AEC/17 (Amended 01/14)

Massey University
Animal Ethics Committee

To: The Secretary
Animal Ethics Committee
Research Ethics Office
Room 1.25
Courtyard Complex
Manawatu Campus PN221

Please provide one original single-sided application plus 15 copies
Application due Wednesday of week prior to meeting

APPLICATION FOR APPROVAL OF PROPOSED RESEARCH, TESTING OR TEACHING PROCEDURES USING LIVE ANIMALS

1. CHIEF APPLICANT: *(Staff Member only)*

(a) **Name** [redacted]
Qualifications BVSc, PhD
Position Senior Lecturer
Inst/Sch/Dept IVABS/mEpiLab

2. OTHER APPLICANTS: *(refer Code of Ethical Conduct, Item 3.2, for those who should be listed)*

(a) **Name** [redacted]
Qualifications BVSc, MVSc
Position Postgraduate student researcher

(b) **Name** [redacted]
Qualifications BSc, MSc, PhD
Position Postdoctoral researcher

OFFICE USE ONLY

RECEIVED
06 MAY 2015
Research Ethics

Date Received:

Copy for:
Applicant
Head of Institute/Department
Office

Date sent: 30-6-15

Protocol No: 15/35

Decision:

MASSEY UNIVERSITY ANIMAL ETHICS COMMITTEE
APPROVED
Date: 15-5-15

[redacted]

3. DETAILS OF PROJECT:

(a) Title *(maximum 20 words)*: Is the family pet a risk for multidrug-resistant bacterial infections?

(b) Type of project

Research	[x]
Testing	[ ]
Teaching	[ ]

Paper Number(s):

(c) Commercial sensitivity status

No	[x]
Yes	[ ]

(d) Does the project involve use of native species?

No	[x]
Yes	[ ]

If yes:

Has DoC approval been:

Sought but not yet granted	[ ]
Granted	[ ]

Permit Number(s):

Māori consultation:
(must be by applicant(s) directly with iwi)

(For guidance, click here)

Has been undertaken	[ ]
Is currently being undertaken	[ ]

The project is approved by iwi*:

Yes [ ] No [ ]

*(*Please refer to guidance opposite)*

4. JUSTIFICATION OF PROJECT:

(a) What are the expected benefits of the proposed work and how will the new knowledge be communicated to others? *(Benefits may include improved basic knowledge, improved animal health, teaching)*

Aims of research: The emergence and transmission of antimicrobial resistant bacteria between companion animals (pets) and people has been recognised as an area requiring further research (Gandolfi-Decristophoris *et al.* 2013; Huber *et al.* 2013; Schmiedel *et al.* 2014). This is especially true for extended-spectrum beta-lactamase producing Enterobacteriaceae (ESBL-E) and plasmid-mediated AmpC beta-lactamase producing Enterobacteriaceae (PMACBL-E), which constitute the focus of this proposed study. The objectives of this study are to:

- Assess risk factors for community ESBL-E/ PMACBL-E infections that are not linked to healthcare facilities or residential homes, via a case-control study
- Assess the population genetics, diversity and transmission of ESBL-E / PMACBL-E in humans, companion animals, and wild birds
- Develop effective public health interventions that will decrease the prevalence and transmission of ESBL-E / PMACBL-E and transmission within the community

Benefits of research: This study will provide important information for designing public health interventions in order to minimise the spread of multidrug-resistant bacteria both within households and between animals and people. Further, the findings could potentially be applied to other close-

contact infectious or zoonotic diseases. The implications for animal health are related to the potential for recommendations to improve antimicrobial stewardship in companion animals.

Dissemination of research: Findings of this study will be used for the generation of a PhD (for [redacted], and will also be disseminated through peer-reviewed literature and conference presentations, including national and international public health conferences. In addition, the extended research team includes highly experienced veterinary and human health researchers based in two universities and a Crown Research Institute. This team is well placed to disseminate results of this work through their advisory roles to the Ministry of Health and Ministry of Primary Industries, as well as through their professional networks in human and veterinary public health. We anticipate a number of peer-reviewed publications will arise from this work and, given the subject area we will aim to publish in high-ranking international journals.

(b) **Why is it necessary to use animals for this activity?** *(The term "animal" is defined in the Code of Ethical Conduct, Item 10)*

This study will compare human cases infected with resistant bacteria to a population of healthy human controls, to investigate the sources of multidrug-resistant bacteria within our communities. We will also assess the roles played by family pets in spreading ESBL/ PMACBL-E within the household. The collection and testing of faecal samples from companion animals is required to compare ESBL/ PMACBL-E isolated from humans with those isolated from their pets. This will address the main research questions of this study, *are pets a source of multi-drug resistant bacteria?*

5. DESCRIPTION OF PROCEDURES AND MANIPULATIONS: *("Manipulation" is defined in the Code of Ethical Conduct, Item 10)*

(a) **Give a brief description of your trial design/teaching demonstration.** *(One or two paragraphs) (For complex protocols, it may be beneficial to provide information as a timeline or in tabulated form)*

For the purpose of this study, a case is defined as a person with a newly acquired ESBL-E / PMACBL-E clinical infection not associated with hospitalisation or long-term care in the past 12-months. These cases will be recruited from four district heath boards: Auckland DHB, Counties Manukau DHB, Northland DHB and Waitemata DHB. Human control participants will be identified by telephone using random digit dialling, by UMR Research, a specialist telephone survey provider. Human controls will be excluded if they have had an overnight admission to a healthcare facility or lived in a residential care home within the 12 months preceding their recruitment.

Human participants will be asked to complete a health based telephone questionnaire, where information will be collected on the pets and people who live in the house with the participant. Human participants and their household contacts will be invited to submit faecal samples to test for ESBL/ PMACBL-E.

Consent will be asked for pets to be included in the study. Participants will be invited to submit one-time faecal samples from pets of the household, and a pooled faecal sample from wild bird feeding/watering sites. A maximum of five pets per household will be included in the study, and one pooled sample from wild birds. Animal sampling will consist of:

- Cat faecal samples *(collected by owners from litter trays)* OR cat faecal samples *(collected under the direct supervision of a veterinarian using an appropriate method without chemical restraint)*
- Dog faecal samples *(collected by owners after dog has passed a fresh bowel motion)*
- Pet bird faecal samples *(collected by owners from cage or aviary floor)* and/or wild bird samples *(collected from outdoor bird feeders or baths by case or control participant)*

Sample submission packs sent to households will include: participant information sheets, consent forms, and all sampling materials for both pet owners and human participants. Information regarding the safe collection of samples by participants will be found on the reverse of the submission form.

Faecal samples from both humans and pets will be collected at the same time, ensuring all samples are sent by prepaid courier to Massey University together.

(b) Describe the statistical methods that you will use to analyse these data.

Human exposures (assessed through the questionnaire) will be measured against disease status of people in this study, and quantified using odds ratios. This will be done via a multivariate logistic regression model, adjusting for confounders (such as demographic factors or pre-existing comorbidities).

The analysis of *E. coli* and *Klebsiella* genotypes isolated from human and animal will enable evaluation of potential transmission of given strains and genes (especially antimicrobial resistance genes) between humans and animals. Data gained from the case-control study will also contribute to the development of a stochastic, compartmental mathematical model of the transmission dynamics of ESBL/ PMACBL-E within households. The model will be framed by estimating the rates of transfer from human to human, animal to animal, and between humans and animals; the shedding-recovery rate; and the lag times between non-shedding and shedding. Forward simulation of the model will also be used to assess public health measures that could decrease the probability of transmission. In particular assessments will be made of the effect of reducing 'risky' behaviours between people and animals on the transmission dynamics of infection.

(c) Provide justification for the group sizes that you propose.

The number of animal samples collected as part of this project will largely be chosen based owners willingness (a convenience sample), as submission of animal faecal samples is not required for participation in the case-control study. To collect an appropriate amount of information regarding animal contact, the numbers of owner households have been selected as follows:

- The human case population will derive from the Auckland and Northland regions. We estimate from the number of ESBL/PMACBL-E cases currently processed by Labtests NZ (approximately 2-3 per day for the Northland and Auckland regions) and the proportion of these that are community acquired (approximately 50%) (Heffernan and Woodhouse 2013; Drinkovic *et al.* 2014), and new infections, that 175 cases can be recruited within 12 months of the start of the study.
    - An estimated number of likely pet faecal submissions by cases can be made assuming 60% of participants agree to have at least one of every type of pet (cat, dog, bird) sampled. For this, we assume 48%, 29% and 6% of the case population are in households owning a cat, dog and bird respectively (Anonymous 2011). This results in an estimate of at least 50 cat samples, 30 dog samples and 5 bird samples from cases. It is likely that for convenience reasons, the true number collected will be higher for dogs and lower for cats. It is expected that only one sample per household will be submitted from pet birds.
    - Assuming 47 % of households feeding wild birds (Galbraith *et al.* 2014), and 60% of control participants submitting a sample, we estimate 70 pooled samples from wild bird baths/feeders.
- The human control population size is based on the above likely number of cases. This is assuming that 48%, 29% and 6% of the control population are in households owning a cat, dog and bird respectively (Anonymous 2011), 525 controls would detect an odds ratio of 2.5 at a significance level of 0.05 with a power of 99% (cats and dogs) and 84% (bird exposure). Over the 12-months of the study, between 40 and 45 controls will be recruited each month by the telephone survey provider.
    - Using the same method for estimating the number of pet submissions, we estimate 40% of control respondents willing to submit a sample from pets. This results in at least 100 cat samples, 60 dog samples, 10 bird samples submitted from control households. As with the case population, it is likely that the true number collected

will be higher for dogs and lower for cats.

- Assuming 47% of households feed wild birds (Galbraith *et al.* 2014), and 40% of control participants submitting a sample, we estimate 100 pooled samples from wild bird baths/feeders.

- The overall maximum number of pet submissions we are likely to process [assuming 68% of households owing a pet (Anonymous 2011), with a maximum of 5 samples submitted] will be 2380.

  - The maximum number of wild bird samples we are likely to process [assuming 47% of households with wild bird feeders/baths (Galbraith *et al.* 2014), and a maximum of 1 sample submitted] will be 330.

(d) Describe the manipulations to be performed on the animals.

The experience of animals involved in this study will vary. Please see the appended submission form for instructions that will be provided to pet owners for the collection of faecal samples. All materials will be provided to all participants for the safe collection of faeces. The differing manipulations of animals involved in this study are outlined below.

- Cats: Outdoor cats will be restrained by a person experienced in handling. A veterinarian will collect a sample from the cat using a small lubricated curette inserted into the rectum. If no sample is obtained, or the sample is not able to be obtained within two minutes, a record will be made that collection was attempted but not successful. No repeat sampling will be done. No direct animal contact is expected in the sample collection from indoor cats. Faecal samples from indoor cats will be collected from the cat litter tray by the cat owner.
- Dogs: The collection of faecal samples from dogs will be performed by the owner. Sampling will occur after the dog has passed a bowel motion, and the owner will collect part of the voided faeces for submission.
- Birds: The collection of faecal samples from birds will be performed by the owner. Faeces will be collected from a cage or aviary surface, or from a wild bird bath or feeder.

(e) How will the proposed manipulation affect the well-being of the animals?

There are unlikely to be any direct effects on the long-term well-being of animals involved in this study.

(f) Describe any restraint applied to the animals.

This section applies to outdoor-living household cats, where the owner consents to collection at the time of case/control participant signing a consent form.

Collection of all outdoor-cat faecal samples by direct means will require restraint of the cat for the duration of faecal collection, up to a maximum of two minutes. This manual restraint will be performed by a person trained in animal handling and under the supervision of a veterinarian registered with the NZVC (named [redacted]. Restraint will be conducted in the presence of the owner, inside the home of the owner, and with concern for both handler and animal safety and welfare.

6. CARE OF ANIMALS:

(a) What access will the animals have to water?

Not applicable.

(b) Describe the feeding regimen for the animals.

Not applicable.

(c) **From where will the animals be sourced?** *(Refer Code of Ethical Conduct, Item 2.15)*
(Where animals are personally owned, consent forms must be obtained)

Animals used in this study will be owned by someone living in the house with people who have participated in a voluntary questionnaire as part of a case-control study on ESBL/PMACBL – Enterobacteriaceae. Please see appended consent form.

