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OGA Only Good Antibodies

Defining the role of antibodies in improving research reproducibility

NC3Rs and Only Good Antibodies community meeting report

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Introduction

1. This report summarises the discussions and outcomes from the "Defining the role of antibodies in improving research reproducibility" meeting held on 27 February 2024. The meeting was co-organised by the NC3Rs[1] and the Only Good Antibodies community (OGA)[2]. The meeting brought together stakeholders from across the biosciences to define a strategy to improve the integrity and reproducibility of biomedical research that relies on commercial antibodies and to explore how this supports efforts to reduce the use of animals in research. Stakeholder groups represented included antibody manufacturers and end-users, the pharmaceutical industry, academic researchers, publishers, research funders and experts in research culture and research improvement. Key objectives of the meeting were to:

    - Identify actions needed to improve the reproducibility and integrity of biomedical research that relies on antibodies.
    - Determine the type and level of support required from each stakeholder group to deliver these actions.
    - Share perspectives on any barriers that might prevent stakeholder groups from taking these actions.

Background

2. An estimated $28.2B per year is spent in the US on preclinical research which is not reproducible, with issues relating to biological reagents likely being the biggest contributor [1, 2]. Similar analyses of the impact of reproducibility on the economics of basic research are hard to come by, but the scale of the problem is likely to be the same. Antibodies are one of the most common and important biological reagents used to understand drug targets in preclinical research and identify and isolate molecules of interest more generally, yet around $1B annually in the US alone is wasted on poor-performing

[1] The NC3Rs is the UK's national organisation that provides scientific leadership to the development and implementation of new models and tools that minimise the use of animals in research and testing and/or improve animal welfare (the 3Rs).

[2] OGA is a collaboration of biomedical and behavioural scientists working with diverse stakeholders to improve research that uses antibodies

antibodies [3, 4]. This represents a significant waste in animals used in the production of these antibodies and in the research studies where they are employed.

3. How well an antibody performs in binding to its target (defined as 'characterisation') will vary between applications, protocols and the cell or tissue types used for each experiment. As such, it is important that their performance qualities are assessed for each context of use (defined as 'validation') and it is the responsibility of the researcher to carry out these studies for their specific protocol. Consensus approaches for the validation of antibodies have been described [5].

4. The complexity and scale of antibody use may have contributed to a marketplace that contains a high proportion of poorly performing antibodies [6-8]. The YCharOS initiative (see paragraph 8) recently quantified for the first time the likely scale of the problem [9, 10]. Greater than 50% of 614 commercial antibodies against 65 neuroscience-related proteins failed characterisation experiments in at least one of three commonly used applications (western blotting, immunofluorescence and immunoprecipitation; Figure 1). It was also found that each of these 65 proteins was linked to an average of ~12 published papers that presented data using poorly performing antibodies, perpetuating the use of these reagents and the associated issues of reproducibility. Moreover, 88.4% of papers using such antibodies in immunofluorescence alone did not present any relevant validation data (Figure 1).

