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STANDARD OPERATING PROCEDURE PACKET

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STANDARD OPERATING PROCEDURE

SUBJECT: Short Term Fast (<8hr) – V1

SOP Number: SOP0175	**To be reviewed:**	
Author(s):	Signed:	Date:
Editor:	Signed:	Date:
Risk Assessor:	Signed:	Date:
Date of Implementation:		

INTRODUCTION:

The purpose of this procedure is to remove mouse access to diet.

ABBREVIATIONS:

DCF = Data Capture Form
IVC = Individually Ventilated Cage
LAA = Laboratory Animal Allergens
NACWO = Named Animal Care and Welfare Officer
PAF = Project Authorisation Form
PIL = Procedure Individual Licence
PPE = Personal Protective Equipment
PPL = Procedure Project Licence
QC = Quality Control
RA = Risk Assessment
RSF = Research Support Facility
SLT = Scientific Leadership Team
SMP = Sick Mouse Procedure
SOP = Standard Operating Procedure

QUALITY CONTROL (QC) DURING PROCEDURE:

Refer to the table below for approved QC fail comments steps to be used during procedures.
If a value has been collected leave on the Data Capture Form (DCF) and then apply the fail reason from below;

In-Life Procedures:

Problem / Issue	QC fail reason
At any point during the procedure the mouse is deemed sick and processed through Sick Mouse Procedure (SMP)	Fail whole DCF as 'Sick mouse' – for all tests that day

Mouse incorrectly scheduled at wrong week	Fail whole DCF as 'Scheduling Issue'
Insufficient anaesthesia level affects the whole test DCF	Fail whole DCF as 'Anaesthesia Issue'
Insufficient anaesthesia level affects specific parameter(s)	Fail parameter(s) as 'Anaesthesia issue'
A welfare issue makes it impossible to collect specific parameters	Fail parameter(s) as 'Welfare issue'
Parameters affected by delays or noise due to fire alarms	Fail parameter(s) as 'Fire alarm'
An equipment failure affecting specific parameters	Fail parameter(s) as 'Equipment failure'
A software issue affecting specific parameters	Fail parameter(s) as 'Software failure'
A procedural error which affects data collection	Fail parameter(s) as 'Manual error'
Parameter cannot be assessed	Fail parameter(s) as 'Readout not possible'
Wrong value has been entered which cannot be re-measured or accounted for	Fail parameter(s) as 'Erroneous data'
Glucose meter unable to record high blood values	Fail parameter(s) as 'Meter reading HI'
Glucose meter unable to record low blood values	Fail parameter(s) as 'Meter reading LO'
Fighting occurs prior to or during data collection	Fail parameter(s) as 'Fighting during procedure'
Parameter on the current DCF is not required for that specific test/pipeline	Fail parameter(s) as 'Not required'

HEALTH & SAFETY:

This procedure is covered by the following Risk Assessment (RA):

Name: WTSI-3332
Assessment Title: Basic Mouse Procedures
Assessor:
Approver:

- Appropriate Personal Protective Equipment (PPE) is to be worn at all times when handling animals. This includes:
  - Overshoes
  - Gown
  - Gloves
- In addition to the above, when sources for Laboratory Animal Allergens (LAA) (animals or soiled cages) are not contained within Local Exhaust Ventilation Systems (change stations, fume hoods or down flow tables), a respiratory mask, for which you have passed a face fit test, must be worn.
- Lone worker alarms should be used when working alone.
- This procedure can only be performed during Research Support Facility (RSF) core hours (7:30am-7:30pm).
- All electrical equipment is to be inspected for damage before use.
- Sharps named in this procedure remain sheathed and do not pose a hazard.

RESPONSIBILITIES:

All staff performing this procedure are responsible for ensuring that this Standard Operating Procedure (SOP) and accompanying Risk Assessment have been read, understood and where applicable is followed in accordance with the relevant Procedure Project Licence (PPL). All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure with a valid Procedure Individual Licence (PIL).