(d) **Where will the animals be kept throughout the study period?**

Outdoor cats will be sampled inside their owner's house.

(e) **Who is responsible for the routine care and health surveillance of the animals?**

[redacted]

(f) **If the Chief Applicant is unavailable, who will make decisions if emergency care is required?**

[redacted]

7. FATE OF ANIMALS:

Note: If any animal is either euthanased or dies due to the unexpected side effects of approved manipulations, the animal should be subjected to a post-mortem examination by an experienced person. The results of the post-mortem must be communicated to the Massey University Animal Ethics Committee along with any modifications put in place to minimise the occurrence of similar events to other animals.

(a) **What will happen to the animals at the completion of the study?**

Outdoor cats will be released into their owner's house, and observed for 5-10 minutes, while the remaining samples and paperwork are processed.

In this study all animals (pets) will remain with owners, as this is a cross-sectional component of the research project, and ongoing physical care is not required for the purposes of the study. However, full contact information will be provided to the owners and they will be able to speak with a veterinarian [redacted] if they have any concerns about their animals after sampling has taken place.

(b) **Will any animals be euthanased, either as part of the study, or in the event of untoward outcomes?**

No	**x**
Yes	

If yes:

Applicants must be familiar with the resource material on supporting staff involved with animal euthanasia at the following link:
http://www.massey.ac.nz/massey/staffroom/national-shared-services/health-safety-for-managers/responding-to-stress-complaint.cfm

The Chief Applicant must also confirm that he/she understands his/her obligations in regard to discussing the availability of this material with all people listed on the application on a per-project basis. ☐

Tick Box

Describe the euthanasia method you will use: Not Applicable

(c) **What level of losses do you expect to occur during this work and how will you investigate any unexpected deaths?** *(refer Code of Ethical Conduct, Items 2.20-2.22)*

No deaths or losses are expected as part of this study, as it is a one-time sample submission from available animals. In the unlikely event of an adverse event following sampling, veterinary advice by phone will be available for the full duration of the study. If any outdoor cats are euthanized or die in the one month following sample collection, a full post-mortem at Massey University will be offered.

8. ALLEVIATION OF IMPACT OF MANIPULATIONS:

(a) **What features of the manipulations minimise their impact on the animals?**

Apart from outdoor cats, animal faecal samples will be collected soon after a recent deposit by the animal. Indirect collection in this manner prevents any impact on the animals involved.

The collection of samples from outdoor cats will be done by a veterinarian, with a small curette and lubricated in a similar manner a faecal sample for intestinal helminth detection may be obtained. Cats will be restrained in a manner described in **5. (f)**, to minimise any physical impact of procedure. Owners of pets will be given a full participant information sheet prior to collection, and consent form for this will be signed by them, indicating that they will require a visit from the research team to collect samples (see appended documents).

All participating pet owners will have the option to be informed if their pet cultures an ESBL/PMACBL *E. coli* or *Klebsiella*. If they wish, an informative letter will also be addressed to their veterinarian, and contact information of a veterinarian on the research team will be provided if the owner or veterinarian has any questions regarding the pet. This is to mitigate any negative consequences of a positive culture for ESBL or PMACBL *E. coli* or *Klebsiella*.

(b) If blood samples are to be collected, stipulate volume per sample and frequency of sampling.

Not applicable.

(c) Stipulate the use (and dose rate and route of administration) of any anaesthesia, analgesia, sedative, tranquilliser or other pharmacological agent applied to reduce the impact of manipulations on the animals.

Not applicable.

(d) What frequency of monitoring is to be maintained?

Outdoor cats will be monitored by the handler and veterinarian during handling for signs of stress.

(e) What advice regarding identification of any expected adverse effects will be given to staff responsible for the ongoing care of the animals?

Not applicable.

9. EXPERIENCE OF APPLICANTS:

(a) What is the experience of the applicants with the techniques being used in this project?

This project involves sampling of faeces from cats is a technique familiar to veterinary researcher [redacted] who has previous experience in practice as a small-animal predominant veterinarian. The person restraining the cat for faecal collection will be suitably trained in handling.

(b) If an applicant is using a technique with which he/she has no previous experience, what training will be provided?

Not applicable.

(c) List the people providing professional services and the services provided. *(refer Code of Ethical Conduct, Item 3.2) (These personnel need not be applicants)*

Not applicable.

Supplementary documentation:

- Participant information sheet and consent form for pet owners
- Submission form with instructions to owners on the appropriate collection of faecal samples
- Draft questionnaire (concurrently submitted to the Health and Disabilities Ethics Committee)

References:

Anonymous. Companion Animals in New Zealand. *The New Zealand Companion Animal Council Inc*, 2011

Drinkovic D, Morris AJ, Dyet K, Bakker S, Heffernan H. Plasmid-mediated AmpC beta-lactamase-producing Escherichia coli causing urinary tract infection in the Auckland community likely to be resistant to commonly prescribed antimicrobials. *The New Zealand Medical Journal* 128, 50-9, 2014

Galbraith JA, Beggs JR, Jones DN, McNaughton EJ, Krull CR, Stanley MC. Risks and drivers of wild bird feeding in urban areas of New Zealand. *Biological Conservation* 180, 64-74, doi:http://dx.doi.org/10.1016/j.biocon.2014.09.038, 2014

Gandolfi-Decristophoris P, Petrini O, Ruggeri-Bernardi N, Schelling E. Extended-spectrum β-lactamase-producing Enterobacteriaceae in healthy companion animals living in nursing homes and in the community. *Am J Infect Control* 41, 831-5, doi:http://dx.doi.org/10.1016/j.ajic.2012.11.013, 2013

Heffernan H, Woodhouse R. Annual survey of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, 2013. Institute of Environmental Science and Research Limited (ESR), Porirua, N.Z., 2013

Huber H, Zweifel C, Wittenbrink MM, Stephan R. ESBL-producing uropathogenic Escherichia coli isolated from dogs and cats in Switzerland. *Veterinary Microbiology* 162, 992-6, doi:http://dx.doi.org/10.1016/j.vetmic.2012.10.029, 2013

Schmiedel J, Falgenhauer L, Domann E, Bauerfeind R, Prenger-Berninghoff E, Imirzalioglu C, Chakraborty T. Multiresistant extended-spectrum β-lactamase-producing Enterobacteriaceae from humans, companion animals and horses in central Hesse, Germany. *BMC Microbiol* 14, 2014

10. USE OF RESTRICTED DRUGS:

(a) Personnel who are **not** registered veterinarians and who wish to administer restricted veterinary or human medicines must read and comply with the Operating Plan for the Use of Restricted Veterinary Medicines according to specific Veterinary Operating Instructions at Massey University.

The code can be downloaded here.

Personnel must also complete the following:

I/We declare that I/we have read the above code and will comply with its requirements.

N/A

Date: _____

11. SIGNATURES OF APPLICANTS:

(a) I have read the Massey University Code of Ethical Conduct for the Use of Live Animals for Research, Testing and Teaching and agree to comply with its requirements throughout the duration of the proposed procedures;

(b) To the best of my knowledge, this protocol or one substantially like it has not been declined by another Animal Ethics Committee.

Note: Carefully read (a) and (b) above before signing

(Original signatures only – must not be electronically inserted)

Signature(s) of Applicant(s)

[redacted]

Printed Name(s) of Applicant(s)

[redacted]

Date: 5/5/15

12. APPLICANT CHECKLIST:

The following checklist must be completed by the applicant prior to arranging for endorsement of the protocol by the Designated Signing Authority: *(A list of current authorising personnel for each institute is available from the Secretary of MUAEC)*

- [x] Appropriate research title when evaluated against methodology
- [x] Justification and methodology written in terms readily understood by lay members of committee
- [x] Clear distinction between justification and research methodology
- [x] Concise wording and information relevant to animal ethics
- [x] Clearly explained experimental design
- [x] Complete power analysis to determine necessary number of animals required
- [x] Provision of all signatures in correct sections
- [x] Provision of heading details (chief applicant, institute, project title) (statistics form)
- [x] Grading of manipulations (statistics form)
- [x] Provision of completion date (statistics form)

13. APPROVAL BY DESIGNATED SIGNING AUTHORITY:

(This signature must be obtained by the applicant **prior to** submission to MUAEC for consideration and must **not** be that of one of the applicants)

(Original signature only – must not be electronically inserted)

I have read this application and agree that it meets the intent and spirit of the Massey University Code of Ethical Conduct for the Use of Live Animals for Research, Testing and Teaching:

Signed: [redacted] Date: 5/5/15

Name: [redacted]

Institute: IVABS - INSTITUTE OF VETERINARY, ANIMAL + BIOMEDICAL SCIENCES.

14. ANIMAL USE STATISTICS:

Please ensure that an "Animal Use Statistics" form (following) is completed and attached.

NOTES:

(a) The staff member with signing authority delegated from MUAEC must not sign his/her own application in Section 13 above. Please obtain the signature of another staff member with delegated authority.

(b) Any departure from an approved protocol that adversely affects the welfare or increases the number of animals or reduces the validity of the study must be approved by the Chair of MUAEC acting with authority vested through Items 3.13-3.15 of the Code of Ethical Conduct. A description of such modifications shall be submitted to the Secretary of MUAEC who will attach it to the original protocol and note it on the agenda for the next meeting. Further copies shall be attached to the protocols held by the Institute and the Chief Applicant.

ANIMAL USE STATISTICS
APPLICATION/FINAL RETURN FORM
(Amended 01/14)

Protocol ID 15/35

If more than one animal type is required, then fill in one form for each type.

Application: When applying to MUAEC for approval of a manipulation, the applicant should complete Box 1 and enter in Boxes 2 to 7, in the *'Planned'* column (P), the appropriate figures for the number of animals required.

Final Return: When the manipulation is concluded, Boxes 2 to 10 should then be completed in the *'Used'* column (U) by entering appropriate figures for the number of animals which were actually used.

NOTE: **Boxes 2, 3, 4, 5, 6, 8-9 and 10 must add up to the same number.**

Chief Applicant: [redacted]

Inst/Sch/Dept: [redacted]

Title of Project: Is the family pet a risk for multidrug-resistant bacterial infections?

1. Animal type: Other Domestic Mammals *(see bottom of this form)* Code: 1 o

2. Source of animals (number)

		P	U
Breeding unit	a		
Commercial	b		
Farm	c		
Born during project	d		
Captured	e		
Imported	f		
Public sources	g	150	
TOTAL = A			

3. Status of animals (number)

		P	U
Normal/conventional	a	150	
*SPF/germ free	b		
Diseased	c		
Transgenic/chimaera	d		
Protected species	e		
Unborn/pre-hatched	f		
Other	g		

* *Specific pathogen free*

4. Main category of manipulation/use (enter the total from 2 above in one box only)

		P	U			P	U			P	U
Teaching	a			Basic biological research	e			Production of biological agents	j		
Species conservation	b			Medical research	f	150		Development of alternatives	k		
Environmental management	c			Veterinary research	g			Other	m		
Animal husbandry	d			Testing	h						

5. Any re-use of animals (number to be inserted)

		P	U			P	U	
No prior use	a	150		Previously used	b			Total a + b = 150

6. Grading of manipulations (number in each grade to be inserted)

(Download guidelines for selecting appropriate categories)

	Grade	P	U
A manipulation or use that causes no stress or pain or virtually no stress or pain. **No impact or virtually no impact.**	A		
A manipulation or use that causes stress or pain of a minor intensity for a short duration. **Little impact.**	B	150	
A manipulation or use that causes stress or pain of a minor intensity for a long duration or of a moderate intensity for a short duration. **Moderate impact.**	C		
A manipulation or use that causes stress or pain of a moderate intensity for a long duration or of a severe intensity for a short duration. **High impact.**	D		
A manipulation or use that causes stress or pain of a severe intensity for a long duration or of a very severe intensity for any duration. **Very high impact.**	E		

7. Expected Date of Completion (maximum three years): One year for animal use: August 2016

ANIMAL DISPOSITION/FATE AT CONCLUSION OF RESEARCH, TESTING OR TEACHING OUTLINED IN THIS PROTOCOL

The data in Boxes 8 to 10 refer only to the animals noted in this protocol which actually entered the project and were manipulated - they do not refer to those it was proposed to manipulate but which were never used. This information is to be provided only when the research, testing or teaching has been completed and the animals have been disposed of as below.