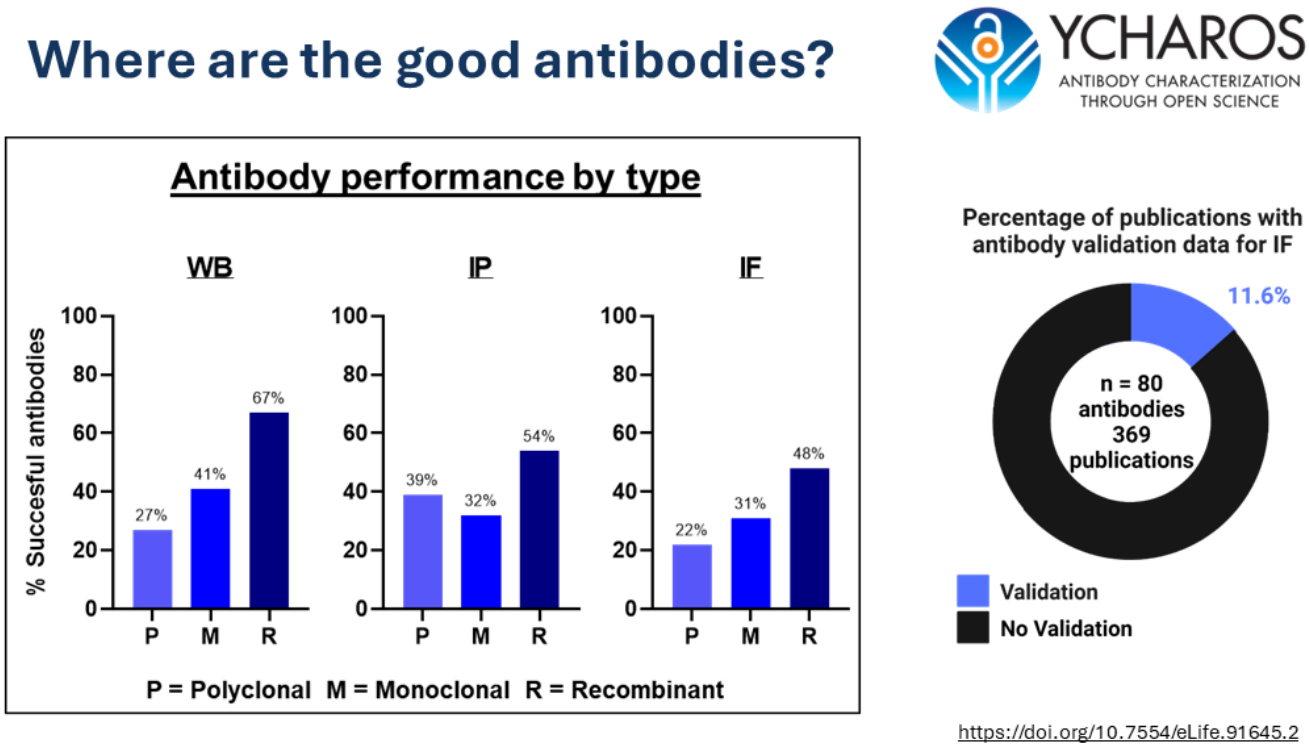

Figure 1. Adapted from Ayoubi *et al* [9]. Performance of antibodies tested by a third party independent open science entity (YCharOS, left). Frequency of presentation of validation data in the literature (right). WB, western blot. IP, immunoprecipitation. IF, immunofluorescence.

5. The problem is complex and includes issues with quality control of the reagents, inherent lot-to-lot variation in some types of antibodies and a lack of appropriate validation experiments performed by researchers [11]. Overcoming this represents a significant challenge but has the potential to make biomedical research more efficient, reliable and reproducible. It will also limit the number of animals used both in antibody production and those wasted in research that uses unsuitable antibodies.

Workshop summary

6. The workshop was made up of three sessions:

    - Presentations on the status of research involving antibodies. This included a summary of the different technologies used to produce antibodies (traditional animal-derived polyclonal and monoclonal antibodies and non-animal derived antibodies and affinity reagents), evidence on the relative performance of antibodies produced using each approach, and issues around end-user adoption of more robust antibodies and best practices in antibody validation.

    - Perspectives on potential methods for improving the reproducibility of research using antibodies from stakeholders in different sectors, including academia, research funding and the pharmaceutical industry.

    - Open discussion sessions to explore potential actions each stakeholder group could take to improve reproducibility of research using antibodies and any perceived barriers to these.

Session 1 – Antibody technologies and an introduction to the antibody reliability crisis

Accelerating the replacement of animal-derived antibodies (Dr Rachel Eyre, NC3Rs, UK)

7. The NC3Rs has established a programme of work to accelerate the replacement of animal-derived antibodies. This was initiated with a meeting in June 2023 [12] that identified several barriers to the uptake of non-animal derived antibodies (NADAs) and affinity reagents (ARs). These included a lack of awareness of NADA/ARs, inertia amongst scientists to deviate from tried and tested reagents that have been published in the peer-reviewed literature and concerns over the validation status of NADA/ARs and the costs and time required to adopt these. An alignment was evident between promoting the uptake of non-animal derived technologies such as recombinant antibodies[3] and

