

## HARRY PERKINS INSTITUTE OF MEDICAL RESEARCH

### ANIMAL ETHICS COMMITTEE (AEC)

#### Application to Transfer a Research Project that Uses Animals

**Project Title:**

**Establishing the role of sodium glucose co-transporter 2 (SGLT-2) in diabetic retinopathy.**

**AEC Permit Number:**


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**/2019**

#### DECLARATION BY AEC CHAIRMAN

I certify that this project has been considered and approved for transfer to the Harry Perkins Institute of Medical Research by the AEC on 12/02/19

**The period of approval for this project is 12/02/19 to 31/10/21.**

<b>AEC Chairman Name:</b>	A/Prof Evan Ingley
<b>AEC Chairman Signature:</b>	
<b>Date:</b>	12/02/19

#### CONDITIONS OF APPROVAL

All matters pertaining to the conduct of the approved project are to be reported to the AEC, which maintains oversight in accordance with conditions stated in the Licence to Use Animals for Scientific Purposes, Number U209/2019-2021 issued to the Harry Perkins Institute of Medical Research by the Department of Primary Industries and Regional Development (DPIRD).

Any variation proposed to the project, and the reasons for that change, must be submitted to the AEC for approval and must not be implemented until approval is granted.

A record of details of any animals requested and used in the project must be retained.

The project should only be conducted in approved premises nominated on the licence.

The AEC must also be notified in writing of;

- Any changes to approved investigators
- Any unexpected incidents or complications that result in deaths, euthanasia or pain and suffering for the animals used in the project. Details of the steps taken to deal with adverse incidents must be included in the notification.

#### OTHER CONDITIONS:

This approval is subject to the following special conditions;

- Where SOPs are noted in the original approved protocol, the equivalent Perkins SOP (if available) will be utilised for work conducted in the Perkins Bioresources Facility.
- Perkins animal monitoring records will be used for all animals in the Perkins facility. Bioresources staff will be able to assist in the preparation of project-specific monitoring records and initial training on the Perkins SharePoint monitoring system.
- A member of the Bioresources staff will observe each investigator the first time that a procedure is performed, to confirm competency.

This form is to be used for existing projects that currently have the approval of another AEC, which the Chief Investigator wishes to transfer to the Harry Perkins Institute Buildings under the jurisdiction of the Perkins Institute AEC.

Investigators are required to provide summary information (see following pages) and attach all documentation related to the currently approved project (approved application form, monitoring sheets, amendments, phenotype reports, annual reports, risk assessments, any adverse event reports (if applicable)). You will also need to complete a simple one-page After Hours information sheet.

**Sections 1-3** are a project summary specifically to assist the AEC in evaluating the transfer request, and tell us the current state of play for the project including animal numbers and staff.

**Sections 4-5** will further assist Bioresources Facilities staff in managing your project needs.

**Sections 6-8** relate to risk management and permits and are to assist both you and us to ensure the appropriate permits are in place to operate within the Perkins buildings.

The Perkins Institute AEC will review the transfer request and associated documentation and decide either to:


- approve the project for transfer with the currently approved protocol; or
- approve the project for transfer subject to minor adjustments to the protocol; or
- not approve the project for transfer in its current state, but rather require a new project application to be completed.

No transfer of animals may occur until Perkins AEC approval is granted.

The Chief Investigator should complete the attached form and provide the necessary documentation to the Perkins AEC Executive Officer, Caroline Kerr, using the AEC email address: [AEC@perkins.uwa.edu.au](mailto:AEC@perkins.uwa.edu.au)

- The application **MUST be reviewed by the Bioresources Manager** or her delegate at least **one week prior** to submission to the AEC, and signed to this effect in the declaration section.  
Email: [simone.ross@perkins.uwa.edu.au](mailto:simone.ross@perkins.uwa.edu.au)
- If the protocol incorporates **imaging at the ACRF Cancer Imaging Facility** then the imaging requirements must be discussed with the Facility Manager, Penny Maton, at least **one week prior** to submission, to ensure that the work is feasible. This is also of great assistance in planning your imaging study. Sign-off must be obtained prior to submission.  
Email: [cif@perkins.uwa.edu.au](mailto:cif@perkins.uwa.edu.au).
- If the protocol incorporates **imaging at CMCA@Perkins** then the imaging requirements must be discussed with the Head of the Bioimaging Facility, Kirk Feindel, at least **one week prior** to submission, to ensure that the work is feasible. This is also of great assistance in planning your imaging study. Sign-off must be obtained prior to submission.  
Email: [kirk.feindel@uwa.edu.au](mailto:kirk.feindel@uwa.edu.au).

If you are unsure of how best to proceed, please discuss with Caroline Kerr at: [AEC@perkins.uwa.edu.au](mailto:AEC@perkins.uwa.edu.au)  
Or call: 6151 0719 or 0432 567 259

 <p>HARRY PERKINS INSTITUTE OF MEDICAL RESEARCH</p>	ANIMAL ETHICS COMMITTEE
	<b>AEC004: PROJECT TRANSFER FORM</b>

<b>Application date:</b>	20/01/2019
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## 1. CURRENT DETAILS

<b>Existing approval granted by which AEC?</b>	<input checked="" type="checkbox"/> RPH AEC <input type="checkbox"/> UWA AEC <input type="checkbox"/> ARC AEC <input type="checkbox"/> Murdoch AEC <input type="checkbox"/> Other AEC (please specify):
<b>Existing Approval Number:</b>	R540/18-21
<b>Project Title:</b>	Establishing the role of sodium glucose co-transporter 2 (SGLT-2) in diabetic retinopathy.
<b>Project Chief Investigator:</b>	Dr Vance Matthews
<b>Contact details (phone &amp; email):</b>	0412723581 or 9224 0239/vance.matthews@uwa.edu.au

<b>Project Start date:</b>	October 2018 (As per document 6b)	<b>Project Finish date:</b>	October 2021
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## 2. PROJECT LOCATION

2.1 Which facility is the project moving from, and which facility do you wish to move it to?		
FROM:	Animal facility(s) currently approved for project:	RPH Animal Facility.
TO:	Perkins <i>Biorensources</i> Facility:	<input checked="" type="checkbox"/> Perkins North (QEll campus) <input type="checkbox"/> Perkins South (Fiona Stanley Hospital campus)

2.2 Will any part of this project remain at its original location?		
<input checked="" type="checkbox"/>	<b>No</b>	<b>Full Project Transfer</b>
<input type="checkbox"/>	<b>Yes</b>	<b>Partial Transfer</b> Note that if a project is to be conducted partially in a Perkins Institute facility and partially in another institute's facility then the approval process differs. In such cases, a Primary AEC and a Secondary AEC need to be assigned, with negotiation between the two AECs to ensure full compliance. In such cases, please consult with the Perkins AEC Executive Officer, Caroline Kerr, to determine the best path forward.

### 3. CURRENT STATUS OF PROJECT

#### 3.1 Please provide a brief overview of the project, to provide the AEC with context.

*(Please make clear which aims will be transferred by listing below and highlighting the relevant sections in the original approved document. Please also state which aims have been fully or partially completed.)*

**Ensure that this is written in LAY LANGUAGE.**

All forms of diabetes can lead to the development of diabetes-specific complications including **diabetic retinopathy (DR)** which, affects the pathology of the small blood vessels and the neural structure of the retina.

Mouse models that mimic human DR-features are pivotal for the development of therapeutic interventions for DR. In particular, this study will utilise three well-characterised mouse models of DR to investigate the underlying mechanisms of DR and potentially decrease the rate of disease progression. The mouse models used are:

- a) The Akita (Ins2Akita) mouse - a naturally occurring diabetes model that carries a dominant mutation in the **Mody4 locus** on chromosome 7 in the insulin 2 gene. The **heterozygous** Akita male mice develop **hyperglycemia** (high blood glucose) and features of DR.
- b) The Kimba (trVEGF029) mouse - a transgenic mouse model in which photoreceptors transiently overexpress **human vascular endothelial growth factor (hVEGF)**. These mice show changes associated with DR but lacks the hyperglycemic background.
- c) The Akimba (trVEGF029/Ins2Akita) mouse – a mouse model generated by crossing Akita and Kimba mice, where the interplay between high blood glucose levels and VEGF leads to the development of DR.

Our current project focuses on the Sodium Glucose Co-transporter 2 (SGLT2) protein. This protein is responsible for glucose reabsorption and was believed to be exclusively expressed in the proximal tubules of the kidney. Excitingly, we have discovered that the eye is a novel source of SGLT2. We have now demonstrated that SGLT2 expression is increased in the eye, with the development of DR.

#### **Hypothesis:**

Inhibition of SGLT2 function can decrease the development and progression of DR.

#### **Aim:**

- 1) To determine whether downregulation of SGLT2 activity using SGLT2 inhibition reduces the progression of DR in mice.

#### **Experiments:**

Mice (WT, Kimba, Akita or Akimba) will be given SGLT2 inhibitors (Dapagliflozin, Empagliflozin or Canagliflozin) at 25mg/kg/day (Nagareddy et al., 2013 and Al-Sharea et al., 2018) via their drinking water for 8 weeks starting at either 6 or 10 weeks of age. Individual experimental sample sizes will be based on availability of animals being bred at ARC. Having started our work with the 6 week and 10 week old mice, we found no signs of toxicity with the administration of Dapagliflozin. Urine will be tested weekly using a urine glucose stick test (Perkins SOP 1.05.15 - Mouse Urine collection) to establish the development of glucosuria. A glucose tolerance test (GTT) will be conducted at the end of week 7 and an insulin tolerance test (ITT) at the start of week 8. At the end of the experiment (end of week 8) serum, eye, kidneys, pancreas, intestines and colon will be collected from the animals for protein analysis and immunohistochemistry.

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**Measurement of body weight**

Body mass will be recorded for all mice weekly for the duration of the experiment (Perkins SOP 1.01.01 - Mouse handling and restraint). *As suggested by Simone Ross, we ask for a loss of weight greater than 15% from the animals starting weight be considered criteria for euthanasia.*

In our pilot studies, using Dapagliflozin, no mouse lost more than 10% body weight. All mice gained weight by the end of the 8 weeks.

**Urine glucose testing**

To establish the development of **glucosuria** induced by the proper function of SGLT2 inhibition, urine will be tested using a urine glucose stick test (SOP 1.05.15) on a weekly basis until the end of the experiment (week 8).

This has been partially completed with Kimba and Akimba mice administered Dapagliflozin. Our pilot study showed that consumption of Dapagliflozin in drinking water resulted in marked glucose secretion in urine as expected.

**Glucose tolerance test (GTT)**

GTT is a straightforward technique where after administration of an initial injection of glucose into the peritoneal space, glucose clearance from the blood is measured. Mice are fasted for 5 hours prior to the test. Following, an intraperitoneal glucose injection (1g/kg; 100-200µl volumes injected) using a 27-gauge needle (Perkins SOP 1.05.02 - Mouse Injection – Intraperitoneal), tail blood samples (~10µl) will be collected at 0, 15, 30, 45, 60, 90, 120 minutes and blood glucose is measured immediately in a glucometer. The initial blood collection is made via a minor tail cut (removal of the very tip of the tail; 0.5mm) and subsequent samples are taken from the same cut by gently removing the scab that forms. *This procedure has been discussed with Simone Ross.* The mice will be returned to their cages between bleeds and monitored for changes in mobility. The procedure will be performed by or under the supervision of staff trained in the techniques required.

This has been partially completed with Kimba and Akimba mice administered Dapagliflozin. In our pilot study, compared to the mice on the vehicle (n=3), there was a pronounced glucose tolerance observed in the Dapagliflozin (DAPA) treated mice (n=3) after 7 weeks.

**Insulin tolerance test (ITT)**

We will conduct insulin tolerance testing (ITT) at the start of week 8 of treatment. The ITT is a straightforward technique where after administration of an initial injection of insulin into the peritoneal space, glucose clearance from the blood is then measured. Mice will be fasted for 5 hours prior to the test. Following, an intraperitoneal insulin injection (0.75U/kg) using a 27-gauge needle (SOP 1.05.02), tail blood samples (~10µl) will be collected at 0, 15, 30, 45, 60, 90, 120 minutes and blood glucose is measured immediately in a glucometer. The initial blood collection is made via a minor tail cut (removal of the very tip of the tail; 0.5mm) and subsequent samples are taken from the same cut by gently removing the scab that forms. The mice will be returned to their cages between bleeds and monitored for changes in mobility and will be monitored closely during the procedure for signs of low blood sugar. The procedure will be performed by or under the supervision of staff trained in the techniques required.

This has been partially completed with Kimba and Akimba mice administered Dapagliflozin. In the pilot study, mild insulin sensitivity was observed in the Dapagliflozin treated mice (n=3) after 8 weeks of treatment in comparison to mice on the vehicle (n=3). This was particularly evident at the latter time-points of the ITT.

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**Cardiac puncture and tissue collection**

At the end of the experiment, the mouse is anaesthetised using methoxyflurane (Perkins SOP 1.03.01 - Inhalation Anaesthesia – Methoxyflurane) or isoflurane (Perkins SOP 1.03.02 - Inhalation Anaesthesia – Isoflurane). Once deeply anaesthetised, the mouse is removed from the anaesthetic chamber and placed on its back on a bench. Blood is then collected following Perkins SOP 1.04.05 - Blood collection in the rodent – Cardiac. Once an adequate amount of blood is collected, the needle is withdrawn and the mouse immediately euthanised by cervical dislocation (Perkins SOP 1.08.01 - Euthanasia of the mouse - Cervical Dislocation). The serum will be obtained and used for measuring inflammatory and metabolic markers. The procedure will be performed by or under the supervision of staff trained in the techniques required. The eye will be collected at the end of experiment and processed in several methods: 1) the eye will be removed and the retina will be dissected followed by immunofluorescence staining to visualize the retinal vasculature; 2) the eye will be removed and placed in fixative followed by wax embedding, sectioning and staining with standard histology methods to examine pathological features of the neural retina; 3) the eye will be removed and the retina will be dissected and followed by the analysis of SGLT2 expression via molecular techniques. In addition to the eye, kidneys, pancreas, intestines and colon will also be collected. Kidneys and pancreas will be sectioned and stained with haematoxylin and eosin and examined for any pathological features. All tissues will be analysed via molecular techniques for key proteins involved in diabetes.

**Observations from Pilot Study**

The conditioning of the mice used in our pilot study did not change based on the criteria outlined on our approved monitoring sheets. They displayed a normal intensity of activity, normal posture and a normal responsiveness to touch. Their coats remained smooth and there was no evidence of any mouse being in pain. There were no discernible differences in the amount of water consumed by mice on the vehicle and those treated with Dapagliflozin. All mice produced plentiful amounts of urine throughout the 8 weeks of the study. Mice on vehicle consumed a similar level of food compared to those mice treated with Dapagliflozin.

All procedures outlined above will be transferred to the Perkins Bioresources Facility. There are no issues or variables expected from the change in location.

3.2 Please provide a summary of animals approved and used to date:

Species (and common name)	Strain (Incl. background strain information)	GM strain? (y/n)	Sex	Age	Number Approved on project	Number Used to date	Number left/intend ed for use at Perkins
<i>e.g. Mus musculus (Mice)</i>	<i>C57Bl/6: E6AP KO</i>	<i>Y</i>	<i>m/f</i>	<i>6-8 wks</i>	<i>300</i>	<i>75</i>	<i>225</i>
Mus musculus (Mice)	C57Bl/6	N	M	6 wks	80	0	80
Mus musculus (Mice)	C57Bl/6: Akita	N	M	6 wks	80	0	80
Mus musculus (Mice)	C57Bl/6: Kimba	Y	M	6 wks	80	3	77
Mus musculus (Mice)	C57Bl/6: Akimba	Y	M	6 wks	80	4	76
Mus musculus (Mice)	C57Bl/6	N	M	10 wks	80	0	80
Mus musculus (Mice)	C57Bl/6: Akita	N	M	10 wks	80	0	80
Mus musculus (Mice)	C57Bl/6: Kimba	Y	M	10 wks	80	10	70
Mus musculus (Mice)	C57Bl/6: Akimba	Y	M	10 wks	80	6	74
Total number of all animals:					640	23	617

**3.3 Please provide a summary listing of project amendments requested and approved to date.**

*Approved amendment applications should be attached to this form.*

*Insert more lines as necessary.*

Amendment title	Date approved
Minor modification request to add Dr Aaron Magno as a co-investigator on this project (Document 4b).	21 <sup>st</sup> August 2018

**3.4 Please provide a current list of staff approved to work on this project and indicate if the investigator has a current Permission to Use Animals (PUA).**

1) Dr Vance Matthews	PUA <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
2) Dr Lakshini Herat	PUA <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
3) Dr Aaron Magno	PUA <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO

**3.5 Please detail any issues that have arisen on this project to date, or any notes that would assist both the AEC and the Bioresources Facility Staff in their management of the welfare of animals on this project.**

**If any adverse events have occurred, please attach the report forms.**

We have included 1 adverse event that we experienced.

**4 HOUSING REQUIREMENTS**

Perkins Bioresources Facilities house animals in groups on aspen bedding with a range of enrichment items, which may include cotton nestlets, aspen chew sticks, paper towels, tissues, custom cardboard rolls, cardboard mouse houses or red houses. Animals are housed in Tecniplast IVC with a limit of 5 mice or 3-4 rats per cage.