8. Alive	Used	
Retained by your institution's farms/colonies	a	
Returned to owner	b	
Released to the wild	c	
Disposed of to others	d	
TOTAL ALIVE	**=B=**	

9. Dead	Used	
Killed for dissection, sampling, taking organs	a	
Died/destroyed in the course of the manipulation/use	b	
Euthanased after manipulation or use	c	
Died/destroyed for reason not associated with manipulation/use	d	
TOTAL DEAD	**=C=**	

10. GRAND TOTAL MANIPULATIONS/USED = B + C =

Check on the final return that B + C = A in the "Used" column of Box 2.

BOX 1: ANIMAL TYPE CODES

Animal Type	Code	Animal Type	Code
Rodents	l a = Mice	Birds	l p = Fowls, Chickens
	l b = Rats		l q = Pigeons
	l c = Guinea Pigs		l r = Other Birds
	l d = Hamsters	Miscellaneous	l s = Marine Mammals
Rabbits	l e = Rabbits		l t = Possums
Farm Animals	l f = Sheep		l u = Reptiles
	l g = Cattle		l w = Amphibia
	l h = Goats		l x = Fish
	l j = Deer		l z = Octopus, Squid, Crab, Lobster, Crayfish
	l k = Pigs	Other	l y = Other Species (*name)
Other Domestic Mammals	l m = Horses		
	l n = Dogs		
	l o = Cats		

Massey University
Animal Ethics Committee

To: The Secretary
Animal Ethics Committee
Research Ethics Office
Room 1.25
Courtyard Complex
Manawatu Campus PN221

Please provide one original single-sided application plus 14 copies
Application due Wednesday of week prior to meeting

APPLICATION FOR APPROVAL OF PROPOSED RESEARCH, TESTING OR TEACHING PROCEDURES USING LIVE ANIMALS

1. CHIEF APPLICANT: *(Staff Member only)*

(a) **Name** [redacted]
Qualifications [redacted]
Position [redacted]
Inst/Sch/Dept [redacted]

2. OTHER APPLICANTS: *(refer Code of Ethical Conduct, Item 3.2, for those who should be listed)*

(a) **Name** [redacted]
Qualifications [redacted]
Position [redacted]

(b) **Name** [redacted]
Qualifications [redacted]
Position [redacted]

(c) **Name** [redacted]
Qualifications [redacted]
Position [redacted]

OFFICE USE ONLY

RECEIVED
24 NOV 2016
Research Ethics

Date Received:

Copy for:
Applicant
Head of Institute/Department
Office ✓

Date sent: 27-2-17

Protocol No: 16/127

Decision:

MASSEY UNIVERSITY ANIMAL ETHICS COMMITTEE
APPROVED
Date: 9-12-16

[redacted]

(d) **Name** [redacted]

Qualifications [redacted]

Position

(e) **Name**

Qualifications

Position

(f) **Name**

Qualifications

Position

---

3. DETAILS OF PROJECT:

(a) **Title** *(maximum 20 words)* Spatiotemporal mapping, pressure recording and electrophysiology of the motility of the partially obstructed rabbit bladder

(b) **Type of project**

Research	x
Testing	
Teaching	

Paper Number(s):

(c) **Commercial sensitivity status**

No	x
Yes	

(d) **Does the project involve use of native species?**

No	x
Yes	

If yes:

Has DoC approval been:

Sought but not yet granted	
Granted	

Permit Number(s):

Māori consultation:
(must be by applicant(s) directly with iwi)

(For guidance, click here)

Has been undertaken	
Is currently being undertaken	

The project is approved by iwi*:

Yes ☐ No ☐

*(*Please refer to guidance opposite)*

4. JUSTIFICATION OF PROJECT:

(a) **What are the expected benefits of the proposed work and how will the new knowledge be communicated to others?** *(Benefits may include improved basic knowledge, improved animal*

health, teaching)

Our group have recently concluded a body of work on pig bladders maintained ex vivo. With the aid of spatiotemporal mapping we have discovered that tonic contraction of the bladder of an animal whose action is said to resemble that of the human bladder is phasic at low frequency. Hence patches of contractile activity are seen to slowly migrate around the surface of the bladder to maintain internal pressure. The findings from the pig have been widely accepted to be of potential relevance in investigation, diagnosis and treatment of overactive bladder (OAB), a world-wide therapeutic problem as is exemplified by recent coverage in Nature reviews urology (DOI 10.1038/nrurol 2015.78). We have since extended this work to rabbit bladders and found similar results and developed sophisticated mathematical methods for quantifying the evolution of patch contractions. We now wish to extend the latter work to examine contractile behaviour in the partially obstructed rabbit bladder model. This model has been widely used in the investigation of the bladder over-activity syndrome but not in the context of spatiotemporal mapping (ST). The necessary procedure will be carried out under general anaesthetic by a senior urological surgeon. The animal will be euthanased 2-4 weeks after the procedure, the bladder excised and its contractile activity mapped and analysed before and after treatment with various pharmaceutical agents. Hence the effects of each of four classes of pharmacological agents on the excised bladder namely a cholinergic agonist, a sympathomimetic agonist, a RhO A antagonist and gap junction blocker (these agents represent the principal possible modalities of treatment for OAB) will be explored. The patterns of motility and their parameters will subsequently be compared to those in the unobstructed bladder as will the responses to pharmacological agents.

(b) **Why is it necessary to use animals for this activity?** *(The term "animal" is defined in the Code of Ethical Conduct, Item 10)*

It would not be possible to conduct this trial on human subjects with overactive bladder as surgical exposure of the bladder for image acquisition (laparoscopy, laparotomy) is not clinically indicated in the investigation or treatment of OAB. The rabbit partial bladder outlet obstruction (PBOO) model is an accepted method (over 1,360 publications) of replicating smooth muscle hypertrophy/pathology associated with overactive bladder syndrome. Reported incidences of rabbit PBOO model surgical and postoperative complications are extremely rare.

5. **DESCRIPTION OF PROCEDURES AND MANIPULATIONS:** *("Manipulation" is defined in the Code of Ethical Conduct, Item 10)*

(a) **Give a brief description of your trial design/teaching demonstration.** *(One or two paragraphs) (For complex protocols, it may be beneficial to provide information as a timeline or in tabulated form)*

An upper limit of 12 rabbits will be used as this is the general size used in many studies. However, the study will be halted once it is apparent that there are similar differences in contraction dynamics determined by ST mapping or in the response to pharmacological agents compared with those of untreated bladders (previous study). The rabbits will be anaesthetised by a veterinary anaesthetist [redacted]. Surgery will be conducted at IVABS and rabbits will be transported (one at a time), from the holding facility (SAPU) to IVABS. On the day of surgery, and every 2 days thereafter, rabbits will be weighed to check condition.

Anaesthesia will be induced with isoflurane in oxygen and nitrous oxide and a laryngeal mask airway placed. An IV catheter will be placed and fentanyl given as necessary, along with Hartmann's solution. Full non-invasive monitoring will be used during anaesthesia. Morphine will be given IM before recovery and meloxicam afterwards.

The operation will in each case be conducted by a senior specialist urological surgeon [redacted]

Under sterile conditions, the urinary bladder is catheterised with an 8-F Foley catheter and exposed through a mid-line incision. Partial BOO is initiated by placing a 00-silk ligature loosely around the catheterised urethra. The catheter is removed and the incision closed in layers with 3-0 sutures. (Method described in M Jock et. al. (2014) "Effect of partial bladder outlet obstruction and reversal on rabbit bladder physiology and biochemistry: duration of recovery period and severity of function"; BJU International and S. Mastsumoto et. al. (2010) "Eviprostat Suppresses Urinary Oxidative Stress in a Rabbit Model of Partial Bladder Outlet Obstruction and in Patients with Benign Prostatic Hyperplasia"; Phytotherapy Research.)

Once a rabbit has recovered from surgery it will be immediately transported from IVABS to [redacted] where their surveillance will begin. The rabbits will be subsequently maintained in cages at [redacted] with a sheet of filter paper inserted beneath the cage. Animals will be closely monitored (every 3 hrs) by staff during the first 48 hrs to ensure that they are able to void urine. Veterinary professionals will be immediately notified at any sign of discomfort or stress. Any animals which do not void urine will be presumed to have acute occlusion of the urethra and euthanased.

After a period of between 2 and 4 weeks, each rabbit will be anaesthetised with halothane in oxygen and nitrous oxide, the bladder removed via a ventral midline incision and transferred to an organ bath. Immediately after this procedure the rabbit will be euthanased with pentobarbitone given intravenously.

(b) **Describe the statistical methods that you will use to analyse these data.**

Simple comparisons of the area, frequency and direction of propagation of tonic contractions within subjects and between treatments will be made. There are currently no data available as to the variance of these parameters with respect to the normal bladder we are the first to describe them.

(c) **Provide justification for the group sizes that you propose.**

This is a feasibility study the numbers arise from the need to check for consistency of response in the model and in response to four pharmacological modalities of treatment as detailed above

(d) **Describe the manipulations to be performed on the animals.**

Transport to and from care facility to IVABS. Induction of general anaesthesia, establishment of PBOO using standard procedures. Subsequent maintenance in the small animal facility for 2-4 weeks before anaesthesia, cystectomy and euthanasia.

(e) **How will the proposed manipulation affect the well-being of the animals?**

Transport and the induction of anaesthesia may be stressful. PBOO, as achieved by the standard procedure, has not been reported to have any ill effects other than increasing intravesical pressure during micturition and causing reactive smooth muscle hypertrophy.

(f) **Describe any restraint applied to the animals.**

The animals will be transported from [redacted] in a cage. They will be anesthetised for administration of PBOO on arrival at Massey University and subsequently maintained in the [redacted] for 2-4 weeks before a further anaesthetic and euthanasia .

6. **CARE OF ANIMALS:**

(a) **What access will the animals have to water?**

Rabbits will have access to water *ad libitum*

(b) **Describe the feeding regimen for the animals.**

The rabbits will maintained on their normal regime of pelleted food and water before anaesthesia.

(c) **From where will the animals be sourced?** *(Refer Code of Ethical Conduct, Item 2.15)*
(Where animals are personally owned, consent forms must be obtained)

The animals will be obtained from a commercial unit

(d) **Where will the animals be kept throughout the study period?**

IVABS for surgery and at [redacted] during the study period.

(e) **Who is responsible for the routine care and health surveillance of the animals?**

Care will be provided by professional animal technician's and staff at [redacted] (as required). Veterinary care by [redacted]

(f) **If the Chief Applicant is unavailable, who will make decisions if emergency care is required?**

[redacted]

7. FATE OF ANIMALS:

Note: If any animal is either euthanased or dies due to the unexpected side effects of approved manipulations, the animal should be subjected to a post-mortem examination by an experienced person. The results of the post-mortem must be communicated to the Massey University Animal Ethics Committee along with any modifications put in place to minimise the occurrence of similar events to other animals.

(a) **What will happen to the animals at the completion of the study?**

The rabbits will be euthanased immediately following the removal of the bladder

(b) **Will any animals be euthanased, either as part of the study, or in the event of untoward outcomes?**

No	☐
Yes	x

If yes:

Applicants must be familiar with the resource material on supporting staff involved with animal euthanasia at the following link:

http://www.massey.ac.nz/massey/staffroom/national-shared-services/health-safety/for-managers/responding-to-stress-complaint.cfm

The Chief Applicant must also confirm that he/she understands his/her obligations in regard to discussing the availability of this material with all people listed on the application on a per-project basis. x *Tick Box*

Describe the euthanasia method you will use.

Pentobarbitone (125mg/kg) intravenously

(c) **What level of losses do you expect to occur during this work and how will you investigate any unexpected deaths?** *(refer Code of Ethical Conduct, Items 2.20-2.22)*

No premature deaths are expected. If there are any unexplained deaths, cadaver will be sent to IVABS for a post mortem

8. ALLEVIATION OF IMPACT OF MANIPULATIONS:

(a) **What features of the manipulations minimise their impact on the animals?**

The animals will be in a cage and handled quietly and gently. All transport will be undertaken using a covered, but well ventilated transport cage.

