The ASC
Animals in Science Committee

ANIMALS IN SCIENCE COMMITTEE

REPORT OF THE

PROJECT LICENCE STRATEGIC REVIEW SUBGROUP

REVIEW OF ANTIBODY LICENCES

1. Introduction

In 2021, the Animals in Science Committee (ASC) set up the Project Licence Strategic Review Subgroup. This was in response to a commissioning letter from the Minister asking for a strategic overview of granted licences. The purpose of this group is to carry out reviews of selected groups of project licences in order to provide strategic advice on specific topics to the Animals in Science Regulation Unit of the Home Office (ASRU).

In 2020, following an Opinion received from its Scientific Advisory Committee, EURL ECVAM[1] produced a report[2] concluding that '*non-animal-derived antibodies are mature reagents generated by a proven technology that are not only equivalent to animal derived antibodies, but in many respects can offer significant scientific advantages and economic benefits*'. The publication of the report has generated much interest within the scientific community and has prompted active discussions regarding the report's conclusions and its assessment of the feasibility of avoiding the use of animals in antibody production. The recommendation that competent authorities '*should no longer authorise the development and production of antibodies through animal immunisation, **where robust, legitimate scientific justification is lacking***' (emphasis added) is a clear ethical principle and legislative requirement that should in any case be being applied across all areas of proposed animal use for scientific purposes.

Following the publication of EURL ECVAM's report on antibody production, ASRU asked the group to prioritise a review of antibody licences, with outputs that should include (but not be restricted to) a set of principles to be used by ASRU in their assessment of project licence applications. The main remit was to look for themes or learnings relating to how applicants are currently providing evidence that they are complying with the requirement to ensure that the 3Rs are implemented.

The ASC agreed that a review of current licences in Great Britain authorising the use of animals for the development or production of antibodies would be useful in assessing how applicants are providing 'robust, legitimate scientific justification' for the continued use of animals in this area. This document presents the group's assessment of that process.

The 31 licences reviewed by the group were held by a range of establishments, including universities, contract research organisations, large pharmaceutical companies and small specialist antibody producers. They had all been granted during the last five years. Many of the licences assessed for this review pre-date the EURL ECVAM report, but at least one acknowledged the report, which was published during the applicant's previous licence.

In reviewing the licences, the group identified several common themes. These included a number relating to the main issue of how the licences were demonstrating that replacement technologies could not be used in place of animals. We also reviewed how the applicants were demonstrating that: the numbers of animals used had been optimised; how the experiments had been refined as far as possible; and how the 3Rs would be applied as fully

[1] European Union Reference Laboratory – European Centre for the Validation of Alternative Methods
[2] https://publications.jrc.ec.europa.eu/repository/handle/JRC120199

as possible during the course of the licence, taking account of contemporary developments in the field. In addition, we noted how applicants had explained the purpose of the work and the expected benefits it would bring and looked at governance issues, such as responsibility for ensuring that no non-animal alternative method is feasible, and the role of the AWERB in the approval process.

The group's review found inconsistency in the amount and quality of information provided by the applicants in seeking to demonstrate their justification for using animals in place of non-animal alternatives for antibody production. There were several good examples of the sort of justifications that we would expect, and which would also be essential for a harm / benefit analysis. We highlight some of these in this review.

Some licences, however, could have given a much clearer case for the use of animals. In this report we set out how we would expect applicants to justify the use of animals in antibody production. We also provide some recommendations for how future licence applications could better show the evidence for their implementation of Reduction, Refinement and the 3Rs, more broadly, as well as explaining the purpose and expected benefits of the work, and the governance systems in place.

2. Replacement

The *Animals (Scientific Procedures) Act 1986* requires (Section 2A) that '*wherever possible, a scientifically satisfactory method or testing strategy not entailing the use of protected animals must be used instead of a regulated procedure*'. Specifically, Schedule 2C (Standard Conditions) places a direct duty on project licence holders '*to ensure*' this happens. In addition, a formal project evaluation is also required to be undertaken by the Secretary of State (Section 5B) who '*must assess the compliance of the programme of work with the principles of replacement, reduction and refinement'* (our emphases).