**If you have special requirements that require a variation to the standard, please specify this below.**



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This might include certain enrichment items NOT being included, or some animals to be housed individually. If this is mentioned in the original approval application, please also include the page number for quick reference for the AEC.

☒ Standard housing arrangements

☐ Variation to standard – specify below:

We currently dilute our treatment chemicals in acidified drinking water.

**5 TRAINING OF RESEARCH PERSONNEL**

Do any investigators require training in any procedures?

☐ Yes, training required by Bioresources staff

(Please allow sufficient time to co-ordinate training sessions and for competency to be signed off.)

☐ Yes, training to be provided by other investigator(s) approved on the project

(Please be aware that Bioresources staff must be present for the first procedure and to sign off competency.)

☒ No, training is not required

**6 RISK MANAGEMENT**

Please identify risk areas associated with your application.

Each identified risk area requires a risk assessment be undertaken.

Please contact the Perkins Institutional Biosafety Committee (IBC) for further details (contact: Kathy Davern, [kathleen.davern@perkins.uwa.edu.au](mailto:kathleen.davern@perkins.uwa.edu.au), 6151 0739)

Note that a handling and storage permit is required for handling of S6 to S9 chemicals/ drugs from the Therapeutic Goods Administration, Australian Committee for Chemicals Scheduling (ACGC). If applicable to this project, the permit should be submitted with the application.


If isotopes are to be used, this must be discussed with the Perkins Radiation Safety Committee.

Contact Chair, Penny Maton, [penny.maton@health.wa.gov.au](mailto:penny.maton@health.wa.gov.au)

Ensure that the Bioresources Manager receives a copy of the risk assessment prior to submission.

☐

BioHazard

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<input type="checkbox"/>	<b>Radiation</b>
<input type="checkbox"/>	<b>Chemical (including Cytotoxic drug)</b>
<input checked="" type="checkbox"/>	<b>Other</b> (We have already completed a risk assessment (document 3b). There are no major risks.

## 7 PERMITS – GMO CATEGORY

**Is the acquisition, holding, or use of the animals/ organisms subject to any permit, law or regulation of the State or Commonwealth (e.g. OGTR, protected native or imported)?**

Please note that the Perkins IBC has oversight of all GMO dealings within Perkins buildings, including dealings related to GM animals. You must obtain approval from the Perkins IBC prior to transferring any GMOs (contact: Kathy Davern, [kathleen.davern@perkins.uwa.edu.au](mailto:kathleen.davern@perkins.uwa.edu.au), 6151 0739)

<input type="checkbox"/>	<b>No</b>	
<input checked="" type="checkbox"/>	<b>Yes</b>	If yes, please specify the permit number below:

Organism / strain	Permit/dealing Number	Type of dealing
Mus musculus / Kimba and Akimba	NLRD 007/2018	NLRD category

## 8 USE OF SCHEDULE 8 DRUGS

**Will you require Schedule 8 drugs for your project?**

The use of Schedule 8 drugs requires a police clearance. Note that this is a legal requirement and is also standard at all veterinary clinics. This category of drugs cannot be dispensed to any person who does not possess this clearance. A copy must be provided to the Bioresources Manager prior to ordering or dispensing of these drugs. All S8 drugs must be maintained in the drug safe and are dispensed upon request.

<input checked="" type="checkbox"/>	<b>No</b>	
<input type="checkbox"/>	<b>Yes</b>	State specific drug(s) require

## 9 CHECKLIST:


Have you attached to this transfer form:

Original approved project protocol: ☒ YES ☐ NO

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Monitoring sheets:	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
Perkins After-hours information sheet:	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
Any project amendments:	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> N/A – no amendments
Any annual reports produced for this project to date:	<input type="checkbox"/> YES <input type="checkbox"/> NO <input checked="" type="checkbox"/> N/A – no annual reports
Any adverse event reports for this project to date:	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> N/A – no adverse events
Any risk assessments related to the project:	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
Phenotype reports for the strains on this project:	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO

*(Please consult the Bioresources Manager to confirm whether a Phenotype Report is required.)*


 <p>HARRY PERKINS INSTITUTE OF MEDICAL RESEARCH</p>	ANIMAL ETHICS COMMITTEE
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## 10 SIGNATURES

### Chief Investigator Declaration:

I hereby declare that:


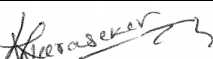
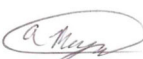
- The above is a true and accurate representation of this project to date.
- I have read the Act, the Regulations and the current version of the Code and accept the responsibilities detailed therein.
- I understand that scientific activities involving the use of animals must not start in Perkins Bioresources Facilities before written approval from the Perkins AEC is received.
- I accept responsibility for the conduct of all experimental procedures detailed in this application, and of the actions of all investigators listed on this project, in accordance with requirements of the Act, the Regulations, the Code, and the Animal Ethics Committee.
- Each person engaged in this project has the qualifications, experience and training appropriate for their role in the project, and are competent to perform procedures described to the extent of their role. If any person is not already skilled in the procedures, I will ensure that they obtain all necessary training in advance of performing any procedure independently. All personnel have been made aware of their role and responsibilities in this project, and have been given copies of all necessary documentation.
- Sufficient and adequate resources will be available to undertake the project.
- I have discussed the transfer of this project with the Bioresources Manager (or their delegate).

Chief Investigator Signature:	
Date:	18/01/19

### Other Investigators' Declaration



I hereby declare that:

- I am familiar with the Act, the Regulations and the *Australian Code for the care and use of animals for scientific purposes (8<sup>th</sup> edition, 2013)* and accept the responsibilities detailed therein to extent of my involvement in this project.
- I accept responsibility for the conduct of all experimental procedures detailed in this application that I undertake, in accordance with the requirements of the Act, the Regulations and the Code and the Animal Ethics Committee.

Investigator Name	Investigator Signature	Date
Vance Matthews		18/01/19
Lakshini Herat		18/01/19
Aaron Magno		18/01/19

### Bioresources Manager Declaration:

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 HARRY PERKINS INSTITUTE OF MEDICAL RESEARCH	<b>ANIMAL ETHICS COMMITTEE</b>	
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<b>Bioresources Manager Signature:</b>		
<b>Date:</b>	22/01/19	

*For projects incorporating imaging in the ACRF Cancer Imaging Facility or CMCA@Perkins, it is necessary to discuss requirements with the Facility Manager and receive sign-off to ensure it is feasible:*

**Facilities Manager, ACRF Cancer Imaging Facility (CIF):**

I (or my delegate) have discussed the imaging requirements of this project with the Chief Investigator and confirm that the component of the protocol outlining imaging at CIF is feasible.

<b>ACRF CIF Facility Manager Signature:</b>  <b>Penny Maton</b>  <b>Email:</b> <a href="mailto:cif@perkins.uwa.edu.au">cif@perkins.uwa.edu.au</a>	
<b>Date:</b>	

**Head, Bioimaging Facility, CMCA@Perkins:**

I (or my delegate) have discussed the imaging requirements of this project with the Chief Investigator and confirm that the component of the protocol outlining imaging at CMCA@Perkins is feasible.

<b>CMCA@Perkins – Head, Bioimaging Facility Signature:</b>  <b>Kirk Feindel</b>  <b>Email:</b> <a href="mailto:kirk.feindel@uwa.edu.au">kirk.feindel@uwa.edu.au</a>	
<b>Date:</b>	

## GLOSSARY

- 1) **Diabetic Retinopathy:** When tiny blood vessels inside the retina at the back of the eye are damaged as a result of diabetes.
- 2) **Mody4 locus:** Maturity onset diabetes of the young gene.
- 3) **Heterozygous:** Genes come in pairs called alleles. Heterozygous is when the cells of an organism contain two different alleles.
- 4) **Hyperglycemia:** When too much glucose is circulating in the blood.
- 5) **Human Vascular Endothelial Growth Factor (hVEGF):** A potent angiogenic factor.
- 6) **Glucosuria:** The excretion of glucose into the urine.



## ***Establishing the role of sodium glucose co-transporter 2 (SGLT-2) in diabetic retinopathy.***

### **1. Contact Details**

<b>AEC Protocol</b>	<b>AE141</b>	
	<b>Name</b>	<b>Mobile</b>
<b>Chief Investigator</b>	Dr Vance Matthews	0412723581
<b>Emergency Contact:</b>	Dr Vance Matthews	0412723581
<b>Monitor</b>	Dr Vance Matthews	0412723581
<b>Monitor</b>	Dr Lakshini Herat	0402620220

### **2. Species/Phenotype/Model issues**

Mus musculus / C57Bl/6 / None  
 Mus musculus / Akita (Ins2Akita) / Diabetes  
 Mus musculus / Kimba (trVEGF029) / Retinopathy  
 Mus musculus / Akimba (trVEGF029/Ins2Akita) / Diabetes and retinopathy

### **3. Monitoring Criteria**

<b>General criteria</b>	<b>0</b>	<b>1</b>	<b>2</b>
Body Posture	Normal	Somewhat and /or intermittent hunched appearance	Moderate/continuous hunching and still
Social behaviour or proximity to other	Normal	shows lack of curiosity in cage mates or surrounding activity	isolated from cage mates no interest in any surrounding activity
Activity/Movement	Normal - active	somewhat active with intermittent stillness compared to others, slight gait abnormality	Will only move when approached or reluctant to move when touched, trembling, paresis in limb
<b>Specific criteria</b>	<b>0</b>	<b>1</b>	<b>2</b>
Respiration	Normal	Rapid respiration, respiratory difficulty	Whole body, abdominal breathing
Eye condition	Normal	Partial closure of eyelids or blinking	Redness, swelling of eyelids, eyes closed
Surgery site	Normal	Redness in immediate surgery area	Redness, swelling around the surgery area
Coat condition	normal flat and glossy	Somewhat ruffled, mild piloerection	Moderately ruffled, severe piloerection
Grimace scale*	Normal	Scored as moderate pain in any or all of the facial actions	Scored as obvious pain in any or all of the facial actions
Body condition* (as per chart)	Normal (BC 3)	Slightly under conditioned	Moderately under conditioned

Weight loss	None	<10%	10% - 15%
<b>Other condition</b> not documented above, but impacting on health and we- being	Normal	Slight or intermittent deviation from normal	Moderate or consistent deviations from normal

\*Refer to chart in procedure rooms for assessment

#### **4. Monitoring Frequency (adjust to fit experiment)**

General:

Mice will be monitored twice per week (Mondays and Fridays) for weight and body condition by the research team.

Mice will be monitored at the end of the day following blood sampling, and again the next morning.

Monitoring increased to daily if an animal scores 1 or more following discussion between the research team and the Perkins Bioresources facility staff.

Monitoring increased to twice daily if an animal scores 2-3.

#### **5. Actions and Interventions**

Score	Assessment	Actions/Interventions
0	Animal within normal limits	No interventions required
1	Animal demonstrates slightly or intermittently deviated from normal	Relevant and specific actions that maintain animal welfare while ensuring experimental outcomes are met. <ul style="list-style-type: none"> <li>○ Increase frequency of monitoring to daily until recovery or endpoint</li> <li>○ Closer and more careful monitoring</li> <li>○ Buprenorphine pain relief administered</li> <li>○ Obtain advice from bioresources</li> </ul>
2-3	Animal demonstrates moderate deviation from normal	Assess for euthanasia and/or seek expert advice. Increase monitoring to twice daily
>3	Animal demonstrates significant deviation from normal or is obviously unwell and/or distressed	Immediate euthanasia and contact Bioresources Manager and/or the Perkins Veterinary Surgeon.

#### **6. Instructions**

1. Each animal is examined and observed for abnormalities at each monitoring point.
2. Each criterion is scored and the score marked on SharePoint. Training may be required to ensure all personnel are consistent in terms of scoring.
3. SharePoint will calculate the total score.




4. Appropriate to the total score actions or interventions are undertaken.
5. Comments concerning abnormalities are recorded in the "Comments" section.
6. Any other abnormalities are recorded in the "Other" section and advice sort from the Bioresources manager or Perkins on-call veterinary surgeon.
7. Any abnormality that is observed to be of greater severity that the descriptions provided above will require immediate euthanasia, notification to the Bioresources Manager/Co-ordinator or Perkins on-call veterinary surgeon and external PM.
8. **All unexpected deaths will be reported immediately to the Bioresources Manager/Co-ordinator/Perkins Veterinary Officer and the AEC office.**

**Independent of overall level scoring the following animals will be euthanased:**

Animals who lose more than the Animal Ethics Committee approved percentage of bodyweight (as recommended by Simone Ross, we are asking for greater than 15% body weight loss to be the criteria used for euthanasia).

Animals who cannot right themselves (cannot stand on own).

	<b>BIORESOURCES FACILITY</b>
	<b>PROJECT AFTER HOURS INFORMATION &amp; EMERGENCY CONTACT DETAILS</b>
	<b>AEC Number: AE141</b>

**Project Title:** Establishing the role of sodium glucose co-transporter 2 (SGLT2) in diabetic retinopathy.

**Chief Investigator:**      **Name:** Dr Vance Matthews  
**Work phone:** 9224 0239  
**Mobile:** 0412723581  
**Email:** vance.matthews@uwa.edu.au

**Contact details of investigators responsible for monitoring the animals:**

*Please list these in the order they should be contacted in the event of an emergency.*

	Name	Work phone	Mobile	Email
Contact 1	Vance Matthews	9224 0239	0412723581	vance.matthews@uwa.edu.au
Contact 2	Lakshini Herat	9224 0239	0402620220	lakshini.weerasekera@uwa.edu.au
Contact 3	Aaron Magno	9224 0230	0488739012	aaron.magno@uwa.edu.au
Contact 4				

*Investigators are responsible for ensuring the welfare of their animals at all times.*

*Please be aware that if Bioresources staff find an animal in a critical condition and they cannot contact the investigators listed above, then under instruction from the Bioresources Manager or their delegate they will euthanise the animal if it is deemed necessary. You are advised to provide details of any samples you require or instructions for the storage of the deceased animals in the unlikely event that this occurs.*

**If an animal needs to be euthanised, which (AEC-approved) method should be used?**

Cervical dislocation (Perkins SOP 1.08.01 - Euthanasia of the mouse - Cervical Dislocation)

**If an animal needs to be euthanised, how should the deceased animal be stored?**

*e.g. bag and place in fridge; bag and place in freezer.*

Bag in freezer

**Any other notes for facility staff:**

N/A



**ANIMAL ETHICS COMMITTEE**

Telephone: (08) 9224 2814

Facsimile: (08) 9224 2981

Asst. Prof. Vance Matthews, Research Fellow  
School of Medicine & Pharmacology  
3rd Floor MRF Building  
Royal Perth Hospital

17<sup>th</sup> October 2018

Dear Vance,

**RPH-AEC Decision: 16<sup>th</sup> October 2018 – Full Project Approval.**

**Application #: R540/18-21 (Matthews):** *Establishing the role of sodium glucose co-transporter 2 (SGLT2) in diabetic retinopathy.*

The Royal Perth Hospital Animal Ethics Committee has reviewed your progress report and gives approval for the full project to proceed with the understanding that project approval may be reviewed at any time.

In keeping with AEC and Animal Facility policy, all animals are to be monitored daily (or more frequently as required) by the Animal Services Coordinator (ASC; Mr. Nicholas Grainger), who has the authority to euthanase distressed animals if deemed necessary. It is also an AEC & NHMRC requirement that any adverse event or unexpected death be reported to the ASC immediately, with the report to be tabled and minuted at the next AEC meeting. In addition, any non-compliance with the Code of Practice will be reported immediately to the AEC and may result in the withdrawal of project approval and possible disciplinary action by the Hospital (Guidelines – Attachment II).

You are reminded that you are legally and ethically responsible for all matters related to the welfare of the animals assigned to this project (Section 3; Code of Practice). It is your responsibility to maintain your own animal records and to supply annually to the AEC, a Project Report and completed Animal Usage Tables. The ASC is available to assist you with these requirements.

Please ensure that you use the above AEC approval number whenever you order animals for this project. All animal orders must be made through the RPH Research Centre Animal Facility.

We wish you the very best in your endeavour.

Yours sincerely

**Prof Kevin Croft**  
**Chairman**  
**ANIMAL ETHICS COMMITTEE**

**Dr L.S. Manning**  
**Executive Officer**  
**ANIMAL ETHICS COMMITTEE**



**Royal Perth Hospital**

IN CONFIDENCE

**RESEARCH APPLICATION FORM  
ANIMAL ETHICS COMMITTEE****R****APPROVAL INFORMATION:****FOR OFFICE USE ONLY**

The RPH-AEC has considered this proposal and has approved it for the period: \_\_\_\_\_  
to \_\_\_\_\_

Title of Application: \_\_\_\_\_

Signature of AEC Chair: \_\_\_\_\_ Date: \_\_\_\_\_

This form is to be completed electronically; hand-written applications will not be accepted. All sections of the application must be retained and addressed. Use "NA" to complete sections which do not apply to the project.

