Home Office

PROJECT LICENCE

The Production of Laboratory Animal Bio-Products

Project licence holder

REDACTED

This **PROJECT LICENCE** permits the licence holder to carry out a programme of scientific procedures on living animals under the **ANIMALS (SCIENTIFIC PROCEDURES) ACT 1986**.

The project licence holder may carry out the specified programme of work, subject to the restrictions and provisions contained within the Act and any limitations and conditions specified within this licence or by the Secretary of State.

This licence does not authorise the holder or any other person to carry out procedures on any animals unless they hold an appropriate personal licence issued under the Act.

Granted authority

This licence has been granted based on the information provided during the application process.

This licence authorises, only:

- work to meet the specified project aims
- use of specified animals and procedures
- work at the specified places

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Introductory details

Project title

The Production of Laboratory Animal Bio-Products

Key words that describe this project

- Dog
- REDACTE
- D
  REDACTE
  D
- Blood products

Licence duration

Years: **5**
Months: **0**

This is a project continuation

From the licence REDACTED
Expiring on **02 October 2018**

Experience

What relevant scientific knowledge or education do you have?

REDACTED

REDACTED

Qualifications

REDACTED

Resources

State the expertise and other resources available to you, whether this work has been peer-reviewed, and how the project will be funded.

REDACTED

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Establishments

Primary establishment

Establishment name: REDACTED
Licence number: REDACTED

Will your project use any additional establishments?

Yes

Additional establishment 1

Select an establishment where work will be carried out

MBR Acres Limited

Why do you need to use this additional establishment?

Transfer of protocol 1 and 2 to MBR Acres X6BB92AF1, Sawtry way, Wyton, Cambridgeshire, PE28 2DX

REDACTED.
REDACTED

REDACTED, REDACTED, REDACTED and REDACTED to become PILs

The Donor dogs will be a selection from dogs already held REDACTED, the group will comprise of some ofthe Stud males or any suitable grade 2 males and the females will be grade two females or retired breeders, these are all relatively young adult dogs.

We have a veterinarian on site to regularly assess the dogs and approve of their fitness for each reuse.

We have a full veterinary suite on site suitable for the euthanasia and terminal bleeds, live donor bleeds.

We also have a suitable procedure area where we could do the live bleeds and or process bloods,

Colony management wise we have a procedure in pace to grade the dogs on a regular basis, on-site we have the ability to treat any dog condition (full vet suite)

These dogs will be group housed amongst our general population of colony dogs, Daily care and routine husbandry will be provided by our experienced and trained animal technicians, the dogs will be regularly moved and re- grouped to keep them socially compatible and interactive.

They will have regular access to exercise areas away from their home pens, where they can interact with technicians and other dogs

Their home pens provide a variety of environmental enrichment in the form of raised platforms, sleeping barrels, suspended toys, good visibility of the other pens and private areas too.

The data for all animals is managed on an Electronic colony management tool called REDACTED orspread sheets.

The animals have a full prophylactic treatment plan maintained in REDACTED.

Each dog bred at Wyton is micro- chipped at 6 weeks of age and has it own individual record from birth, there is full traceability of all animals on site.

Who will supervise work at this additional establishment?

REDACTED
null

REDACTED

Places other than a licensed establishment (POLES)

List the POLEs where you intend to carry out regulated procedures.

No answer provided

Why do you need to carry out regulated procedures at these POLEs.

No answer provided

Transfer and movement of animals

Will any animals undergoing regulated procedures be moved between licensed establishments?

No answer provided.

Background

What is the current state of knowledge or product availability on which the proposed project intends to build?

Fundamental biochemical and physiological research, bio-analytical assay in pharmacokinetic study and some aspects of human and veterinary diagnostic methods require the use of 'normal' animal blood or animal plasma or sera for control or reference purposes. For example, prior to the measurement of the concentration of a drug in experimental samples customers are required to perform a validation of the method and sampling conditions. As part of this validation they have to assess drug stability in the whole blood of the target species. Also, regulators require the level of binding of candidate drugs by red blood cells is taken in account since binding to red blood cells affects the partitioning of the compound between blood and plasma and may lead to high blood to plasma ratio. If not taken into account, high blood to plasma ratio may result in incorrect calculation of the pharmacokinetic parameters as they are typically determined by analysis of compound concentrations in the plasma. The degree of red blood cell binding is elucidated by incubating the study compound in fresh whole blood and plasma. The blood is centrifuged to plasma and the ratio of study compound observed in plasma in comparison to reference plasma sample will be used to calculate red blood cell binding and blood to plasma partitioning ratio. Such studies require the availability of freshly drawn whole blood and the shelf life for effective use in these studies can be very short (1-2 days from collection).

REDACTED

The project aims to provide a service for the supply of blood products (whole blood, plasma, serum and preserved tissues) (such as musculoskeletal, intestinal, pancreatic and liver) and body fluids (such as urine, cerebrospinal fluid and bile) for use by customers in the scientific community who require such

products for use in basic research, bio-analytical and diagnostic assays for both human and veterinary medicine.

REDACTED

REDACTED

When regulatory toxicology studies require the use of the Beagle dog, REDACTED for the safety assessment of drugs intended for use in humans or in companion or agricultural animals, the biopharmaceutical companies or contract research companies performing theresearch frequently require a supply of blood products or normal tissues from the same animal modeland we seek to serve this need.

In 2016 we supplied 10,942ml of whole dog blood and 41515ml of dog plasma to our customers.

This was used in part, as support for analysis, calibration and method development/validation. For example, normal donor plasma is required to calibrate the apparatus used in many *in vivo* DMPK studies, including quantitative bioanalytical methods using techniques including LC/MS/MS and LC/HR-MS systems can be used and are chosen depending on the nature and need for each study. A variety of sample preparation techniques (SPE, ion exchange, LLE, SLE, protein precipitation) and different levels of method prequalification/validation can be applied, depending on the biological matrix,analyte properties, and the requirements for each candidate drug. Analysis of radio-labelled compounds with UPLC-online-radio detection may also be required.