In both the spirit of A(SP)A and its specific requirements, the use of animals should not be considered the default option. Rather, potential alternatives should be explored rigorously before submission of the application and, again, before the work starts. With regard to the potential for replacement, we believe that in order to comply with the requirements of A(SP)A 1986, applicants should demonstrate *how* they have searched for, and considered, alternative approaches not involving animals. In the licences we reviewed there was wide variation in how well applicants did this. In many cases, only limited, broad or generic statements were included, such as: '*due to the complex nature of the immune system it is not possible to generate antibodies to a novel protein without inoculating an entire animal.'*

In our view, many licences did not provide sufficient *demonstration* that:

- They have actively searched for, sought access to, and interrogated third-party suppliers and libraries to confirm whether or not the antibody they require is already available,
- The antibodies that the applicant needs cannot be isolated from third-party, animal-free antibody libraries, or they cannot or will not be provided to the applicant,

- It is not currently scientifically possible for the antibodies that the applicant needs to be produced by non-animal methods.

A minority of applicants did provide a level of information that should be regarded as the standard requirement. This includes an evidence-based and reasoned explanation for the factors that are currently barriers to the use of non-animal alternatives, together with their applicability to their planned work. This should apply to each of the potential non-animal methods, such as display platforms of affinity reagents (e.g. naive libraries of antibody fragments, synthetic aptamers and affimers), or other non-antibody applications such as PCR or mass spectrometry.

In some cases, applicants simply deferred to, or relied on, their clients to make a judgement as to whether or not a non-animal method was available, stating, for example, that the client confirms or states they cannot achieve their objectives without the use of animals, and also whether they have checked for alternatives. As described in the section below on governance, it is the legal duty of the project licence holder to ensure that an alternative method is used whenever possible. We would therefore expect the licence holder to require the client to demonstrate how they have come to the conclusion that non-animal methods could not be used.

In order for applicants to demonstrate compliance with the principle of replacement, and for project evaluators to assess this meaningfully, it would also be necessary for applicants to discuss whether or not they have access to the necessary resources to enable the uptake and use of alternative methods where these are available. We noted that some applicants mentioned that certain animal-free antibody libraries were currently accessible only to academic rather than commercial entities.

A handful of applicants highlighted cost as a potential barrier, noting that non-animal derived animal friendly affinity (AFA) reagents that could replace efficiently the production of antibodies in animals are not available ‘off the shelf' and therefore can be cost prohibitive in terms of both time and financial resource. However, we noted that one applicant provided a surprisingly high estimate of the difference in relative cost, which we think should have been challenged.

A further key area we expect to be considered before the authorisation of the use of animals for antibody development or production, is the extent to which applicants have demonstrated that their attempts to develop and use animal-free antibodies have failed (e.g. for scientific issues that cannot currently be resolved). Where this is the case, there should be some evidence of ‘who tried’ and ‘what didn’t work’? Generic statements such as “*there aren’t any alternatives*” or “*we tried and it didn’t work*” should not be considered sufficient justification.

3. Recommendations

To demonstrate compliance with the principle of replacement, applicants should provide a *meaningful discussion* of the purported scientific limitations of the animal-free methods in terms of how they relate to the objectives of the study. There should also be a robust

justification of *why* other methods cannot be used to provide the results required, and evidence of communication with specialist providers of animal-free antibodies in order to confirm that the antibodies they seek are not available and cannot be produced using a non-animal approach.

We would expect that for each of the libraries used their characterisation and validation is confirmed and for each new antigen, the applicant should not only describe explicitly the intended application of the antibody, but they should also be able to provide information to show the reasons why attempts to use animal-free antibodies have not been successful so far. For example, if animal-free antibodies were isolated, but were functionally inadequate, the applicant should describe what measures were taken to try to ameliorate the functional limitations (e.g. affinity maturation or other genetic modifications).

The applicant should also be able to describe the measures taken to try to resolve any other issues experienced with the use of animal-free antibodies: e.g. did they review and consider the reagents used during the panning rounds; the panning conditions; bioinformatics/sequencing information; antigen characteristics and immobilization etc? For most of the licence applications we reviewed, there was little or no mention or evidence of the use, or attempted use, of novel methods, such as phage display technologies. Doing so would provide reassurance within the regulatory framework that due regard had been paid to the requirement to avoid the use of animals '*wherever possible*'.

