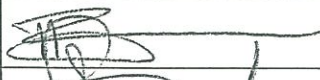
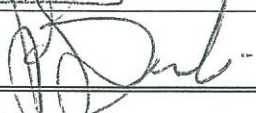


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|-----------------------|--------------------------------------|----------------------|-------------|
| <b>Title</b>          | Blood sample collection from rodents |                      |             |
| <b>SOP no</b>         | SOP_Viv_Anim 26                      | <b>Issue no</b>      | 1           |
| <b>Issue date</b>     | 30-06-2014                           | <b>Revision date</b> | 30-06-2016  |
| <b>Effective date</b> | 04-07-2014                           | <b>Page no</b>       | Page 1 of 6 |


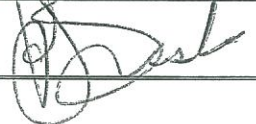
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**1. COMPILATION AND AUTHORISATION**

| Action         | Designated Person | Signature  | Date       |
|----------------|-------------------|--|------------|
| Compiled by:   | Mr H Buntting     |  | 27-06-2014 |
| Authorised by: | Mr CJJ Bester     |  | 27-06-2014 |

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**2. DISTRIBUTION**

| Department       | Name       | Signature  | Date       |
|------------------|------------|--|------------|
| PCDDP QA Manager | L Scholtz  |  | 02-07-2014 |
| Head: Vivarium   | CJJ Bester |  | 02-07-2014 |

**3. DOCUMENT HISTORY**

| Date       | Issue no | Reason for revision |
|------------|----------|---------------------|
| 30-06-2014 | Issue 1  | First issue of SOP  |



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|-----------------------|--------------------------------------|----------------------|-------------|
| <b>Title</b>          | Blood sample collection from rodents |                      |             |
| <b>SOP no</b>         | SOP_Viv_Anim 26                      | <b>Issue no</b>      | 1           |
| <b>Issue date</b>     | 30-06-2014                           | <b>Revision date</b> | 30-06-2016  |
| <b>Effective date</b> | 04-07-2014                           | <b>Page no</b>       | Page 2 of 6 |

**4. POLICY and OBJECTIVE**

The purpose of this Standard Operating Procedure (SOP) is to illustrate the correct methods of humanely collecting blood samples from rodent that limits any negative impacts that the collection may have on the animals.

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**5. SCOPE**

This SOP encompasses all rat, mouse, rabbit and guinea pig blood collection methods employed at the vivarium.

**6. REFERENCE DOCUMENTS**

- SANS 10386:2008 The Care and Use of Animals for Scientific Purposes
- SOP\_Viv\_Anim 2 - Anaesthesia of rats for Surgical Procedures.
- SOP\_Viv\_Anim 4 - Isoflurane Anaesthesia in Mice.
- SOP\_All\_Wast 1 – Waste management.
- SOP\_Viv\_Eqp\_Opr 2 - Weighing of Animals and Materials.

**7. ABBREVIATIONS and/or DEFINITIONS**

| <b>Abbreviation</b> | <b>Description</b>                     |
|---------------------|--|
| °C                  | degree Celsius                         |
| cc                  | cubic centimetre                       |
| cm                  | centimetre                             |
| DST                 | Department of Science and Technology   |
| G                   | gauge                                  |
| GLP                 | Good laboratory practice               |
| mL                  | millilitre                             |
| mm                  | Millimetre                             |
| N/A                 | Not applicable                         |
| NWU                 | North West University                  |
| PCDDP               | Pre-Clinical drug development platform |
| PPE                 | Personal Protective Equipment          |
| QA                  | Quality assurance                      |
| SANS                | South African National Standard        |
| SOP                 | Standard operating procedure           |
| SPF                 | Specific pathogen free                 |



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|-----------------------|--------------------------------------|----------------------|-------------|
| <b>Title</b>          | Blood sample collection from rodents |                      |             |
| <b>SOP no</b>         | SOP_Viv_Anim 26                      | <b>Issue no</b>      | 1           |
| <b>Issue date</b>     | 30-06-2014                           | <b>Revision date</b> | 30-06-2016  |
| <b>Effective date</b> | 04-07-2014                           | <b>Page no</b>       | Page 3 of 6 |

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Superseded on 13-05-2018

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## 8. RESPONSIBILITIES

This SOP must be followed by all staff members/researchers who have been assessed by the Vivarium head or designate, as fully competent to apply the procedure below following a period of training and experience. This SOP applies mainly to staff employed and researchers using the Vivarium. Staff in the process of gaining competence may undertake the procedure if supervised by a member of the Vivarium who is fully competent in the procedure.

## 9. TEST PRINCIPLES

N/A

## 10. MATERIALS and EQUIPMENT

- Sterile Blood collection tubes
- Sterile needles – 25G and 23G
- Sterile syringes – 1cc, 3cc, 5cc and 10cc
- Gauze
- Hair dryer/Heating lamp
- Rodent restrainer

## 11. SAFETY

### 11.1. Required Qualifications

- a) All personnel performing blood sampling must be appropriately trained and the training recorded.

### 11.2. Required Safety Equipment

- a) Standard prescribed GLP PPE.

