Home Office

NON-TECHNICAL SUMMARY

Production of antibodies, antisera and blood products III

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants
  - (ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

Blood products, Tissue samples, In-vitro diagnostic reagents, Controlled environment, Antibodies

Animal types	Life stages
Rabbits	adult, juvenile
Llamas and Alpacas	juvenile, adult
Pigs	juvenile, adult

Animal types	Life stages
Goats	juvenile, adult
Sheep	juvenile, adult
Cattle	juvenile, adult
Guinea pigs	juvenile, adult
Mice	juvenile, adult
Rats	juvenile, adult

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

This service provided under this project licence will supply in-vitro diagnostic reagents and animal tissues to research groups and biomedical companies, nationally or internationally to further their fundamental and applied research for the development and application of research projects and new treatments in support of human and animal healthcare.

This includes:

- Provision of blood products
- Provision of tissue samples
- Production of antibodies to microorganisms e.g. bacterial / viral antigens for use in diagnostic tests
- Production of antibodies to purified peptides / proteins

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

This service provided under this project licence will supply in-vitro diagnostic reagents and tissues for ex-vivo studies to laboratories, nationally or internationally and underpins research ranging from fundamental or specific disease focused basic research to the commercial development of new treatments in support of human and animal healthcare.

Antibodies play a part in many aspects of today's drug discovery and development. The antibodies can be then used in a multitude of applications, including but not limited to western blot (WB), immunoprecipitation (IP), immunofluorescence (IF), immunohistochemistry (IHC), chromatin immunoprecipitation (ChIP), and flow cytometry (FC). As a cornerstone of the body's immune response, antibodies can provide significant data to support scientists' research.

Drug discovery starts with identification and validation of targets, and antibodies are the gold standard when it comes to the specific detection of an interesting biomolecule or pathway. Most life science laboratories use antibodies in some way. Due to their outstanding specificity they make exquisite tools that allow researchers to identify molecules that cannot otherwise be identified. Thus enabling conclusions to be drawn about the target molecule and pathway of interest. In addition, the management of infections, in humans and animals, due to microorganisms is aided by the use of appropriate antibody diagnostic tests. Polyclonal antibodies are invaluable tools for research and diagnostics. The main reason for this is their ability to provide signal amplification. These antibodies can bind to several epitopes of the same antigen. Thus, several antibodies can bind to the same target antigen, leading to a strong signal or more effective capture of the target antigen. The use of antibodies in research has been the cornerstone of numerous discoveries and they will continue for the foreseeable future.

The use of whole blood and its products is a key step in large numbers of research projects. Be this as a source of immune cells, all major immune cells can be found in the most natural condition in freshly drawn peripheral blood. In addition, peripheral blood is increasingly used to obtain stems cells. Or as a reagent within a particular in-vitro test. For example for:

- Addition to microbiological media to grow fastidious microorganisms
- Production of standard reagents for laboratory procedures or diagnosis
- Developing techniques for determination of immunological response (e.g. ELISA or RIA)
- Normal negative controls for diagnostic assays
- Dilution of antisera for diagnostic assay kits

This licence will provide whole blood and its products to aid research and development projects. Where possible, this will be obtained from other sources (e.g. abattoir, butcher). On occasions the use of abattoir blood isn't appropriate. For example, if it is not possible to guarantee the sterility of blood obtained from an abattoir. As it is not possible to sterilise whole blood products, blood must be collected aseptically from a live animal.

Isolated tissue and organ preparations allow researchers to investigate the physiology and pharmacology of various tissue samples in a controlled environment without the complications of an intact animal model. These in vitro/ex vivo experiments can be performed using a variety of tissues and organs including smooth muscle, skeletal muscle, cardiac muscle, gastrointestinal and urogenital tissue samples Because of the high metabolic activity of certain tissues, especially hearts it is not possible to obtain these tissues from other sources or after the animal has been killed using a schedule 1 method, due to the prolonged times and crude extraction procedures of non-research facility removal protocols.

For hearts it is necessary for the explantation process to be carried out under controlled conditions by trained personnel with immediate cardioplegic arrest (minutes), in order for the samples to remain alive and viable for the ex vivo studies. These tissue samples must be removed under terminal anaesthesia.

What outputs do you think you will see at the end of this project?

Research undertaken using tissue samples or antibodies supplied via this project licence will be used to advance the science of the respective projects.

This will either be by the progression of the strongest candidates into clinical development with the intention of developing marketable products or the development of new processes or equipment to aid recovery or quality of life of patients.

They will lead to an increase in the understanding of the underlining biology behind the diseases investigated.

The data generated from these studies will be used to help the researcher understand the biology behind the diseases of interests. Data will also be used for filing new patents and thus disseminated through the patent publication pathways.

In addition, to patent applications, scientific publications and conference presentations will be used to disseminate key scientific findings and promote the general advancement of the research studied.

Who or what will benefit from these outputs, and how?

In the first instance the tissue samples and antibodies generated from studies will aid the researchers in their continued research. Allowing for the continued research for new treatments for diseases which will lead to their further development (e.g. in clinical trials) and could potentially lead to new therapies being introduced to the market.