4. Reduction

The approval of a licence to use animals in order to produce antibodies is predicated on both the lack of any viable non-animal alternative (as discussed in the previous section) and also on the justification for the number of animals that will be required to carry out the work.

For commercial suppliers of antibodies, we recognise that the total number of animals needed during the lifetime of the licence can be difficult to estimate because that will depend on the number of commissions, which cannot be predicted. Nevertheless, all licence applications should provide a thorough description of the protocol for producing each type of antibody, including the number of animals typically required within each stage of the process, as is the case for other scientific procedures. On that basis, we would expect applications to avoid using general statements such as: "[*Estimation of the number required] rests on previous experience*", or "*The number of animals used in each assay will be the minimum required to produce the volume of antiserum needed and will depend on the species chosen and the antisera titres*."

We acknowledge that, for new antibodies, it will be hard to predict the success (or not) of the procedure. Nevertheless, good practice involves specifying in the licence both the number of animals needed for each stage of the first attempt at producing the required antibody and also a prediction of the likelihood of success. An example of a good practice was a proposal to use three animals, in the first instance (as a quasi-pilot): animal(s) with the most vigorous (hyperimmune) response would then be used for the next stage of the process. Only if no adequate immune response was achieved from that cohort, would more animals be used.

This approach was described as an acceptable compromise on the basis that animal numbers are kept low and there is a good chance of success: a view that we endorse.

Recommendations

We believe that a detailed justification of the number of animals required to produce each specific antibody should be included in the licence, and that applicants should demonstrate that they have considered incorporating into the programme of work any procedures that could reduce the number of animals needed to produce an antibody.

Other approaches aimed at facilitating reduction should be discussed where appropriate. Examples include:

- Exploring the possible benefits of challenging the animals with multiple antigens,
- Investigating the parameters for each stage of the process, in order to develop an optimal procedure for the production of antibodies (polyclonal and monoclonal),
- Choice of species: for instance, the use of large animals that would tolerate multiple harvesting of samples, with less detriment to their welfare.

5. Refinement

Many of the licences provided good information on how the procedures would be refined and the animals would be cared for. The licences reviewed stated that the protocols involved were expected to be 'mild' or 'moderate', or, in one case, 'non-recovery'. No licences were expected to involve 'severe' suffering.

There was some inconsistency in the justification for a decision to classify a protocol prospectively as mild or moderate. For instance, some licences stated that the use of Freund's Complete Adjuvant (FCA) meant that the protocol limit would need to be moderate, in recognition of the fact that it may induce localised granulomas or ulceration at the site of the injection. However, other licences that stated they would use FCA were classified as mild.

Similarly, the route of injection appeared to be viewed differently across different licences. For instance, one licence classified as moderate a procedure in which animals would receive antigen via the intraperitoneal (IP) route, and only under exceptional circumstances. By contrast, other licences classified that same approach (i.e., IP administration) as mild.

Other reasons for a prospective moderate classification included protocols producing polyclonal antibodies using bacteria and *treponema pallidum*.

Recommendations

Given the inconsistencies observed in the licences around the justification given for the severity classification both for the routes of injection and use of adjuvants, we recommend applicants would benefit from clear guidance on both. It would also be useful for AWERBs to

consider from their local perspective how they view the severity of certain adjuvants, techniques, or routes of injection.

6. 3Rs more broadly

In addition to specific information on how each of the 3Rs has been considered in the proposed programme of work, applicants should also demonstrate how they are proactively seeking to ensure the 3Rs will continue to be applied as fully as possible during the lifetime of the licence.

Most of the licences that the group assessed represented ongoing licensed activity. Only some provided significant detail about the steps being taken to implement the 3Rs continually and proactively.

We looked at two areas of the licences in connection with this section and asked: What are the learnings from past licences? And: Are there plans to implement 3Rs advances in future/over the course of this licence?

We found that less than half of the licences mentioned learnings from previous licences. Learnings noted included general mentions of appraising each experiment in order to improve the next, but it would have been more useful if more detail had been supplied.