**Please send one (1) electronic copy as a RTF file via email, and thirteen, signed hardcopies (the original and twelve (12) copies) of your application to:**

**RPH Animal Ethics Committee Executive Officer**  
**Research Centre**  
**Royal Perth Hospital**  
[linda.manning@health.wa.gov.au](mailto:linda.manning@health.wa.gov.au)

**This application will not be considered by the RPH Animal Ethics Committee unless:**

- It is typed, written in **LAY-LANGUAGE** and all sections are complete.
- The RPH Animal Services Coordinator (RPH-ASC) has reviewed and signed the application (**page 2**)
- All signatures required on the declaration page (**page 15**) are provided (eg. CI, Co-CIs, HOD)
- A copy of the RPH Authority to Use Animals Form (**page 16**) for EACH listed investigator/co-investigator directly involved in conducting animal experimentation **is attached to the original application only.**

Projects will not be approved until reports (annual or final) for all current and previous projects have been received. Deadlines for the RPH-ASC application review (new requirement) and for application submission to the RPH-AEC are provided below. The RPH-AEC 2018 meeting dates are also provided.

**REVIEW & SUBMISSION DEADLINES AND MEETING DATES:**

<b>DEADLINE FOR RPH-ASC APPLICATION REVIEW</b>	<b>DEADLINE FOR SUBMISSION TO THE RPH-AEC</b>	<b>RPH-AEC MEETING DATES 2018</b>
Tuesday, 28 <sup>th</sup> Nov. 2017	Tuesday, 5 <sup>th</sup> December 2017	<b>Tuesday, 19<sup>th</sup> December 2017</b>
Tuesday, 30 <sup>th</sup> January 2018	Tuesday, 6 <sup>th</sup> February 2018	<b>Tuesday, 20<sup>th</sup> February 2018</b>
Tuesday, 27 <sup>th</sup> March 2018	Tuesday, 2 <sup>nd</sup> April 2018	<b>Tuesday, 17<sup>th</sup> April 2018</b>
Tuesday, 29 <sup>th</sup> May 2018	Tuesday, 5 <sup>th</sup> June 2018	<b>Tuesday, 19<sup>th</sup> June 2018</b>
Tuesday, 31 <sup>st</sup> July 2018	Tuesday, 7 <sup>th</sup> August 2018	<b>Tuesday, 21<sup>st</sup> August 2018</b>
Tuesday, 25 <sup>th</sup> Sept. 2018	Tuesday, 2 <sup>nd</sup> October 2018	<b>Tuesday, 16<sup>th</sup> October 2018</b>
Tuesday, 27 <sup>th</sup> Nov. 2018	Tuesday, 4 <sup>th</sup> December 2018	<b>Tuesday, 18<sup>th</sup> December 2018</b>

**APPLICATIONS RECEIVED AFTER THE DEADLINE DATES WILL NOT BE ACCEPTED FOR CONSIDERATION AT THE CORRESPONDING AEC MEETING DATE.**

**A. PROJECT DETAILS:**

<b>Research Project Title (as per the front page): Establishing the role of sodium glucose co-transporter 2 (SGLT2) in diabetic retinopathy.</b>	
<b>Application reviewed by the RPH Animal Services Coordinator (Nicholas Grainger)</b>	
Date: _____	Signature: _____
<i>This information must be provided prior to the application being submitted to the RPH-AEC.</i>	
Is this project FULLY funded? <i>If yes, please indicate source(s) of funding and whether this AEC approval is required for the grant application:</i> This project is funded by a MRF 2018 grant. (title: Investigating the role of sodium glucose co-transporter 2 in diabetic retinopathy) <i>If no, please indicate how the costs for this project be covered.</i>	Yes [X]      No [ ]
Is there a commercial aspect to this project? <i>If yes, provide details:</i>	Yes [ ]      No [X]
Proposed starting date: July 2018	Expected completion date: July 2021 (3 years maximum)

**B. PRIOR OR OTHER APPROVALS:**

Has this proposal, or aspects of this proposal, had prior approval at RPH? <i>If yes, previous approval no:</i>	Yes [ ]      No [X]
Has this proposal, or aspects of this proposal, been submitted to any other AEC? <i>If yes, please provide details:</i> Name of the Institution: Outcome of the application: Approved [ ]    Not Approved [ ]    Under consideration [ ] <i>If approved, please provide Approval Number:</i>	Yes [ ]      No [X]
If this proposal, or aspects of this proposal, have been approved by another AEC, justify why approval by the RPH-AEC is also being requested.	

**C. OUTSTANDING NON-COMPLIANCES:**

<b>Has the CI or co-investigator(s) been associated with any AEC-approved project for which non-compliance issues have not been closed out?</b> (eg. non-submission of an annual/final report; non-reporting of adverse events, conduct of procedures not approved by the AEC, non-payment of project costs etc...)	Yes [ ]      No [X]
<i>If yes, this project cannot be approved until the non-compliance has been addressed. Objective evidence to this effect must be provided (eg. letter from the approving AEC and/or Animal Care Service).</i>	

**D. ALL OTHER CURRENT APPROVALS (for CIs and co-investigators):** Provide the following information for all other animal-based projects, including those under consideration, on which the CI and/or co-investigators are associated.

**Project Title:** Establishing the role of the sodium glucose co-transporter 2 (SGLT-2) on sympathetic nervous system activation and blood pressure.

**Name of Institution:** Royal Perth Hospital.

**Approval #:** R537/17-20

**Staff listed on project:** Vance Matthews, Caroline Rudnicka and Lakshini Herat.

**Capacity of staff:** Vance Matthews (CI) and all others are co-investigators.

**Project duration:** 3 years (2017-2020).

**Project Title:** Potential of 68 Gallium Dotatate PET for detecting high risk atherosclerosis in mice.

**Name of Institution:** Royal Perth Hospital.

**Approval #:** R538/17-20

**Staff listed on project:** Carl Schultz, Vance Matthews, Caroline Rudnicka and Lakshini Herat.

**Capacity of staff:** Carl Schultz (CI) and all others are co-investigators.

**Project duration:** 3 years (2017-2020).

## E. COLLABORATIONS:

<p><b>Does this project involve collaboration with another institution?</b></p> <p><i>If yes, attach a copy of all external Institutional approval letters pertaining to any aspect of this project, and provide written evidence that an inter-Institutional agreement is in place between all Institutions involved with this project.</i></p>	<p>Yes [ <input type="checkbox"/> ]      No [X]</p>
<p><b>Does this project involve collaboration with an overseas laboratory?</b></p> <p><i>If yes, provide details of the proposed arrangements:</i></p>	<p>Yes [ <input type="checkbox"/> ]      No [X]</p>

## F. CHIEF INVESTIGATOR:

<p>Title, first name, last name, qualifications: Dr Vance Matthews (PhD)</p>		<p>Current appointment: Research Fellow</p>
<p>Department: School of Biomedical Science</p>		
<p>Telephone no: 9224 0239</p>	<p>Mobile no: 043 452 7697</p>	<p>Email address: vance.matthews@uwa.edu.au</p>
<p>Completed RPH Authority to Use Animals Form attached?</p> <p>A completed copy of the Authority Form is required unless the CI is not directly involved in conducting animal procedures.</p> <p><b>Tick NA</b> – if not directly involved in conducting animal procedures.</p>		<p>Yes [X]      No [ <input type="checkbox"/> ]      NA [ <input type="checkbox"/> ]</p>
<p>Date UWA PAWes course (or equivalent) completed successfully?</p> <p>A PAWes course (or equivalent) must have been completed within the last 5 years.</p>		<p>Original course: March 2010 Refresher course: June 2015</p> <hr/> <p>(date of completion)</p>

**G. CO-INVESTIGATORS (must include all persons working with animals):**

1. INVESTIGATOR: Qualifications: Current Appointment: Department:	Mrs Lakshini Herat. BSc. Research Assistant School of Biomedical Sciences		
Telephone: 9224 0239/0402 620 220      Fax: N/A      Email: lakshini.weerasekera@uwa.edu.au			
Completed RPH Authority to Use Animals Form attached? A completed copy of the Authority Form is required unless the investigator is not directly involved in conducting animal procedures. <b>Tick NA</b> – if not directly involved in conducting animal procedures.		Yes [X]      No [ ]      NA [ ]	
Date UWA PAWes course (or equivalent) completed successfully? A PAWes course (or equivalent) must have been completed within the last 5 years.		Original course: 2010 Refresher course: June 2015  _____ (date of completion)	
2. INVESTIGATOR: Qualifications: Current Appointment: Department:	Dr Aaron Magno BSc (Hons) PhD Research Assistant Research Centre, RPH.		
Telephone: 9224 0239      Fax: N/A      Email: aaron.magno@uwa.edu.au			
Completed RPH Authority to Use Animals Form attached? A completed copy of the Authority Form is required unless the investigator is not directly involved in conducting animal procedures. <b>Tick NA</b> – if not directly involved in conducting animal procedures.		Yes [ X ]      No [ ]      NA [ ]	
Date UWA PAWes course (or equivalent) completed successfully? A PAWes course (or equivalent) must have been completed within the last 5 years.		__ Refresher course: Feb 2017 __ (date of completion)	
3. INVESTIGATOR: Qualifications: Current Appointment: Department:			
Telephone:      Fax:      Email:			
Completed RPH Authority to Use Animals Form attached? A completed copy of the Authority Form is required unless the investigator is not directly involved in conducting animal procedures. <b>Tick NA</b> – if not directly involved in conducting animal procedures.		Yes [ ]      No [ ]      NA [ ]	

Date UWA PAWes course (or equivalent) completed successfully? A PAWes course (or equivalent) must have been completed within the last 5 years.	_____ (date of completion)
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**ALL INVESTIGATORS WORKING WITHIN THE ANIMAL FACILITY MUST COMPLETE AN RPH-AUTHORITY TO USE ANIMALS FORM (provided with this document) FOR EACH APPLICATION.**

**THE RPH ANIMAL SERVICES COORDINATOR IS REQUIRED TO MAINTAIN COMPETENCY RECORDS FOR ALL STAFF WORKING WITH ANIMALS.**

**EMERGENCY CONTACT PERSONNEL (and day-to-day contact personnel)**

1. Name of Investigator: Dr Vance Matthews	
9224 0239	043 452 7697
_____ (Phone)	_____ (Mobile)
2. Name of Facility Staff: Nick Grainger (RPH-ASC) or trained delegate	
08-9224-2814/2825	0424 261 110 OR 0451 052 589
_____ (Phone)	_____ (Mobile)

**PERSONNEL RESPONSIBLE FOR ANIMAL MONITORING – (ANAESTHESIA, SURGERY & POST- OP RECOVERY PERIOD)**

1. Name of Investigator: Dr Vance Matthews	
9224 0239	043 452 7697
_____ (Phone)	_____ (Mobile)
2. Name of Facility Staff: Nick Grainger (RPH-ASC) or trained delegate	
08-9224-2814/2825	0424 261 110 OR 0451 052 589
_____ (Phone)	_____ (Mobile)

**PERSONNEL RESPONSIBLE FOR EUTHANASIA**

1. Name of Investigator: Dr Vance Matthews	
9224 0239	043 452 7697
_____ (Phone)	_____ (Mobile)
2. Name of Facility Staff: Nick Grainger (RPH-ASC) or trained delegate	
08-9224-2814/2825	0424 261 110 OR 0451 052 589
_____ (Phone)	_____ (Mobile)



## H. ANIMALS:

Projects conducted at more than one site must indicate the site where the animals will actually be used and the institution responsible for reporting animal usage.

SPECIES (common name)	STRAIN	SOURCE*	AGE RANGE/ GENDER	SITE WHERE ANIMALS USED**	# OF ANIMALS /YR	TOTAL # REQUESTED
Mice	<i>Akita</i> ( <i>Ins2<sup>Akita</sup></i> )	ARC	6 week; male	RPH	20-30	80
Mice	<i>Kimba</i> ( <i>VEGF<sup>+/+</sup></i> )	ARC	6 week; male	RPH	20-30	80
Mice	<i>Akimba</i> ( <i>Ins2<sup>Akita</sup></i> <i>VEGF<sup>+/-</sup></i> )	ARC	6 week; male	RPH	20-30	80
Mice	WT type	ARC	6 week; male	RPH	20-30	80
Mice	<i>Akita</i> ( <i>Ins2<sup>Akita</sup></i> )	ARC	10 week; male	RPH	20-30	80
Mice	<i>Kimba</i> ( <i>VEGF<sup>+/+</sup></i> )	ARC	10 week; male	RPH	20-30	80
Mice	<i>Akimba</i> ( <i>Ins2<sup>Akita</sup></i> <i>VEGF<sup>+/-</sup></i> )	ARC	10 week; male	RPH	20-30	80
Mice	WT type	ARC	10 week; male	RPH	20-30	80

\* Source is to indicate the actual location from which the animals are being delivered to the Research Centre Animal Facility (eg. ARC, Murdoch, PMH, UWA).

\*\* As per the inter-institutional agreement and/or approval letter.

## I. GENETICALLY MODIFIED ANIMALS:

Are genetically modified animals to be used in this project?	Yes [X] <sup>#</sup> No [ ]
Name of modified gene	vascular endothelial growth factor (VEGF)
Type of genetic modification (KO, Tg etc)	Tg
What level of containment is required for the animals to be used in this project?	PC-1 [X]      PC-2 [ ]*

<sup>#</sup>If yes, a copy of OGTR/Biosafety Committee approval (as appropriate) must be provided.

**Note: An application will be submitted shortly.**

**\*The RPH Research Centre Animal Facility is certified for PC-1 activities only; it currently cannot accommodate projects which require PC-2 or higher containment.**

## J. PERMIT REQUIREMENTS:

Is the acquisition, retention or use of these animals, subject to any permit, law or regulation of the State or Commonwealth (such as the importation of non-indigenous animals or the use of native animals)?	NO
--	----

**If YES, attach a copy of the permit.**

## K. HEALTH AND SAFETY CONCERNS:

<b>1. Does this project involve the use of:</b>	
Potent teratogens and carcinogens.	Yes [ ]*      No [X]
Recombinant DNA molecules/genetically modified animals.	Yes [X]*      No [ ]
Potentially infectious or hazardous agents that might pose a health risk to other animals and/or staff.	Yes [ ]*      No [X]

S8 drugs	Yes [ <input type="checkbox"/> ]*      No [X]
Ionising radiation.	Yes [ <input type="checkbox"/> ]*      No [X]
<p><i>* If yes to any of the above, attach appropriate permits and/or approval, if required. (eg. Biosafety Committee, Infection Control; Police Clearance; Medical Physics approval)</i></p> <p><b>Note: An application will be submitted shortly.</b></p>	
<p><b>2. Risk Assessment:</b> (identify potential risks to staff and/or animals, and how they will be managed and minimised):</p> <p>Based on previous similar experiments conducted by the CI, the CI believes there are no major risks to staff or animals. The staff will be extremely careful when handling scalpel blades and needles to avoid cuts and needle stick injuries. These have not occurred in the past.</p>	

**L. EXPERIMENTAL PROTOCOL IN LAY LANGUAGE:****1. PROJECT JUSTIFICATION:** (including pilot studies)

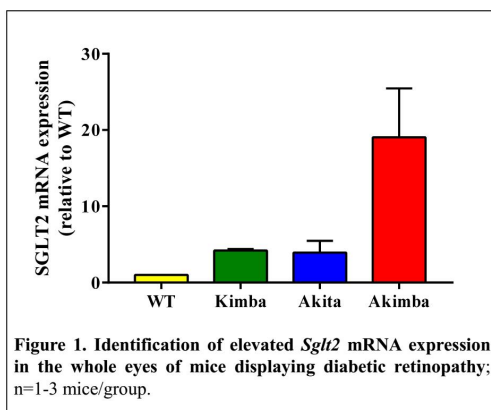
**USE LAY LANGUAGE.** In one page or less, clearly state what this project intends to achieve and the significance and/or benefits of the project outcomes.

### 1.1 Objectives and aims of project:

All forms of diabetes can lead to the development of diabetes-specific complications. Diabetes induced eye disease, also known as Diabetic retinopathy (DR), affects the pathology of the small blood vessels and the neural structure of the retina. Diabetic retinopathy is the most common vascular complication leading to blindness among working age adults (20-74 years) in developed countries. Clinically, DR is marked by features such as: pericyte ghosts, acellular capillaries, microaneurysms and the growth of new blood vessels on the retinal surface.

Mouse models that mimic a wide array of human DR-features are pivotal for the development of therapeutic interventions for DR. In particular, this study will utilise three well-characterised mouse models of DR to investigate the underlying mechanisms of DR and potentially decrease the rate of disease progression. The mouse models used are:

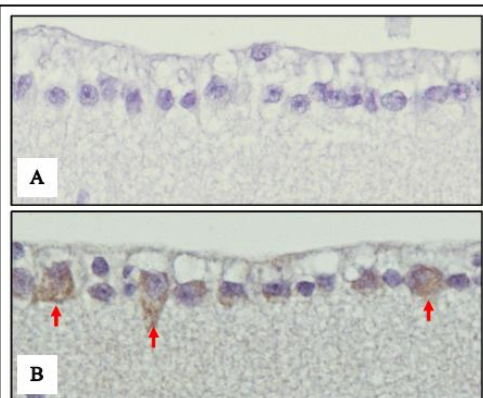
1) The Akita (Ins2Akita) mouse - a naturally occurring diabetes model that carries a dominant mutation in the Mody4 locus on chromosome 7 in the insulin 2 gene. The heterozygous Akita male mice develop hyperglycemia and features of DR.