[3] Recombinant antibodies are produced using recombinant DNA technologies. This relies on determining the DNA sequence that encodes an antibody. These sequences are cloned into transfection plasmids and delivered into cells *in vitro*. The cells then produce the antibodies, which are secreted into the cell culture media.

initiatives such as OGA [11] and the YCharOS platform [9] which aim to promote the use of the most reliable and reproducible reagents for biomedical research. Outcomes from the NC3Rs workshop, including a series of next steps for moving the programme forwards, are available in the meeting report [12].

Antibody characterisation in a trusted open science ecosystem (Prof Aled Edwards, Structural Genomics Consortium and University of Toronto, Canada)

8. The YCharOS (Antibod**Y Char**acterization by **O**pen **S**cience) platform is an open science initiative where antibody manufacturers have partnered with research funders and the YCharOS team[4] to perform standardised antibody characterisation experiments [10]. To date YCharOS has evaluated the performance of ~1000 antibodies directed at ~100 human protein targets working with, but independently from, major reagent antibody manufacturers. Performance of each antibody was defined as their ability to correctly bind to the protein of interest in western blot, immunoprecipitation and immunofluorescence experiments. This work has shown that:
    - Many scientific publications use antibodies which do not correctly identify the protein of interest.
    - Recombinant antibodies perform better than traditional animal-derived monoclonal and polyclonal antibodies.
    - For many targets, recombinant antibodies already exist that are suitable for western blot, immunoprecipitation and immunofluorescence.
9. YCharOS' work is increasing the use of more reproducible tools and leading to new discoveries. For example, C9ORF72, a gene strongly implicated in driving Motor Neurone Disease (MND), had been studied almost exclusively using non-selective antibodies until 2019 [13]. However since YCharOS' discovery of highly selective reagents for this gene and their application in MND research programmes, these now make up 35% of cited anti-C9ORF72 antibodies in publications from 2023.
10. Scaling the YCharOS platform is a current challenge as there are over 20,000 human proteins and over six million commercial antibodies [14]. YCharOS is exploring the possibility of overcoming this by

[4] YCharOS is an independent third-party entity comprising academic labs in Canada and the UK who perform characterisation experiments on antibodies received from commercial manufacturers.

federating or franchising the platform, with different sites focused on specific areas of the genome or proteome.

End-user perspectives and stakeholder engagement (Dr Harvinder Virk, OGA and University of Leicester, UK)

11. OGA has conducted focus groups and surveys to understand how end-users select antibodies to use in their research. These demonstrated that researchers often select products based on the number or perceived quality of citations using an antibody in the literature, the reputation of a vendor and previous use of an antibody by colleagues. Barriers that prevent best antibody practices include a lack of awareness of the high frequency of poorly selective antibodies in catalogues and the published literature, how to perform antibody validation studies, and the time and cost associated with conducting robust validation experiments. The initiative aims to bring together different stakeholders to create an action plan that makes best practices in antibody selection and use possible, easy and more rewarding [11].

Session 2 – Models of research improvement

Lessons from implementation of the ARRIVE guidelines for *in vivo* research (Professor Emily Sena, University of Edinburgh, UK)

12. Cases of research malpractice are often the basis for implementing research integrity initiatives, however there is greater potential for impact through improving normal research practice across the whole research community. Experience with the ARRIVE guidelines[5] has demonstrated that despite widespread endorsement by the scientific community, implementing new research practice can be difficult and researchers can be reluctant to adopt new initiatives. To test methods of improving compliance to the ARRIVE guidelines Professor Sena and colleagues established a randomised controlled trial of researchers submitting manuscripts for publication [16], where some participants were asked to submit an ARRIVE checklist along with their manuscript. Results showed that simply asking authors to submit an ARRIVE checklist, without implementing additional checks by the editorial team, did not significantly improve compliance to the ARRIVE guidelines, it only ensured that they submitted a checklist. Complying with the guidelines was perceived as a high administrative burden