(b) **If blood samples are to be collected, stipulate volume per sample and frequency of sampling.**

N/A

(c) **Stipulate the use (and dose rate and route of administration) of any anaesthesia, analgesia, sedative, tranquilliser or other pharmacological agent applied to reduce the impact of manipulations on the animals.**

Initially, the rabbits will anaesthetized with 5% isoflurane in 33% oxygen and 66% nitrous oxide then maintained on 2% isoflurane given via a laryngeal mask airway. Fentanyl will be given IV at 1µg/kg as necessary. Post-operative morphine 0.5mg/kg will be given IM, followed by meloxicam 1mg/kg SC for 3 days, or longer if necessary.

For removal of the bladder, rabbits will be induced with 5% halothane in 33% oxygen and 66% nitrous oxide, and maintained on 1.5% halothane in oxygen and nitrous oxide given through a face mask attached to a Bain's circuit during the surgery.

The rabbit will be euthanased with intravenous pentobarbitone (125mg/kg) given immediately after removal of the bladder.

(d) **What frequency of monitoring is to be maintained?**

Rabbits will be monitored intensively during surgery and recovery. During the first 48 hrs post-surgery, rabbits will be monitored every 3 hours. Normal daily rabbit husbandry procedures for the remainder of study thereafter.

(e) **What advice regarding identification of any expected adverse effects will be given to staff responsible for the ongoing care of the animals?**

Animals showing evidence of distress or which are not passing urine will be identified and appropriate measures taken including (if necessary) euthanasia. It is important to know that rabbits evolved as a prey species, an animal that normally needs to hide any handicap in order to escape predation. Signs of distress may be subtle, such as an increase in respiration, reluctance to move, sudden aggression, persistently squinting the eyes, a loss of interest in the surroundings or an inability to rest or sleep normally. Loud tooth grinding can indicate pain, particularly if it is associated with the other signs listed above. However, rabbits can normally exhibit quieter, infrequent tooth grinding as a sign of contentment. It is unusual for rabbits to vocalize, but when they experience sudden pain or anxiety they may give a high-pitched squeal. Due to the abdominal surgery, the rabbits posture is assumed to be hunched and they will protect area. There will also be, licking, rubbing or scratching at an area. Water and food intake will be closely monitored. Staff given the responsibility of health monitoring will be well versed in identifying signs of discomfort and veterinary advice will be sort. Weights will be taken every 2 days to monitor condition. Cachexia (weight loss) in rabbits is 10% of normal body weight.

9. EXPERIENCE OF APPLICANTS:

(a) **What is the experience of the applicants with the techniques being used in this project?**

[redacted] is experienced in the administration of anaesthetics to animals

[redacted] are experienced in the surgical removal of elements the digestive tract and their maintenance in organ baths.

[redacted] has extensive experience in the measurement of EMG, pressure and its analysis

[redacted] is a specialist urological surgeon

(b) **If an applicant is using a technique with which he/she has no previous experience, what training will be provided?**

N/A. All staff have considerable experience in their roles

(c) **List the people providing professional services and the services provided.** *(refer Code of Ethical Conduct, Item 3.2) (These personnel need not be applicants)*

[redacted]

10. USE OF RESTRICTED DRUGS:

(a) Personnel who are **not** registered veterinarians and who wish to administer restricted veterinary or human medicines must read and comply with the Operating Plan for the Use of Restricted Veterinary Medicines according to specific Veterinary Operating Instructions at Massey University.

The code can be downloaded here.

Personnel must also complete the following:

I/We declare that I/we have read the above code and will comply with its requirements.

_____	_____
_____	_____
_____	_____
_____	_____

Date: _____

11. SIGNATURES OF APPLICANTS:

(a) I have read the Massey University Code of Ethical Conduct for the Use of Live Animals for Research, Testing and Teaching and agree to comply with its requirements throughout the duration of the proposed procedures;

(b) To the best of my knowledge, this protocol or one substantially like it has not been declined by another Animal Ethics Committee.

Note: Carefully read (a) and (b) above before signing

(Original signatures only – must not be electronically inserted)

Signature(s) of Applicant(s) [redacted]

Printed Name(s) of Applicant(s) [redacted]

Date: 21.11.16.

12. APPLICANT CHECKLIST:

The following checklist must be completed by the applicant prior to arranging for endorsement of the protocol by the Designated Signing Authority: *(A list of current authorising personnel for each institute is available from the Secretary of MUAEC)*

- ☑ Appropriate research title when evaluated against methodology
- ☑ Justification and methodology written in terms readily understood by lay members of committee
- ☑ Clear distinction between justification and research methodology
- ☑ Concise wording and information relevant to animal ethics
- ☑ Clearly explained experimental design
- ☑ Complete power analysis to determine necessary number of animals required
- ☑ Provision of all signatures in correct sections
- ☑ Provision of heading details (chief applicant, institute, project title) (statistics form)
- ☑ Grading of manipulations (statistics form)
- ☑ Provision of completion date (statistics form)

13. APPROVAL BY DESIGNATED SIGNING AUTHORITY:

(This signature must be obtained by the applicant **prior to** submission to MUAEC for consideration and must **not** be that of one of the applicants)

(Original signature only – must not be electronically inserted)

I have read this application and agree that it meets the intent and spirit of the Massey University Code of Ethical Conduct for the Use of Live Animals for Research, Testing and Teaching:

Signed: [redacted] Date: 24/11/16

Name: [redacted]

Institute: IFNHH

14. ANIMAL USE STATISTICS:

Please ensure that an "Animal Use Statistics" form (following) is completed and attached.

NOTES:

(a) The staff member with signing authority delegated from MUAEC must not sign his/her own application in Section 13 above. Please obtain the signature of another staff member with delegated authority.

(b) Any departure from an approved protocol that adversely affects the welfare or increases the number of animals or reduces the validity of the study must be approved by the Chair of MUAEC acting with authority vested through Items 3.13-3.15 of the Code of Ethical Conduct. A description of such modifications shall be submitted to the Secretary of MUAEC who will attach it to the original protocol and note it on the agenda for the next meeting. Further copies shall be attached to the protocols held by the Institute and the Chief Applicant.

ANIMAL USE STATISTICS
APPLICATION/FINAL RETURN FORM
(Amended 01/14)

Protocol ID 16/127

If more than one animal type is required, then fill in one form for each type.

Application: When applying to MUAEC for approval of a manipulation, the applicant should complete Box 1 and enter in Boxes 2 to 7, in the '*Planned*' column (P), the appropriate figures for the number of animals required.

Final Return: When the manipulation is concluded, Boxes 2 to 10 should then be completed in the '*Used*' column (U) by entering appropriate figures for the number of animals which were actually used.

NOTE: **Boxes 2, 3, 4, 5, 6, 8-9 and 10 must add up to the same number.**

Chief Applicant: [redacted]

Inst/Sch/Dept: IFNHH

Title of Project: Spatiotemporal mapping, pressure recording and electrophysiology of the motility of the rabbit bladder

1. Animal type: Rabbit Code: 1e
(see bottom of this form)

2. Source of animals (number)

		P	U
Breeding unit	a		
Commercial	b	12	
Farm	c		
Born during project	d		
Captured	e		
Imported	f		
Public sources	g		
TOTAL = A			

3. Status of animals (number)

		P	U
Normal/conventional	a	12	
*SPF/germ free	b		
Diseased	c		
Transgenic/chimaera	d		
Protected species	e		
Unborn/pre-hatched	f		
Other	g		

* *Specific pathogen free*

4. Main category of manipulation/use (enter the total from 2 above in one box only)

		P	U			P	U			P	U
Teaching	a			Basic biological research	e	12		Production of biological agents	j		
Species conservation	b			Medical research	f			Development of alternatives	k		
Environmental management	c			Veterinary research	g			Other	m		
Animal husbandry	d			Testing	h						

5. Any re-use of animals (number to be inserted)								
		P	U			P	U	
No prior use	a	12		Previously used	b			Total a + b =

6. Grading of manipulations (number in each grade to be inserted)

(Download guidelines for selecting appropriate categories)

	Grade	P	U
A manipulation or use that causes no stress or pain or virtually no stress or pain. **No impact or virtually no impact.**	A		
A manipulation or use that causes stress or pain of a minor intensity for a short duration. **Little impact.**	B		
A manipulation or use that causes stress or pain of a minor intensity for a long duration or of a moderate intensity for a short duration. **Moderate impact.**	C		
A manipulation or use that causes stress or pain of a moderate intensity for a long duration or of a severe intensity for a short duration. **High impact.**	D	12	
A manipulation or use that causes stress or pain of a severe intensity for a long duration or of a very severe intensity for any duration. **Very high impact.**	E		

7. Expected Date of Completion (maximum three years): Feb 2018

ANIMAL DISPOSITION/FATE AT CONCLUSION OF RESEARCH, TESTING OR TEACHING OUTLINED IN THIS PROTOCOL

The data in Boxes 8 to 10 refer only to the animals noted in this protocol which actually entered the project and were manipulated - they do not refer to those it was proposed to manipulate but which were never used. This information is to be provided only when the research, testing or teaching has been completed and the animals have been disposed of as below.

8. Alive	Used	
Retained by your institution's farms/colonies	a	
Returned to owner	b	
Released to the wild	c	
Disposed of to others	d	
TOTAL ALIVE	**=B=**	

9. Dead	Used	
Killed for dissection, sampling, taking organs	a	
Died/destroyed in the course of the manipulation/use	b	
Euthanased after manipulation or use	c	
Died/destroyed for reason not associated with manipulation/use	d	
TOTAL DEAD	**=C=**	

10. GRAND TOTAL MANIPULATIONS/USED = B + C =

Check on the final return that B + C = A in the "Used" column of Box 2.

BOX 1: ANIMAL TYPE CODES

Animal Type	Code	Animal Type	Code
Rodents	1 a = Mice	Birds	1 p = Fowls, Chickens
	1 b = Rats		1 q = Pigeons
	1 c = Guinea Pigs		1 r = Other Birds
	1 d = Hamsters	Miscellaneous	1 s = Marine Mammals
Rabbits	1 e = Rabbits		1 t = Possums
Farm Animals	1 f = Sheep		1 u = Reptiles
	1 g = Cattle		1 w = Amphibia
	1 h = Goats		1 x = Fish
	1 j = Deer		1 z = Octopus, Squid, Crab, Lobster, Crayfish
	1 k = Pigs	Other	1 y = Other Species (*name)
Other Domestic Mammals	1 m = Horses		
	1 n = Dogs		
	1 o = Cats		

AEC/17 (Amended 01/14)

Massey University
Animal Ethics Committee

To: The Secretary
Animal Ethics Committee
Research Ethics Office
Room 1.25
Courtyard Complex
Manawatu Campus PN221

Please provide one original single-sided application plus 14 copies
Application due Wednesday of week prior to meeting

APPLICATION FOR APPROVAL OF PROPOSED RESEARCH, TESTING OR TEACHING PROCEDURES USING LIVE ANIMALS

1. CHIEF APPLICANT: *(Staff Member only)*

(a) **Name** [redacted]
Qualifications [redacted]
Position [redacted]
Inst/Sch/Dept IVABS

2. OTHER APPLICANTS: *(refer Code of Ethical Conduct, Item 3.2, for those who should be listed)*

(a) **Name** [redacted]
Qualifications [redacted]
Position

(b) **Name** [redacted]
Qualifications [redacted]
Position

(c) **Name** [redacted]
Qualifications [redacted]
Position

OFFICE USE ONLY

RECEIVED
07 MAR 2017
Research Ethics

Date Received:

Copy for:
Applicant
Head of Institute/Department
Office ✓

Date sent: 2-5-17

Protocol No: 17/19

Decision:

MASSEY UNIVERSITY ANIMAL ETHICS COMMITTEE
APPROVED
Date: 16-3-17

[redacted]

(d)	**Name**	[redacted]
	Qualifications	[redacted]
	Position	
(e)	**Name**	
	Qualifications	
	Position	
(f)	**Name**	
	Qualifications	
	Position	

3. DETAILS OF PROJECT:

(a) Title *(maximum 20 words)*: Collection of feline plasma for validation of an ELISA for feline vasopressin

(b) Type of project

- **Research** [X]
- **Testing** [ ]
- **Teaching** [ ]

Paper Number(s):

(c) Commercial sensitivity status

- **No** [X]
- **Yes** [ ]

(d) Does the project involve use of native species?