Normal whole blood, plasma or serum can also be required in research applications in the fields of oncology, vaccine production and studies of ophthalmic products, pulmonary system, cardiovascular system, pain and urology. Small volumes of blood from dogs are also used to prepare toxicology matrixes that are used to asses drug stability. Fresh dog blood is also used to support PK and TK studies over many disciplines. FDA and MHRA guidelines state blood must be fresh as possible. The enzyme levels present whole blood is drawn are important and may not be preserved in frozen samples. However in other studies, this may not be a factor and such customers can use frozen tissue and blood products.

REDACTED

REDACTED

Benefits

What are the expected benefits of your programme of work? Why are these benefits worthwhile?

REDACTED aims to maintain a supply of biological materials to the research community. This allows many companies to draw from our pool of animals which are of a high health status, reducing the need for duplication of donor animals at their facilities. With our current facilities, expertise and breeding programmes, we are ideally suited for this role.

We carefully monitor and manage production levels of animals on a month by month basis looking at

animals produced vs sales. Ex-breeding, stock unsuitable for sale and surplus stock animals can also be utilised to provide a pool of tissues and biological fluids since these are required in a wide range of *in vitro* and *in vivo* studies. Some examples are detailed below.

We are able to harvest multiple types of bio-products from single donor animals that can be used for different studies and customers. This contributes to a reduction in the numbers of animals required.

Similarly, we are able to harvest bioproducts from unsold animals and these products can often be frozen for sale when demand occurs – thereby avoiding the need to sacrifice additional animals.

We also contribute to a reduction in the numbers of animals required by the 'end users' such that they can avoid the need to obtain their own specific live animal donors for harvesting the specific bio-product they require.

We have developed a team of people that is highly skilled at the removal of tissues and fluids over the course of the last project license. We have developed a high level of technical ability which means that the techniques can be performed in the most refined way thereby reducing animal wastage.

Bio-analysis is a major component of exploratory and preclinical drug evaluation by distribution, metabolism and pharmacokinetic (DMPK) screening that often requires the use of animal bio-products for method validation. New chemical entities failing certain DMPK characteristics early in drug development do not proceed to further evaluation in laboratory animals therefore this work as a whole reduces overall animal use in the drug evaluation process. (EMEA guidelines).

In supplying normal body fluids to the scientific community for supportive validation and calibration of analytical systems and bioassays, it is expected that the need to repeat work will be reduced thus keeping the number of animals used to a minimum. Equally, the supply of body fluids assists the research and toxicology scientists in their studies. REDACTED

The provision of these products enables other laboratories which do not have the facilities, expertise or capacity to do the production themselves to advance their research and other activities as quickly as possible thereby bringing the advantages of new treatments to patients as quickly as possible.

REDACTED

References

List up to 10 key references that support the need for your proposed project, or refer to any specific models that you propose using.

https://www.nc3rs.org.uk/3rs-resources/blood-sampling Removal of blood from laboratory mammals and birds. First report of the BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement 1994 LASA Good Practice Guidelines: collection of blood samples http://www.piqo.se/pdf/lasa_blood_sampling.pdf The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals, 8th Edition, Editors R Hubrecht & J Kirkwood, Various authors The Effects of Acute Blood Loss for Diagnostic Bloodwork and Fluid Replacement https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4485629/ Morton DB. Ethical aspects of the use of animal models of infection. In: Zak O, Sand MA, editors. Handbook of animals models of infection. San

Diego: Academic Press; 1999. p.30-48 Festing MF, Altman DG. Guidelines for the design and statistical analysis of experiments using laboratory animals. ILAR J. 2002;43:244-58. Removal of blood from laboratory mammals and birds First report of the BVA/FRAME/RSPCA/UFAW Joint working group on refinement (1993) Interpreting stress responses during routine toxicity studies: a review of the biology, impact, and assessment. Toxicol Pathol. 2013;41(4):560-614. doi: 10.1177/0192623312466452. Epub 2013 Mar 7.

Purpose

Which purposes apply to your project?

- (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.
  - (iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
- (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 aims mentioned in

purpose (b)

Aims and objectives

What do you aim to achieve, establish, or produce by undertaking the proposed programme of work?

REDACTED are aiming to continue to offer a centralised service for the provision of products for those with no facilities, capacity or expertise for the benefit of biomedical science. With an emphasis onthe following fundamental areas.

1. Efficient service providing continuity of supply to biomedical science.
2. High level of animal welfare, ethical consideration and regard for the 3R principles.
3. Skilled and trained personnel.
4. Traceable, consistent and high-quality products of blood, serum, plasma and whole blood and animal tissue (ISO 9001-2015).

Project plan

Provide an overview of the project.

The company uses a procedure where every customer placing product requests is asked to fill in a justification form describing the intended use of the specified tissue(s) or matrices. This form has been developed and approved by the REDACTED Animal Welfare Ethical Review Body (AWERB) to incorporate questions which positively justify the use of animal tissues compared to non-animal models. This formalso determines if requests for tissues or blood products are ethically justified and fit within the purposes of this licence.

Retrospective analysis of the previous bio-products Project License was undertaken at our AWERB on 5th June 2018 and a copy was sent to the Home Office.