2) The Kimba (VEGF<sup>+/+</sup>) mouse - a transgenic mouse model in which photoreceptors transiently overexpress human vascular endothelial growth factor (hVEGF). These mice show changes associated with DR but lacks the hyperglycemic background.

3) The Akimba (Ins2AkitaVEGF<sup>+/-</sup>) mouse – a mouse model generated by crossing Akita and Kimba mice, where the interplay between high blood glucose levels and VEGF leads to the development of DR.

These mouse models were all generated in Perth by our collaborator Prof. Elizabeth Raczky at the Lions Eye Institute.



**Figure 2. SGLT2 is expressed in the diabetic retina.** Negative control staining (A) and SGLT2 positive staining (red arrows) (B) in the retinal ganglion cell layer. Magnification 300X.

Our current project focuses on the Sodium Glucose Co-transporter 2 (SGLT2) protein. This protein is responsible for glucose reabsorption and is believed to be exclusively expressed in the proximal tubules of the kidney. Excitingly, we have discovered that the eye is a novel source of SGLT2. As shown in Figures 1 and 2, we have now demonstrated that SGLT2 expression is increased in the eye, with the development of DR.

#### Aim:

To determine whether downregulation of SGLT2 activity using SGLT2 inhibition reduces the progression of DR in mice.

#### Hypothesis:

Inhibition of SGLT2 function may be a novel approach to decrease the development and progression of DR.

A key therapeutic goal of treating diabetic complications including DR is by improving glycaemic control. The main effect of SGLT-2 inhibitors in this study is the reduction of hyperglycaemia by inhibiting glucose reabsorption and causing glucosuria. The SGLT2 inhibitors have been shown to have protective effects, targeting two of the main complications of diabetes which are cardiovascular disease and kidney related complications. However, to date, none of the studies have looked at the retinal vascular and neural pathology in detail. Hence, studies are urgently needed to establish the role of SGLT2 in DR and to test the effectiveness of SGLT2 inhibitors in mouse models with retinal and neural damage associated with DR.

**1.2 Potential benefits/anticipated outcomes of the project:** (for example the benefits to humans, animals, or the environment):

This study is of high importance as we will be able to investigate for the first time in Akita, Kimba and Akimba mice, the *in vivo* effects of SGLT-2 inhibition on the development and progression of DR. Our work may also highlight novel molecular pathways involved in DR.

**1.3 What instruction will be provided to co-investigators on the projects in terms of ethical and legal responsibilities involved in the use of animals for scientific purposes, as well as in the appropriate methods for animal care and use?** (eg Code of Practice 2.4.5; 2.4.6(vi); 2.4.8(xviii))

All staff working with animals have completed the PAWES course. In addition, all staff will read the following two documents: 1) Royal Perth Hospital Animal Ethics Committee 2018 Operational Policies and Guidelines for Applicants and 2) Australian Code of Practice for the Care and Use of Animals for Scientific Purpose

## **2. ANIMAL DETAILS:**

### **2.1 Justification for use of animals\*:**

**replacement** (eg describe the alternatives to animal use that you have considered and/or adopted. Explain the reason for use of animals and why the choice of species):

**reduction** (eg. describe the ways that you propose to minimise the use of animals): and,

**refinement** (Please see reference to refinement below, within details of procedures)

**REPLACEMENT:** We are also performing extensive *in vitro* studies of the effect of SGLT-2 regulation in cell lines in culture. However, these studies are not informative with regard to the effect of SGLT-2 activation *in vivo*. For this purpose, whole animal studies are required to investigate the role of SGLT2 in DR and how the inhibition of SGLT2 may prevent the progression of DR. In addition, pathological changes cannot be evaluated in *in vitro* studies.

**REDUCTION:** The number of mice required for the studies outlined in this application have been determined with the assistance of Mrs Sally Burrows (Biostatistician, Royal Perth Hospital). To reduce animal numbers, we will use the same animals for glucose tolerance tests, insulin tolerance tests, retinal assessments and SGLT2 activity studies. In this manner, statistically valid endpoints are expected to be achieved with the minimum number of animals required.

**REFINEMENT:** The most appropriate and accurate methods are being used to measure the main end-points in this study. Methods used to assess glucose levels use very small volumes of blood to minimize the impact on the animal.

\* **ANIMAL ETHICS AWARENESS:** To ensure that both the researcher and the Ethics Committee are aware of the actual cost to the animal compared to the benefits to be achieved, **IF** project specific breeding programs are required to generate the experimental animals requested, attach phenotype data and provide an estimate of the total number of animals actually required to generate the number of experimental animals requested. Justify the total number (eg. breeding animals; foetus/neonates; genotypes not suitable for experimental use etc..).

### **2.2 Statistical Comparisons: Provide information on the comparisons evaluated for each experimental procedure, the power to be achieved for the comparison to be considered statistically significant, and the number of animals/group required to achieve this level of significance.**

A Statistician must be consulted to confirm that the study is adequately powered to detect the expected changes in each of the outcomes of interest.

Within type comparisons will be made between vehicle and each of the inhibitor groups at 18 weeks. With a sample size of 20 per group, differences of 89% of the sd or larger will be detectable with 80% power and alpha of 0.05.

Name & Position of statistician consulted: \_\_\_\_\_

Signature (Statistician)\_\_\_\_\_

Date:\_\_\_\_\_

### **3. EXPERIMENTAL PROCEDURES:**

#### **3.1 Non-Surgical Experimental Procedures:**

Explain what is being done and why. **USE LAY LANGUAGE.** **All experimental procedures MUST be supported by a diagram, flowchart and/or table.**

Provide details of the generic constituents (not the trade name), the dose rate as mg/kg, the route of administration, the frequency and duration of administration, the timeframe of the experiments, intra-procedural use of analgesics, the number of animals in each group/step and refinement methods, etc: Include competency & training of staff responsible for administering the drugs, monitoring the animals during the procedure and conducting the procedures.

NB: for information on RPH AEC approved pre-meds, anaesthetics, analgesics and support materials for each species, refer to the Animal Facility SOPs (available from the animal facility). If any anaesthetics, analgesics or support materials vary from approved RPH substances, please provide details and a brief explanation of why it is required.

#### **Effect of SGLT2 inhibitors (SGLT2i) on DR**

We would like to use WT, Akita, Kimba and Akimba strains to test the effect of SGLT2i on the development and progression of DR. Only male mice will be used in experiments due to the variability of the DR phenotype in females. All mice will be on a standard chow diet for the full duration of the experiment. The SGLT2i and vehicle will be administered via drinking water.

#### **Group 1 – Experiment starts at 6 weeks of age and ends at 14 weeks of age (8 weeks of treatment)**

- 1) WT (6 week old mice); Vehicle (n=20)
- 2) WT (6 week old mice); Dapagliflozin (25mg/kg/day for 8 weeks; n=20)
- 3) WT (6 week old mice); Empagliflozin (25mg/kg/day for 8 weeks; n=20)
- 4) WT (6 week old mice); Canagliflozin (25mg/kg/day for 8 weeks; n=20)
- 5) Akita (6 week old mice); Vehicle (n=20)
- 6) Akita (6 week old mice); Dapagliflozin (25mg/kg/day for 8 weeks; n=20)
- 7) Akita (6 week old mice); Empagliflozin (25mg/kg/day for 8 weeks; n=20)
- 8) Akita (6 week old mice); Canagliflozin (25mg/kg/day for 8 weeks; n=20)
- 9) Kimba (6 week old mice); Vehicle (n=20)
- 10) Kimba (6 week old mice); Dapagliflozin (25mg/kg/day for 8 weeks; n=20)
- 11) Kimba (6 week old mice); Empagliflozin (25mg/kg/day for 8 weeks; n=20)
- 12) Kimba (6 week old mice); Canagliflozin (25mg/kg/day for 8 weeks; n=20)
- 13) Akimba (6 week old mice); Vehicle (n=20)
- 14) Akimba (6 week old mice); Dapagliflozin (25mg/kg/day for 8 weeks; n=20)
- 15) Akimba (6 week old mice); Empagliflozin (25mg/kg/day for 8 weeks; n=20)
- 16) Akimba (6 week old mice); Canagliflozin (25mg/kg/day for 8 weeks; n=20)

#### **Group 2 – Experiment starts at 10 weeks of age and ends at 18 weeks of age (8 weeks of treatment)**

- 1) WT (10 week old mice); Vehicle (n=20)
- 2) WT (10 week old mice); Dapagliflozin (25mg/kg/day for 8 weeks; n=20)
- 3) WT (10 week old mice); Empagliflozin (25mg/kg/day for 8 weeks; n=20)
- 4) WT (10 week old mice); Canagliflozin (25mg/kg/day for 8 weeks; n=20)
- 5) Akita (10 week old mice); Vehicle (n=20)
- 6) Akita (10 week old mice); Dapagliflozin (25mg/kg/day for 8 weeks; n=20)
- 7) Akita (10 week old mice); Empagliflozin (25mg/kg/day for 8 weeks; n=20)
- 8) Akita (10 week old mice); Canagliflozin (25mg/kg/day for 8 weeks; n=20)
- 9) Kimba (10 week old mice); Vehicle (n=20)

- 10) Kimba (10 week old mice); Dapagliflozin (25mg/kg/day for 8 weeks; n=20)
- 11) Kimba (10 week old mice); Empagliflozin (25mg/kg/day for 8 weeks; n=20)
- 12) Kimba (10 week old mice); Canagliflozin (25mg/kg/day for 8 weeks; n=20)
- 13) Akimba (10 week old mice); Vehicle (n=20)
- 14) Akimba (10 week old mice); Dapagliflozin (25mg/kg/day for 8 weeks; n=20)
- 15) Akimba (10 week old mice); Empagliflozin (25mg/kg/day for 8 weeks; n=20)
- 16) Akimba (10 week old mice); Canagliflozin (25mg/kg/day for 8 weeks; n=20)

#### Note:

- 1) All mice will arrive 1 week before the commencement of the experiment in order to facilitate acclimatization.
- 2) All animal handling procedures will be performed by staff trained in the techniques required.
- 3) All strains of mice used in the protocol have been studied previously for periods greater than 25 weeks of age. Hence, we should not experience any possible risk of death during the time-frame of this protocol.
- 4) The SGLT2i Canagliflozin, Empagliflozin, Dapagliflozin (25mg/kg/day) and vehicle will all be administered via drinking water and at daily doses indicated in published literature (Nagareddy et al. [2013] Cell Metab. 17(5): 695-708 and Al-Sharea et. al. [2018] Atherosclerosis. 271: 166-176.).
- 5) We are examining the effectiveness of SGLT2 inhibitors as a treatment for diabetic retinopathy. The younger age of 6 wks has been used previously by others to test novel diabetic retinopathy treatments, which is why we have selected this time-point. At this age the Kimba and Akimba demonstrate features of diabetic retinopathy that we aim to reduce with SGLT2 inhibitors. However, in the clinical setting it is not always possible to capture patients in the early onset stages of diabetic retinopathy. Therefore we will also treat 10 wk old mice with SGLT2 inhibitors to determine if this is a viable treatment in patients at a more advanced stage of diabetic retinopathy. Ideally the treatment should be effective in both age groups but might be less effective or ineffective in more advanced stages of diabetic retinopathy.
- 6) While all 3 SGLT2 inhibitors are related they have been shown to have different pharmacokinetics, pharmacodynamics, and pharmacological effects. All 3 SGLT2 inhibitors are currently being used in clinical trials for the treatment of diabetes. However, no trials have directly compared the 3 SGLT2 inhibitors, with the only analysis comparing the 3 being cost-effectiveness. Therefore we would like to test all 3 in order to establish which one has the greatest potential in the treatment of diabetic retinopathy.

#### SGLT2 inhibitor treatment

As indicated above, 6 week (n=20/group) and 10 week (n=20/group) old male mice will be placed on normal chow diet for 8 weeks with free access to drinking water containing vehicle or SGLT2i (Canagliflozin, Empagliflozin or Dapagliflozin; 25mg/kg/day). Drinking water containing the SGLT2i or vehicle will be freshly prepared and replaced on a weekly basis.

#### Measurement of drinking water with SGLT2 inhibitor

Volume specific bottles will be used per cage. On a weekly basis, the remaining liquid volume will be measured and the total consumed volume will be calculated.

#### Measurement of body weight

Body mass will be recorded for all mice weekly for the duration of the experiment.

#### Urine glucose testing

To establish the development of glucosuria induced by the proper function of SGLT2i, urine will be tested using a urine glucose stick test on a weekly basis until the end of the experiment.

#### Fasting

Food will be withdrawn from mice 5 hours prior to glucose testing and insulin tolerance testing. Mice will be placed into clean cages and treatment water will remain.



### Glucose tolerance test

We will conduct glucose tolerance testing (GTT) at the end of week 7 of treatment. The intraperitoneal GTT is a straightforward technique where after administration of an initial injection of glucose (sugar) into the peritoneal space, glucose clearance from the blood is measured. The expected frequency of adverse impacts such as potential perforation of organs during injection is minimal. Mice will not be allowed to eat for 5 hours prior to the test. Following, an intraperitoneal glucose injection (1g/kg; 100-200µl volumes injected) using a 27-gauge needle, tail blood samples (~10µl) will be collected at 0, 15, 30, 45, 60, 90, 120 minutes and blood glucose is measured immediately in a glucometer. The initial blood collection is made via a minor tail cut (removal of the very tip of the tail; 1mm) and subsequent samples are taken from the same cut by gently removing the scab that forms. The mice will be returned to their cages between bleeds and monitored for changes in mobility. The procedure will be performed by or under the supervision of staff trained in the techniques required.

### Insulin tolerance test

Insulin stimulates sugar to be taken up into tissues. The sensitivity of tissues to insulin is determined by measuring the absence of sugar in the blood. We will conduct insulin tolerance testing (ITT) at the start of week 8 of treatment. The intraperitoneal ITT is a straightforward technique where after administration of an initial injection of insulin into the peritoneal space, glucose clearance from the blood is measured. The expected frequency of adverse impacts such as potential perforation of organs during injection is minimal. Mice will not be allowed to eat for 5 hours prior to the test. Following, an intraperitoneal insulin injection (0.75U/kg) using a 27-gauge needle, tail blood samples (~10µl) will be collected at 0, 15, 30, 45, 60, 90, 120 minutes and blood glucose is measured immediately in a glucometer. The initial blood collection is made via a minor tail cut (removal of the very tip of the tail; 1mm) and subsequent samples are taken from the same cut by gently removing the scab that forms. The mice will be returned to their cages between bleeds and monitored for changes in mobility and will be monitored closely during the procedure for signs of low blood sugar. If glucose levels decrease to 2mmol, glucose will be administered to mice. In this case, mice will receive an intraperitoneal injection of 100µl of glucose (25% glucose solution in saline). This is expected to be a rare event. The procedure will be performed by or under the supervision of staff trained in the techniques required.

### Cardiac puncture

At the end of the experiment, the mouse is anaesthetised using methoxyflurane (FSOP 200/15-18; 6.2(d) – General Anaesthetics). Once deeply anaesthetised, the mouse is removed from the anaesthetic chamber and placed on its back on a bench. The heart beat is palpated and a 26G needle inserted between the ribs from the side and directly into the heart. When blood is seen in the hub of the needle, the plunger on the syringe is slowly pulled back to draw blood into the syringe. Once an adequate amount of blood is collected, the needle is withdrawn and the mouse immediately euthanised by cervical dislocation. The serum will be obtained and used for measuring inflammatory and metabolic markers. The procedure will be performed by or under the supervision of staff trained in the techniques required.

### Tissue collection

In particular, the eye will be collected at the end of experiment and processed in several methods: 1) the eye will be removed and the retina will be dissected followed by immunofluorescence staining to visualize the retinal vasculature; 2) the eye will be removed and placed in fixative followed by wax embedding, sectioning and staining with standard histology methods to examine pathological features of the neural retina; 3) the eye will be removed and the retina will be dissected and followed by the analysis of SGLT2 expression via molecular techniques.

In addition to the eye, kidneys, liver and heart tissue will also be collected. All tissues will also be sectioned and stained with haematoxylin and eosin and examined for any pathological features.

### **3.1.1 Steps taken to ensure the welfare of the animal: (eg. potential impact and methods employed to reduce impact).**

refinement\*: step-by-step description of what will happen to animals and their tissues identifying actions taken at each step to minimise suffering and loss.

### **Glucose tolerance test (GTT) and Insulin tolerance test (ITT): Intraperitoneal injections, minor tail cut**

(removal of the very tip of the tail; 1mm) and repeated tail blood sampling (7 x 10ul, from same site following disturbance of scab) are routinely carried out on conscious animals. Mice will be returned to their cages between bleeds and carefully monitored for any changes in mobility. We feel that repeated anaesthesia is unnecessary and detrimental to the health and wellbeing of the animal. These techniques are expected to cause low-level pain and minimal distress. We have routinely used 5-6 hr fasting as it is adequate to ensure the hormonal and metabolic milieu is consistent between mice and will aid recovery and limit weight loss (Ayala et al. [2006] Diabetes 55[2]: 390-397). Hence, mice will be fasted for 5-6 hours prior to beginning of the GTT or ITT.