- **No** [X]
- **Yes** [ ]

If yes:

Has DoC approval been:

- **Sought but not yet granted** [ ]
- **Granted** [ ]

Permit Number(s):

Māori consultation:
(must be by applicant(s) directly with iwi)

(For guidance, click here)

- **Has been undertaken** [ ]
- **Is currently being undertaken** [ ]
- **The project is approved by iwi*:**
  - Yes [ ] No [ ]

*(*Please refer to guidance opposite)*

4. JUSTIFICATION OF PROJECT:

(a) What are the expected benefits of the proposed work and how will the new knowledge be communicated to others? *(Benefits may include improved basic knowledge, improved animal health, teaching)*

The most important stimulus of vasopressin (VP) secretion is plasma osmolality (Bouby and Fernandes, 2003) and the rate at which the kidneys excrete water is regulated by VP. The higher the concentration of VP the more water will be retained leading to a more concentrated urine. Several studies (such as Bouby et al., 1990 and Bolignano and Zoccali, 2010) suggest that high concentrations of VP could have deleterious effects on the nephrons in the kidney, therefore reducing VP concentrations could be expected to enhance preservation of nephrons, at least during the early stages of kidney disease.

As cats ingest less water when exclusively fed a dry diet compared to a high moisture diet, it is hypothesised that VP concentrations are high in cats exclusively fed a dry diet. We have previously found support for this hypothesis by finding an increase in plasma osmolarity, blood pressure, and urine protein excretion (protein:creatinine ratio) in cats fed a dry diet compared with when fed a high moisture diet, which suggests that dry food may lead to excessive secretion of VP. This may not be a problem for healthy cats, but might contribute to disease progression in chronic kidney disease.

We attempted to validate an ELISA to assay copeptin, which is co-secreted with VP and a surrogate marker for VP (MUAEC #14-30). However, assay performance was poor and to date, all our efforts at identifying the feline copeptin peptide have been unsuccessful. Therefore we have now decided to measure actual VP levels in feline blood via the ELISA method.

Commercial VP ELISA kits are available, however none have been validated for use in cats. We would like to validate an ELISA to measure feline VP, which would enable us to test our hypotheses regarding the concerns of exclusive dry food feeding. It will also enable us to evaluate VP concentrations in naturally occurring disease where water balance is disturbed. This application covers the production of sufficient plasma that contains VP within the normal physiological ranges to be used as a positive control reagent in the development, validation, and continued use of the ELISA. We wish to produce plasma with a "normal high" and a "normal low" concentration of VP.

Results will be published in peer reviewed scientific literature and form part of a PhD thesis.

References:
Bolignano D, Zoccali C. Vasopressin beyond water: implications for renal diseases. Current Opinion in Nephrology and Hypertension. 19 (5): 499-504, 2010.

Bouby N, Bachmann S, Bichet D, Bankir L. Effect of water-intake on the progression of chronic-renal-failure in the 5/6 nephrectomized rat. American Journal of Physiology. 258 (4): F973-F979, 1990.

Bouby N, Fernandes S. Mild dehydration, vasopressin and the kidney: animal and human studies. European Journal of Clinical Nutrition. 57: S39-S46, 2003.

(b) **Why is it necessary to use animals for this activity?** *(The term "animal" is defined in the Code of Ethical Conduct, Item 10)*

The establishment and validation of an ELISA requires samples collected form live cats.

5. **DESCRIPTION OF PROCEDURES AND MANIPULATIONS:** *("Manipulation" is defined in the Code of Ethical Conduct, Item 10)*

(a) **Give a brief description of your trial design/teaching demonstration.** *(One or two paragraphs) (For complex protocols, it may be beneficial to provide information as a timeline or in tabulated form)*

The normal maintenance diet of the cats at the [redacted] is a commercial canned (wet) diet. For this study 8 cats will be group housed and blood will be collected while the cats remain on their wet diet ("normal low VP"). The cats will then be switched onto a commercial dry diet, which will be fed once a day for two days (ad libitum). Approximately 2-3 hours after receiving their meal on the second day, blood will again be collected from each cat ("normal high VP"). We have previously shown that cats switched from wet to dry diets take 48 hours to reach maximal urine concentration and voluntary

water intake in response to dietary change. Thus at 24 hours after switching to the dry diet, it is proposed that VP secretion will be near maximal within normal healthy ranges.

Following the second blood sample the cats will return to their normal canned (wet) diet.

The collected blood will be separated and plasma will be stored at -80°C until assay for VP.

(b) **Describe the statistical methods that you will use to analyse these data.**

Although we will measure the 'high' and 'low' VP values within each cat, this application is to produce "reagent" and does not require statistical analysis.

(c) **Provide justification for the group sizes that you propose.**

The number of cats required is determined by our estimate of the volume of plasma needed for ELISA establishment, validation, and use as an internal standard for future experiments. We estimate that 10mL of plasma in each state will suffice. This equates to 40mL of whole blood, collected on two occasions (80mL total). Thus, 8 cats would be sufficient (2 x 5mL blood), without requiring excessive bleeding or excessive numbers of cats.

(d) **Describe the manipulations to be performed on the animals.**

Each cat will be subjected to:

Blood collection. Two blood samples (2 x 5ml) jugular venous blood sample collected using a 25 gauge needle.

(e) **How will the proposed manipulation affect the well-being of the animals?**

Blood collection is routinely performed for health checks, veterinary diagnostics etc. Discomfort will be minimised by quick and gentle handling. Any cat requiring excessive restraint or sedation will not be included in the trial.

(f) **Describe any restraint applied to the animals.**

Standard manual restraint for jugular venous sampling and intravenous injection.

6. **CARE OF ANIMALS:**

(a) **What access will the animals have to water?**

Cats will have access to water at all times.

(b) **Describe the feeding regimen for the animals.**

Cats will receive a commercial dry biscuit diet once a day (*ad libitum*).

(c) **From where will the animals be sourced?** *(Refer Code of Ethical Conduct, Item 2.15)*
(Where animals are personally owned, consent forms must be obtained)

[redacted]

(d) **Where will the animals be kept throughout the study period?**

[redacted]

(e) **Who is responsible for the routine care and health surveillance of the animals?**

[redacted]

(f) **If the Chief Applicant is unavailable, who will make decisions if emergency care is required?**

[redacted]

7. FATE OF ANIMALS:

Note: If any animal is either euthanased or dies due to the unexpected side effects of approved manipulations, the animal should be subjected to a post-mortem examination by an experienced person. The results of the post-mortem must be communicated to the Massey University Animal Ethics Committee along with any modifications put in place to minimise the occurrence of similar events to other animals.

(a) **What will happen to the animals at the completion of the study?**

Remain at the [redacted]

(b) **Will any animals be euthanased, either as part of the study, or in the event of untoward outcomes?**

No [x]

Yes [ ]

If yes:

Applicants must be familiar with the resource material on supporting staff involved with animal euthanasia at the following link:

http://www.massey.ac.nz/massey/staffroom/national-shared-services/health-safety/for-managers/responding-to-stress-complaint.cfm

The Chief Applicant must also confirm that he/she understands his/her obligations in regard to discussing the availability of this material with all people listed on the application on a per-project basis. [ ] *Tick Box*

Describe the euthanasia method you will use.

(c) **What level of losses do you expect to occur during this work and how will you investigate any unexpected deaths?** *(refer Code of Ethical Conduct, Items 2.20-2.22)*

No animal loss expected. Any unexpected death will be investigated by a post-mortem examination carried out by a Massey University veterinary pathologist as per normal [redacted] policy.

8. ALLEVIATION OF IMPACT OF MANIPULATIONS:

(a) **What features of the manipulations minimise their impact on the animals?**

Experienced veterinarians and staff from the [redacted] will minimise animal restraint and the impact of the procedure. For blood collection a 25 gauge needle will be used and a local anaesthetic (Xylocaine jelly) will be applied approximately 5 minutes before the collection of the sample.

The protocol induces a period of mild-subclinical dehydration that is part of normal feeding practices. This prevents the need to deliberately cause dehydration by water depravation.

(b) **If blood samples are to be collected, stipulate volume per sample and frequency of sampling.**

Two blood samples, 5ml per sample, which means a total of 10ml will be collected. All cats will have a minimum weight of 3.0 kilogram; therefore the maximum amount of blood taken will be 0.3% of body weight. Cats will only be selected if they have not been bled in the prior 28 days.

(c) **Stipulate the use (and dose rate and route of administration) of any anaesthesia, analgesia, sedative, tranquilliser or other pharmacological agent applied to reduce the impact of manipulations on the animals.**

N/A

(d) **What frequency of monitoring is to be maintained?**

The cats will be monitored twice a day (morning and late afternoon).

(e) **What advice regarding identification of any expected adverse effects will be given to staff responsible for the ongoing care of the animals?**

N/A

9. **EXPERIENCE OF APPLICANTS:**

(a) **What is the experience of the applicants with the techniques being used in this project?**

Jugular venipuncture is a routine procedure regularly performed by the applicants.

(b) **If an applicant is using a technique with which he/she has no previous experience, what training will be provided?**

N/A

(c) **List the people providing professional services and the services provided.** *(refer Code of Ethical Conduct, Item 3.2) (These personnel need not be applicants)*

10. USE OF RESTRICTED DRUGS:

(a) Personnel who are **not** registered veterinarians and who wish to administer restricted veterinary or human medicines must read and comply with the Operating Plan for the Use of Restricted Veterinary Medicines according to specific Veterinary Operating Instructions at Massey University.

The code can be downloaded here.

Personnel must also complete the following:

I/We declare that I/we have read the above code and will comply with its requirements.

_____	_____
_____	_____
_____	_____
_____	_____

Date: _____

11. SIGNATURES OF APPLICANTS:

(a) I have read the Massey University Code of Ethical Conduct for the Use of Live Animals for Research, Testing and Teaching and agree to comply with its requirements throughout the duration of the proposed procedures;

(b) To the best of my knowledge, this protocol or one substantially like it has not been declined by another Animal Ethics Committee.

Note: Carefully read (a) and (b) above before signing

(Original signatures only – must not be electronically inserted)

[redacted]

Printed Name(s) of Applicant(s)

[redacted]

Date: 7/3/17

12. APPLICANT CHECKLIST:

The following checklist must be completed by the applicant prior to arranging for endorsement of the protocol by the Designated Signing Authority: *(A list of current authorising personnel for each institute is available from the Secretary of MUAEC)*

- ☑ Appropriate research title when evaluated against methodology
- ☑ Justification and methodology written in terms readily understood by lay members of committee
- ☑ Clear distinction between justification and research methodology
- ☑ Concise wording and information relevant to animal ethics
- ☑ Clearly explained experimental design
- ☑ Complete power analysis to determine necessary number of animals required
- ☑ Provision of all signatures in correct sections
- ☑ Provision of heading details (chief applicant, institute, project title) (statistics form)
- ☑ Grading of manipulations (statistics form)
- ☑ Provision of completion date (statistics form)

13. APPROVAL BY DESIGNATED SIGNING AUTHORITY:

(This signature must be obtained by the applicant **prior to** submission to MUAEC for consideration and must **not** be that of one of the applicants)

(Original signature only – must not be electronically inserted)

I have read this application and agree that it meets the intent and spirit of the Massey University Code of Ethical Conduct for the Use of Live Animals for Research, Testing and Teaching:

Signed: [redacted] Date: 7-3-17

Name: [redacted]

Institute: [redacted]

14. ANIMAL USE STATISTICS:

Please ensure that an "Animal Use Statistics" form (following) is completed and attached.

NOTES:

(a) The staff member with signing authority delegated from MUAEC must not sign his/her own application in Section 13 above. Please obtain the signature of another staff member with delegated authority.

(b) Any departure from an approved protocol that adversely affects the welfare or increases the number of animals or reduces the validity of the study must be approved by the Chair of MUAEC acting with authority vested through Items 3.13-3.15 of the Code of Ethical Conduct. A description of such modifications shall be submitted to the Secretary of MUAEC who will attach it to the original protocol and note it on the agenda for the next meeting. Further copies shall be attached to the protocols held by the Institute and the Chief Applicant.

ANIMAL USE STATISTICS
APPLICATION/FINAL RETURN FORM
(Amended 01/14)

Protocol ID 17/19

If more than one animal type is required, then fill in one form for each type.

Application: When applying to MUAEC for approval of a manipulation, the applicant should complete Box 1 and enter in Boxes 2 to 7, in the *'Planned'* column (P), the appropriate figures for the number of animals required.