[5] The ARRIVE guidelines (Animal Research: Reporting of *In Vivo* Experiments), developed by the NC3Rs, are a checklist of recommendations to improve the reporting of research involving animals – maximising the quality and reliability of published research, and enabling others to better scrutinise, evaluate and reproduce it. The guidelines have been endorsed by over 1,000 journals across the life sciences in the UK and internationally. Further information about ARRIVE can be found on the ARRIVE guidelines website.

for authors and editors and with a lack of effective enforcement measures in place authors were not motivated to comply. Subsequent work [17] has identified opportunities to improve compliance, such as by simplifying the guidelines to focus on key essential items to facilitate their use in practice, providing a rationale and explanation for each item to improve understanding and ensuring compatibility with other reporting standards, such as those developed by the National Institutes of Health (NIH, USA) [18].

Research culture change strategies and research improvement (Dr Tim Errington, Centre for Open Science, USA)

13. Adoption of any new technology among a population is variable, with different groups taking up new practices at different rates. The groups have been described as “innovators”, “early adopters”, “mainstream” and “laggard” and different strategies may be needed to motivate each of these to adopt new practices. To enable large scale changes in practice among a community there must be (a) an advantage to the user, (b) the change must be easy and compatible with the user’s motivations and current practices, and (c) the change should be observable to others in the scientific community to encourage its wider adoption.

14. An example of changing practice in the scientific community is the widespread adoption of preprints, which can be viewed within the framework of “make it possible, make it easy, make it normal, make it rewarded, make it required” (Figure 2). Table 1 describes each of the actions that occurred to address each of the steps within the framework and support the acceptance of preprints as a normal part of the publication process. This approach should not be taken to be linear or sequential, but alignment between these levels is critical and should be considered when encouraging adoption of any new research practice.

Figure 2. Developing an approach to improve the use of antibodies in research by making best practices possible, easy and rewarding. Reproduced from Biddle *et al.* [11], adopted from the Centre for Open Science Strategy for Culture Change [15].

Culture change framework step	Action to support the acceptance of preprints
Make it possible	The establishment of Biorxiv
Make it easy	Preprints compatible with traditional publishing workflows
Make it normal	Social media sharing of uptake among scientists
Make it rewarded	Preprints recognised as valid outputs by funding organisations
Make it required	Funders introducing policies to make it required

Table 1. The actions needed to support the more widespread adoption of preprints as part of the normal process for publishing research outputs.

Insights from a pharmaceutical company (Dr Andrew Buchanan, AstraZeneca, UK)

15. AstraZeneca has standard approaches for reagent antibody validation [19, 20]. The approach used can vary by the stage of development of a project and the potential value of the target, but the focus is on finding an antibody that is suitable for use for the specific application and the scientific question rather than taking a 'one size fits all' approach. In the pharmaceutical industry, time and resources are dedicated to finding the correct antibody, because reagents that are not fit for purpose can lead to misdirection of drug development efforts and this can have both financial and ethical implications. Dr Buchanan highlighted that the end goal in industry is getting a product to patients rather than publishing research in journals, and this is better aligned with best practices in antibody validation.

Insights from a research funder's approach to preclinical tools and reproducibility (Dr Nicole Polinski, Michael J. Fox Foundation, USA)

16. The Michael J. Fox Foundation supports the development of robust preclinical tools, including appropriately characterised antibodies, through a specific tools programme. The Foundation made a strategic decision to develop this program to ensure that their funded research, and research in the field more generally, does not fail to reach robust outcomes towards a cure for Parkinson's disease due to poorly characterised reagents. One of the initiatives the Foundation has introduced is to fund studies to reproduce key Parkinson's research findings published in academic journals. It was important to the Foundation to ensure that key findings that would likely impact future research were reproducible. This initiative has faced many challenges that reflect the difficulty of trying to incentivise and promote reproducible research. These include a lack of interest from the scientific community to apply for funding to perform replication studies and a lack of support from the teams that originally published the research.