For secondary phenotyping, seek confirmation with project manager for deviations from this SOP. Any deviation will be detailed in the Project Authorisation Form (PAF).

RESOURCES:

Equipment:

1. Fasting details sheet or relevant procedure worksheet
2. Weekly weight template sheet or relevant procedure worksheet
3. Balance
4. Pen
5. Tecniplast Interactive Cage Change Station
6. Tecniplast Individually Ventilated Cage (IVC) rack
7. Transport rack
8. 70% Ethanol – **Hazardous substance: highly flammable**
9. Hydrex Pink - **Hazardous substance: highly flammable**
10. Hydrex Hard Surface Spray - **Hazardous substance: highly flammable**

For Single Housed Fasting:

1. A clean cage with lid and water bottle; 1 per mouse
2. Nestlet; 1 per mouse
3. Fun tunnel; 1 per mouse
4. “Experimental mice removed for short term fast followed by XXX” labels
5. Procedure/fasting labels as appropriate

For Group Housed Fasting:

1. A clean cage with lid and water bottle; 1 per cage
2. Nestlet; 1 per cage
3. Fun tunnel; 1 per cage
4. Short Term Fast Labels

Associated SOPs/Documentation:

- **EQ18** – Use of Tecniplast Interactive Cage Change Stations
- **SOP0045** – Weigh Mice for Phenotyping Procedures
- Relevant procedure SOP
- **Fasting labels** –\Short Term Fasting Label – Whole Cage
- Procedure labels (see relevant SOP)
- **Weekly weight template**

Staff: This test can be carried out by one phenotyper.

NOTE:

This SOP can be performed by either singly housing or group housing mice for the duration of the fast depending on the procedure that follows. Check with the relevant SOP, the primary phenotyper for the procedure or a member of the Scientific Leadership Team (SLT) as to which type of fast must be performed.

If, 8 hours after the mice were put on fast, there are mice that haven't undergone their follow-up procedure; they must be provided with food. QC fail all data points using the relevant QC fail comment. Report this to the appropriate primary phenotyper.

PROCEDURE:

Before performing any tests verify this is the correct set of procedures at this time point in the pipeline or project, by consulting the cage card(s).

1. Preparation:
   - 1.1 Print the procedure or fasting labels and collect the corresponding fasting details sheet or relevant procedure worksheet.
   - 1.2 Prepare the change station with a balance, nestlets and fun tunnels, procedure or fasting labels, worksheet and pen. Add "Experimental mice removed for short term fast followed by XXX" labels if any mice are to remain in the home cage unfasted.
   - 1.3 For single housed fasting: prepare an individual cage for each mouse undergoing short term fasting. Set-up each with a water bottle and a procedure/fasting label as appropriate.
     For group housed fasting: prepare a cage for each cage undergoing short term fasting. Set-up each with a water bottle and a fasting label.
   - 1.4 For single housed fasting without specific procedure fasting labels, split the mice on the database to generate individual cage cards.
   - 1.5 For group housed fasting: Log the 'Short term fast (<8hrs)' procedure on the database.

2. Prepare the change station for use (refer to EQ18 – Use of Tecniplast Interactive Cage Change Station).

3. Collect scheduled mice from the animal room, if appropriate transport them to the procedure room and register them to the correct rack (Refer to SOP0101 – Taking and Returning Cages for Procedures). Place the cages on the IVC holding rack.

4. Place 'Phenotyping in progress' sign on the outside of the door.

5. Wearing the correct PPE, perform a welfare check and weigh each mouse to be tested (refer to SOP0045 – Weigh mice for Phenotyping Procedures). Record the pre-fasted weight on the procedure record sheet against the correct mouse barcode.
   - 5.1 If a mouse seems unfit to undergo the procedure or becomes so at any point, contact a Named Animal Care and Welfare Officer (NACWO).
     - 5.1.1 If the mouse is deemed unfit to start or finish the test, follow QC procedures and either return the mouse to its home cage or

continue to individually house the mouse, providing food, as per the protocol being followed.