Looking at the recent demand for bio-products closely and looking at historical data over the last five years covered by the previous project license we aim to utilise our ex-breeding or surplus stock animals or we may make a small but deliberate breeding increase to provide extra availability of stock animals to act as donors from which the required biological samples can be obtained to fulfil orders. All orders are checked for validity (REDACTED) Bio resources request form, requirements checked against stored levels of product or availability of the donor animals (where number of recent bleeds, intervals between and volumes removed are observed), before confirming the order. Stock product is continually monitored to avoid depletion and increased stock levels. In times of low demand the use of the live donors is discouraged. All of the animals held REDACTED are health screened at quarterly intervals and this ensures the bio-products we supply are removed from healthy normal animals. For some research

applications, customers are able to use tissues that have been frozen and stored for long periods and thus we are able to utilise 100% of the animal tissues we take and thereby avoid any waste. As there are no generally accepted methods to assess frozen product viability (or shelf-life) for the numerous ways the products may be used by our customers, we rely on customers to perform such tests in their own experimental setting. Therefore, there is currently no accepted "use by date" for products stored at under -160 degrees Celsius and many customers accept bioproducts that have been stored frozen at these temperatures for up to two years or more. Accordingly, our frozen stock is offered to customers in preference to freshly sampled stock as this contributes to a reduction in the numbers of donors required.

Customers who require our bioproducts in applications where fresh blood is required or when enzyme activity must be unimpaired are generally not able to utilise frozen bioproducts since the process of freezing and thawing can denature some proteins and can affect the activity of many enzymes or the viability of the thawed blood cells. Similarly customers who are purchasing tissues for cell culture applications will demand 'fresh' tissues since the viability of cells after freezing and thawing may be seriously impaired.

We rely on our customers to justify the choice of species to be used as the donor for their bioproduct requirements. Where possible, we propose the supply of material obtained after humane killing of the donor. A live donor is only chosen if the customer has a need for fresh (not frozen) material from a living donor.

Frozen stock is only replenished when we are satisfied that there will be future demand for the bioproduct or if the stock is no longer fit for customer use (this is determined by customer feedback).

Where possible, products will be collected following humane killing by a Schedule 1 method, but in order to harvest maximum volumes and meet certain customer specifications such as 'must be barbiturate free' (barbiturates can cause severe haemolysis in blood samples) or 'fresh and never frozen'; procedures will be carried out on live donor animals (protocol 2) or on animals under terminal general anaesthesia (protocol 1). Blood products will be collected in response to customers who request immediate availability that are unable to accept frozen product but donor animals will also be routinely used to collect products for storage in accordance with Protocols 1 and 2.

When large orders for blood products are required (within a short period of time), such volumes exceed what can be supplied by the donor colony. In these circumstances, it may be possible to make use of an 'otherwise surplus or unsaleable animal and in this case the donor will be exsanguinated under terminal general anaesthesia. This enables the harvest of maximum possible volumes and therefore reduces the total numbers of animals required for obtaining large volumes without barbiturate contamination. All blood products collected are managed for quality by our bioproducts department. Colour and viscosity are checked against known good samples to ensure only good quality samples are sent to the customer

Thus, terminal bleeding is also undertaken when the donor pool cannot supply sufficient quantities by collection under protocol 2, or when animal tissues or fluids are required (e.g. liver, bile, bone) such that an invasive procedures would be required (harvesting CSF or bile) and it is only performed when suitable surplus donor animals are available. Dogs, REDACTED may be terminally anaesthetised prior to their blood being removed using standard phlebotomy techniques and appropriate equipment (which will include materials for the cannulation of an artery or vein, cardiac

puncture or venepuncture) dependant on the donor species and withdrawal site accessibility. Animals will be humanely killed using a schedule 1 method following completion of the terminal bleeding procedure. The animals chosen for donation of bio-products can be ex-breeders, surplus stock or those deemed unsuitable for sale (e.g. have a mild congenital malformation such as mono-orchidism) that would otherwise have been humanely killed under schedule 1 without any positive use.

Dogs, REDACTED , REDACTED and REDACTED may be anaesthetised and bled out fully as a non-recovery procedure, (protocol 1 AC). The anaesthetic regime used is under the direction of the NVS and is suitable for each species. REDACTED .

We supplied an average of 8300 ml of whole dog blood per year under Protocol 2 1rom 10-15 donor dogs during the previous 5 year project. Under Protocol 1, an average of 14156 ml per year of dog whole blood was taken and used to harvest canine plasma and serum from an average of 40 dogs per year. We expect an increase in demand over the next 5 years since one provider of dog blood products has recently ceased to provide this service.

Species	*Year*	*Number of Procedures* — *Live bleeds*	*Volume of whole blood (ml)*	*Number of procedures* — *Terminal bleeds*	*Volume of whole blood (ml)*
Dog	*2014*	*156*	*6239*	*66*	*23100*
Dog	*2015*	*123*	*4513*	*7*	*3100*
Dog	*2016*	*209*	*10942*	*117*	*41515*
REDACTED					
Dog	*2017*	*139*	*15019*	*7*	*2718*
REDACTED					
Dog	*2018*	*172*	*5062*	*1*	*350*
REDACTED					

The donor's suitability for use/reuse is assessed prior to being assigned 'donor status' and this status will then be assessed on a continuing basis by the NVS, personal licensee's, Named Animal Care and

Welfare Officers and the animal technicians responsible for the animal's care and welfare. Due attention is given to the Home Office Advice Note 02/2015: Animals (Scientific Procedures) Act 1986 Use, Keeping Alive and Re-use Date: October 2015.

An animal's suitability for use/reuse as a donor is determined by its behaviour during the course of a procedure, its ability to be socially housed with other donors, size, and temperament, condition of vessels and outcome of veterinary clinical examination and routine haematology testing. Animals will be familiarised with the procedure area and handlers prior to procedures being performed and animals reacting unfavourably to this will, if necessary, be exposed to an extended familiarisation and socialisation programme in an attempt to overcome the unfavourable issues presenting a problem to the handler or causing distress to the animal. The training involves training to stand on a table top, hear and feel clippers and dummy bleeds using capped needles and capped shavers. Animals that are declared as unsuitable to continue as donors can be re-used on a terminal procedure (Protocol 1) or humanely killed using a schedule 1 method. Consideration will also be given to whether the dog would be deemed suitable for rehoming according to the Marshall rehoming policy.

The attached 'Decision Tree' for reuse shows the decision-making process that is used to determine if reuse of a donor animal can be permitted.