Some licences gave more specific details on the changes to the current licence that had been based on learnings from the previous one. One stated that as a refinement from the previous licence, neither Freund's Complete nor Freund's Incomplete Adjuvant would be used in the current licence. One said that improvements enabled more serum to be obtained from a single animal, leading to an overall reduction in the number of animals used. Another stated that archived hybridomas would be used when feasible.

We acknowledge that the nature of the work and the species generally involved meant that most of these licences are not required by the legislation to go through the formal process of 'retrospective assessment'. Nevertheless, it was surprising and somewhat disappointing that only two applicants mentioned their likely plans to retrospectively review, in conjunction with others within the organisation such as the AWERB. Yet, review of a licence in terms of the outcomes, learnings and opportunities to improve is generally considered good practice for any programme of work.

We found that the majority of licences provided some mention of plans to implement advances in the 3Rs over the course of the current licence and some provided details of how the licence holder would continue to look for 3Rs developments and advances. Again, these plans ranged widely, including:

- General statements that if any new *in vitro* or *ex vivo* technologies emerge during the lifetime of the licence, they would be adopted,
- 'Continuous improvement' approaches,
- Requirements that each new study would require justification for the use of animals,
- Some very specific examples of 3Rs advances that would be pursued.

Several licences mentioned literature reviews, resources from the NC3Rs and discussion with colleagues in the field as ways of keeping up to date with developments in the 3Rs that might arise during the course of the licence. Two licences mentioned ongoing work that aimed to reduce the number of animals used by up to 50%.

Some licences included a specific protocol which allowed for exploratory and development work in assessing new 3Rs approaches.

One licence, granted since the publication of the ECVAM report, noted the potential welfare and scientific advantages in using non-animal derived antibodies. It provided detailed examples of how 3Rs advances would be monitored and applied and who (by role) within the establishment would be involved in this process. It stated that formal requests for the generation of antibodies under the licence would now require justification as to why non-animal derived antibodies could not be used. This licence also included further information on how the licence holder intends to keep up to date with 3Rs and other developments and about plans to create non-animal derived antibodies.

We also found examples giving detailed information on the possibility of using non-animal-derived antibodies in future. In one it was acknowledged that the anti-complex antibody may not need as high an affinity and therefore could be made via an *in vitro* display method. The applicant had successfully used third parties for this in the past and was also developing an in-house replacement method.

Recommendations

We would expect to see the inclusion in licence applications of meaningful information that demonstrates an applicant's commitment to, and plan for, identifying and incorporating 3Rs advances in the field that take place during the life of the licence being applied for.

We would also like to see how learnings from the animal work to be undertaken will be captured and considered in order to help replace, reduce or refine the use of animals in any future potential work. As examples of this process working effectively, it would also be appropriate for specific learnings, and advances from any past licences held, to be highlighted and fully described: in particular where successful approaches have been taken to reduce the number of animals used, or the severity of the techniques or procedures involved.

The discovery of the availability of a given antibody depends on open literature (including databases) and commercial catalogues. Regarding the former, there is clearly scope for encouraging scientists to populate databases, conscientiously, together with confirmation of their willingness to share the resource. Such a co-operative effort would make a substantial contribution to replacement and reduction.

Finally, we would encourage, wherever possible, the publication and wider sharing of relevant progress towards using non-animal derived antibodies. Informing the NC3Rs and other relevant scientific stakeholders in the sector of relevant advances could lead to further

opportunities for the 3Rs to be more fully implemented by others applying for licences. The AWERB Knowledge Hub could also be a repository for this information.

7. Purpose and Benefit

All the licences included the stated purpose of the work, as categorized on the annual 'Return of Procedures' statistics form. Most licences identified the work's relevance to more than one category. The most frequent was a combination of translational disease and basic research. Other categories were 'Translational detection etc.', 'Drug/food/feed Development' and 'Forensic'.

The stated aims and benefits of the projects were many and varied across a wide spectrum of applications. About one third gave only a cursory description: for example, to treat disease and the production of high-quality, non-commercially available polyclonal and monoclonal antibodies for use in scientific study. There is room for improvement here, while not over-stating the likely benefits of the work.