5.1.2 Make a note of the mouse not to be tested on the record sheet (including the reason for not testing) and clearly label the individual cage card if still being single housed.

5.1.3 Add the health concern in the database and complete a health concern cage label for the home cage.

5.1.4 If the mouse must be culled, commence the SMP.

6. Place the mouse into the cage prepared for it, ensuring the cage is devoid of diet and add a nestlet and fun tunnel.
   6.1 For single housed fasting: note the time on the procedure/fasting label and return the cage to the ventilated rack.
   6.2 For group housed fasting: transfer all mice, including stuffers, to the cage, add the newly printed cage card and note the time on the fasting label. Tear the old cage card in half and place the half with the barcode on the old home cage. Return both cages to the ventilated rack.

7. If stuffers are present:
   7.1 For single housed fasting: leave them in the home cage, label the cage with an "Experimental mice removed for short term fast followed by XXX" label and return to the holding rack.
   7.2 For group housed fasting: transfer them to the fasting cage with the test mice.

8. When all weights have been taken, clean the weight scale with 70% Ethanol/Hydrex Hard Surface Spray and remove from the working area.

The time by which the mice must be fasted will depend on what procedure they are undergoing. See the procedure SOP, the primary phenotyper for the procedure or a member of the STL for the necessary timings.

9. For single housed fasting: Log the 'Short Term Fast (<8hrs)' procedure on the database.

10. Clean all equipment, surfaces and the floor. **Transfer all waste to a yellow offensive waste bag or clearly labelled waste container.**

11. Check the relevant procedure SOP for any additional set-up that may be required.

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STANDARD OPERATING PROCEDURE

SUBJECT: Intraperitoneal Glucose Tolerance Test (ip-GTT) – V1

SOP Number: SOP0026	**To be reviewed:**	
Author(s):	Signed:	Date:
Editor:	Signed:	Date:
Risk Assessor:	Signed:	Date:
Date of Implementation:		

INTRODUCTION:

The purpose of this procedure is to investigate glucose tolerance and clearance in wild-type and genetically altered mice.

ABBREVIATIONS:

DCF = Data Capture Form
ip-GTT = intraperitoneal Glucose Tolerance Test
IVC = Individually Ventilated Cage
LAA = Laboratory Animal Allergens
PAF = Project Authorisation Form
PIL = Procedure Individual Licence
PPE = Personal Protective Equipment
PPL = Procedure Project Licence
QC = Quality Control
RA = Risk Assessment
RSF = Research Support Facility
SLT = Senior Leadership Team
SMP = Sick Mouse Procedure
SOP = Standard Operating Procedure

QUALITY CONTROL (QC) DURING PROCEDURE:

Refer to the table below for approved QC fail comments steps to be used during procedures.
If a value has been collected leave on the Data Capture Form (DCF) and then apply the fail reason from below;

In-Life Procedures:

Problem / Issue	QC fail reason
At any point during the procedure the mouse is deemed sick and processed through Sick Mouse Procedure (SMP)	Fail whole DCF as 'Sick mouse' – for all tests that day

Mouse incorrectly scheduled at wrong week	Fail whole DCF as 'Scheduling Issue'
Insufficient anaesthesia level affects the whole test DCF	Fail whole DCF as 'Anaesthesia Issue'
Insufficient anaesthesia level affects specific parameter(s)	Fail parameter(s) as 'Anaesthesia issue'
A welfare issue makes it impossible to collect specific parameters	Fail parameter(s) as 'Welfare issue'
Parameters affected by delays or noise due to fire alarms	Fail parameter(s) as 'Fire alarm'
An equipment failure affecting specific parameters	Fail parameter(s) as 'Equipment failure'
A software issue affecting specific parameters	Fail parameter(s) as 'Software failure'
A procedural error which affects data collection	Fail parameter(s) as 'Manual error'
Parameter cannot be assessed	Fail parameter(s) as 'Readout not possible'
Wrong value has been entered which cannot be re-measured or accounted for	Fail parameter(s) as 'Erroneous data'
Glucose meter unable to record high blood values	Fail parameter(s) as 'Meter reading HI'
Fighting occurs prior to or during data collection	Fail parameter(s) as 'Fighting during procedure'
Parameter on the current DCF is not required for that specific test/pipeline	Fail parameter(s) as 'Not required'