Long term, the research projects have the potential to significantly enhance the quality of life for people and animals suffering from diseases or potentially cure them. This will benefit the whole society via reduction in absenteeism from work or school and reduction in demand on health services.

How will you look to maximise the outputs of this work?

Our commercial client will, where not confidential, look to publish the information via scientific publications and conference presentations in addition to patent applications.

One of the key goals of our academic clients will be to publish the results via scientific publications conference presentations, in order to promote the general advancement of the fields studied.

Species and numbers of animals expected to be used

- Cattle: 300
- Sheep: 375
- Goats: 135
- Pigs: 450
- Camelids: No answer provided
- Mice: 350
- Rats: 350
- Rabbits: 110
- Guinea pigs: 275
- : 150
- : 150

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

All animals will be juvenile or adult. These two categories are included as the immune system changes during development and specific ages of animals maybe required.

This species included in this licence are: Camelid, Cattle, Pig, Sheep, Goat, Poultry, Guinea pig, Rabbit, Rat and Mouse.

In the first instance the species selected are the most relevant to the scientific aims of the particular project. Each individual requestor will be required to justify their species of choice as part of the formal request. These requests are often very specific in the nature of the samples or antibodies required and they are not available commercially. We are able to meet these very specific needs.

While we may use multiple species in a number of the protocols, we predict that pigs will be the species used the most to supply tissue samples. For example, pig hearts are very similar to humans and therefore the pig would be the species of choice for this research area. Because of the high metabolic activity of certain tissues, especially hearts, it is not possible to obtain these tissues from other sources. This is due to the prolonged times it takes to collect these samples and the complicated process required to successfully remove the organs.

For antibody production, camelids, sheep and goats will be the species used most often. Camelids produce specific antibodies called VHH fragments. The unique size and structure of these antibodies mean they are able to bind to hidden antigens that are not accessible to whole antibodies, for example the active sites of enzymes. Sheep and goats' antibodies have a high affinity and sensitivity, and are

particularly adapted to small antigens or small epitopes. In addition sheep and goats allow the production of larger volumes compared to smaller species and the animal does not necessarily need to be culled as part of the process.

Typically, what will be done to an animal used in your project?

After arrival all animals will be allowed at least 7 days to become acclimatised to the unit.

Then depending on the protocol:

Blood sampling: One or more blood samples will be taken. The animal will be assessed between each sampling and only sampled if considered to be fit and healthy. At the end of the blood sampling period the animal will either be re-used for tissue sample collection, returned to stock or re-homed.

Tissue collection: The animal will be terminally anesthetised and, the tissue sample collected. . After which the animal, still under terminal anaesthesia, is euthanised .

Antibody production: The animal will be immunised with the antigen, given a booster injection if required and blood samples taken for antibody extraction. The animal may be used in one or more rounds of antibody production. The animal will be assessed between each round and only used if considered to be fit and healthy. At the end, the animal will either be re-used for tissue sample collection, returned to stock or re-homed.

What are the expected impacts and/or adverse effects for the animals during your project?

The provision of tissue samples should have no adverse effects on the animals. The collection will either be a simple blood sample or collection under terminal anaesthesia.

Antibody production should also have little or no impact on the animals. This will involve simple injections and blood sample collection. There is the potential for an animal to have an allergic reaction to the antigen which may induce some adverse effects. This is expected to be transient and have no lasting effect on the animal.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

For the majority of animals, the severity level will be mild. However, as stated above, in some studies the animals may experience some adverse effects. These would only cause the animal a moderate level of distress which will in most cases be transient.

What will happen to animals at the end of this project?

- Killed
- Kept alive
- Rehomed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

At a certain point in research projects there is a point where in vitro studies cannot completely answer the question being asked. This is due to the complexities of the microenvironment of tissues and the involvement of the immune system. This cannot be fully replicated in a laboratory setting.

Because of the high metabolic activity of certain tissues, especially hearts, it is not possible to obtain these tissues from other sources or after the animal has been killed using a schedule 1 method, due to the prolonged times and crude extraction procedures of non-research facility removal protocols. For hearts It is necessary for the explantation process to be carried out under controlled conditions by trained personnel with immediate cardioplegic arrest (minutes), in order for the samples to remain alive and viable for the ex vivo studies. These tissue samples have to be removed under terminal anaesthesia.

Blood is required as a source of primary cells for in-vtro/ex-vivo studies. Wherever possible, blood products will be obtained from abattoirs or after when animals have been put down for other reasons. In many cases, abattoir samples may not be suitable due to contamination or lack of information and/or traceability on the specific animal being used. Also, it is not possible to sterilise whole blood products, blood must be collected aseptically from a live animal.

It is also now possible to generate antibodies using non-animal derived methods, thanks to developments of new technologies. There are however still cases where there are simply no non-animal derived antibodies available as a suitable alternative. Particularly in the generation of polyclonal antibodies. This is the area of antibody production that we focus on.

Which non-animal alternatives did you consider for use in this project?

The very nature of the blood and tissue sample supply included in this project licence means that at the point we are approached, the use of non-animal alternatives has been completed or cannot answer the questions being asked.