Final Return: When the manipulation is concluded, Boxes 2 to 10 should then be completed in the *'Used'* column (U) by entering appropriate figures for the number of animals which were actually used.

NOTE: **Boxes 2, 3, 4, 5, 6, 8-9 and 10 must add up to the same number.**

Chief Applicant: [redacted]

Inst/Sch/Dept: IVABS

Title of Project: Collection of feline plasma for validation of an ELISA for feline vasopressin

1. Animal type: Cat Code: 1 o
(see bottom of this form)

2. Source of animals (number)

		P	U
Breeding unit	a	8	
Commercial	b		
Farm	c		
Born during project	d		
Captured	e		
Imported	f		
Public sources	g		
TOTAL = A		8	

3. Status of animals (number)

		P	U
Normal/conventional	a	8	
*SPF/germ free	b		
Diseased	c		
Transgenic/chimaera	d		
Protected species	e		
Unborn/pre-hatched	f		
Other	g		

* *Specific pathogen free*

4. Main category of manipulation/use (enter the total from 2 above in one box only)

		P	U			P	U			P	U
Teaching	a			Basic biological research	e			Production of biological agents	j	8	
Species conservation	b			Medical research	f			Development of alternatives	k		
Environmental management	c			Veterinary research	g			Other	m		
Animal husbandry	d			Testing	h						

5. Any re-use of animals (number to be inserted)								
		P	U			P	U	
No prior use	a			Previously used	b	8		Total a + b = 8

6. Grading of manipulations (number in each grade to be inserted)			
(Download guidelines for selecting appropriate categories)	Grade	P	U
A manipulation or use that causes no stress or pain or virtually no stress or pain. **No impact or virtually no impact.**	A		
A manipulation or use that causes stress or pain of a minor intensity for a short duration. **Little impact.**	B	8	
A manipulation or use that causes stress or pain of a minor intensity for a long duration or of a moderate intensity for a short duration. **Moderate impact.**	C		
A manipulation or use that causes stress or pain of a moderate intensity for a long duration or of a severe intensity for a short duration. **High impact.**	D		
A manipulation or use that causes stress or pain of a severe intensity for a long duration or of a very severe intensity for any duration. **Very high impact.**	E		

7. Expected Date of Completion (maximum three years): August 2017

ANIMAL DISPOSITION/FATE AT CONCLUSION OF RESEARCH, TESTING OR TEACHING OUTLINED IN THIS PROTOCOL

The data in Boxes 8 to 10 refer only to the animals noted in this protocol which actually entered the project and were manipulated - they do not refer to those it was proposed to manipulate but which were never used. This information is to be provided only when the research, testing or teaching has been completed and the animals have been disposed of as below.

8. Alive	Used	
Retained by your institution's farms/colonies	a	
Returned to owner	b	
Released to the wild	c	
Disposed of to others	d	
TOTAL ALIVE	**=B=**	

9. Dead	Used	
Killed for dissection, sampling, taking organs	a	
Died/destroyed in the course of the manipulation/use	b	
Euthanased after manipulation or use	c	
Died/destroyed for reason not associated with manipulation/use	d	
TOTAL DEAD	**=C=**	

10. GRAND TOTAL MANIPULATIONS/USED = B + C =

Check on the final return that B + C = A in the "Used" column of Box 2.

BOX 1: ANIMAL TYPE CODES

Animal Type	Code	Animal Type	Code
Rodents	l a = Mice	Birds	l p = Fowls, Chickens
	l b = Rats		l q = Pigeons
	l c = Guinea Pigs		l r = Other Birds
	l d = Hamsters	Miscellaneous	l s = Marine Mammals
Rabbits	l e = Rabbits		l t = Possums
Farm Animals	l f = Sheep		l u = Reptiles
	l g = Cattle		l w = Amphibia
	l h = Goats		l x = Fish
	l j = Deer		l z = Octopus, Squid, Crab, Lobster, Crayfish
	l k = Pigs	Other	l y = Other Species (*name)
Other Domestic Mammals	l m = Horses		
	l n = Dogs		
	l o = Cats		

AEC/17 (Amended 01/14)

Massey University
Animal Ethics Committee

To: The Secretary
Animal Ethics Committee
Research Ethics Office
Room 1.23
Courtyard Complex
Manawatu Campus PN221

Please provide one original single-sided application plus 15 copies
Application due Wednesday of week prior to meeting

APPLICATION FOR APPROVAL OF PROPOSED RESEARCH, TESTING OR TEACHING PROCEDURES USING LIVE ANIMALS

1. **CHIEF APPLICANT:** *(Staff Member only)*
   - (a) **Name** [redacted]
   - **Qualifications** [redacted]
   - **Position** [redacted]
   - **Inst/Sch/Dept** [redacted]

2. **OTHER APPLICANTS:** *(refer Code of Ethical Conduct, Item 3.2, for those who should be listed)*
   - (a) **Name** [redacted]
   - **Qualifications** [redacted]
   - **Position** [redacted]
   - (b) **Name** [redacted]
   - **Qualifications** [redacted]
   - **Position** [redacted]
   - (c) **Name**
   - **Qualifications**
   - **Position**

OFFICE USE ONLY

RECEIVED
06 SEP 2016
Research Ethics

Date Received:

Copy for:
Applicant
Head of Institute/Department
Office ✓

Date sent: 2-11-16

Protocol No: 16/90

Decision:

MASSEY UNIVERSITY ANIMAL ETHICS COMMITTEE APPROVED
Date: 16-9-16

[redacted]

(d)	**Name**	.....
	Qualifications	.....
	Position	.....
(e)	**Name**	.....
	Qualifications	.....
	Position	.....
(f)	**Name**	.....
	Qualifications	.....
	Position	.....

3. DETAILS OF PROJECT:

(a) Title *(maximum 20 words)*: Determination of the digestible indispensable amino acid score (DIAAS) of dietary protein-based foods.

(b) Type of project

Research	☐
Testing	☒
Teaching	☐
Paper Number(s):	

(c) Commercial sensitivity status

No	☐
Yes	☒

(d) Does the project involve use of native species?

No	☒
Yes	☐

If yes:

Has DoC approval been:

Sought but not yet granted	☐
Granted	☐
Permit Number(s):	

Māori consultation:
(must be by applicant(s) directly with iwi)

(For guidance, click here)

Has been undertaken	☐
Is currently being undertaken	☐
The project is approved by iwi*:	
Yes ☐	No ☐

*(*Please refer to guidance opposite)*

4. JUSTIFICATION OF PROJECT:

(a) What are the expected benefits of the proposed work and how will the new knowledge be communicated to others? *(Benefits may include improved basic knowledge, improved animal health, teaching)*

In January 2013, the FAO endorsed the "digestible indispensable amino acid score" DIAAS method as the method of choice for determining dietary protein quality for use with human nutrition. This replaced the "protein digestibility-corrected amino acid score" (PDCAAS) method that had been used previously. The DIAAS scores are based on the ratio between the true ileal digestible amino acid content of a protein source (for all indispensable amino acids) and the amino acid requirement for each indispensable amino acid. A score greater than 1 indicates that the protein source will meet the nutritional requirements for the amino acid in question while a score below 1 indicates that the requirements will not be met for that amino acid. The Riddet Institute was one of the drivers of the adoption of the new method by the FAO and has been determining the DIAAS of protein-based products for different companies over the last few years (Animal Ethics Committee Protocol 13/24). We continue to be approached by further companies that need to have their products assessed using the new DIAAS method. Most of these requests involve the determination of DIASS for only a few samples at a time and the companies require their results quickly. This means that it is not practical to have to apply for ethics approval on a case-by-case basis.

Given that the essence of DIAAS is to evaluate the protein quality of protein sources that are consumed by humans then the protein-based foods that we will test will be foods for human consumption. Therefore it is safe to conclude that the foods will also be safe for consumption by rats and pigs.

The animal model to be used will be chosen based on the food being tested and the preference of the supplier of the products that we will evaluate. For example, infant formulas would be tested using the 3-week old piglet model while protein sources intended to be consumed by children or adults would be tested using either the growing pig or growing rat models. The growing pig is a superior model for humans as compared to the growing rat. However, the rat is a cheaper and very suitable second choice. The choice of model will depend on the requirements of the company commissioning the work. When the pig is chosen for the model animal, digesta samples can be collected following anaesthesia of the animal or via a cannula inserted in the small intestine. Whereas the cannulated pig is considered the best method for this (FAO, 2014, Research Approaches and Methods for Evaluating the Protein Quality of Human Foods, http://www.fao.org/3/a-i4325e.pdf), when a small number of protein sources is to be tested, sampling from the euthanized pig is preferred as this method is more simple and cost-effective when done for a small number of protein sources.

The method we will continue to use (which is the same as that approved in Animal Ethics Committee Protocol 13/24) is essentially the same for all the protein-based foods we test (ie true ileal amino acid digestibility assay conducted in either rats or pigs). Therefore, in this application, we want to apply for an overarching approval that will allow us to conduct the true ileal amino acid digestibility assay either in the growing rat, the 3-week-old piglet (for testing infant formulas) or the growing pig with digesta samples collected following euthanasia of the animals, on protein-based foods destined for human consumption on an "as required basis" using up to 500 pigs or rats over the next three years. The actual numbers of animal used would entirely depend on how many requests for analysis we get within the timespan of this application (3 years) and at this stage that cannot be forecast.

The types of samples we would test are not known at this stage and therefore it is not possible to provide a list of the foods that will be tested. However, these samples may range from refined protein sources from dairy, plant or animal origin (eg, casein, whey powder, soy protein isolate, rice protein isolate, potato protein concentrate) to whole foods (eg, rice, wheat, corn, meat, eggs, milk products).

(b) **Why is it necessary to use animals for this activity?** *(The term "animal" is defined in the Code of Ethical Conduct, Item 10)*

Data generated from the study is applicable to humans. Digestibility studies, however, require a whole system analysis, and therefore, using human subjects is not possible. *In vitro* studies are not practical because one cannot replicate the various metabolic processes of digestion accurately. Therefore an animal model is the most suitable system. The 3-week-old piglet is a suitable model for the 3-month-old infant and the growing pig and growing rat are suitable models for the adult human when investigating nutritional aspects.

5. **DESCRIPTION OF PROCEDURES AND MANIPULATIONS:** *("Manipulation" is defined in the Code of Ethical Conduct, Item 10)*

(a) **Give a brief description of your trial design/teaching demonstration.** *(One or two paragraphs) (For complex protocols, it may be beneficial to provide information as a timeline or in tabulated form)*

This application relates to determining the true ileal amino acid digestibility of a range of protein-based foods in order to determine the DIAAS of those foods. The methods described below are described for a single protein-based food but the same protocol will be used for all the foods we test under this application.

Determining true ileal amino acid digestibility in rats

The protocol described here has been previously approved by the ethics committee (Animal Ethics Protocol 13/24) and used repeatedly over the past 18 years with little negative impact on the rats. The methods were agreed on by an FAO Expert Working Group (in which I participated) and are now considered to be the standard methods for determining DIAAS of food products when the rat is used (FAO 2014).

Twelve Sprague-Dawley male rats (200 to 250g body weight) will be housed individually in stainless steel cages in a room maintained at 21 ± 2°C with a 12 h:12 h light/dark cycle ([redacted] Water will be continuously available. During the experimental period (14 days), the animals will be fed test diets consisting of sugar (100 g/kg), purified cellulose (50 g/kg), vitamins and minerals (50 g/kg), soyabean oil (100 g/kg), titanium dioxide (3 g/kg; indigestible marker) and the test protein-based food (the amount of the test food added to the diet will vary depending on the protein content of the food but the diets will be formulated to contain 100 g/kg protein). The diets will be made up to 1 kg with purified starch. The test diets will meet the nutrient requirements of the rats for all nutrients except protein as prescribed by the National Research Council (1995). There will be twelve rats per dietary treatment. The rats will either receive the test diet for the entire two week trial or if the amount of the test product is limited (ie, if importation of the protein-based foods is difficult) then the rats will receive a casein-based basal diet (the same as the test diet except casein will be substituted for the test product) for the first 12 days and the test diet for the last 2 days. The rats will receive their daily ration as 9 meals fed hourly between 0800h and 1600h each day. During each meal, the rats will have unrestricted access to their respective diets for a 10 min period, after which, the food will be removed. On the final day of the trial, five hours after the start of feeding, the rats will be exposed to a rising plane of CO_2 and euthanized. The abdomen will be opened and the terminal ileum removed and digesta flushed out then immediately frozen at -20°C and used for subsequent analysis of amino acids and titanium. The digesta samples will be pooled across pairs of rats within the same treatment group such that there will be 6 digesta samples per treatment. This is to ensure that there is sufficient digesta material for the subsequent analysis of amino acids and titanium. The true ileal amino acid digestibility of the protein-based food will be determined and the DIAAS calculated.