HEALTH & SAFETY:

This procedure is covered by the following Risk Assessment(s) (RA):

Name: WTSI-1201
Assessment Title: Intra peritoneal glucose tolerance testing (ipGTT)
Assessor:
Approver:

- Appropriate Personal Protective Equipment (PPE) is to be worn at all times when handling animals. This includes:
  - Overshoes
  - Gown
  - Gloves
- In addition to the above, when sources for Laboratory Animal Allergens (LAA) (animals or soiled cages) are not contained within Local Exhaust Ventilation Systems (change stations, fume hoods or downflow tables), a respiratory mask, for which you have passed a face fit test, must be worn.
- Lone worker alarms should be used when working alone.
- This procedure can only be performed during Research Support Facility (RSF) core hours (7:30am-7:30pm).
- All electrical equipment is to be inspected for damage before use.

RESPONSIBILITIES:

All staff performing this procedure are responsible for ensuring that this Standard Operating Procedure (SOP) and accompanying RA have been read, understood and

where applicable is followed in accordance with the relevant Procedure Project Licence (PPL). All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure with a valid Procedure Individual Licence (PIL).

For secondary phenotyping, seek confirmation with project manager for deviations from this SOP. Any deviation will be detailed in the Project Authorisation Form (PAF).

RESOURCES:

Equipment:

1. Transport rack
2. Tecniplast Interactive Cage Change Station
3. ip-GTT record sheet
4. Pen
5. 70% Ethanol **- Hazardous substance: highly flammable**
6. Hydrex Pink hand spray **- Hazardous substance: highly flammable**
7. Hydrex Hard Surface spray - **Hazardous substance: highly flammable**
8. Tecniplast Individually Ventilated Cage (IVC) holding rack
9. Diet (as defined by pipeline)
10. 2x ACCU-CHEK Aviva Kit *(Supplier name; Phoenix. Supplier product code;* 3171261*)*
11. ACCU-CHEK Aviva Test Strips (50 Pack) *(Supplier name; Phoenix. Supplier product code;* 3171253*)*
12. ACCU-CHEK Aviva Control Solution (2 Pack) *(Supplier name; Phoenix. Supplier product code;* 3171246*)*
13. Timers; 1 per 4 mice
14. BD Plastipak 1 ml syringes; 1 for each mouse
15. BD Microlance 3 needles ½" 27 gauge; 1 for each mouse **Sharps risk: Needles are never to be re-sheathed**
16. Marker pen
17. Corkboard
18. Disposable single edge razor blade **Sharps risk**
19. Yellow sharps/offensive waste container
20. Cauterising device and charger **Burn risk**
21. 20% glucose in NaCl solution
22. Two paper towels
23. Swing bin liner
24. A clean base to rehouse mice; 1 for each home cage
25. Nestlets; 1 for each home cage
26. A clean cage with lid to replace those used during the procedure; 1 for each mouse
27. 'Post Procedure Check Required' labels

Associated SOPs/Documentation:

- **EQ18** – Use of Tecniplast Interactive Cage Change Stations
- **SOP0045** – Weigh mice for Phenotyping Procedures
- **SOP0059** – Preparation of 20% Glucose in NaCl (0.9%)
- **SOP0093** – Overnight Fast
- **SOP0101** – Taking and Returning Cages for Procedures
- **SOP0175** – Short Term Fast (<8h)
- **ip-GTT record sheet**
- **Procedure labels**

Staff: For 2 setups; 3 phenotypers, reduced to 2 phenotypers later in the procedure.