Session 3 – Open discussion session: Defining an action plan to making best practices in research using antibodies possible, easy and rewarding

17. The open discussion session included a round table panel discussion to introduce high-level concepts and opportunities for improved antibody practice, and stakeholder-specific breakout groups to explore these in more detail and develop action plans.

18. The round table panel discussion included individuals from each stakeholder group represented at the meeting. Participants were asked to provide a personal perspective on how their sector might contribute to making best practices for research using antibodies possible, easy and rewarding. The discussion was chaired by Dr Harvinder Virk (OGA) and was interactive between the panel members and meeting delegates. Round table panel participants were:

    - Dr Nicole Polinski (Michael J. Fox Foundation, USA)
    - Dr Lynne Howells (University of Leicester, UK)
    - Dr Hannah Cable (Abcam, UK)
    - Professor Anita Bandrowski (Research Resource Identification Initiative (RRID), USA)
    - Dr Andrew Chalmers (CiteAb, UK)
    - Dr Simon Goodman (The Antibody Society, USA)
    - Professor Aled Edwards (YCharOS initiative and the Structural Genomics Consortium, Canada)
    - Dr Catriona MacCallum (Wiley, UK)

    This was followed by breakout groups where concepts and action points for research funders, institutions and industry and publishers raised during the panel session were discussed in further detail.

19. The key points that emerged from the combined open discussion session were:

    a. Each stakeholder group has a role to play in improving the reproducibility of research using antibodies.

    b. Education on the important scientific, economic and animal welfare benefits of improving reproducibility of research involving antibodies is crucial for obtaining buy-in from researchers to successfully enable the adoption of new standards.

    c. Research institutions are well placed to provide the infrastructure and education needed to enable scientists to undertake good research practice. However, new initiatives require funding and it is unclear if existing institutional budgets would be sufficient to cover this. Convincing established principal investigators to change research methods can be difficult, but early career researchers are more likely to be receptive to improving research reproducibility. Research institutes could establish institutional strategies and policies on

improving research reproducibility to guide scientists, networks to support training and sharing best practice and experience and pilot funding schemes to enable researchers to explore new approaches. Capacity could be built in research institutions by providing education to early career researchers via doctoral training centres on methods for improving research reproducibility.

d. Funders are an important influence in changing research practice as end-user behaviour is strongly informed by funder requirements. The Michael J. Fox Foundation and the NC3Rs were highlighted as exemplars of funders who have developed tailored initiatives aimed at improving research reproducibility. In addition to the ARRIVE guidelines to support reproducibility in *in vivo* research, NC3Rs has also recently published their RIVER (Reporting *In Vitro* Experiments Responsibly) recommendations [21]. These are specifically tailored to reporting *in vitro* experiments, such that manuscripts describe the minimum information necessary for a reader to assess the methodological rigour and reliability of the study and facilitate reproduction of the methods and results. The recommendations include specific guidance on how the use of antibodies should be reported (e.g. using RRIDs), including a description of how they were validated. These initiatives could be used as models for other funders to adopt, in the way that ARRIVE has been, however it was noted that different funding organisations will have different levels of flexibility around the initiatives they can implement.

e. The potential for funders to request additional information on antibodies and research reproducibility in funding applications was agreed as important, but would need careful consideration and community buy-in. Completing funding applications is already a considerable time burden and additional work in this process may result in some pushback. However, this should not prevent advances being made in supporting research reproducibility at this important point in the research process. Funders have been successful in engaging applicants in the importance of justifying their animal use, their experimental design and the use of both sexes of animals, tissues and cells, all of which require further information to be included in applications, but ultimately enable more reproducible and translatable research. Following their workshop in June 2023, the NC3Rs has committed to reviewing their own policies and application processes as a research funder and to work with other funders to raise standards across the board. Applicants to NC3Rs funding schemes will be encouraged to consider applying more reproducible non-animal derived antibodies and affinity reagents and expected to justify their continued use of animal-derived antibodies, including how the antibodies have been/will be validated. In 2024 the NC3Rs launched a non-animal derived product validation grants scheme to support the studies that are required to build the experience and confidence necessary to encourage the adoption of these reagents and products for use in *in vitro* research.