Note: While the intention of an insulin tolerance test is to only create a fall in blood glucose of approximately 50% of normal levels, sometimes blood sugars fall lower than anticipated in mice which are unusually insulin sensitive. Mice whose blood sugars fall rapidly and are at risk of hypoglycaemia, are monitored constantly and blood sugars measured at least every 15 minutes. Glucose is administered immediately if animals show signs of hypoglycaemia and/or blood sugars drop below 2 mmol. A 100µl volume of glucose (25% solution in saline) will be administered intraperitoneally. It should be noted that based on the experience of the chief-investigator, Vance Matthews, an additional ip injection of glucose is very seldom required ( $\leq 1\%$  of cases) due to an insulin tolerance test inducing hypoglycaemia.

### **3.1.2 Physical restraint of animals: (details of physical restraints used)**

#### **Manual restraint**

A mouse is manually restrained in one hand by scruffing the loose skin at the back of its neck with forefinger and thumb and restraining the tail with the little and ring fingers. The bladder will be gently pushed to encourage urination in order to be tested for the presence of glucose. Most mice urinate during handling. Therefore, this manual restraining is required only if the mouse does not urinate when handled.

### **3.1.3 Pre-Meds & Anaesthetics:**

#### **Anaesthetising using methoxyflurane (FSOP 200/15-18; 6.2 (d) – General Anaesthetics)**

A clean anaesthetic induction chamber is lined with cotton wool. 1ml of methoxyflurane is added to the cotton wool and allowed to soak in. A tissue is placed over the cotton wool and the mouse placed on top of the tissue.

### **3.1.4 Monitoring of anaesthesia:**

To check that the mouse is adequately anaesthetised, its breathing is watched. Once the mouse has stopped moving around the chamber and its breathing has slowed and become rhythmic, it is removed from the cage and its pedal reflex checked by pinching each of its back feet. If the mouse doesn't pull back on either foot, a short procedure such as cardiac puncture can be performed. If there is a reflex, then the mouse is placed back in the chamber and checked again.

### **3.1.5 Intra-procedure analgesics:**

N/A

### **3.1.6 Other preparative procedures (eg. support substances, neuromuscular junction blocker)**

N/A

## **3.2 Surgical Experimental Procedures:**

Explain what is being done and why. **USE LAY LANGUAGE. Each procedure MUST be supported by a diagram, flowchart and/or table**

Provide details of the generic constituents (not the trade name), the dose rate as mg/kg, the route of administration, the frequency and duration of administration, the timeframe of the experiments, intra-procedural use of analgesics, the number of animals in each group/step

and refinement methods, etc: Include competency & training of staff responsible for administering the drugs, monitoring the animals during the procedure and conducting the procedures.

NB: for information on RPH AEC approved pre-meds, anaesthetics, analgesics and support materials for each species, refer to the Animal Facility SOPs (available from the animal facility). If any anaesthetics, analgesics or support materials vary from approved RPH substances, please provide details and a brief explanation of why it is required.

N/A

### **3.2.1 Steps taken to ensure the welfare of the animal: (eg. potential impact and methods employed to reduce impact).**

refinement\*: step-by-step description of what will happen to animals and their tissues identifying actions taken at each step to minimise suffering and loss.

N/A

### **3.2.2 Physical restraint of animals: (details of physical restraints used)**

N/A

### **3.2.3 Pre-Meds & Anaesthetics:**

N/A

### **3.2.4 Monitoring of anaesthesia:**

N/A

### **3.2.5 Intra-procedure analgesics:**

N/A

### **3.2.6 Other preparative procedures (eg. support substances, blood collection, volume replacement, neuromuscular junction blocker)**

N/A

## **3.3 Post-Procedural Monitoring: (include training & competency of staff)**

### **3.3.1 Steps taken to ensure the welfare of the animal (eg. potential impact and methods employed to reduce impact).**

refinement\*: step-by-step description of what will happen to animals and their tissues identifying actions taken at each step to minimise suffering and loss.

#### **SGLT2 inhibitors via drinking water:**

During our SGLT2i experiments outlined in this application, mice will be monitored for signs of distress or illness. These include physiological changes (changes in breathing, piloerection, lack of grooming, increased muscle tone, lack of movement), mental changes (unresponsive, aggressive, alertness), loss of weight (**10%** or more) or reluctance to be handled. The level of pain and distress will be scored as indicated in **the attached monitoring sheet**. If the mice are clearly in pain or distress, they will be humanely euthanased.

#### **Glucose tolerance test (GTT) and Insulin tolerance test (ITT):**

The average fasting blood glucose level for a 20 week old mouse on normal chow is approximately 10mmol. While the intention of an insulin tolerance test is to only create a fall in blood glucose of approximately 50%, sometimes blood sugars fall lower than anticipated in mice which are unusually insulin sensitive. Mice whose

blood sugars fall rapidly and are at risk of hypoglycaemia, are monitored constantly and blood sugars measured at least every 15 minutes. Glucose (25% solution in saline; 100ul) is intraperitoneally administered immediately if animals show signs of hypoglycaemia and/or blood sugars drop below 2 mmol during an insulin tolerance test. It should be noted that based on the experience of the co-investigator, Vance Matthews, an additional ip injection of glucose is very seldom required ( $\leq 1\%$  of cases) due to an insulin tolerance test inducing hypoglycaemia.

Mice will be returned to their cages between bleeds and carefully monitored for any changes in mobility. The level of pain and distress will be scored as indicated in the **attached monitoring sheet**. If the mice are clearly in pain or distress, they will be humanely euthanased.

**3.3.2 Post-operative analgesics: (provide details of the generic constituents (not the trade name), the dose rate as mg/kg, the route of administration, and the frequency and duration of administration):**

NB: for information on RPH AEC approved anaesthetics, analgesics and support materials for each species, refer to the Animal Facility SOPs (available from the animal facility). If any anaesthetics, analgesics or support materials vary from approved RPH substances, please provide details and a brief explanation of why it is required.

N/A

**3.3.3 Monitoring schedule (method and frequency after procedure; staff involved):**

While at the Royal Perth Hospital animal facility, animals will be monitored routinely and observations recorded on the attached monitoring sheet. Royal Perth Hospital animal facility staff will check mice daily once in the experimental area. Dr Vance Matthews and Lakshini Herat will monitor mice once **a week** in the experimental area. Throughout the experimental period of administering various treatments outlined in section 3.1, mice will be observed for deviations from normal behavioural patterns, changes in sleeping, feeding, drinking, grooming, exploratory behaviour and social behaviour. The actions taken in response to deviations from normal behaviour/presentation will be dependent upon the observations made and advice from ACU staff.

**3.3.4 Post-procedural pain and distress (identify potential animal welfare concerns, eg. pain and distress, and how they will be monitored and addressed)**

Please refer to the attached monitoring sheet.

**3.3.5 Fate of distressed animals (see criteria for Euthanasia below 3.4.1):**

The Animal Facility ASC has the authority to immediately euthanase animals showing symptoms of pain and distress; (ref: “Guidelines to Promote the Wellbeing of Animals Used for Scientific Purposes” and “Australian Code of Practice for the Care and Use of Animals for Scientific Purposes”). Every effort will be made to notify the contact person prior to euthanasia of the animal.

Please refer to the attached monitoring sheet.

**3.4 Euthanasia: (provide details of the generic constituents (not the trade name), the dose rate as mg/kg, and the route of administration): Include competency & training of staff.**

NB For information on RPH AEC approved euthanasia methods for each species; refer to Animal Facility SOPs (available from the animal facility). If any euthanasia method varies from approved RPH procedures, please provide details and a brief explanation of why it is required.

Cervical dislocation (as per FSOP 202-15-18; 6.3 Euthanasia).

**3.4.1 Criteria for Euthanasia: (details of how animals will be assessed for euthanasia):** The Animal Facility ASC has the authority to immediately euthanase animals showing symptoms of pain and distress; (ref: “Guidelines to Promote the Wellbeing of Animals Used for Scientific Purposes” and “Australian Code of Practice for the Care and Use of Animals for Scientific Purposes”). Every effort

will be made to notify the contact person prior to euthanasia of the animal.

Animals will be euthanized by cervical dislocation should they display changes in behaviour or physical presentation significantly deviating from that normally observed for inbred mice. **A loss of weight of greater than 10% from the animals starting weight will be considered criteria for euthanasia.**

If emergency contact cannot be contacted, ACS staff will euthanise mice if it is required. Cervical dislocation will be performed by Dr Vance Matthews or Lakshini Herat who is experienced in the technique.

### **3.5 Post-Euthanasia Animal Use:**

Blood (for plasma collection) and tissues will be harvested and processed for future investigations as stated in section 3.1.

### **3.6 Method and Details of Carcass Disposal:**

Carcasses will be placed in a freezer in the RPH animal house and will be incinerated later.

## **4. DETAILS OF FACILITIES, HOUSING, CARE AND TRANSPORT OF ANIMALS**

**4.1 Available facilities:**

Royal Perth Hospital animal house (PC1).

**4.2 Animal maintenance staff:**

Dr. Alison Rose.

**4.3 Housing, care & maintenance:**

Changing feed and bedding on a regular basis as per facility standards. However, given the diabetic status in Akita and Akimba mice, the bedding might be wet due to extra urination. This will be monitored and bedding will be changed more frequently if needed.

Drinking water containing SGLT2 inhibitors will be freshly prepared and replaced on a weekly basis by investigators Dr. Vance Matthews or Lakshini Heart.

**4.4 Transport:** (provide details of any proposal to transport animals into or out of Royal Perth Hospital and/or to house animals off site (guidelines sections 3.8 & 3.13).

Mice will be transported to the RPH animal house under specific pathogen free conditions from The Animal Resources Centre.

**4.5 Maximum length of holding in weeks:**

9 weeks

**4.5 Special requirements if dealing with genetically modified organisms:**

N/A

**4.7 Other special requirements:**

Bedding may need frequent changing specially in Akita and Akimba mice due to possible increased urination due to diabetes.

**OTHER RELEVANT DOCUMENTS:**

[ X ] I have attached other relevant documents (please specify number of documents and their titles).

1x Monitoring sheet

4x Flow Diagrams

2x PAWES course related documents

**Type of Project: (circle the appropriate procedure number):**

Procedure Number:	Description:
1	<p><b>Observation Involving No or Minor Interference</b></p> <p>Animals are not interacted with or, where there is interaction, it would not be expected to compromise the animal's welfare any more than normal handling, feeding, etc. There is no pain or suffering involved.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> <li>• <i>Observational study only</i></li> <li>• <i>Breeding or reproductive study with no detriment to the animal</i></li> <li>• <i>Feeding trial, such as Digestible Energy determination of feed in a balanced diet</i></li> <li>• <i>Behavioural study with minor environmental manipulation</i></li> <li>• <i>Teaching of normal, non-invasive husbandry such as handling and grooming</i></li> </ul>
2	<p><b>Animal Unconscious Without Recovery</b></p> <p>Animal is rendered unconscious under controlled circumstances with little or no pain or distress. Any pain is minor and brief and does not require analgesia. Procedures are carried out on the unconscious animal which is then killed without regaining consciousness.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> <li>• <i>Teaching surgical techniques on live, anaesthetised patients which are not allowed to recover following the procedure</i></li> </ul>
3	<p><b>Minor Conscious Intervention</b></p> <p>Animal is subjected to minor procedures which would normally not require anaesthesia or analgesia. Any pain is minor and analgesia usually unnecessary, although some distress may occur as a result of trapping or handling.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> <li>• <i>Injections, blood sampling in conscious animal</i></li> <li>• <i>Minor dietary or environmental deprivation or manipulation, such as feeding nutrient-deficient diets for short periods</i></li> <li>• <i>Trapping and release as used in species impact studies</i></li> <li>• <i>Trapping and humane euthanasia for collection of specimens</i></li> <li>• <i>Trapping and humane euthanasia for feral animal control research</i></li> <li>• <i>Stomach tubing, shearing</i></li> </ul>
4	<p><b>Minor Surgery With Recovery</b></p> <p>Animal is rendered unconscious with as little pain or distress as possible. A minor procedure is carried out and the animal allowed to recover. Depending on the procedure, pain may be minor or moderate and post-operative analgesia may be appropriate.</p> <p>Field capture using chemical restraint methods are also included here.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> <li>• <i>Biopsies</i></li> <li>• <i>Cannulations</i></li> <li>• <i>Sedation/anaesthesia for relocation, examination or injections/blood sampling</i></li> </ul>
5	<p><b>Minor Physiological Challenge</b></p> <p>Animal remains conscious for some or all of the procedure. There is interference with the animal's physiological or psychological processes. The challenge may cause only a small degree of pain/distress or any pain/distress is quickly and effectively alleviated.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> <li>• <i>Minor infection</i></li> <li>• <i>Early oncogenesis</i></li> <li>• <i>Arthritis studies with pain alleviation</i></li> <li>• <i>Induction of metabolic disease</i></li> <li>• <i>Prolonged deficient diets</i></li> <li>• <i>Polyclonal antibody production</i></li> <li>• <i>Antiserum production</i></li> </ul>

Procedure Number:	Description:
6	<p><b>Major Surgery With Recovery</b></p> <p>Animal is rendered unconscious with as little pain or distress as possible. A major procedure, such as abdominal or orthopaedic surgery, is carried out and the animal allowed to recover. Post-operative pain is usually considerable and at a level requiring analgesia.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> <li>• Orthopaedic surgery</li> <li>• Abdominal or thoracic surgery</li> <li>• Transplant surgery</li> </ul>
7	<p><b>Major Physiological Challenge</b></p> <p>Animal remains conscious for some or all of the procedure. There is interference with the animal's physiological or psychological processes. The challenge causes a moderate or large degree of pain/distress which is not quickly or effectively alleviated.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> <li>• Major infection</li> <li>• Major phenotypic modification</li> <li>• Oncogenesis without pain alleviation</li> <li>• Arthritis studies with no pain alleviation</li> <li>• Uncontrolled metabolic disease</li> <li>• Isolation or environmental deprivation for extended periods</li> </ul>
8	<p><b>Death As An Endpoint</b></p> <p>This category only applies in those cases where the death of the animal is a planned part of the procedures and animals die but are not euthanased. Where predictive signs of death have been determined and euthanasia is carried out before significant suffering occurs, they may be placed in category 7.</p> <p><b>Examples</b></p> <ul style="list-style-type: none"> <li>• Lethality testing (including LD<sub>50</sub>, LC<sub>50</sub>)</li> </ul> <p><b>It does not include:</b> death by natural causes; animals which are euthanased as part of the project; animals which are euthanased if something goes wrong; animals euthanased for dissection or for use as museum specimens; or accidental deaths.</p>
9	<p><b>Production of genetically modified animals</b></p> <p>This category is intended to allow for the variety of procedures which occur during the <u>production of genetically modified (GM) animals</u>. As animals in this category may be subjected to both minor <i>and</i> major physiological challenges <i>and</i> surgical procedures, this category reflects the varied nature of the procedures carried out. It effectively includes ALL animals used in GM production other than the final progeny which are used in a different category of procedure.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> <li>• Initial breeding animals for GM production</li> <li>• Animals culled as part of the GM production process</li> <li>• Animals culled during the production of animals that are free of unwanted infectious agents or pathogens</li> </ul>




## APPLICATION DECLARATION

I/we, the undersigned:

- (i) Have read and agree to abide by the conditions and constraints of the *Australian code of practice for the care and use of animals for scientific purposes (8<sup>th</sup> edition, 2013)* and the *RPH-AEC Operational Policies and Guidelines for Applicants and Attachments (current version)*;
- (ii) Certify that the resources in the Animal Facility, including housing and personnel, have been deemed appropriate for the welfare of the animals and the satisfactory completion of the project by the RPH Animal Services Coordinator.



- (iii) Will ensure that the qualifications, competency, experience and/or supervision of all listed personnel are appropriate to the procedures to be performed (and be able to provide evidence) and that only listed personnel will conduct animal-based activities on this project;
- (iv) Will ensure that any adverse event or unplanned death that occurs during the conduct of this project will be reported immediately to the RPH-ASC for tabling and discussion at the next AEC meeting.
- (v) Acknowledge that costs associated with the project are the responsibility of the Chief Investigator and that project approval may be revoked if payment has not been received in a timely manner (i.e. > 60 days from the invoice date).
- (vi) Will ensure that, in addition to the information provided in the annual/final report for the project, the RPH-AEC will be made aware of any publications published or pending for the program being undertaken.
- vii) Agree to provide evidence of funding and an account number for billing purposes prior to the project being initiated.
- viii) Acknowledge that the information contained in this form is a true and accurate record.