NOTE:

If at any point during the procedure, excessive/uncontrolled bleeding from the tail tip incision occurs, this must be stopped using a cauterising device. If the use of a cauterising device is required, follow step 17.1.

20% glucose in NaCl solution falcons are stored at -20ºC. Volumes required for the procedures are removed from frozen storage and stored at 4ºC prior to the test date. **Stock remaining from the previous week is disposed of into a yellow offensive waste bin.**

PROCEDURE:

Before performing any tests verify this is the correct set of procedures at this time point in the pipeline or project, by consulting the cage card(s).

1. See the schedule, the primary phenotyper for the procedure or a member of the Scientific Leadership Team (SLT) for which type of ip-GTT is to be performed:
   1.1 **Overnight Fasted ip-GTT:** on the day before the procedure, fast the mice (refer to SOP0093 – Overnight Fast).
   1.2 **Short Term Fasted ip-GTT:** follow SOP0175 – Short Term Fast (<8hr).
   1.3 **Non-fasted ip-GTT:** follow steps on how to singly house the mice only from SOP0175 – Short Term Fast (<8hr).

2. Collect the ip-GTT record sheets.

3. Take the 20% glucose in NaCl solution from the fridge to allow it to warm to room temperature.

4. Prepare the change station for ip-GTT with:
   4.1 Cork board
   4.2 Razor blade – left in its cardboard sheath until use
   4.3 2x Aviva glucose meters
   4.4 Aviva test solutions, checking expiry date and the date bottles were opened. Replace if opened more than 3 months previously or past expiry date, making sure to write the date on the box.
   4.5 Aviva test strips; 1 box per meter
   4.6 Timers; 1 per 4 mice
   4.7 Needles; 1 per mouse – needles remain sheathed until use
   4.8 Syringes; 1 per mouse
   4.9 Sharps container
   4.10 2x paper towels
   4.11 Marker pen
   4.12 Pen

5. Calculate the volume of glucose solution necessary for each mouse; 0.1ml/10g so give a dose of 2mg/g, (10% of body weight when using prepared 20% solution) and record this on the ip-GTT record sheet.

6. Prepare one syringe per mouse, clearly numbered.

7. Invert the glucose solution well and check that it is clear and fill syringes with the correct dose calculated in step 5.

8. Perform QC of the Accu-Chek blood glucose meters:
   8.1 If a pot of Aviva test strips has been started, finish it before opening a new pot (unless they have expired). If a new pot is being opened write the date and meter number on the lid of the pot.
   8.2 Insert the gold coloured end of the strip into the bottom of the meter. This will activate the meter. The code number for the bottle will appear briefly when a new test strip is inserted.
   8.3 Wipe the tip of the control solution bottle with a clean tissue.
   8.4 Hold the bottle horizontal and squeeze gently so that one drop of solution forms at the opening.
   8.5 Touch the drop to the test strip that has been inserted into the meter. The meter will beep to signify enough solution has been applied and an hour glass symbol will appear on the screen.
   8.6 The meter will display the glucose concentration of that solution, along with a flashing letter *L*.
   8.7 Record the displayed glucose concentration on the ip-GTT record form.
   8.8 Press the right arrow key to select the number of the control solution applied and then press the on/off/set button located at the top right of the meter. If the glucose concentration is within the expected range for that control solution an 'OK' message will be displayed.
   8.9 Repeat steps 8.2-8.8 with the second control solution.
   8.10 QC is successful when both solutions 1 and 2 have 'OK' messages shown. If either is out of the expected range, an error message will be displayed. In such cases:
      8.10.1 Repeat the QC control using a new strip. If this remains unsuccessful;
      8.10.2 Repeat QC control with new control solutions. If no success;
      8.10.3 Perform QC control on a different meter, informing the primary phenotyper responsible for the test or a member of the SLT of the faulty equipment.
   8.11 At all points of the procedure ensure there are 2 meters calibrated and ready for use in case of an error with the first. When a new pot of strips is begun, QC control must be performed.