f. Antibody manufacturers can contribute to education around antibody reproducibility by including instructions within product datasheets on how to perform validation experiments for different applications. They could also provide further information within their product catalogues that describes the characterisation experiments they perform on their own reagents. It was suggested that manufacturers should request that customers provide their validation data back to them so that they can identify and remove from catalogues those reagents that do not perform reproducibly in these studies. Incentives could be offered to facilitate this. It was noted that AbCam has already removed reagents from their catalogue following validation work they have carried out in partnership with YCharOS.

g. Interventions that are easy to implement should be identified and encouraged first to facilitate adoption by end-users or stakeholders. Using RRIDs to report antibodies used in research was cited as a specific example of a straightforward intervention. Interventions targeted at the point of making funding applications are difficult, because a detailed plan including the exact antibodies to be used in the research is unlikely to be finalised at the time of application. Similarly, interventions at the point of publication are difficult as the work has already been completed and it is too late for reagents to be changed. That said, it was noted that at both of these points in the research process it was important to encourage researchers to consider the impact on reproducibility of the reagents they use and how these are reported (e.g. RIVER recommendations).

h. There are opportunities to make some interventions to improve research reproducibility easier to implement through automation. Databases such as the RRID portal and CiteAb[6] could be used to identify antibodies in publications which have performed poorly in validation experiments. This would allow researchers to select from publications only those antibodies which have been sufficiently validated.

i. Trusted third parties, with no perceived conflicts of interest, are powerful in providing independent scrutiny of research reagents. This has already been demonstrated with YCharOS, which has been successful in engaging with manufacturers to identify and remove from the market those antibodies which may not be fit for purpose. Use of YCharOS data could be extended to other stakeholders, for example publishers could ask to see validation

[6] CiteAb is a commercial database that tracks the use of research reagents such as antibodies across the published literature. It helps researchers to understand and find more information about reagents used in research.

data for antibodies in submitted manuscripts, or at least if and how antibodies were validated, as described in the RIVER recommendations.

j. An independent, publicly funded platform to authenticate the characterisation of an antibody (e.g. NCBI mAb), linked to persistent machine readable identifiers (RRIDs) would be a powerful mechanism to ensure characterisation data was utilised by publishers.

k. Publishers could be a stakeholder group able to share awareness and education of best research practices with researchers. Collective action across publishers will be necessary to successfully implement new practices without detriment to individual journals. If a single journal insists that additional assessment of antibodies used in the research is included in submitted manuscripts, authors may choose to submit their work elsewhere. A consortium of publishers working together might overcome the potential competitive disadvantage.

l. The reward landscape in the current research environment represents a challenge to increasing adoption of antibody best practice. Researchers are rewarded for producing publishable findings, and the validation work required to ensure that antibodies are fit for purpose takes time and resources. Publishers and end-users will need to be rewarded for adopting better practices.

m. A roadmap setting out a vision for the development and adoption of consensus standards by 2030 is needed. This should include stakeholder-specific action plans that would enable this vision to be realised.

Summary

20. There was consensus among all stakeholder groups that action is necessary to improve the integrity and reproducibility of biomedical research that uses antibodies. Examples of initiatives already making progress in this area include YCharOS [9], which partners with antibody manufacturers to independently characterise commercially available antibodies. Although the initiative has only evaluated ~1000 antibodies targeting ~100 human proteins, this has already resulted in the removal of some animal-derived polyclonal antibodies from the market and the promotion of more reproducible recombinant alternatives. Data produced by this initiative could also be used to highlight publications that rely on unsuitable antibodies and reduce their use in future studies. Modifications to reagent databases such as the RRID initiative and CiteAb may enable this in future.

21. It was recognised that funders can play an important role in improving the research reproducibility. The Michael J. Fox Foundation's preclinical tools programme was highlighted as an example of excellence from a research funder. It was recognised that this model may not be suitable for all funding agencies, however all funders may cooperate with wider stakeholders to support an ecosystem where best practices in antibody characterisation and validation are promoted. The NC3Rs

is facilitating this through their RIVER recommendations and will work with funders and journals to encourage their widespread adoption in the same way that it has done so successfully for the ARRIVE guidelines. They are also reviewing their policies and application processes to encourage greater consideration of how the reagents used in research may impact reproducibility.