Many, however, gave proper detail of the potential benefits. Among the stated aims and proposed benefits were:

- Clinical diagnostics,
- Anti-venoms,
- Understanding aquatic diseases,
- Work to replace the current animal potency assay required for certain vaccines,
- Potential treatments already in clinical trials,
- A new class of engineered antibody-drug conjugates for cancer treatment,
- Studying the effectiveness of vaccines for Covid-19.

There were several good examples of the description of the purpose. One was an explanation that the antibodies produced under the licence would be used for research into a wide range of inflammatory (complement) disorders to define mechanisms of complement-mediated diseases better in experimental models and other systems. Another was to develop novel diagnostics for human diseases and to guide design of novel therapeutics for these diseases. Others described how producing the antibodies in question was important for the understanding and monitoring of diseases affecting several species of animals. They explained how screening for these diseases is important for the health of the species concerned and the continued trade of animals into and out of the UK.

Recommendations

There is plenty of opportunity for applicants to give a proper description of the intended benefits of the licence. While it may draw upon benefits achieved in past projects, the focus should always be the requirement to justify the specific benefits of the particular project being applied for, while being careful not to over-state them. A succinct account can help in the

assessment of the study, but can also, if translated well into the non-technical summary, give wider publics a helpful insight into the work and why it is considered worthwhile and valid under A(SP)A.

Where an antibody service is being provided commercially or in a regulatory context, it is recognised that it is not possible to be specific about each application for which the product is to be used. Here the emphasis is on demonstrating that appropriate governance measures are in place, as discussed in the following section.

8. Governance

It is important that the AWERB and applicants for project licences to use animals for antibody production can demonstrate strong governance in relation to the operation of the licence. In doing this the AWERB can demonstrate its effectiveness against a number of its assigned tasks including to: *"Follow the development and outcome (retrospective review) of projects carried out in the establishment, taking into account the effect on the animals used; and to identify and advise on elements that could further contribute to the 3Rs."*

The governance should demonstrate that processes are in place to establish that there is no alternative source for the antibody and the applicant should be able to demonstrate why it is not possible to produce the antibody without the use of animals. In the case where antibodies are being supplied to other organisations, appropriate diligence and oversight of client requests is important so as to ensure that the uses are for purposes justified under the licence, and that animals are used only when there is no alternative. It is important to remember that the legal duty for *ensuring* that an alternative method is used wherever possible lies with the project licence holder, not the client.

Good practice in governance can be demonstrated in a number of ways. Several applications described the use of ethical review forms/questionnaires that ensure all avenues have been explored before allowing animal work to proceed. The responses are generally reviewed by the licence holder and/or the Named People and AWERB. This is part of the authorisation process for individual antibody production. One example stated that the AWERB considers every request for animal use under the project licence. Proposals are approved only if the following criteria are met: the scientific background is robust; and an appropriate scientific rationale for why non-animal alternatives are not suitable is submitted for consideration for each request to generate an antibody in animals.

Other examples include the project licence holder signing off each immunisation according to a set of criteria, followed by a regular retrospective update to the AWERB.

As noted in the previous section, when antibodies are being provided as a service to clients, the project licence cannot be specific about the details of each antibody that will be produced over the course of five years. A system for assessing each project and carrying out retrospective review, including noting how the antibodies produced were ultimately used, would demonstrate that the applicant understands the importance of carrying out due diligence and has created a robust governance process. Some licences outlined governance

processes that involved a discussion with the AWERB. These discussions and rationale are often not systematically documented in the way the use of review forms/questionnaires can be.

Recommendations

The involvement of the AWERB and ethical review should be clearly explained – both prospectively, during the course of the licence, and in any retrospective review. An example of good practice would be to provide a set of questions that should be addressed (with reference to the contents of this report) and a process to record the outcome(s) to enable structured retrospective review and provide documentary support of a good governance process for facility audits.

9. Conclusions

The group's review of antibody licences found many examples of good practice, but also several areas where there were inconsistencies in the quality of the applications. The recommendations we have made in this review are intended to help ensure that all applicants are providing meaningful and thorough information, which demonstrates how they intend to, and will, fully implement the principles of the 3Rs in the process of developing and producing antibodies.