If performing fasted ip-GTT, wait until the appropriate period has passed before continuing onto the next step.

9. Turn on power to cauterising device.

10. Bring the first cage into the change station:
    10.1 *Person 1*: Remove the mouse from the cage, checking the earmark before placing it on the corkboard. While holding the mouse at the base of its tail, extend index finger to tail tip to immobilise the end of the tail. Remove the tail tip using a blade, removing as little tissue as possible.
    10.2 *Person 2*: Insert a test strip into the meter. Once this has been done, you have 2 minutes to take the measurement before the meter turns off. If this time elapses, re-insert strip.

11. Take sample for T0 blood glucose concentration measurement:

11.1 *Person 1*: Holding the mouse at the base of its tail, run two fingers laterally up the mouse's tail, applying light pressure, to bring blood to the tip.

11.2 *Person 2*: Bring the meter and inserted strip alongside the tail so that the blood touches the yellow tip of the strip. The blood should completely replace the yellow colour of the strip. The meter will beep and display a turning hourglass as it calculates the glucose level. The calculated blood glucose concentration (mmol/L) will then appear on the screen. Record this number as T0 and **dispose of test strip into the yellow sharps bin.**

12. Administer intraperitoneal injection of glucose solution:
    12.1 *Person 1*: Select the correct syringe for the mouse and inject. **Dispose of the needle and syringe in the sharps bin.**
    12.2 *Person 2*: Press the start button on the timer when the injection is completed and note the time on the procedure label.

13. Return the mouse and the procedure label to the cage and return it to its position on the IVC holding rack. Keep the timer in the change station.

14. Repeat steps 10-13 for all mice, making sure each injection happens between 50 seconds and 1 minute 15 seconds after the previous mouse (use the timer from mouse 1 to gauge the time to inject mouse 2, and so on).

15. Once the mice have started the procedure:
    15.1 *Person 2:* start the DCFs and print the updated cage cards.
    15.2 *Person 1*: continue with the rest of the steps listed below.

16. Monitor timer 1. Fifteen minutes post injection the second reading is to be taken
    16.1 Move the cage into the change station, insert a fresh strip into the meter, and remove the mouse no more than 30 seconds before the 15 minute mark.
        16.1.1 Holding the mouse at the base of its tail, run two fingers laterally up the mouse's tail, applying light pressure, to bring blood to the tip.
        16.1.2 Bring the meter and inserted strip alongside the tail so that the blood touches the yellow tip of the strip from the side. The blood should completely replace the yellow colour of the stripe. The meter will beep and display a turning hourglass as it calculates the glucose level.
        16.1.3 The calculated blood glucose concentration (mmol/L) will then appear on the screen. Record this number as T15.
        16.1.4 **Dispose of test strip into the yellow sharps bin.**

17. Repeat measurements as described in step 16 for all mice at 15, 30, 60 and 120 minutes, being aware that:
    17.1 If at any point during the procedure excessive/uncontrolled bleeding from the tail wound occurs, this must be stopped using a cauteriser as follows;
        17.1.1 Remove hand held base unit from the charging dock.
        17.1.2 Insert the filament.
        17.1.3 Press and hold the black button on the hand held unit until the filament glows red.

17.1.4 Briefly touch this filament to the open wound. Do not apply for prolonged periods of time.
17.1.5 Take no more measurements from this mouse, return it to its home cage and provide food. QC fail data points post cauterisation.
17.1.6 Comment on the requirement for cauterisation in the comments field.

17.2 If at any point during the procedure a measurement is missed or acquired later than the specified time point, enter the blood glucose value (if taken) in the correct field then fail this field. In the text box which is then displayed enter the reason for the erroneous measurement referring to the table in the 'QC DURING PROCEDURE' section for approved QC fail comments to be used during procedures.