22. A roadmap towards improving reproducibility of research using antibodies could be created. This could initially focus on the adoption of RRIDs which would link to characterisation data such as that produced by YCharOS where available. This roadmap would describe the steps that each stakeholder would have to take in creating a research ecosystem that encourages the adoption of more robust reagents and better validation practices. It would outline where collaborative working between stakeholders is necessary and how actions could be co-ordinated.

23. Although it was agreed that improved antibody validation is necessary, further discussion around the best approach for this is needed. The five pillars approach for antibody validation was developed by the International Antibody Validation Working Group [5]. It outlines five different complimentary approaches that can be used to support or refute the selectivity of an antibody for its target. Several stakeholders at the meeting expressed some concern about utilising this framework from a robustness and utility perspective. For example, some felt that pillar three (termed 'independent antibody strategies'), which compared staining patterns (on tissue slices used in immunohistochemistry) of two different antibodies that recognise the same target and confirming they have the same pattern, was not robust enough. However, others felt it represented a reasonable compromise to allow progress.

24. A key gap identified was a lack of awareness of the issue of poorly characterised antibodies amongst end-users, and a lack of education and training available to promote best practices. Community champions will be needed to promote best practices to researchers. These individuals and groups may already exist within current networks, such as the UK Reproducibility Network, and need to be identified and supported by the wider stakeholder community to create an ecosystem where researchers are able to capitalise on current and future opportunities for antibody best practice.

Next steps for OGA and the NC3Rs

25. Based on the discussions held in the meeting, OGA and the NC3Rs will take the following next steps:

    a. Raise awareness of the importance of improving reproducibility of research involving antibodies among the research community. This will be achieved through the promotion of existing resources, for example OGA's free webinars with the UK Reproducibility Network (appropriate for all stakeholders) and with Responsible Research in Practice (aimed at end-users). The NC3Rs is developing an online platform to share information about NADAs/ARs to expedite their adoption and impact on research reproducibility. This will also highlight resources aimed specifically at improving the reproducibility of research using antibodies and

will link to resources developed by OGA and relevant others.

b. OGA will explore piloting an induction education programme with participating Doctoral Training Programs to train PhD students in approaches that can be used to perform antibody validation. They will also look to expand the OGA network of local champions with expertise across the UK and internationally. These champions would be local sources of expertise on antibody best practices. We would advocate for institutions to recognise their champions and provide time and resources to enable them to help their communities to perform antibody validation for their research.

c. OGA will collaborate with YCharOS to accelerate the positive impact that they are making on identifying robust reagents and promoting their use. OGA is piloting the first YCharOS site in the UK which will involve producing independent third-party antibody (or affinity reagent) characterisation data for new targets of potential importance to respiratory sciences. The NC3Rs will continue to support the work of OGA and YCharOS to generate the evidence base necessary to support wider adoption of the most reproducible antibody reagents. NC3Rs-funded researchers will be encouraged to include RRIDs for all antibodies when submitting their work for publication and to publish on the NC3Rs Gateway where RRIDs are already required. The NC3Rs will also drive the uptake of the RIVER recommendations, which describe the minimum information needed to assess the methodological rigour and reliability of *in vitro* experiments.

d. OGA will work with the community to develop consensus, stakeholder-specific and time-defined action plans to enable the implementation of practices to improve research reproducibility. A Delphi process will be used to develop these action plans with the endorsement of each stakeholder group. The aim of the action plan will be to create a roadmap that can be used to improve practices at each stakeholder level, highlighting the steps that are needed, with a shared vision and timeline. The approach will be informed by co-ordination between stakeholder groups and the involvement of experts in behaviour change and research improvement. The NC3Rs will support OGA to undertake the Delphi process, having undertaken a similar exercise during the revision of their ARRIVE guidelines (ARRIVE 2.0) to improve their usability.

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