### 11.3. Hazards

- a) All needles and syringes must be disposed of in an appropriately labelled sharps container.
- b) All material in contact or consisting of biological materials must be disposed of in an appropriately labelled biohazard container.
- c) Animals must be restrained adequately before any collections are performed.

|                       |                                      |                      |             |
|-----------------------|--------------------------------------|----------------------|-------------|
| <b>Title</b>          | Blood sample collection from rodents |                      |             |
| <b>SOP no</b>         | SOP_Viv_Anim 26                      | <b>Issue no</b>      | 1           |
| <b>Issue date</b>     | 30-06-2014                           | <b>Revision date</b> | 30-06-2016  |
| <b>Effective date</b> | 04-07-2014                           | <b>Page no</b>       | Page 4 of 6 |

## 11.4. Disposing of waste

Safely dispose of waste according to SOP\_All\_Wast 1.

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**12. PROCEDURES**

- 12.1. Weigh animal according to SOP\_Viv\_Eqp\_Opr 2 - Weighing of Animals and Materials, and draw maximum blood according to the guidelines stipulated in table 1.

**Table 1: Maximum Blood Collection Volumes**

| Collection Interval | Percent of Blood volume | Percent of body weight | Fluid replacement required |
|---------------------|-------------------------|------------------------|----------------------------|
| One time            | 15%                     | 1.5%                   | Yes                        |
| Every 2 weeks       | 10%                     | 1.0%                   | No                         |
| Weekly              | 7.5%                    | 0.75                   | No                         |
| Terminal            | 30-50%                  | 3-5%                   | N/A                        |

|                                       | Mouse      | Rat          | Hamster     |
|---------------------------------------|------------|--------------|-------------|
| Adult body weight                     | 25-35g     | 250-500g     | 90-120g     |
| Adult Blood volume                    | 2-2.75mL   | 15-30mL      | 5.4-7.2mL   |
| Weekly sample volume (7.5%)           | 0.15-0.21  | 1.125-2.25mL | 0.4-0.54mL  |
| Every two week sampling (10%)         | 0.2-0.28mL | 1.5-3.0mL    | 0.54-0.72mL |
| One time with fluid replacement (15%) | 0.3-0.42mL | 2.25-4.5mL   | 0.81-1.08mL |
| Terminal                              | 1-1.5mL    | 8-12mL       | 3-5mL       |

## 12.2. Tail Nick in Mice

- Place the cage of animals in heated box (~ 35°C) for no more than 5 minutes to warm mice and dilate vessels.
- Once the animals are warmed, place them in an appropriately sized restraint device with the tail extended. An alternative method to warm the tail is to place the animal in a restraint device, then rub the tail for several seconds with a gauze pad soaked in very warm, but not hot, water to dilate the vessels.
- Holding the end of the tail, gently twist the tail to expose the lateral tail vein on either side.
- Approximately 2-3 cm from the tip of the tail, make a small nick over the lateral tail vein using a sterile scalpel blade. When the nick is made deep enough, blood should start welling up from the nick immediately.

|                       |                                      |                      |             |
|-----------------------|--------------------------------------|----------------------|-------------|
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| <b>SOP no</b>         | SOP_Viv_Anim 26                      | <b>Issue no</b>      | 1           |
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| <b>Effective date</b> | 04-07-2014                           | <b>Page no</b>       | Page 5 of 6 |

- e) Blood can be collected either by touching a capillary tube to the bead of blood, or by allowing the blood to drop into a collection tube. The tail can be gently stroked from the base of the tail toward the tip to encourage blood flow.
- f) Once a sufficient amount of blood is collected, apply pressure to the nick with clean gauze for 15-30 seconds to stop the flow of blood.
- g) Release the animal from the restraint device and return it to its cage.

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#### 12.3. Tail Vein Bleed in Rats

- a) Place the animal in a restraint device with the tail extended. Alternatively, the rat can be lightly anesthetized with isoflurane, following the SOP for anesthesia.
- b) Place the rat in a heated box (~ 35°C)/blow with hairdryer for 2-5 minutes to warm the tail and dilate vessels.
- c) Clean the tail with a gauze swab soaked with warm water.
- d) Using a 23-25G needle attached to a syringe, insert the needle into one of the lateral veins, approximately 2-3 cm from the tip of the tail.
- e) When blood appears in the hub retract the plunger slowly to collect the desired amount of blood.
- f) Once blood collection is complete, remove the needle and apply gentle pressure with gauze for 15-30 seconds to stop the flow of blood.
- g) Release the animal from the restraint device and return it to its cage. If the animal was anesthetized, monitor the animal until it is fully awake and able to walk normally.

#### 12.4. Cardiac Puncture

- a) Anesthetize the animal following SOP\_Viv\_Anim 2 - Anaesthesia of rats for Surgical Procedures.
- b) Place the animal on its back and wipe the chest and abdomen with a gauze swab soaked with 75% ethanol.
- c) Attach an appropriately sized needle to a syringe and insert it at a 30 ° angle just below the xyphoid process, angling the needle slightly toward the left shoulder. A 25G 16mm needle is sufficient for mice. For rats, a 23G 25mm or longer needle may be required.

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|-----------------------|--------------------------------------|----------------------|-------------|
| <b>Title</b>          | Blood sample collection from rodents |                      |             |
| <b>SOP no</b>         | SOP_Viv_Anim 26                      | <b>Issue no</b>      | 1           |
| <b>Issue date</b>     | 30-06-2014                           | <b>Revision date</b> | 30-06-2016  |
| <b>Effective date</b> | 04-07-2014                           | <b>Page no</b>       | Page 6 of 6 |

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- d) Retract the plunger slightly to create a vacuum inside the syringe, then advance the needle until blood appears in the hub of the needle.
- e) Slowly retract the plunger to collect the desired amount of blood.
- f) Once the blood collection is complete, withdraw the needle and euthanize the animal.

### 13. ADDENDUM

N/A