	Name	Signature	Date
<b>Chief Investigator</b>	Dr. Vance Matthews		4-6-18
<b>Person(s) performing the animal procedures if other than the Chief Investigator</b>	Lakshini Herat		4-6-18
	Aaron Magno		23-8-18
<b>Head of Research Department</b>	Professor Gerald Watts Acting [ ]		

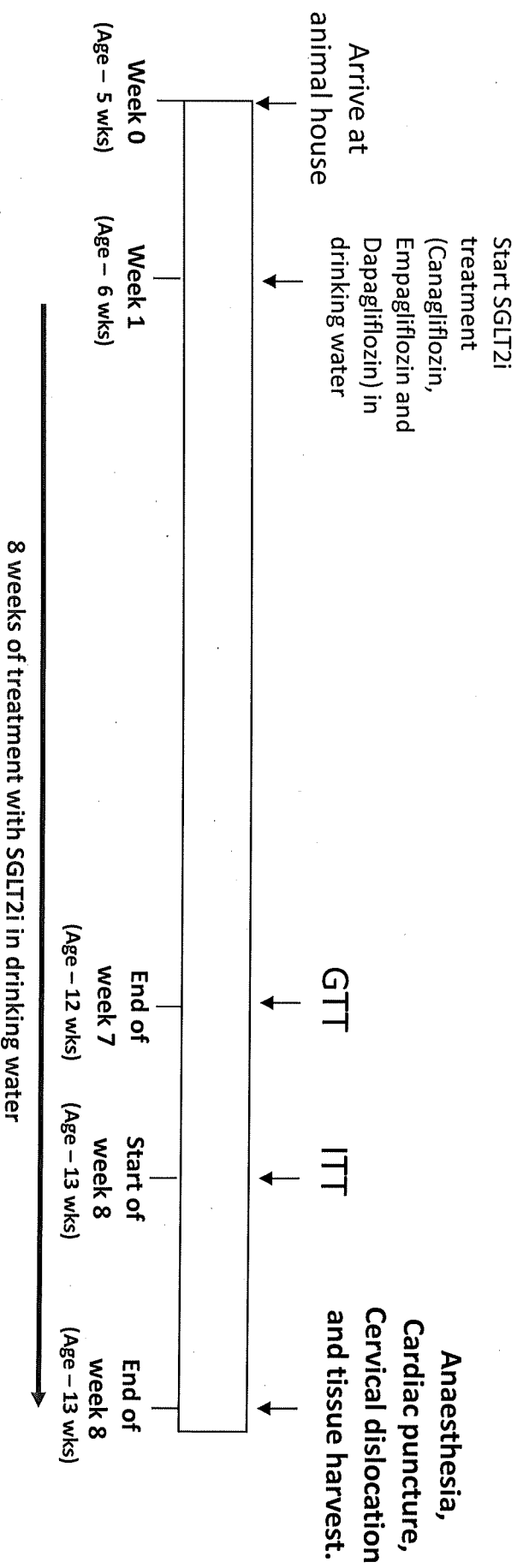
**Evidence of funding and an account number for billing purposes must be provided prior to the project being initiated.**

Account Number: BU: 00885; PG: 51007200

Billing Details: Vance Matthews, UWA, School of Biomedical Sciences.

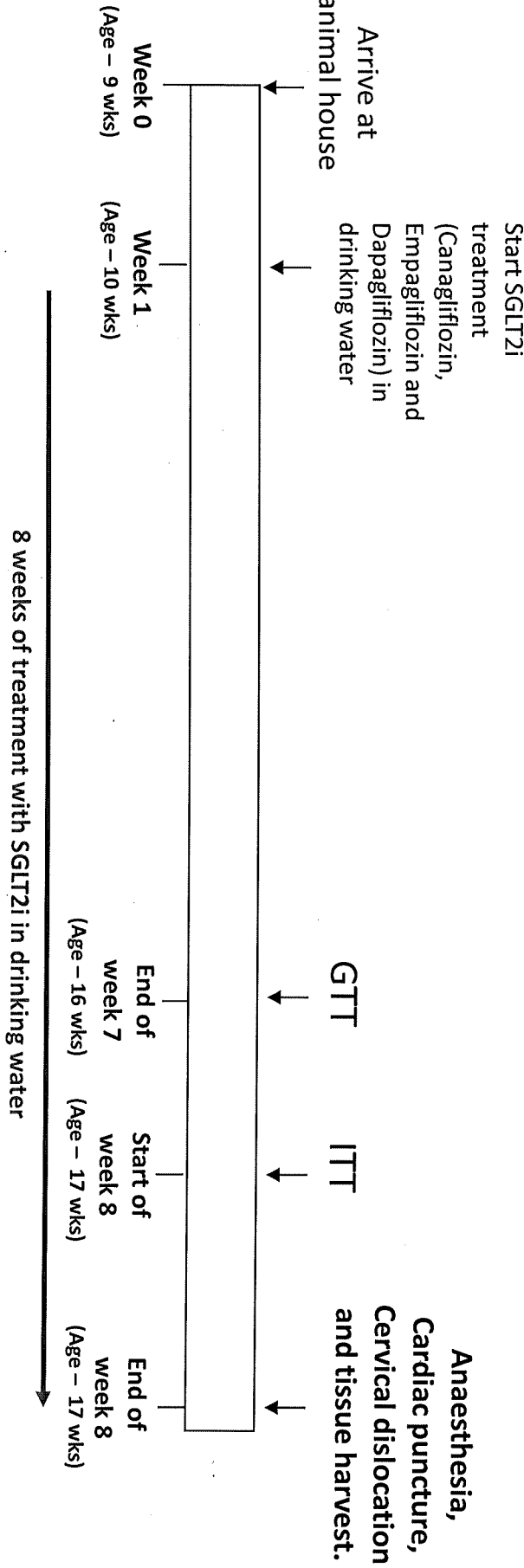
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**Time-line for commencement of treatments, Glucose tolerance tests (GTT),  
Insulin tolerance tests (ITT) and completion of experiment (mice starting at 6 weeks of age)**



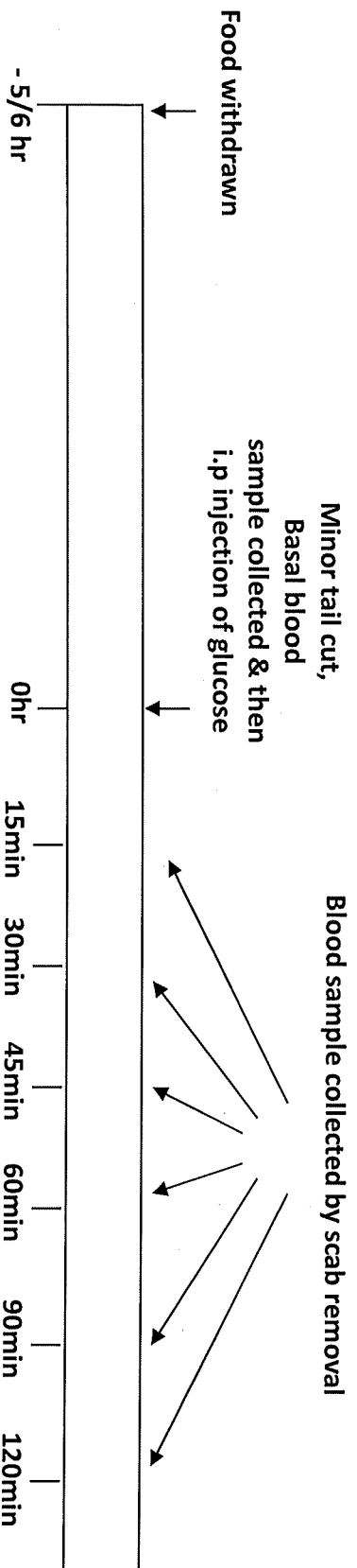
**This time line will be followed for Akita, Kimba, Akimba and WT strains**

**Time-line for commencement of treatments, Glucose tolerance tests (GTT),  
Insulin tolerance tests (ITT) and completion of experiment (mice starting at 10 weeks of age)**

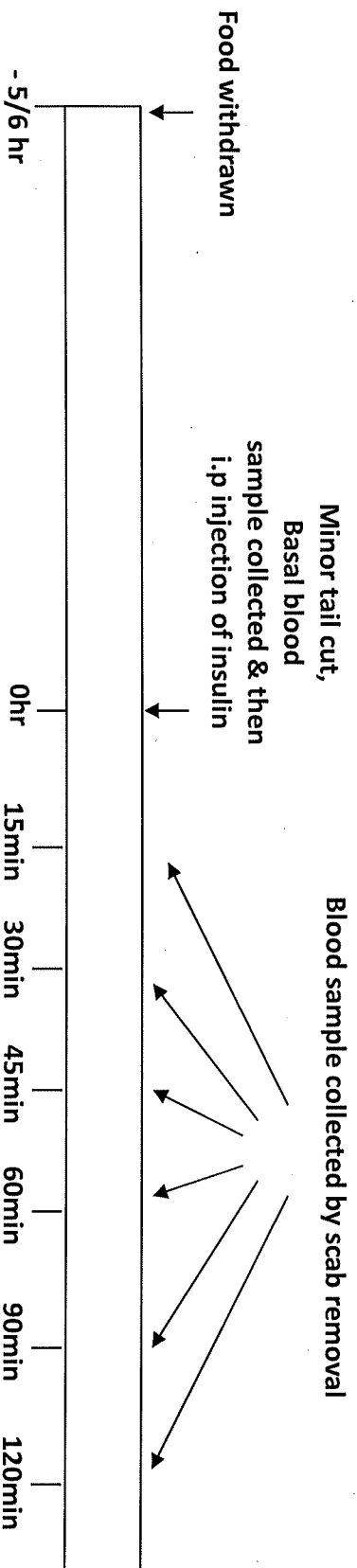


**This time line will be followed for Akita, Kimba, Akimba and WT strains**

Glucose tolerance tests (GTT): fasting periods, i.p injections and tail bleeds  
for an individual mouse



Insulin tolerance tests (ITT): fasting periods, i.p injections and tail bleeds  
for an individual mouse





**ANIMAL ETHICS COMMITTEE**

Telephone: (08) 9224 2814

Facsimile: (08) 9224 2981

Asst. Prof. Vance Matthews, Research Fellow  
School of Medicine & Pharmacology  
3rd Floor MRF Building  
Royal Perth Hospital

22<sup>nd</sup> August 2018

Dear Dr Matthews,

**RPH-AEC Decision: 21<sup>st</sup> August 2018 – Minor modification request - APPROVED.**

**Application #: R540/18-21 (Matthews):** *Establishing the role of sodium glucose co-transporter 2 (SGLT2) in diabetic retinopathy.*

The Royal Perth Hospital Animal Ethics Committee has approved your request to add Dr Aaron Magno as a co-investigator on this project.

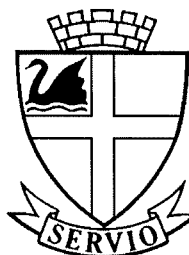
Yours sincerely

**Prof Kevin Croft**  
Chairman  
ANIMAL ETHICS COMMITTEE

**Dr L.S. Manning**  
Executive Officer  
ANIMAL ETHICS COMMITTEE



# IN CONFIDENCE

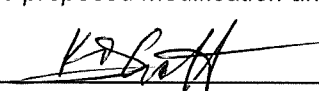


## ROYAL PERTH HOSPITAL ANIMAL ETHICS COMMITTEE

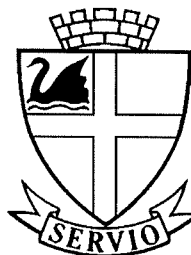
### MINOR PROJECT MODIFICATION FORM

**MINOR MODIFICATION APPROVAL INFORMATION:** This form is to be used for minor modifications (eg. addition or change to co-investigators; duration of project, animal numbers, animal strain etc..) requiring only Executive Committee consideration.

Applicants are to complete this form and submit it electronically to the Executive Officer of the RPH Animal Ethics Committee ([linda.manning@health.wa.gov.au](mailto:linda.manning@health.wa.gov.au)) for submission to the AEC Executive Committee for consideration.

<b><u>FOR OFFICE USE ONLY</u></b>			
The AEC has considered the proposed modification and has approved it for the duration of the project:			
Signature of AEC Chair: <u></u>		Date: <u>21/8/2018</u>	
PROJECT TITLE:	Establishing the role of sodium glucose co-transporter 2 (SGLT2) in diabetic retinopathy.		
AEC Approval #:	R540/18-21	Commencement Date/Project Duration:	August 2018 (3 years)
Brief description of modification and reason(s) for request	Although this project has not been approved in full, we would like to add Dr Aaron Magno as an investigator so that he can assist with monitoring mice, weighing mice, fasting mice, measuring blood glucose levels and also assist with dissection and collection of tissues for the approved pilot study. Provided the full project is approved in future, this request will also need to apply to the full project.		
Date submitted	21/08/18		
Chief Investigator:	Dr Vance Matthews.	Department:	UWA School of Biomedical Sciences.
Person submitting request (if not the CI)	N/A	Project Affiliation:	Royal Perth Hospital
Telephone: 9224 0239      Mobile: 043 452 7697      Email: <a href="mailto:vance.matthews@uwa.edu.au">vance.matthews@uwa.edu.au</a>			

# IN CONFIDENCE



## ROYAL PERTH HOSPITAL ANIMAL ETHICS COMMITTEE

### PILOT STUDY TEMPLATE

#### FOR OFFICE USE ONLY

The AEC has approved the proposed pilot study: 02/08/18

Signature of AEC Chair: \_\_\_\_\_

Date: \_\_\_\_\_

21/08/18

*dated at full Committee meeting*

AEC Approval # and  
PROJECT TITLE:

**R540/18-21: Establishing the role of sodium glucose co-transporter 2 (SGLT2) in diabetic retinopathy.**

Project Commencement:

July 2018

Project Duration:

July 2021

Chief Investigator:

Vance Matthews

Department:

School of Biomedical  
Sciences

Telephone:

Mobile:

Email:

Telephone: 9224 0239

Mobile: 043 452 7697

Email: vance.matthews@uwa.edu.au

Brief description of  
modification and reason(s)  
for request

To address RPH-AEC concerns and AWO comments.

A report on the outcomes of this pilot study is to be submitted to the Committee prior to approval for any additional animals to be used.



## Details of Pilot Study Protocol:

### Description of Pilot Study Protocol:

Body condition scoring is recorded on our approved monitoring sheets.



The pilot study has been approved according to the following protocol:

- The 6 male Akimba mice (8-9wk of age) currently available at the ARC are to be acclimatised for ~ one week (week of the 6<sup>th</sup> of August).
- 3 are to be put on drinking water containing the vehicle with the other 3 put on drinking water containing dapagliflozin (25mg/kg/day for 8 weeks) with testing to be conducted as per the application (e.g. glucose & insulin tolerance).
- The animals are to be monitored daily for the 1<sup>st</sup> 3 days with hydration, fluid and food consumption documented and included in the study report.
- The acceptable weight loss is to remain at 10%.

A report on the outcomes of this pilot study is to be submitted to the Committee prior to approval for any additional animals to be used.

## APPLICATION DECLARATION

I/we, the undersigned agree to abide by all the conditions of the parent project:

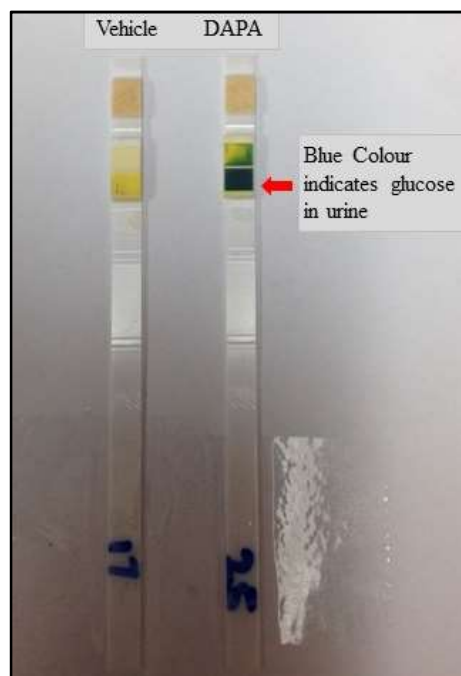
	Name	Signature	Date
Chief Investigator	Vance Matthews		21/8/18
Person(s) performing the animal procedures if other than the Chief Investigator			
RPH Animal Services Coordinator	N. GRANGER		21.8.18

## Pilot Study Progress Report

### **R540/18-21: Establishing the role of sodium glucose cotransporter 2 (SGLT2) in diabetic retinopathy.**

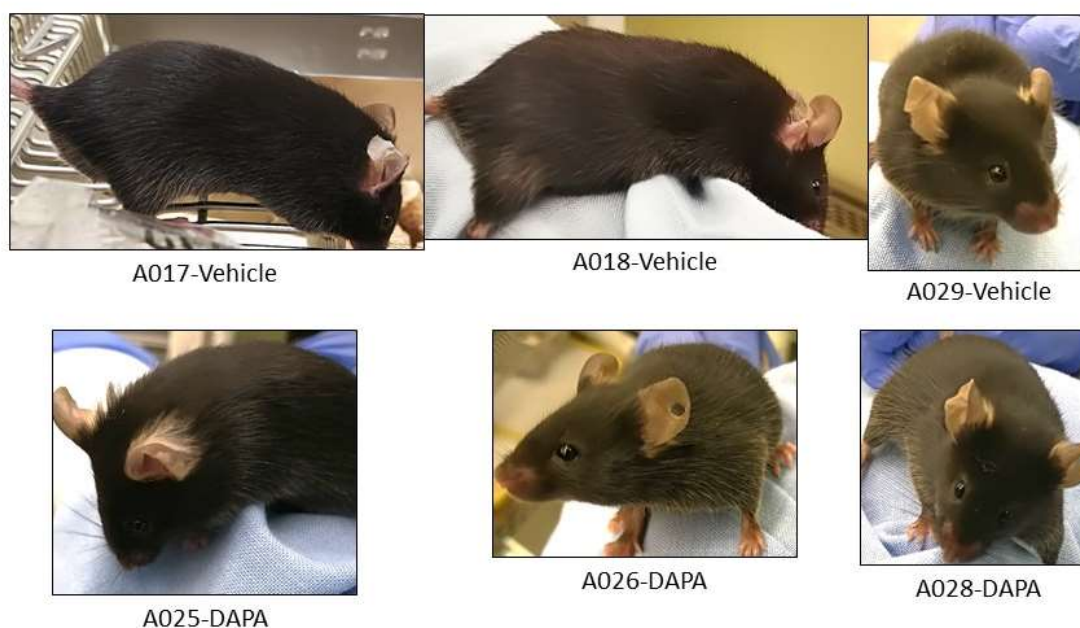
#### **Urinary Glucose**

Consumption of Dapagliflozin (DAPA) in drinking water resulted in marked glucose secretion in urine.