With regard to reuse the NVS has overall responsibility. The NVS will ensure the animals are healthy by routine examinations, observation and laboratory tests,if required. Only animals that are healthy will be reused, according to the parameters set out in the attached Decision Tree. Between reuse the animals will be held in housing suitable for their species will full expression of the 5 freedoms under the care of the NVS

An animal under Protocol 2 may be bled up to four times a month (maximum) to a maximum of 15% of their circulating blood volume (AA). This is around 52 blood samples per year for as many years as they are deemed healthy. This for a 15kg dog is approximately 15% of (85(ml blood/kg) x15 (weight)) = 15%x1275mls of blood =191mls of blood every 4 weeks. Throughout the period of the last project license, donor dogs were, on average, bled twice in a 28-day period, taking around 5-10% of their blood volume. This ranged from 45 to 81ml per bleed). There is no set upper age limit for donor dogs.

Prior to each bleed, dogs are assessed by a suitably qualified technician to verify their suitability for re-use. Under the responsibility of the NVS, general clinical signs and are checked. The re-use check-ups will look at general health. The check up will ensure that any re-use will not cause a beach of the severity limit (i.e. this must remain as 'mild') in respect of the whole life experience of the donor and that the general health and well-being of the donor has returned to normal.

This means that the donor must be able to eat, drink, excrete, respire, move and interact with peers; be free of disease; not be suffering pain and must be free of fear, distress or anxiety.

Blood may be collected from a pool of live animals, such as dogs, as described in Protocol 2 without anaesthesia providing the blood is drawn within specified and recommended limits from superficial veins using equipment that is suitable for the size and species of the animal. Normal circulating blood volumes for each species will be based on recommendations made in the LASA guidelines and the NC3Rs web site:

http://www.verutech.com/pdf/lasa_blood_sampling.pdf

https://www.nc3rs.org.uk/3rs-resources/blood-sampling

Individual donors are weighed before blood is taken to determine the volume that may be drawn on each occasion. Also, full records of volumes and dates of blood donations from individually identified donors are maintained to assure full traceability and to ensure that volume/frequency limits are not exceeded. This is done by making entries in the individual donors' animal history record/donor record and laboratory documentation.

Blood obtained from conscious donor animals has the advantage of being 'drug' free as no sedatives, barbiturate or anaesthetic are required and volumes can be obtained from several live donors to create a pooled sample where the customer requests this. This reduces the necessity to euthanise the donors, therefore reducing the numbers of animals required.

Other advantages of maintaining a pool of live donor animals is that animals with minor defects not affecting their welfare may be utilised for this purpose. Also, the pool of animals may be available on a long-term basis should a scientist wish to conduct repetitive experimental studies over a period of time using the blood products taken from the same animals.

Animals that are held as part of a blood donor pool will typically be bled from the jugular vein according to product demand but also routinely to ensure that they remain accustomed to the minimal restraint techniques, the procedure area and the actual procedure. The technique involves shaving the area, inserting a cannula and withdrawing the sample into appropriate vacutainers. The optional use of a skin anaesthetic is a refinement for nervous animals but this is not routinely required. The cephalic or saphenous vein may also be used for sampling if required.

Since we are supplying blood products obtained under protocol 2 to a range of scientists who each require the blood products for use in their individual projects, each donation of blood is recorded as a re-use of the actual donor(s) on each occasion that blood is taken.

Dogs will be given time to interact with humans on a daily basis (for pen cleaning, habituation etc.) and they will have a period of additional human contact in order to improve socialisation, tractability and familiarity with animals caretakers. Environmental enrichment is provided, in part, through pen design; all enclosures having multilevel raised platforms. A door above the platform provides access to human contact. A mix of solid panels and vertical bars allow for times of privacy and interaction. Enrichment objects are presented in a variety of ways within the pen, they may be given on the floor or suspended. Each pen has an interconnecting hatch to allow additional pen space to be created or to allow extra socialisation with compatible groups of dogs.

The lifetime of an animal used as a donor also depends upon its species. Since many dogs respond very well to the housing and donation regime especially after suitable training, they will continue as donors for several years.

Anaesthesia

Induction and maintenance of general or local anaesthesia, sedation or analgesia to mitigate the pain, suffering or distress associated with the performance of regulated procedures under this licence will be indicated by using the following codes in protocols: AA (no anaesthesia); AB (general anaesthesia with recovery); AB-L (local anaesthesia); or AC (non-recovery general anaesthesia).

Replacement

Why is it not possible to achieve your objectives without using animals?

When synthetic alternatives are not validated as suitable, live animals must be used to derive bio-products.
There are certain studies that must be performed in laboratory animals, therefore it is necessary to use bio-products derived from these same species in order to complete the range of tests, some of which are legally required including: Analytical methods requiring controls for quantifying validation, GLP drug development, TK studies and calibration of OC samples under the EMEA guidelines.

Reduction

How will you ensure that the number of animals used in this project will be kept to a minimum?

We held our previous project license from October 2013 to October 2018 and we have built up a regular customer base for bio-products.

We strongly believe this aids reduction and avoids duplication of other pools of animals that can provide bio-products. We use a request form for products and take steps to maximise the yield from each animal used. For example if we have a request for dog tissues we notify our clients to ensure we take as many tissues from the single animal. Often we will also harvest tissues for which there has been historical demand and store these in our freezer to supply when required. The use of tissue and blood kept frozen at -18 degrees C that are not immediately required reduces the number of animals used in bio-products. At this level of temperature items it is believed can be stored indefinitely. However frozen tissue or blood products may not always be suitable.

Blood products are necessary for the calibration of test systems and some bioassays. Using good quality blood components will improve the significance of test results in studies involving animals and therefore lead to improved scientific knowledge and a reduction in the overall number of animals required.