Phage display library monoclonal antibody production is a valid alternative to using animals to produce monoclonal antibodies.

Why were they not suitable?

Currently the production of polyclonal using phage display libraries is not as effective as using this system to produce monoclonal antibodies. There is a need still to produce polyclonal antibodies, particularly in applications where their heterogenous nature is beneficial for recognition of multiple epitopes.

When monoclonal antibodies are required, phage display libraries are the leading technology to use for the production. However, there are limitations., Tthey can produce large amounts of specific antibodies but may be too specific to detect across a range of species. They are vulnerable to the change of epitope. Even a slight change in conformation may lead to dramatically reduced binding capacity. Developing a monoclonal takes time and requires high technical skills.

There is still a role for polyclonal antibodies in research. Polyclonal antibodies are ideal reagents in diagnostic assays and hemagglutination reactions due to their ability to recognize different epitopes of a target molecule. This makes them more sensitive and able to detect antigens in very low concentrations. The best use of polyclonal antibodies is to detect unknown antigens. Polyclonal antibodies are used as a secondary antibody in immunoassays (e.g. ELISA, western blotting, microarray assays, immunohistochemistry, flow cytometry). Their role is to bind to different epitopes and amplify the signal, leading to better detection.

In addition, with immunohistochemistry the effects of the tissue fixation and processing on the epitope is unknown and highly variable, Polyclonal antibodies can be a better option because their multi-epitope binding allows for antigen recognition even if some of these epitopes are affected by changes in an antigen's structure or accessibility due to the processing of the tissue samples.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

These numbers have been calculated on the numbers used under the previous 2 project licences. The number of possible projects we are aware of, either from our external clients or from academic grants that are being submitted by researchers over the next 5 years.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

To ensure we use minimal number of animals required to obtain meaningful and relevant data, we have extensively consulted available literature, attended experimental design and statistical courses, discussed with statisticians and NC3Rs staff and information provided by the NC3Rs. All requestors will be required to justify the number of animals required and if appropriate show how they came to this number.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

In many cases, the numbers of animals required will be reduced by re-using animals for the mild procedures e.g. blood sampling. There is evidence that the immunisation of animals with multiple antigens at the same time give a good antibody response and the separation/purification of the different antibodies is possible. The animal experience is no different when give one or a multiple antigen immunisation. This can significantly reduce the number of animals required.

When providing tissue samples, we try to coordinate it so that multiple tissues can be taken from the same animal.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

In the first instance the species selected for antibody production and blood products supply are the most relevant to scientific and logistical (e.g. sample volume) requirements of the project and within the constraints of the licence.

Study protocols will be designed to ensure that any harmful effects resulting from any procedure will be detected early. For example, by including specific periods of observation when we know adverse effects are most likely to occur, e.g. the first 15 minutes post-dose. Having a dosing regime that allows time between each animal dosed, if an adverse effect occurs, we can intervene to stop other animals being affected.

Terminal tissue/organ sampling will be conducted under anaesthesia, so the animal feels no pain or suffering during the procedure.

Why can’t you use animals that are less sentient?

The studies, tissue and blood sampling undertaken will be very species specific. The most appropriate ones for reach request will be used. All requests will have to justify why that particular species is required.

The process of whole body anaesthesia, for simple procedures like blood sampling, would be more stressful to the animal than the actual sampling.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

The least invasive route of substance administration will be used. Along with the minimum number of injections to produce the required response and smallest needle gauge appropriate will be used

If the blood sampling can be fulfilled, (e.g. minimum blood volume for the species fits with the scientific aims) with one or more species e.g. sheep or cattle, then the species which is easier to handle (therefore the procedure will be less stressful for the animal) and in ready supply will be chosen.

All animals will receive appropriate operative care in terms of anaesthesia and pain management both during and after the procedure.

When appropriate, blood sampling maybe conducted on farms, thereby reducing the stress to the animal by transporting them and introducing them to a new environment prior to sampling.

In house expertise further enhances animal welfare, by providing close collaboration with dedicated animal care staff and veterinary consultants, and ready access to highly skilled advice.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow the NC3Rs guidelines on the "Responsibility in the use of animals in bioscience research" and consult all the relevant references listed therein. , e.g. NC3Rs Blood sampling resource. (Reference: NC3Rs/BBSRC/Defra/MRC/NERC/Royal Society/Wellcome Trust (2019) Responsibility in the use of animals in bioscience research: expectations of the major research councils and charitable funding bodies. London: NC3Rs).

For substance administration the LASA substance administration guidelines will be consulted (Reference: Administration of Substances to Laboratory Animals: Routes of Administration and Factors to Consider. J Am Assoc Lab Anim Sci. 2011 Sep; 50(5): 600–613).

Animals will continually be monitored for signs of pain and distress, especially post-challenge, by use of the grimace scale; https://www.nc3rs.org.uk/sites/default/files/documents/Guidelines/MGS%20

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will continuously monitor publications and the NC3Rs website for new and alternative models that could be implemented as part of this project. In addition, articles on advances in the 3Rs are regularly published on our internal Users News Forum and other relevant information is circulated by AWERB. Whenever possible we will implement these refinements into our studies.