#### **Body Conditioning**

The conditioning of the six mice did not change during the pilot study based on the criteria outlined on our approved monitoring sheets. They displayed a normal intensity of activity, normal posture and a normal responsiveness to touch. Their coats remained smooth and there was no evidence of any mouse being in pain. The representative images are from mice at week 5 of the dietary regiment.

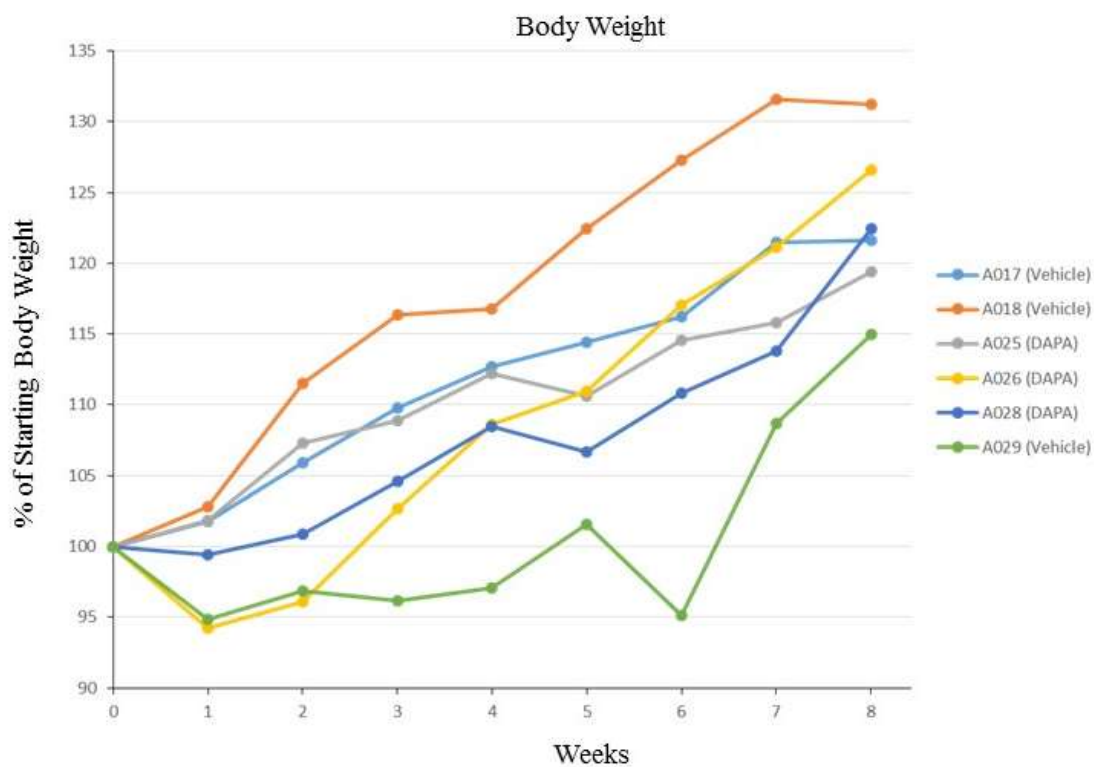


## Pilot Study Progress Report

### **R540/18-21: Establishing the role of sodium glucose cotransporter 2 (SGLT2) in diabetic retinopathy.**

#### **Body Weight**

No mouse lost more than 10% body weight during the pilot study. All mice gained weight by the end of the 8 weeks.

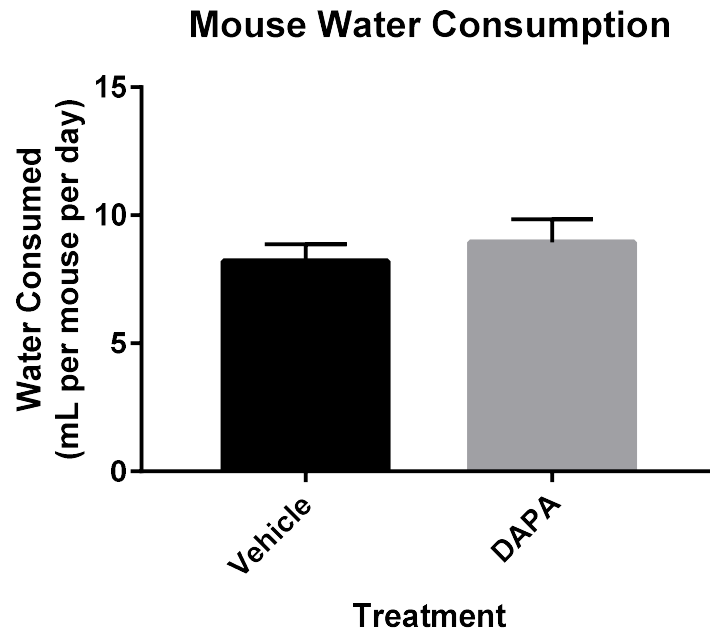


## Pilot Study Progress Report

### **R540/18-21: Establishing the role of sodium glucose cotransporter 2 (SGLT2) in diabetic retinopathy.**

#### **Water Consumption and Hydration**

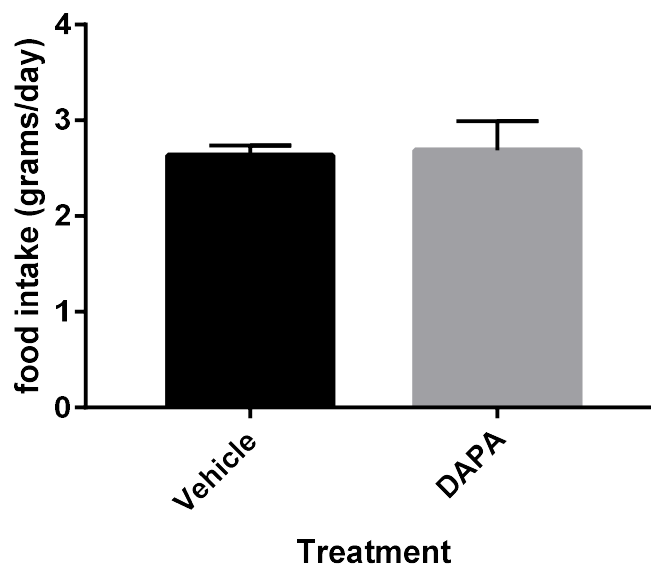
There was no discernible differences in the amount of water consumed by mice on the vehicle (n=3) and those treated with Dapagliflozin (DAPA) (n=3). All mice produced plentiful amounts of urine throughout the 8 weeks of the study.



#### **Food Intake**

Mice on vehicle (n=3) consumed a similar level of food compared to those mice treated with Dapagliflozin (DAPA) (n=3).

#### **Food intake over the first 3 days of the experiment**

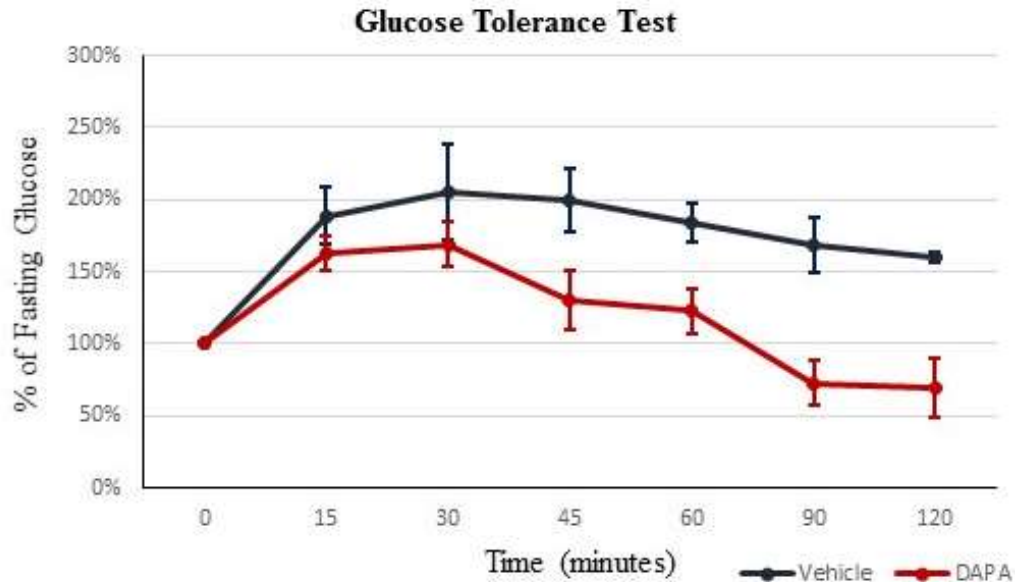


## Pilot Study Progress Report

### **R540/18-21: Establishing the role of sodium glucose cotransporter 2 (SGLT2) in diabetic retinopathy.**

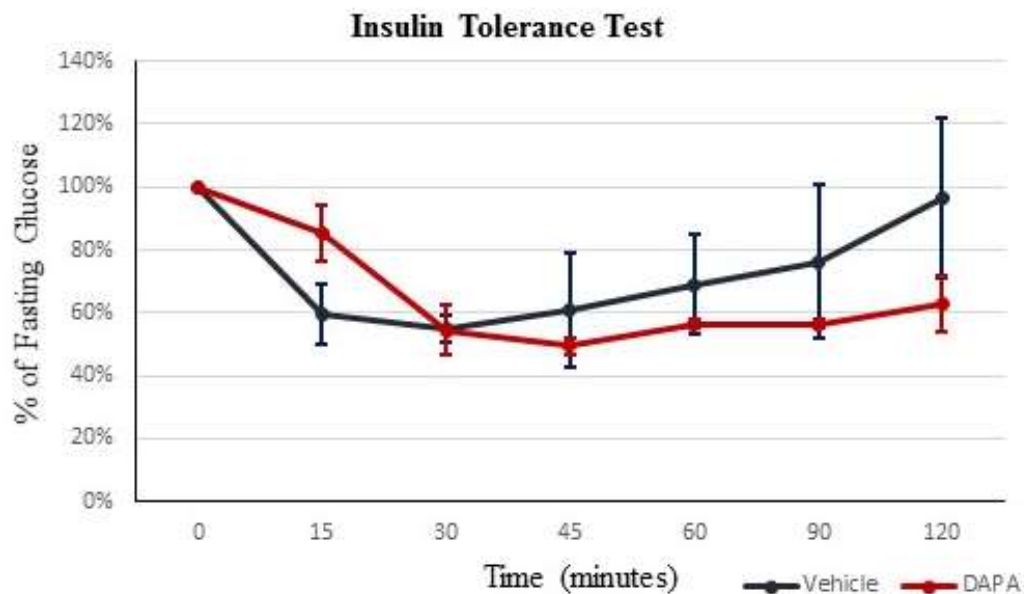
#### **Glucose Tolerance Test (GTT)**

Compared to the mice on the vehicle (n=3), there was a pronounced glucose tolerance observed in the Dapagliflozin (DAPA) treated mice (n=3) after 7 weeks.



#### **Insulin Tolerance Test (ITT)**

Mild insulin sensitivity was observed in the Dapagliflozin (DAPA) treated mice (n=3) after 8 weeks of treatment in comparison to mice on the vehicle (n=3). This was particularly evident at the latter time-points of the ITT.



## RPH AEC - ADVERSE EVENT NOTIFICATION FORM

When completed, this form is to be forwarded as per Adverse Events Reporting Policy.

<b>RPH AEC approval #: R540/18-21</b>		<b>Chief Investigator: Dr Vance Matthews</b>	
<b>Date</b>	23.11.18	<b>Research</b>	xx
<b>Time</b>	17:30	<b>Teaching</b>	
<b>Species/ Strain</b>	AKIMBA	<b>Date of arrival</b>	13.11.18
<b>Gender</b>	mALE	<b>Age / Date of birth</b>	~8WKS
<b>ID/Pen #</b>	Box 2 # AO57	<b>Animal Source</b>	ARC
<b>OGTR Status and IBC #</b>	Nlrd 007/2018	<b>OH&amp;S Risk</b>	Standard
<p><b>Animal's recent history</b> (experimental procedures, diet, last 5 bodyweights, previous health concerns etc.)</p> <p>Arrived ARC 13.11.18  Housed with other 4 cage mates.  Dapagliflozin treatment group. 19.77g at time of treatment.  Animal appeared normal post treatment – eating drinking and generally active.  Weighed 23.11.18 – 13.81g culled by researcher due to excessive weight loss</p> <p>Researcher culled and carried out PM before notifying facility staff – All organs and general appearance appeared normal. Researcher email attached to form.</p>			
<b>Are other animals associated at welfare risk?</b> <i>If yes then AWO and ASC must be notified immediately.</i>		<b>YES [ ]                      NO [x ]</b>	
<b>Type of post mortem required:</b>			
<b>1. Post Mortem Observation</b> – All observations to be recorded on PM records sheet and forwarded to AEC.			
<b>2. Post Mortem Examination</b> – All findings, internal or external, to be recorded on PM records sheet. External reports - pathology, histology etc. to be attached and forwarded to AEC.			<b><i>Xx ATTAC HED</i></b>
<b>Form completed by:</b>	N.GRAINGER	<b>Date:</b>	21.11.18
<b>Form checked by CI/co- investigator:</b>		<b>Date:</b>	

All E mail correspondence relating to any of the above must be kept and forwarded to ASC.

REFERENCE NUMBER – 18/04

Valid 01/03/16

ASC – N.Grainger

Hi Nick,

Further to our quick chat this afternoon, I now provide more information on A057 which was culled today as it exceeded the 10% weight loss criteria. I have logged this mouse out and indicated his absence on the cage card. All other mice in the cage are clearly healthy and not displaying weight loss that is concerning.

The mouse was monitored by Aaron Magno yesterday and the mouse was climbing on the lid of the cage and active.

Lakshini weighed the mouse today and there was clear weight loss compared to it's previous weight measurement. Again, today Lakshini observed the mouse to be active and eating.

Even when I culled the mouse, it was still very active. I did an autopsy and the following organs were all normal in morphology:

Coat appearance, eyes, kidneys, liver, intestines, spleen, lungs and heart.

It is a bit of a mystery as to why this mouse showed the weight loss.

Please let me know if you need any other information.

Best regards,

Vance.

Dr Vance Matthews,

Senior Research Fellow,

UWA School of Biomedical Sciences and Dobney Hypertension Centre,

Level 3, MRF Building, Rear 50 Murray St, PERTH WA 6000,

## Phenotypical Information

### Akita

Mice heterozygous for the Akita spontaneous mutation (Ins2Akita) are viable and fertile. Symptoms in heterozygous mutant mice include hyperglycemia, hypoinsulinemia, polydipsia, and polyuria, beginning around 3-4 weeks of age. Obesity or insulinitis does not accompany diabetes. Aged mice exhibit gait disturbance and decreased sensory nerve conduction velocity, but do not exhibit learning or memory deficits (Choeiri C et al., 2005). However, they can exhibit hyperphagia and anxiety behavior (Asakawa et al., 2007). No other abnormal behaviours are observed in this strain. Progressive retinal abnormalities begin as early as 12 weeks after the onset of hyperglycemia (Barber AJ, et al., 2005). Their mean lifespan of 305 days is significantly shorter than the background strain of C57Bl/6 (690 days). Akita mice have successfully been used by Professor Rakoczy's group (who has provided the mice and is the group from which Dr Magno has come) (Rakoczy et al., 2010) and others (Chaurasia et al., 2018) in experiments to an age of 24 weeks. Current breeding stocks include 31 week old mice. Body weight does not increase in the same age-dependent manner as the C57Bl/6 background strain. At age 8 weeks their body weight is ~17g and ~18g at 24 weeks old. Once animals start to become diabetic they appear more scruffy with piloerection, hunched and sunken eyes get increasingly more obvious. Divided septums are also quite common, reducing numbers available for breeding.


### Kimba

Kimba mice do not show symptoms of diabetes like the Akita. However, they do have a higher incidence than other mouse strains on the same background of sunken eyes but similar to the rates seen in the Akita. There are no abnormal behaviours seen in this strain. Kimba mice have been used experimentally up to age 24 weeks (Rakoczy et al., 2010, Chaurasia et al., 2018). Oldest animal currently being used for breeding is 31 weeks old. Their body weight does increase in an age dependent manner like the C57Bl/6 background strain (~24g at 8 weeks, ~29g at 24 weeks). No other abnormal behaviours are seen in this strain.

### Akimba

Being a cross of Akita and Kimba, these mice have the phenotypic features of both mice, including the diabetes and similar eye problems. As such, Akimba mice have been used experimentally to age 24 weeks and has the same lower body weight and lack of weight increase seen in the Akita (Rakoczy et al., 2010, Chaurasia et al., 2018). No other abnormal behaviours are seen in this strain.