Determining true ileal amino acid digestibility in growing pigs

The protocol described here has been approved previously by the ethics committee (eg. Animal

Ethics Committee Protocol 13/24) and used repeatedly over the past 18 years with little negative impact on the pigs.

Six PIC Camborough 46 x PICboar 356L (20-25 kg BW) pigs will be housed individually in metabolism stalls in a room maintained at 22 ± 2°C with a 12 h: 12 h light/dark cycle [redacted]. Water will be continuously available. During the experimental period (14 days), the animals will be fed test diets consisting of sugar (100 g/kg), purified cellulose (50 g/kg), vitamins and minerals (50 g/kg), soyabean oil (100 g/kg), titanium dioxide (3 g/kg; indigestible marker) and the test protein-based food (the amount of the test food added to the diet will vary depending on the protein content of the food but the diets will be formulated to contain 100 g/kg protein). The diets will be made up to 1 kg with purified starch. The test diets will meet the nutrient requirements of the growing pig for all nutrients except protein as prescribed by the National Research Council (1998). There will be six pigs per dietary treatment. The pigs will either receive the test diet for the entire two week trial or if the amount of the test product is limited (ie, if importation of the protein-based foods is difficult) then the pigs will receive a casein-based basal diet (the same as the test diet except casein will be substituted for the test product) for the first 12 days and the test diet for the last 2 days. The daily ration will be 10% of the metabolic bodyweight and will be adjusted weekly based on the weight of the animals at the beginning of each week. The pigs will receive their daily ration as 9 meals (each $1/9^{th}$ of the daily ration for each meal) fed hourly between 0800h and 1600h each day. On the final day of the trial, 5 hours after the start of feeding, the pigs will be anaesthetised and euthanized. The abdomen will be opened and the terminal ileum removed and digesta flushed out then immediately frozen at -20°C and used for subsequent analysis of amino acids and titanium. The true ileal amino acid digestibility of the protein-based food will be determined and the DIAAS calculated.

Determining true ileal amino acid digestibility in the 3-week-old piglet

The protocol described here is be similar to protocols that have been approved previously by the ethics committee (eg. Animal Ethics Committee Protocol 02/100, 07/07, 13/24) and used by our group. In these studies, the piglets generally remained healthy and the only real issue has been training the piglets to drink from bottles. About 3% of the piglets never adjusted to bottle feeding and had to be fed from a drinking bowl.

This piglet model will only be used for the DIAAS determination of infant formulas. Six 2-week-old PIC Camborough 46 x PICboar 356L piglets will be housed individually in plastic metabolism crates in a room maintained at 28 ± 2°C with a 15 h: 9 light/dark cycle [redacted]. The test infant formula will be reconstituted with tap water as prescribed by the infant formula manufacturer and bottle-fed to the piglets. There will be six pigs per dietary treatment. The piglets will receive the test diet for the entire two-week trial period. The daily ration will be 345 ml of reconstituted formula per kg BW and will be adjusted weekly based on the weight of the animals at the beginning of each week. The daily ration is based on previous work (Rutherfurd, S. M., Darragh, A. J., Hendriks, W. H., Prosser, C. G. and Lowry, D. 2006. True ileal amino acid digestibility of goat and cow milk infant formulas. J. Dairy Sci. 89, 2408-2413; Darragh, A. J., and P. J. Moughan. 1995. The three-week-old piglet as a model for studying protein digestion in human infants. J. Ped. Gastric Nutr. 21:387–393). The daily ration will be fed as 9 meals given every 2 hours between 6:00am and 10:00pm. On the final day of the trial, 5 hours after the start of feeding, the piglets will be anaesthetised and euthanized. The abdomen will be opened and the terminal ileum removed and digesta flushed out then immediately frozen at -20°C and used for subsequent analysis of amino acids and titanium. The true ileal amino acid digestibility of the protein-based food will be determined and the DIAAS calculated.

(b) Describe the statistical methods that you will use to analyse these data.

The aim of this application is not to compare protein sources but rather to determine the DIAAS scores of protein-based foods as an as requested basis.

(c) **Provide justification for the group sizes that you propose.**

Variable (eg, Weight)	Means or Expected Difference	SD	Type 1 error (ά)	Type 2 error (β)	Power	Number of animals needed
True ileal digestible amino acid content	**80% vs 82%**	**1.4%**			**82%**	**6**

The aim of this application is not to compare protein sources but rather to determine the DIAAS scores of protein-based foods as an as requested basis. However, if such comparisons are needed then we know from past studies with n=6 we can detect significant differences in the true ileal digestible amino acid content (and therefore DIAAS) where means differ by 2% or more.

(d) **Describe the manipulations to be performed on the animals.**

The rats will be housed in stainless cages at the [redacted] The rats will be euthanized with carbon dioxide according to the procedure described in the Standard Operating Procedure 09/03, "Procedure for Performing Euthanasia in Mice, Rats, Hamsters and Guinea Pigs using CO_2".

The pigs and piglets will be housed at the [redacted]in metabolism stalls; feed intake and body condition will be monitored daily and the pigs will be weighed weekly. When required, piglets may be gently restrained by hand when bottle-feeding. A combination of xylazine, ketamine, zolazepam, and tiletamine will be administered by intravenous or intramuscular injection for sedation and anaesthesia according to Ko et al., 1993, and Ko et al., 1995. The pigs and piglets will be euthanized using a method compliant with standards published in the New Zealand Animal Welfare (Pigs) Code of Welfare 2010 Minimum Standard Number 19 'Emergency Humane Destruction' ("Animal Welfare (Pigs) Code of Welfare 2010,"). The Standard dictates that:

- When pigs have to be killed it must be done by persons competent in the handling and killing of pigs and death must be confirmed by inspection of the animal; and
- When a pig needs to be killed it must be handled, restrained and killed in such a manner as to minimise unnecessary pain and distress prior to death; and
- Pigs must be rapidly rendered insensible and remain in that state, until death
- Animals rendered insensible by a blow or shot to the brain must be bled out immediately to ensure death occurs before recovery from stunning.

Animal Welfare (Pigs) Code of Welfare 2010, New Zealand Ministry for Primary Industries (December 3, 2010).

Ko, J. C., Williams, B. L., Rogers, E. R., Pablo, L. S., McCaine, W. C., & McGrath, C. J. (1995). Increasing xylazine dose-enhanced anesthetic properties of telazol-xylazine combination in swine. Laboratory Animal Science, 45(3), 290-294.

Ko, J. C., Williams, B. L., Smith, V. L., McGrath, C. J., & Jacobson, J. D. (1993). Comparison of Telazol, Telazol-ketamine, Telazol-xylazine, and Telazol-ketamine-xylazine as chemical restraint and anesthetic induction combination in swine. Laboratory Animal Science, 43(5), 476-480.

(e) **How will the proposed manipulation affect the well-being of the animals?**

The weighing and handling of the pigs and piglets will incur minor stress.

Pigs readily and rapidly acclimatise to changes in feeding regimen and environment and therefore we do not expect any adverse affects on the well-being of the pigs.

On the final day of the trial, the pigs will receive an intramuscular injection that will result in a minor amount of discomfort; otherwise all manipulations will be conducted while the pigs are anaesthetised or after they have been killed.

The caging and handling of the rats will incur minor stress. Asphyxiation will also incur minor stress through shortness of breath.

(f) Describe any restraint applied to the animals.

The pigs and piglets will be gently restrained when receiving the intramuscular injection on the final day.

6. CARE OF ANIMALS:

(a) What access will the animals have to water?

Tap water will be available at all times for the growing pigs and rats. The piglets will receive their water as part of the reconstituted infant formula being tested (340 ml/kg BW/day).

(b) Describe the feeding regimen for the animals.

For the trials involving either rats or growing pigs, the test diets will consist of sugar (100 g/kg), purified cellulose (50 g/kg), vitamins and minerals mix (50 g/kg), soyabean oil (100 g/kg), titanium dioxide (3 g/kg; indigestible marker) and the protein-based food to be tested The amount of the test food included into the diet will depend on the protein content of the food but the amount will be sufficient to maintain a protein concentration in the diet of 100 g/kg. The diets will be made up to 1 kg with purified starch. Where a test food contains less than 100 g/kg protein (eg rice) then the diet will be formulated to contain only the test food, the vitamin and mineral mix and titanium dioxide and the diet will only be fed to the animals on the final two days of the study.

The rats will receive their daily ration as 9 meals fed hourly between 0800h and 1600h each day. During each meal, the rats will have unrestricted access to their respective diets for a 10 min period, after which, the food will be removed.

The daily ration for the pigs will be 10% of the metabolic bodyweight and will be adjusted weekly based on the weight of the animals at the beginning of each week. The pigs will receive their daily ration as 9 meals (each $1/9^{th}$ of the daily ration for each meal) fed hourly between 0800h and 1600h each day.

For the piglets the daily ration will be 345 ml of reconstituted formula per kg BW and will be adjusted weekly based on the weight of the animals at the beginning of each week. The daily ration will be fed as 9 meals (each $1/9^{th}$ of the daily ration for each meal) given every 2 hours between 0600h and 2200h.

(c) From where will the animals be sourced? *(Refer Code of Ethical Conduct, Item 2.15)*
(Where animals are personally owned, consent forms must be obtained)

The pigs will be obtained from a commercial farm [redacted] The rats will be obtained from [redacted]

(d) Where will the animals be kept throughout the study period?

The pigs will be kept at [redacted]. The rats will be kept at [redacted]

(e) Who is responsible for the routine care and health surveillance of the animals?

The daily care of the animals will be conducted by suitably trained staff. This training will be

conducted by [redacted] for the pig trials, with the help of [redacted] for the rat trials.

(f) If the Chief Applicant is unavailable, who will make decisions if emergency care is required?

[redacted] will be the primary contact. If necessary she will contact [redacted] or [redacted] for health problems with the pigs and [redacted] for the rats. If necessary, Dr [redacted] will be consulted for emergency health issues for the rats.

7. FATE OF ANIMALS:

Note: If any animal is either euthanased or dies due to the unexpected side effects of approved manipulations, the animal should be subjected to a post-mortem examination by an experienced person. The results of the post-mortem must be communicated to the Massey University Animal Ethics Committee along with any modifications put in place to minimise the occurrence of similar events to other animals.

(a) What will happen to the animals at the completion of the study?

The animals will be euthanized as part of the study.

(b) Will any animals be euthanased, either as part of the study, or in the event of untoward outcomes?

No	☐
Yes	[x]

If yes:

Applicants must be familiar with the resource material on supporting staff involved with animal euthanasia at the following link:

Animal Euthanasia Support Guidelines.pdf

The Chief Applicant must also confirm that he/she understands his/her obligations in regard to discussing the availability of this material with all people listed on the application on a per-project basis. [x] *Tick Box*

Describe the euthanasia method you will use.

In compliance with the Animal Welfare (Pigs) Code of Welfare 2010, the preferred method for euthanasia in these studies will be by intracardial injection of a lethal dose of pentobarbitone while under anaesthesia. Other methods approved in the Code will be considered when intracardial injection or pentobarbitone interfere with study requirements (e.g. tissue sampling, conflict with an assay method, physiological monitoring processes, etc.). This will be done by [redacted]

The rats will be euthanized with carbon dioxide according to the procedure described in the Standard Operating Procedure 09/03, "Procedure for Performing Euthanasia in Mice, Rats, Hamsters and Guinea Pigs using CO_2". This process will be supervised by [redacted]

(c) What level of losses do you expect to occur during this work and how will you investigate any unexpected deaths? *(refer Code of Ethical Conduct, Items 2.20-2.22)*

We do not expect losses to occur during the study before the animals are euthanized at the end of the trial. In the event of unexpected deaths, the carcasses will be sent to the post mortem room of the Massey Veterinary Clinic for necropsy.