As multiple samples can be obtained from a small number of live donors this reduces the need to individually euthanise animals for the purpose of taking each sample. Where possible an individual animal will be used for more than one purpose, for example, following a procedure for the collection of blood from an animal under anaesthetic from which it is not allowed to recover, tissues and organs may be harvested or the cadaver may subsequently be used for educational purposes.

Re-use means that a small group of animals can be kept at our premises and can be used to donate blood products at appropriate intervals. We are able to pool the samples from our donors and then, by performing regular small volume bleeds, we can limit the number of large or terminal bleeds required (that would be done under protocol 1) and thereby reduce the numbers of animals sacrificed. Also, re-

use of our donors means that customers don't need to keep their own donor animals for this purpose or purchase donors for terminal bleeding thereby also reducing total animal numbers.

Refinement

Why are your choices of animal, model, and method the most refined for the intended purpose?

REDACTED Only blood with a strong scientific need is collected. A new Bioproducts request form has been designed and issued in consultation with our customers. Only customers satisfactorily are completing this form and after internal approval will the request be fulfilled. All orders are approved by the laboratory NACWO and the laboratory manager.
Under our previous project license and this renewal, we will only supply tissues from species which we routinely keep at our site. All the species that we propose to use have well-established roles in preclinical research.

All personnel involved in the blood sampling procedures are fully trained and competent to perform the procedures. Our techniques have been developed so as to cause minimal distress to the animals.

Terminal blood sampling is conducted under a general anaesthetic from which the animals are not permitted to recover from. The techniques we employ cause the minimum amount of discomfort and stress to the animals when we anaesthetise them.

For live dog bleeding, this technique has been refined and we may use local anaesthetic (LA) in sensitive dogs to reduce any stress. Due to the possibility of side effects e.g. dermatitis from prolonged numbing of the skin, it was felt the LA would and should only be applied to dogs that require it.

We also ensure that no more than 10% blood volume is removed in any 24 hour period and no more than 15% in any 28-day period.

http://www.verutech.com/pdf/lasa_blood_sampling.pdf

https://www.nc3rs.org.uk/3rs-resources/blood-sampling

Origin

List the likely origins of animals that will be used in this project.

UK, EU and Non-EU

Protocols

Summary table

No.	Protocol	Animal types	Est. numbers	Life stages	GA	Severity category
1	1 Blood sampling under terminal anaesthesia	Dogs REDACTED REDACTED	250	Adult	No	
2	2 Blood sampling without anaesthesia	Dogs	35	Adult	No	mild

Protocol 1

1 Blood sampling under terminal anaesthesia

Severity: Non-recovery

Animal types	Est. numbers	Life stages
Dogs	250	Adult
REDACTED		
REDACTED		
REDACTED		

Protocol details

Severity category

non-recovery

Type of animals

Animal type

Dogs

Are some of these animals genetically altered?

No

Estimated number of animals

250

Protocol 1

Protocol 1 continued

Life stage of the animals

REDACTED

Animal type

REDACTED

Are some of these animals genetically altered?

REDACTED

Estimated number of animals

REDACTED

Life stage of the animals

REDACTED

Animal type

REDACTED

Are some of these animals genetically altered?

REDACTED

Estimated number of animals

REDACTED

Life stage of the animals

REDACTED

Animal type

REDACTED

Are some of these animals genetically altered?

REDACTED

Estimated number of animals

REDACTED

Life stage of the animals

REDACTED

Continued use/re-use

Describe what has been done to any animals that can be classed as

a) Continued use

N/A

b) Re-use

N/A

Steps

Steps in this protocol

1. Induction and maintenance of general anaesthesia (AC) using a route and agents suitable for species as agreed with the NVS.
2. The level of anaesthesia will be assessed by pedal and eye reflex
3. The maximum blood volume will be obtained through cardiac puncture, cannulation or venepuncture of the femoral artery, cranial vena cava and or other superficial veins.[

Protocol 1 Protocol 1 continued

At the end of the procedure the animal will be killed by Schedule 1 method without regaining consciousness.

Fate of animals

Fate of animals not killed at the end of the protocol

None selected

Give more details about the fate of animals that you are proposing.

All animals in protocol 1 will be humanely killed by a schedule 1 method whilst under anaesthesia.

Adverse effects

Describe the likely adverse effects and the expected incidence in the different animals used.

Terminal blood sampling under anaesthesia is a non-recovery procedure conducted with the animals under an species-specific general anaesthetic regime that has been developed in consultation with the NVS to ensure that the agent(s) and route(s) used are the ones which will cause minimum of discomfort and distress to the animal during the induction of anaesthesia. (AC)

If before or during the sampling procedure, complications arise with the anaesthetic the sampling will immediately cease and the animal will be immediately humanely killed using a schedule 1 method.

In the case of dogs, they will be intubated for this purpose of this procedure and the terminal bleeding process is normally finished within 1 hour from when the initial anaesthetic is given.

The animal will always be humanely killed at the end of the procedure using a schedule 1 method.

Protocol 2

2 Blood sampling without anaesthesia

Severity: Mild

Animal types	Est. numbers	Life stages
Dogs	35	Adult

Protocol details

Severity category

mild

Type of animals

Animal type

Dogs

Are some of these animals genetically altered?

No

Estimated number of animals

35

Life stage of the animals

Adult

Continued use/re-use

Describe what has been done to any animals that can be classed as

a) Continued use

N/A

b) Re-use

Animals that have been used under this protocol and kept alive under the supervision of the NVS, may be re-used. The donor's suitability for use/reuse is assessed prior to being assigned 'donor status' and this status will then be assessed on a continuing basis by the NVS, personal licensee's, Named Animal Care and Welfare Officers and the animal technicians responsible for the animal's care and welfare. Due attention is given to the Home Office Advice Note 02/2015: Animals (Scientific Procedures) Act 1986 Use, Keeping Alive and Re-use Date: October 2015.