	ANIMAL ETHICS COMMITTEE
	<b>AEC005: PHENOTYPE REPORT FOR GENETICALLY MODIFIED ANIMALS</b>

The main purpose of this report is to assist with the monitoring and assessment of the impact of the genetic modification upon the health and welfare of the affected animals. Please provide information consistent with this purpose (i.e. detailed descriptions of *in vitro* methodology are not desired). It is a tool to make it easier for an AEC to appreciate the welfare impact of the genetic modification made to this strain of mouse.

Please use lay language or provide glossary definitions.

### Project Details

1. AEC Project No:	R540(18-21)		
2. Project Title:	Establishing the role of sodium glucose co-transporter 2 (SGLT-2) in diabetic retinopathy.		
3. Start Date:	October 2018	Finish Date:	October 2021
4. Chief Investigator:	Dr Vance Matthews		
Institute/ Department:	UWA School of Biomedical Sciences and Dobney Hypertension Centre		

\* Relates to approved project dates.

### 5. Animal Details

Genetically modified animal species:	Mus musculus
Strain/genetic description: (If there is a common name, please include)	C57Bl/6(Ins2Akita) - Akita
Background strain:	C57Bl/6
Source: (i.e. specified external laboratory source)	ARC
What is the health profile of the current colony? Provide the most recent health report.	Attached


### 6. How much is known about the biological characteristics/phenotype of this strain?

Indicate by selecting one of the following:

☒ Well characterised

☐ Partially-characterised/some information available

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	<b>ANIMAL ETHICS COMMITTEE</b>
	<b>AEC005: PHENOTYPE REPORT</b> <b>FOR GENETICALLY MODIFIED ANIMALS</b>

[ ] Unknown

## GLOSSARY

Word	Lay explanation

## 7. Genetic alteration:

<b>Briefly describe which gene has been added/deleted/altered.</b>	Akita mice contain a naturally occurring mutation in their insulin 2 gene.
<b>Affected organs/tissues: (e.g. gene expressed in liver only)</b>	The mutated insulin 2 gene affects organs/tissues involved in glucose regulation.
<b>Is animal health, welfare, breeding or lifespan affected?</b>	Mice heterozygous for the Akita spontaneous mutation (Ins2Akita) are viable and fertile. Symptoms in heterozygous mutant mice include hyperglycemia, hypoinsulinemia, polydipsia, and polyuria, beginning around 3-4 weeks of age. Their mean lifespan of 305 days is significantly shorter than the background strain C57Bl/6 (690 days).
<b>What abnormalities are known to exist (or do you expect) in these animals?</b>	Akita mice have a high incidence of small or 'squinty' / blue eyes. Divided septums are also quite common, reducing numbers available for breeding.

## 8. Clinical Observations

Comparison of genetically modified animals with non-genetically modified littermates is desirable.

- Supply a record of clinical observations made on a representative sample of the genetically modified animal(s).
- Minimum period for observation record is 3 months; life-long data to be included where possible. If supplying "average" data, indicate number of animals observed and a measure of the variability of the data.

Symptoms in heterozygous mutant mice include hyperglycemia, hypoinsulinemia, polydipsia, and polyuria, beginning around 3-4 weeks of age. Progressive retinal abnormalities begin as early as 12 weeks after the onset of hyperglycemia. Due to their diabetes, Akita mice have only been used experimentally to the age of 25 weeks. Our experiments will end prior to the Akita mice reaching this age. These mice have been housed at the Perkins Bioresources Facility previously.

## 9. Phenotype:

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Briefly detail observations which have been made to characterise the genetically modified animal strain (i.e. behaviour, physiology, reproductive or developmental measures). Your answer to this question should inform the AEC about abnormalities or changes which have a welfare impact.

Symptoms in heterozygous mutant mice include hyperglycemia, hypoinsulinemia, polydipsia, and polyuria, beginning around 3-4 weeks of age (Rakoczy et al., 2010). Aged mice exhibit gait disturbance. They exhibit decreased sensory nerve conduction velocity, but do not exhibit learning or memory deficits (Choeiri C et al., 2005). However, they can exhibit hyperphagia and anxiety behavior (Asakawa et al., 2007). No other abnormal behaviours are observed in this strain. Progressive retinal abnormalities begin as early as 12 weeks after the onset of hyperglycemia. Body weight does not increase in the same age-dependent manner as the C57Bl/6 background strain. At 8 weeks of age their body weight is ~17g and ~18g at 24 weeks (Rakoczy et al., 2010). Once animals start to become diabetic they appear more scruffy with piloerection, hunched and sunken eyes get increasingly more obvious.

#### 10. Minimisation of pain or distress

Describe any adverse effects, pain or distress, and/or unexpected mortality, the causes if known and how these problems were resolved. If none this should be indicated.

No adverse events are expected for the 8 week duration of our planned experiments from investigator experience with the mouse model and associated literature.

#### 11. Special husbandry or animal care requirements specific for the new genetically modified animal strain.

If these are necessary, please provide details.

Bedding will be spot changed when required. A full change will be carried out once per week.  
Wet mash food will be made available.

#### 12. Humane euthanasia and experimental endpoint criteria

What phenotypical issues may result in an animal being humanely killed or removed from an experimental study prematurely?

If a greater than 15% weight loss is observed.

#### CERTIFICATION OF THE CHIEF INVESTIGATOR





**AEC005: PHENOTYPE REPORT  
FOR GENETICALLY MODIFIED ANIMALS**

- V. B. Quiller*


.....18-1-19.....  
Date

OFFICE USE ONLY:

I certify that this report has been considered and accepted by the Harry Perkins Institute Animal Ethics Committee at the meeting on ..... 12/02/19.....(date)



..... 12/02/19.....  
Chair's signature AEC Date

	ANIMAL ETHICS COMMITTEE
	<b>AEC005: PHENOTYPE REPORT FOR GENETICALLY MODIFIED ANIMALS</b>

The main purpose of this report is to assist with the monitoring and assessment of the impact of the genetic modification upon the health and welfare of the affected animals. Please provide information consistent with this purpose (i.e. detailed descriptions of *in vitro* methodology are not desired). It is a tool to make it easier for an AEC to appreciate the welfare impact of the genetic modification made to this strain of mouse.

Please use lay language or provide glossary definitions.

#### Project Details

1. AEC Project No:	R540(18-21)		
2. Project Title:	Establishing the role of sodium glucose co-transporter 2 (SGLT-2) in diabetic retinopathy.		
3. Start Date:	October 2018	Finish Date:	October 2021
4. Chief Investigator:	Dr Vance Matthews		
Institute/ Department:	UWA School of Biomedical Sciences and Dobney Hypertension Centre		

\* Relates to approved project dates.

#### 5. Animal Details

Genetically modified animal species:	Mus musculus
Strain/genetic description: (If there is a common name, please include)	C57Bl/6(trVEGF029/Ins2Akita) - Akimba
Background strain:	C57Bl/6
Source: (i.e. specified external laboratory source)	ARC
What is the health profile of the current colony? Provide the most recent health report.	Attached


#### 6. How much is known about the biological characteristics/phenotype of this strain?

Indicate by selecting one of the following:

☒ Well characterised

☐ Partially-characterised/some information available

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 HARRY PERKINS INSTITUTE OF MEDICAL RESEARCH	ANIMAL ETHICS COMMITTEE
	<b>AEC005: PHENOTYPE REPORT  FOR GENETICALLY MODIFIED ANIMALS</b>

[ ] Unknown

## GLOSSARY

Word	Lay explanation

## 7. Genetic alteration:

<b>Briefly describe which gene has been added/deleted/altered.</b>	Akimba mice were produced by mating Kimba (trVEGF029) mice with Akita (Ins2Akita) mice. They overexpress human VEGF165 gene and contain a naturally occurring mutation in their insulin 2 gene.
<b>Affected organs/tissues: (e.g. gene expressed in liver only)</b>	The human VEGF165 gene is expressed only in the eye. The mutated insulin 2 gene affects organs/tissues involved in glucose regulation.
<b>Is animal health, welfare, breeding or lifespan affected?</b>	Being a cross of Akita and Kimba, these mice have the phenotypic features of both mice, including the diabetes and similar eye problems. As such, Akimba mice have a mean lifespan of 305 days and have been used experimentally to the age 24 weeks and have the same lower body weight and lack of weight increase seen in the Akita (Rakoczy et al., 2010, Chaurasia et al., 2018).
<b>What abnormalities are known to exist (or do you expect) in these animals?</b>	Akimba mice have a higher incidence than other mouse strains on the same background of small or 'squinty' / blue eyes. Divided septums are also quite common, reducing numbers available for breeding. No other abnormalities are expected excluding those outlined earlier.

## 8. Clinical Observations


Comparison of genetically modified animals with non-genetically modified littermates is desirable.

- Supply a record of clinical observations made on a representative sample of the genetically modified animal(s).
- Minimum period for observation record is 3 months; life-long data to be included where possible. If supplying "average" data, indicate number of animals observed and a measure of the variability of the data.

Symptoms in heterozygous mutant mice include hyperglycemia, hypoinsulinemia, polydipsia, and polyuria, beginning around 3-4 weeks of age. Progressive retinal abnormalities begin as early as 12 weeks after the onset of hyperglycemia. Due to their diabetes Akimba mice have only been used experimentally to the age of 25 weeks. Our experiments will end prior to the Akimba mice reaching this age. These mice have been housed at the Perkins Bioresources Facility previously.

Form: AEC005 Version: 1.1 July 2017	Harry Perkins Institute of Medical Research Animal Ethics Committee	Page 2 of 4
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	ANIMAL ETHICS COMMITTEE
	<b>AEC005: PHENOTYPE REPORT FOR GENETICALLY MODIFIED ANIMALS</b>

## 9. Phenotype:

<p>Briefly detail observations which have been made to characterise the genetically modified animal strain (i.e. behaviour, physiology, reproductive or developmental measures). Your answer to this question should inform the AEC about abnormalities or changes which have a welfare impact.</p>
<p>Symptoms in heterozygous mutant mice include hyperglycemia, hypoinsulinemia, polydipsia, and polyuria, beginning around 3-4 weeks of age (Rakoczy et al., 2010). Aged mice exhibit gait disturbance. Progressive retinal abnormalities begin as early as 12 weeks after the onset of hyperglycemia. Body weight does not increase in the same age-dependent manner as the C57Bl/6 background strain. At 8 weeks of age their body weight is ~17g and ~18g at 24 weeks (Rakoczy et al., 2010). Once animals start to become diabetic they appear more scruffy with piloerection, hunched and sunken eyes get increasingly more obvious.</p>

## 10. Minimisation of pain or distress

<p>Describe any adverse effects, pain or distress, and/or unexpected mortality, the causes if known and how these problems were resolved. If none this should be indicated.</p>
<p>We have experienced one adverse event with this strain for the 8 week duration of our planned experiments.</p>

## 11. Special husbandry or animal care requirements specific for the new genetically modified animal strain.

<p>If these are necessary, please provide details.</p>
<p>Bedding will be spot changed when required. A full change will be carried out once per week. Wet mash food will be made available.</p>


## 12. Humane euthanasia and experimental endpoint criteria

<p>What phenotypical issues may result in an animal being humanely killed or removed from an experimental study prematurely?</p>
<p>If a greater than 15% weight loss is observed.</p>

## CERTIFICATION OF THE CHIEF INVESTIGATOR


- I understand the requirements of legislation and the *Australian code of practice for the care and use of animals for scientific purposes* (2004) governing the use of animals for research and teaching.
- I will continue to conduct the project in full compliance with the aforementioned requirements.

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	ANIMAL ETHICS COMMITTEE
	<b>AEC005: PHENOTYPE REPORT FOR GENETICALLY MODIFIED ANIMALS</b>

 ..... 18-1-19 .....  
 Signature of Chief Investigator Date

....Vance Matthews.....  
 Please Print Name

OFFICE USE ONLY:	
<b>DECLARATION BY CHAIR OF AEC</b>	
I certify that this report has been considered and accepted by the Harry Perkins Institute Animal Ethics Committee at the meeting on ..... 12/02/19.....(date)	
 .....	12/02/19
Chair's signature AEC	Date



	ANIMAL ETHICS COMMITTEE
	<b>AEC005: PHENOTYPE REPORT FOR GENETICALLY MODIFIED ANIMALS</b>

The main purpose of this report is to assist with the monitoring and assessment of the impact of the genetic modification upon the health and welfare of the affected animals. Please provide information consistent with this purpose (i.e. detailed descriptions of *in vitro* methodology are not desired). It is a tool to make it easier for an AEC to appreciate the welfare impact of the genetic modification made to this strain of mouse.

Please use lay language or provide glossary definitions.

### Project Details

<b>1. AEC Project No:</b>	R540(18-21)		
<b>2. Project Title:</b>	Establishing the role of sodium glucose co-transporter 2 (SGLT-2) in diabetic retinopathy.		
<b>3. Start Date:</b>	October 2018	<b>Finish Date:</b>	October 2021
<b>4. Chief Investigator:</b>	Dr Vance Matthews		
<b>Institute/ Department:</b>	UWA School of Biomedical Sciences and Dobney Hypertension Centre		

\* Relates to approved project dates.

### 5. Animal Details

<b>Genetically modified animal species:</b>	Mus musculus
<b>Strain/genetic description: (If there is a common name, please include)</b>	C57Bl/6(trVEGF029) - Kimba
<b>Background strain:</b>	C57Bl/6
<b>Source: (i.e. specified external laboratory source)</b>	ARC
<b>What is the health profile of the current colony? Provide the most recent health report.</b>	Attached


### 6. How much is known about the biological characteristics/phenotype of this strain?

Indicate by selecting one of the following:

☒ Well characterised

☐ Partially-characterised/some information available

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	<b>ANIMAL ETHICS COMMITTEE</b>
	<b>AEC005: PHENOTYPE REPORT</b> <b>FOR GENETICALLY MODIFIED ANIMALS</b>

[ ] Unknown

## GLOSSARY

Word	Lay explanation

## 7. Genetic alteration:


<b>Briefly describe which gene has been added/deleted/altered.</b>	The human VEGF165 gene under the control of a truncated mouse opsin promoter was added.
<b>Affected organs/tissues: (e.g. gene expressed in liver only)</b>	Eye only
<b>Is animal health, welfare, breeding or lifespan affected?</b>	No
<b>What abnormalities are known to exist (or do you expect) in these animals?</b>	They have a higher incidence than other mouse strains on the same background of small or 'squinty' / blue eyes. Some mice may show a slightly hunched appearance. There are no abnormal behaviours seen in this strain.

## 8. Clinical Observations

Comparison of genetically modified animals with non-genetically modified littermates is desirable.

- Supply a record of clinical observations made on a representative sample of the genetically modified animal(s).
- Minimum period for observation record is 3 months; life-long data to be included where possible. If supplying "average" data, indicate number of animals observed and a measure of the variability of the data.

Kimba mice have been used experimentally up to age 24 weeks (Rakoczy et al., 2010, Chaurasia et al., 2018). Our experiments will end prior to the mice reaching this age. Their body weight does increase in an age dependent manner like the C57Bl/6 background strain. There are no abnormal behaviours seen in this strain. These mice have been housed at the Perkins Bioresources Facility previously.

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### 9. Phenotype:

<p>Briefly detail observations which have been made to characterise the genetically modified animal strain (i.e. behaviour, physiology, reproductive or developmental measures). Your answer to this question should inform the AEC about abnormalities or changes which have a welfare impact.</p>
<p>Kimba mice do not exhibit a phenotype different from their background strain, C57Bl/6, aside from retinopathy as the genetic modification is isolated to the eye.</p>

### 10. Minimisation of pain or distress

<p>Describe any adverse effects, pain or distress, and/or unexpected mortality, the causes if known and how these problems were resolved. If none this should be indicated.</p>
<p>We have not experienced any adverse events with this strain for the 8 week duration of our planned experiments.</p>

### 11. Special husbandry or animal care requirements specific for the new genetically modified animal strain.

<p>If these are necessary, please provide details.</p>
<p>Bedding will be spot changed when required. A full change will be carried out once per week. Wet mash food will be made available.</p>

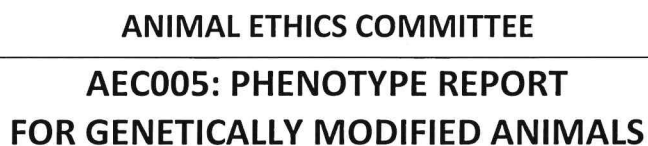
### 12. Humane euthanasia and experimental endpoint criteria

<p>What phenotypical issues may result in an animal being humanely killed or removed from an experimental study prematurely?</p>
<p>If a greater than 15% weight loss is observed.</p>

### CERTIFICATION OF THE CHIEF INVESTIGATOR

- I understand the requirements of legislation and the *Australian code of practice for the care and use of animals for scientific purposes* (2004) governing the use of animals for research and teaching.
- I will continue to conduct the project in full compliance with the aforementioned requirements.

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.....18-1-19.....  
Date

OFFICE USE ONLY:

I certify that this report has been considered and accepted by the Harry Perkins Institute Animal Ethics Committee at the meeting on ..... 12/02/19.....(date)

..... 12/02/19.....  
Chair's signature AEC Date