8. ALLEVIATION OF IMPACT OF MANIPULATIONS:

(a) What features of the manipulations minimise their impact on the animals?

All animals will be handled gently at all times, so that routine procedures such as weighing cause minimal stress. Pigs will be anesthetized before being euthanized.

(b) **If blood samples are to be collected, stipulate volume per sample and frequency of sampling.**

No blood will be taken.

(c) **Stipulate the use (and dose rate and route of administration) of any anaesthesia, analgesia, sedative, tranquilliser or other pharmacological agent applied to reduce the impact of manipulations on the animals.**

The preferred method for euthanasia for the pigs/piglets in these studies is as follows. Anaesthesia with Zoletil 100 (zolazepam and tiletamine, both 50 mg/mL) reconstituted with 2.5ml Ketamine and 2.5ml Xylazine, both 100mg/ml. Final solution contains 50mg/ml of each drug. It is then administered at a dose rate of 0.4mL of the mixed solution /10kg BW by intramuscular injection. The pigs will be euthanized by an intravenous or intracardiac injection of a lethal dose (0.3 ml/kg BW) of pentobarbitone (Pentobarb 300).

(d) **What frequency of monitoring is to be maintained?**

The growing pigs and rats will be monitored periodically between 0800h to 1700h daily, piglets between 0600h and 2200h.

(e) **What advice regarding identification of any expected adverse effects will be given to staff responsible for the ongoing care of the animals?**

Pigs and rats will be monitored daily by staff when they are fed. [redacted] will be notified if any pigs show signs of ill health. [redacted] will be notified if any rats show signs of ill health.

9. EXPERIENCE OF APPLICANTS:

(a) **What is the experience of the applicants with the techniques being used in this project?**

We will be using personnel who have numerous years of experience with animals. [redacted] has a vast experience in overviewing animal studies. [redacted] has been directly involved in animal studies, including studies using the same methods that are proposed here for over 20 years. [redacted] has vast experience with rat trials, including the methods proposed here.

(b) **If an applicant is using a technique with which he/she has no previous experience, what training will be provided?**

No untrained personnel will be used.

(c) **List the people providing professional services and the services provided.** *(refer Code of Ethical Conduct, Item 3.2) (These personnel need not be applicants)*

All pigs and piglets will be anesthetized and euthanized by [redacted] He will also provide assistance if any pigs of piglet show signs of ill health.

10. USE OF RESTRICTED DRUGS:

(a) Personnel who are **not** registered veterinarians and who wish to administer restricted veterinary or human medicines must read and comply with the Operating Plan for the Use of Restricted Veterinary Medicines according to specific Veterinary Operating Instructions at Massey University.

The code can be downloaded here.

Personnel must also complete the following:

I/We declare that I/we have read the above code and will comply with its requirements.

n/a.	

Date: _____

11. SIGNATURES OF APPLICANTS:

(a) I have read the Massey University Code of Ethical Conduct for the Use of Live Animals for Research, Testing and Teaching and agree to comply with its requirements throughout the duration of the proposed procedures;

(b) To the best of my knowledge, this protocol or one substantially like it has not been declined by another Animal Ethics Committee.

Note: Carefully read (a) and (b) above before signing

Signature(s) of Applicant(s) [redacted]

Printed Name(s) of Applicant(s) [redacted]

Date: 01/09/2016

12. APPLICANT CHECKLIST:

The following checklist must be completed by the applicant prior to arranging for endorsement of the protocol by the Designated Signing Authority: *(A list of current authorising personnel for each institute is available from the Secretary of MUAEC)*

- ☑ Appropriate research title when evaluated against methodology
- ☑ Justification and methodology written in terms readily understood by lay members of committee
- ☑ Clear distinction between justification and research methodology
- ☑ Concise wording and information relevant to animal ethics
- ☑ Clearly explained experimental design
- ☑ Complete power analysis to determine necessary number of animals required
- ☑ Provision of all signatures in correct sections
- ☑ Provision of heading details (chief applicant, institute, project title) (statistics form)
- ☑ Grading of manipulations (statistics form)
- ☑ Provision of completion date (statistics form)

13. APPROVAL BY DESIGNATED SIGNING AUTHORITY:

I have read this application and agree that it meets the intent and spirit of the Massey University Code of Ethical Conduct for the Use of Live Animals for Research, Testing and Teaching:

Signed: [redacted] Date: 11/1/16

Name: [redacted]

Institute: [redacted]

14. ANIMAL USE STATISTICS:

Please ensure that an "Animal Use Statistics" form (following) is completed and attached.

NOTES:

(a) The staff member with signing authority delegated from MUAEC must not sign his/her own application in Section 13 above. Please obtain the signature of another staff member with delegated authority.

(b) Any departure from an approved protocol that adversely affects the welfare or increases the number of animals or reduces the validity of the study must be approved by the Chair of MUAEC acting with authority vested through Items 3.13-3.15 of the Code of Ethical Conduct. A description of such modifications shall be submitted to the Secretary of MUAEC who will attach it to the original protocol and note it on the agenda for the next meeting. Further copies shall be attached to the protocols held by the Institute and the Chief Applicant.

ANIMAL USE STATISTICS
APPLICATION/FINAL RETURN FORM
(Amended 01/14)

Protocol ID 16/90

If more than one animal type is required, then fill in one form for each type.

Application: When applying to MUAEC for approval of a manipulation, the applicant should complete Box 1 and enter in Boxes 2 to 7, in the *'Planned'* column (P), the appropriate figures for the number of animals required.

Final Return: When the manipulation is concluded, Boxes 2 to 10 should then be completed in the *'Used'* column (U) by entering appropriate figures for the number of animals which were actually used.

Chief Applicant: [redacted]

Inst/Sch/Dept: [redacted]

Title of Project: Determination of the digestible indispensable amino acid score (DIAAS) of dietary protein-based foods

1. Animal type: Pigs Code: 1K
(see bottom of this form)

2. Source of animals (number)

		P	U
Breeding unit	a		
Commercial	b		
Farm	c	500	
Born during project	d		
Captured	e		
Imported	f		
Public sources	g		
TOTAL = A			

3. Status of animals (number)

		P	U
Normal/conventional	a	500	
*SPF/germ free	b		
Diseased	c		
Transgenic/chimaera	d		
Protected species	e		
Unborn/pre-hatched	f		
Other	g		

* *Specific pathogen free*

4. Main category of manipulation/use (enter the total from 2 above in one box only)

		P	U			P	U			P	U
Teaching	a			Basic biological research	e			Production of biological agents	j		
Species conservation	b			Medical research	f			Development of alternatives	k		
Environmental management	c			Veterinary research	g			Other	m		
Animal husbandry	d			Testing	h	500					

5. Any re-use of animals (number to be inserted)

		P	U			P	U	
No prior use	a	500		Previously used	b			Total a + b =

6. Grading of manipulations (number in each grade to be inserted)

(Download guidelines for selecting appropriate categories)

	Grade	P	U
A manipulation or use that causes no stress or pain or virtually no stress or pain. **No impact or virtually no impact.**	A		
A manipulation or use that causes stress or pain of a minor intensity for a short duration. **Little impact.**	B	500	
A manipulation or use that causes stress or pain of a minor intensity for a long duration or of a moderate intensity for a short duration. **Moderate impact.**	C		
A manipulation or use that causes stress or pain of a moderate intensity for a long duration or of a severe intensity for a short duration. **High impact.**	D		
A manipulation or use that causes stress or pain of a severe intensity for a long duration or of a very severe intensity for any duration. **Very high impact.**	E		

7. Expected Date of Completion (maximum three years): September 2019

ANIMAL DISPOSITION/FATE AT CONCLUSION OF RESEARCH, TESTING OR TEACHING OUTLINED IN THIS PROTOCOL

The data in Boxes 8 to 10 refer only to the animals noted in this protocol which actually entered the project and were manipulated - they do not refer to those it was proposed to manipulate but which were never used. This information is to be provided only when the research, testing or teaching has been completed and the animals have been disposed of as below.

8. Alive	Used	
Retained by your institution's farms/colonies	a	
Returned to owner	b	
Released to the wild	c	
Disposed of to others	d	
TOTAL ALIVE	**=B=**	

9. Dead	Used	
Killed for dissection, sampling, taking organs	a	
Died/destroyed in the course of the manipulation/use	b	
Euthanased after manipulation or use	c	
Died/destroyed for reason not associated with manipulation/use	d	
TOTAL DEAD	**=C=**	

10. GRAND TOTAL MANIPULATIONS/USED = B + C =

Check on the final return that B + C = A in the "Used" column of Box 2.

ANIMAL USE STATISTICS
APPLICATION/FINAL RETURN FORM
(Amended 01/14)

Protocol ID 16/90

If more than one animal type is required, then fill in one form for each type.

Application: When applying to MUAEC for approval of a manipulation, the applicant should complete Box 1 and enter in Boxes 2 to 7, in the *'Planned'* column (P), the appropriate figures for the number of animals required.

Final Return: When the manipulation is concluded, Boxes 2 to 10 should then be completed in the *'Used'* column (U) by entering appropriate figures for the number of animals which were actually used.

Chief Applicant: [redacted]

Inst/Sch/Dept: [redacted]

Title of Project: Determination of the digestible indispensable amino acid score (DIAAS) of dietary protein-based foods

1. Animal type: Rats Code: 1b
(see bottom of this form)

2. Source of animals (number)

		P	U
Breeding unit	a	500	
Commercial	b		
Farm	c		
Born during project	d		
Captured	e		
Imported	f		
Public sources	g		
TOTAL = A			

3. Status of animals (number)

		P	U
Normal/conventional	a	500	
*SPF/germ free	b		
Diseased	c		
Transgenic/chimaera	d		
Protected species	e		
Unborn/pre-hatched	f		
Other	g		

* *Specific pathogen free*

4. Main category of manipulation/use (enter the total from 2 above in one box only)

		P	U			P	U			P	U
Teaching	a			Basic biological research	e			Production of biological agents	j		
Species conservation	b			Medical research	f			Development of alternatives	k		
Environmental management	c			Veterinary research	g			Other	m		
Animal husbandry	d			Testing	h	500					

5. Any re-use of animals (number to be inserted)								
		P	U			P	U	
No prior use	a	500		Previously used	b			Total a + b =

6. Grading of manipulations (number in each grade to be inserted)			
(Download guidelines for selecting appropriate categories)	Grade	P	U
A manipulation or use that causes no stress or pain or virtually no stress or pain. **No impact or virtually no impact.**	A		
A manipulation or use that causes stress or pain of a minor intensity for a short duration. **Little impact.**	B	500	
A manipulation or use that causes stress or pain of a minor intensity for a long duration or of a moderate intensity for a short duration. **Moderate impact.**	C		
A manipulation or use that causes stress or pain of a moderate intensity for a long duration or of a severe intensity for a short duration. **High impact.**	D		
A manipulation or use that causes stress or pain of a severe intensity for a long duration or of a very severe intensity for any duration. **Very high impact.**	E		

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TOTAL ALIVE	**=B=**	

9. Dead	Used	
Killed for dissection, sampling, taking organs	a	
Died/destroyed in the course of the manipulation/use	b	
Euthanased after manipulation or use	c	
Died/destroyed for reason not associated with manipulation/use	d	
TOTAL DEAD	**=C=**	

10. GRAND TOTAL MANIPULATIONS/USED = B + C =

Check on the final return that B + C = A in the "Used" column of Box 2.

BOX 1: ANIMAL TYPE CODES

Animal Type	Code
Rodents	1 a = Mice
	1 b = Rats
	1 c = Guinea Pigs
	1 d = Hamsters
Rabbits	1 e = Rabbits
Farm Animals	1 f = Sheep
	1 g = Cattle
	1 h = Goats
	1 j = Deer
	1 k = Pigs
Other Domestic Mammals	1 m = Horses
	1 n = Dogs
	1 o = Cats
Birds	1 p = Fowls, Chickens
	1 q = Pigeons
	1 r = Other Birds
Miscellaneous	1 s = Marine Mammals
	1 t = Possums
	1 u = Reptiles
	1 w = Amphibia
	1 x = Fish
	1 z = Octopus, Squid, Crab, Lobster, Crayfish
Other	1 y = Other Species (*name)