Animals that are kept alive are maintained at the establishment under the supervision of the

Named Veterinary Surgeon (NVS) until they are humanely killed, re-used, re-homed or moved to another establishment

Steps

Steps in this protocol

1. The animal will be restrained and the area surrounding the withdrawal site may be shaved to allow improved access.
2. Local anaesthesia and or vein dilation topical creams may be applied (AA). Blood samples will be drawn from superficial vessels using cannulas or needles. This may be repeated provided that no more than 10% blood volume is removed in any 24 hour period and no more than 15% in any 28-day period.
3. Kept alive at the establishment for prospective reuse under this procedure.
4. Animals may be killed by schedule 1 method or may be terminally anaesthetised and blood collected prior to being killed by a schedule 1 method. (AC)

Protocol 2 Protocol 2 continued

5. The withdrawal sites will be rotated and must be fully recovered from any abnormal reaction before re-use is undertaken at the same site

Fate of animals

Fate of animals not killed at the end of the protocol

- Kept alive at the establishment
- Set free/re-homed

Give more details about the fate of animals that you are proposing.

The majority of animals sampled under this protocol, provided they have suffered no more than mildly during the course of procedures and which are not suffering or likely to suffer as a result, may be kept alive in accordance with Standard Condition 11. These animals may then be re-used under this protocol as detailed in the project plan.

Animals intended for rehoming will be carefully assessed, and only those that are likely to adapt and thrive in a new home should be considered. They should be in good health; the most suitable animals are also confident and adaptable. In this context it is important to be aware that beagles are relatively tolerant of changes in their surroundings and have well-developed passive coping strategies, which means that overt demonstrations of stress are less frequent in these animals than in some other strains of dog. Nervous animals adapt less readily to new environments and in consequence may suffer more stress during the process.

They will therefore require more time and effort to re-home successfully.

The age of the animal is a consideration though not necessarily a determining factor. Younger dogs are likely to adapt to a new home more readily, though older dogs can be rehomed successfully as well

Adverse effects

Describe the likely adverse effects and the expected incidence in the different animals used.

Animals will be continuously assessed in consultation with the NVS for their suitability as donor animals.

Protocol 2

Protocol 2 continued

The protocol provides limits for the maximum volumes that may be collected, consequently neither hypervolemia nor anaemia are expected. These levels are monitored on a regular basis by haematology, clinical examination and individual records.

Infection of sampling sites is extremely rare. However, should this occur, animals will be withdrawn from the protocol, referred to the NVS for treatment and not returned to the protocol until the infection is resolved.

Collection sites will be rotated to avoid damage to vessels.

An animal under Protocol 2 may be bled four times a month (maximum) to a maximum of 15% of their circulating blood volume. This is around 52 blood samples per year for as many years as they are deemed fit to remain in the donor pool. The blood volume of a Beagle dog is calculated as approximately 85ml blood per kg of body weight (1275ml). Thus 15% of the blood volume equates to191ml. This volume of blood may be drawn every 4 weeks.

There is no upper age limit for protocol 2 and retirement of the donors will be at the discretion of the NVS who's regular check-ups will determine the suitability for the re-use of the donor (as detailed in the project plan and re-use sections above).

Refinement control measures

Withdrawal of blood will be undertaken using a combination of volumes, routes and frequencies that will result in no more than transient discomfort and no lasting harm. Should there be a failure to collect the sample another superficial vessel would be accessed or another licensee would collect the sample. No more than three attempts will be made at a single site for all species will be allowed. If the bleed was unsuccessful it will be recorded as such but the volume taken will count towards the animal's running total.

If any procedure results in any suffering that is greater than mild and transient or in any way compromises normal behaviour the animal will be humanely killed unless, in the opinion of our veterinary surgeon, the complications can be remedied quickly by minor interventions including altering diet (e.g. moistened diet) or treatments. Additional monitoring will be maintained following NACWO/NVS advice until the animal fully recovers or if no prompt improvement is seen the animal will be humanely killed.

At the end of their useful lives the NVS will decide their fate. The beagles may be sold to a customer or kept alive after retirement from the protocol if they meet re-homing requirements and re-homing can be arranged or euthanised under schedule 1 or they may be terminally bled as required. Re-homing will be applied for on an individual basis.

Cats, dogs, primates, and equidae

Explain why no other species is either suitable for the purpose or practically available.

From 2013 to 2018 under the previous service project license, we have had consistent requests for dog whole blood and plasma. On Average requests per year, we would receive requests for 1,250 ml of whole dog blood and 10,000ml of dog plasma. The requests are accompanied by a completed justification form which asks the customer to justify the use of animal products compared to non-animal models, along with the benefits of the project and the purpose.

The date and source of dog are in recorded in the company's proprietary (REDACTED) or the data is storedin an Excel database as required by the Standard Conditions (9 & 10) of the establishment Licence.

We are providing dog bio-products as a service to our customers and dogs are only used for this project due to their historical and expected future demand for canine blood products and tissues. Holding a pool of dogs REDACTED reduces the need for duplication of donor animals at multiple customers' animal facilities. Demands from customers are based on scientific needs such as: dogs have a similar gastric mucosal membrane to that of humans. The dog is also the target species for veterinary products being tested, or the dog model is chosen as it is the only species that most closely reflects the situation in man for a particular area of research.

Endangered animals

Explain why the programme of work cannot be achieved without using endangered animals.

N/A

Animals taken from the wild

Why can't your aims and objectives be achieved without using animals taken from the wild. How will you minimize harms that arise during their capture and release?

N/A

Marmosets

Why can't you achieve your aims and objectives without using marmosets that have been bred in captivity or obtained from a self-sustaining colony?

N/A

Feral animals

Why can't you achieve your aims and objectives without using feral animals of a domestic species?

N/A

Neuromuscular blocking agents (NMBAs)

Detail the use of NMBAs in any part of this project?

N/A

Project summary

Describe the aims and objectives of the project.

To use animals to provide blood and tissues to generate data to support the development of effective and safe medicines to treat diseases where there is currently a clinical unmet need e.g. cancer & heart disease.

