvesting	<ul style="list-style-type: none"> • Sample list (tissues and fluids) • Tests performed (clinical chemistry, haematology, histopathology, morphometry, etc.) • Interval between death and specimen collection / initial tissue fixation • Temperature at which the body was held until specimen collection / initial tissue fixation
4	Fixation	<ul style="list-style-type: none"> • Fixative type(s): chemical (cross-linking vs. coagulating), physical (e.g., freezing) • Fixative composition: single agent vs. multi-agent (e.g., Karnovsky's) • Tissue: Fixative volume ratio • Number of fixative changes (and the interval between each exchange) • Method of fixation: immersion vs. perfusion • Length of storage in fixative • Temperature of fixation • Use of post-fixation (e.g. for decalcification)
5	Processing technique	<ul style="list-style-type: none"> • Reagents for tissue dehydration and clearing • Sequence of dehydration and clearing steps • Lengths of dehydration and clearing steps • Temperatures of dehydration and clearing steps
6	Conventional/Tinctorial stain	<ul style="list-style-type: none"> • Reagents (including vendor) • Procedure (technical details and/or literature citation)
7	Molecular techniques	<p>Immunohistochemistry (IHC)</p> <ul style="list-style-type: none"> • Antibody properties: class/subclass, nature (monoclonal vs. polyclonal), host species, clone, lot, vendor • Antibody manufacturing: affinity purification, conjugate labelling • IHC procedure: antigen retrieval methodology, blocking procedures, antibody concentration, incubation times and temperatures, detection methodology, counterstain, process (manual vs. automated), special considerations (e.g., mouse-on-mouse kits)

		<ul style="list-style-type: none"> Control materials: reagents (specific vs. spurious) and tissues (positive vs. negative) <p>In situ hybridisation/cytogenetic (ISH)</p> <ul style="list-style-type: none"> Probe properties: type (DNA, RNA, peptide nucleic acid (PNA)), length (oligomer vs. longer sequence), and sequence (including database accession no.), and if applicable lot and vendor Probe manufacturing: conjugate labelling ISH procedure: probe sequences and concentrations, cocktail recipes, incubation times and temperatures, detection methodology, counterstain, process (manual vs. automated) Control materials: reagents (specific vs. spurious) and tissues (positive vs. negative)
8	Lesion terminology and scoring system used	<ul style="list-style-type: none"> Terminology: definitions, ontology, and/or literature citation Scoring system: detailed criteria (text and ideally visual depictions) and/or literature citation Digital imaging algorithms
9	Data processing and statistical analysis	<ul style="list-style-type: none"> Experimental unit Reasons for data exclusion and how many samples were affected Statistical tests used
10	Blind (coded) review	<ul style="list-style-type: none"> Endpoint(s) subject to blind analysis Timing of blind analysis (<i>a priori</i> vs. <i>post hoc</i>) Rationale for approach taken (blind review from the beginning or blind or non-blind initial evaluation followed by blind review)
11	Data interpretation	<ul style="list-style-type: none"> Qualifications and/ or training of person acquiring, interpreting, and reporting pathology data, to be included in author list or acknowledgements section
12	Preparation and presentation of figures	<ul style="list-style-type: none"> Nature of lesions depicted (representative vs. exceptional) <u>Global</u> image manipulations: white and colour balance, conversion (e.g. colour to grayscale), image sharpening Scale bar

Table 2. The CLIP Principles for documenting pathology images in publications (adapted from Lang et al 2012 [60])*

The following data regarding pathology images should be reported:

1. The subject of the image
2. Details of the acquisition of the image
3. Details of the selection of the image
4. Details of any modifications to the image
5. Important details of the image itself
6. Details of the analysis or interpretation
7. Implications of the image

*It is recommended that potential authors consult the full publication by Lang et al 2012 [60] for further details.

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