What are the potential benefits that will derive from this project?

Bio-products provided will contribute invaluable scientific information to support and progress potential new medicines where there is currently an unmet clinical need. Conducting investigations using blood and/or tissues taken from animals reduces the number of potential new medicines requiring evaluation in living animals and can be used to establish whether conducting experiments on living animals would be beneficial.
The products are also used to aid the development of new medicines in man or animals when it is necessary to calibrate and validate many of the machines or testing systems used to support research. They may also be used to support other methods in research as an alternative to live animals.

What types and approximate numbers of animals will you use over the course of this project?

Over a 5 year period:
Dogs: 275
REDACTED

REDACTED

What are the expected adverse effects and endpoints for animals used in this project?

More than 85% of the animals used under this licence will be kept under general anaesthesia throughout the sampling procedures and will not be brought back to consciousness. They will be humanely killed while still under anaesthesia with an overdose of anaesthetic. Therefore these animals are not expected nor likely to experience any adverse effects.
The remainder (mainly dogs) are trained to donate small volumes of fresh whole blood at weekly or monthly intervals and these donors are not expected to suffer adverse effects as a result of the project (similar to taking a blood sample from a human). These dogs will continue to be used as donors for several years until they are retired. The dogs receive full clinical health checks by a veterinarian and experienced animal technicians. They will be retired if there are signs that their, normal health state is affected by the project, their age or health issues.
Training of donors is however not always possible and in the case of REDACTED. Adverse effects from repeated blood collection are not expected under this project but could (rarely) include slight bruising, anaemia or uncontrolled bleeding. Any animal with anaemia or poor clotting mechanisms will be removed from the bio-products donor pool. Adverse effects of the sedation are not expected. Any adversely affected animals will cease to be used and will be referred to the responsible veterinary surgeon who will determine the need for any treatment, consider its suitability for rehoming orif the animal should be humanely killed.

Replacement

Why can’t your project use non-animal alternatives?

Drug research programmes rely, in part, on biological materials obtained from human or animal sources to validate and confirm disease-associated drug targets and the mechanisms of action for potential new medicines. This programme of work supports the replacement of using living animals by enabling the supply of high quality blood and tissue samples where living cells are needed for experiments due to the lack of appropriate cells from existing alternative sources or instances where it is not possible to use cell culture techniques.

There are a number of promising technologies in development which aim to utilise human cells to recreate the physiological functions of organs without using animals. However, these *in vitro* approaches do not yet offer an alternative to totally replace the need for research animals and authentic blood, blood products, body fluids and tissues to enable their use in all the investigations required to support the research and development of new medicines.

Reduction

How will you ensure that the number of animals used will be kept to a minimum?

The number of animals used is minimised by using proven collection techniques including taking blood under non-recovery anaesthesia to ensure that large volume, non-clotted samples can be obtained.

Tissue requests will be co-ordinated in order to supply multiple samples from one animal (e.g. whole blood, pancreas, femurs and liver) to a number of requesters for their individual purposes. This reduces the total number used.

The project aims to provide blood components and tissues of the highest quality as this improves the significance of test results in studies involving animals and can often lead to improved scientific knowledge and a reduction in the overall number of animals.

The project can reuse animals and this means that multiple samples can be obtained from a smaller number of donors thereby reducing the need to kill animals for the purpose of taking each sample.

The use of blood products, tissues and organs that are obtained from animals that are not suitable for direct use in research reduces the total numbers of live animals required for research

Refinement

Why are your choices of animal, model, and method the most refined?

Where there is scientific need to preserve tissue integrity/architecture or obtain high volume and quality blood samples, then taking samples under appropriate and well maintained non-recovery anaesthesia is considered the most refined approach and we have refined the technique so that we will cause the minimum amount of discomfort and distress to the animal when we anaesthetise it.

Persons taking samples are well trained in the techniques involved to ensure high quality samples are obtained quickly & effectively with minimal impact on animal welfare.

The choice of donor species is driven by the scientific needs of research scientists.

When it is prudent to sedate the donor prior to sampling (if the donor cannot be readily trained or if it would be hazardous for the person taking the sample), then a second drug is used to reverse the sedative and thereby speed up recovery from sedation.

By only using donor dogs from the colonies we house, we ensure they are kept in appropriate long-term housing. The reuse of animals in a donor pool means they are used for a minimum 1 year in REDACTED and several years for dogs. The animals benefit in the long term by being housed in appropriate socially enriched housing, cared for by trained staff. This housing is in the holding rooms of the general population and they therefore benefit from being in busy, familiar surroundings with social contacts of other dogs.

Additional conditions

Special conditions for this licence

No answer provided

Export of animals (transfer)

Genetically altered rodents, genetically altered zebra fish and genetically altered Xenopus sp. bred and/or maintained under the authority of this project may be transferred to scientific establishments outside the United Kingdom only if:

1. The transfer will be made to a recognised scientific research establishment with a scientific requirement for genetically altered animals (or their controls) of that type; and where appropriate veterinary care can be provided as necessary; and
2. Sending tissue, gametes or embryos is not practicable or carries a higher potential welfare cost than moving live animals; and
3. Animals will be transported in accordance with all relevant regulations regarding welfare of animals in transit or the import or export of animals; and
4. Animals will be inspected by a competent person before transfer; and
5. A veterinary surgeon will confirm that he/she is not aware of any reason why these animals might suffer by virtue of the fact of being moved to another recognised scientific establishment.
6. Any transport related problems with the welfare of the animals will be notified to the Home Office promptly.

Standard conditions

1. The licence holder is responsible for the overall implementation of the programme of work specified in this licence and for ensuring that the programme of work is carried out in compliance with the conditions of the licence.

2. The licence holder shall ensure that the specified programme of work does not involve the application of any regulated procedure to which there is a scientifically satisfactory alternative method or testing strategy not entailing the use of a protected animal.

3. The licence holder shall ensure that regulated procedures are not applied to an animal as part of the specified programme of work if the data to be obtained from the application of those procedures is already available in a Member State and has been obtained there by procedures which satisfy any relevant regulatory requirements of the EU.

4. The licence holder shall ensure that the regulated procedures applied as part of the programme of work specified in this licence are those which to the greatest extent use the minimum number of animals; involve animals with the lowest capacity to experience pain, suffering, distress or lasting harm; cause the least pain, suffering, distress or lasting harm; and are most likely to provide satisfactory results.

5. The licence holder shall ensure that the regulated procedures applied as part of the programme of work specified in this licence are designed so as to result in the death of as few protected animals as possible; and to reduce to the minimum possible the duration and intensity of suffering caused to those animals that die and, as far as possible, ensure a painless death.

6. The licence holder shall ensure that the appropriate level of supervision is provided for all personal licensees carrying out regulated procedures under the authority of this licence.

7. The licence holder shall ensure that a regulated procedure is not applied to an animal as part of the programme of work specified in this licence if the procedure may cause the animal severe pain, suffering or distress that is likely to be long-lasting and cannot be ameliorated.

8. The licence holder shall ensure that where a regulated procedure is being applied to an animal as part of the programme of work specified in this licence, any unnecessary pain, suffering, distress or lasting harm that is being caused to the animal shall be stopped.

9. The licence holder shall ensure that where a regulated procedure is applied to an animal as part of the specified programme of work, death as the end-point of the procedure is avoided as far as possible and is replaced by an early and humane end-point; and as soon as the purpose of the procedure has been achieved, the procedure is stopped and appropriate action is taken to minimise the suffering of the animal.

10. The licence holder shall ensure that where a regulated procedure has been applied to an animal as part of the programme of work specified in this licence, a suitably qualified person classifies the severity of the procedure as “non-recovery”, “mild”, “moderate” or “severe” using the criteria in Annex 8 of the Animals Directive. For the purposes of this condition, a series of regulated procedures applied to an animal for a particular purpose is to be treated as constituting a single regulated procedure.

11. Where a series of regulated procedures are applied to an animal for a particular purpose the licence holder shall ensure that the animal is killed at the end of the series unless a veterinary surgeon or other competent person has determined that the animal is not suffering and is not likely to suffer adverse effects, as a result of the regulated procedures.

12. Regulated procedures shall not be carried out on any stray animal of a domestic species as part of the programme of work specified in this licence.

13. Except with the authorisation of the Secretary of State, regulated procedures shall not be carried out as part of the programme of work specified in this licence on any of the following type of animal:

    (a) any feral animal of a domestic species;
    (b) any animal taken from the wild;
    (c) a marmoset unless it is the offspring of marmosets bred in captivity or has been obtained from a self-sustaining colony of marmosets;
    (d) any animal of a description specified in Schedule 2 to the Act unless it has been bred for use in procedures.

14. If the application of regulated procedures to animals taken from the wild is authorised inthis licence the holder shall ensure:

    (a) that animals taken from the wild are captured by a competent person using a method which does not cause the animal avoidable pain, suffering, distress or lasting harm; and

(b) that an animal taken from the wild which is found to be injured or in poor health is not subjected to a regulated procedure unless and until it has been examined by a veterinary surgeon or other competent person; and, unless the Secretary of State has agreed otherwise, action has been taken to minimise the suffering of the animal.

15. The licence holder must give any necessary assistance to inspectors carrying out visits by virtue of section 18(2A)(b); and to experts of the European Commission carrying out duties under Article 35 of the Animals Directive.

16. If the licence holder becomes aware of a failure to comply with any conditions of the licence the holder must take appropriate steps to rectify the failure (if it is capable of being rectified); and keep a record of the steps taken.

17. All authorised procedures shall be carried out under general or local anaesthesia unless—

(a) anaesthesia would be more traumatic to the animal concerned than the procedures themselves; or
(b) anaesthesia would be incompatible with the purposes of the procedures.

18. The licence holder shall ensure adherence to the severity limits as specified in the project licence and observance of any other controls described in the licence. If these constraints appear to have been, or are likely to be, breached, the holder shall ensure that the Secretary of State is notified as soon as possible.

19. The licence holder shall maintain a contemporaneous record of all animals on which procedures have been carried out under the authority of the project licence. This record shall show the procedures used and the names of personal licensees who have carried out the procedures. The record shall, on request, be submitted to the Secretary of State or made available to an Inspector.

20. The licence holder shall send to the Secretary of State, before 31 January each year (and within 28 days of the licence having expired or been revoked), a report in a form specified by the Secretary of State, giving details of the number of procedures and animals used, and the nature and purpose of the procedures performed under the authority of the project licence during the calendar year.

21. The licence holder shall maintain a list of publications resulting from the licensed programme of work and a copy of any such publication shall be made available to the Secretary of State on request. The list shall, on request, be submitted to the Secretary of State or made available to an Inspector, and it shall be submitted to the Secretary of State when the licence is returned to him on expiry or for revocation.

22. The project licence holder shall submit such other reports as the Secretary of State may from time to time require.

23. The project licence holder shall ensure that details of the programme of work and regulated procedures specified in the licence, and any additional conditions imposed on those procedures, are known to

(a) all personal licensees performing those procedures;

(b) the Named Person Responsible for Compliance;
(c) the Named Animal Care and Welfare Officers responsible for the day to day care of the animals;
(d) the Named Veterinary Surgeon, on request; and
(e) the Named Information Officer and Named Training and Competency Officer, on request.

24. The licence holder must obtain the permission of the Secretary of State before—

(a) any animal undergoing regulated procedures is moved from a place specified in one section 2C licence to a place specified in another section 2C licence; or
(b) any animal is released for slaughter, unless this is already explicitly authorised by the project licence.

25. The licence remains the property of the Secretary of State, and shall be surrendered to him on request.