17.3 If blood glucose from T15 onwards does not rise (or rises slowly, or with a flat curve), highlight this data to the primary phenotyper responsible for the test or to a member of the SLT during the procedure so it can be QC failed if necessary.

17.4 At later time points it may be necessary to remove scabbing from the wound to yield a sample.

18. Prior to T120, prepare the new home cages in the change station:
    18.1 Using clean bases and the pre-fasting home cages, transfer the cage lids from the pre-fasting home cages with food (topping up as necessary) to the clean bases and label with the updated cage cards.
    18.2 Transfer any stuffers into the corresponding new home cages, seed with nesting material from the pre-fasting home cage and add 1x new nestlet. Place all remaining dirty bedding, into the swing bin liner.
    18.3 Return the new home cages to the rack before removing the dirty cage from the change station and immediately covering it with an empty cage base.

19. When time allows or at the end of the procedure, record results and observations according to the current DCF:
    19.1 Include the meter numbers and QC check related comments. For example: "Meter 1, L1=2.4, L2=17.8, T0-T30; Meter 2, L1=2.4, L2=17.1, T60-T120" in the field for QC related comment.
    19.2 If the meter has displayed 'HI' at any time point, enter the value as 33.4 then QC fail as described in the QC fail section of this SOP.

20. During the collection of the T120 reading, return the experimental mice to their home cage, containing stuffers if present, and take apart the glucose cage.
    20.1 Remove the nestlet, fun tunnel and individual cage label.
    20.2 Put the water bottle to one side and remove the cage lid.
    20.3 Place the cage base on the floor beside the change station immediately covering it with an empty cage base.
    20.4 Transfer used cage bases and lids to the dirty cage collection point.

21. Clean all equipment, surfaces and the floor. **Transfer all waste to a yellow offensive waste bag or clearly labelled waste container. Single edge razor blades, needles and syringes are to be disposed of in a designated sharps bin.**

22. Turn off power to the cauterising device.

23. **All cages must display the updated cage card. Place a 'POST PROCEDURE CHECK REQUIRED' label on all cages, record the number of cages returned in the day book and register them to the correct rack whilst returning them to their destination/home rack in the animal room. (Refer to SOP0101 – Taking and Returning Cages for Procedures).**

24. Using a clean cage base and lid, prepare clean, empty cages to replace those used from the IVC holding rack.

SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Preparation of 20% Glucose in 0.9% Sodium Chloride (NaCl) Solution – V1

SOP Number: SOP0059	**To be reviewed:**	
Author(s):	Signed:	Date:
Editor:	Signed:	Date:
Risk Assessor:	Signed:	Date:
Date of Implementation:		

INTRODUCTION:

The purpose of this procedure is to prepare a sterile 20% glucose in NaCl (0.9%) solution for use in Glucose Tolerance Testing (GTT).

ABBREVIATIONS:

GTT = Glucose Tolerance Test
ipGTT = intra peritoneal Glucose Tolerance Testing
NaCl = Sodium Chloride
PAF = Project Authorisation Form
PIL = Procedure Individual Licence
PPE = Personal Protective Equipment
PPL = Procedure Project Licence
RA = Risk Assessment
RSF = Research Support Facility
SLT = Scientific Leadership Team
SOP = Standard Operating Procedure

HEALTH & SAFETY:

This procedure is covered by the following Risk Assessment (RA):

Name: WTSI-3333
Assessment Title: Intra peritoneal glucose tolerance testing (ipGTT)
Assessor:
Approver:

- Where possible use plastic equipment over glassware.
- Appropriate Personal Protective Equipment (PPE) is to be worn at all times when working in sterile conditions. This includes:
    - Gloves
- Lone worker alarms should be used when working alone.

- This procedure can only be performed during Research Support Facility (RSF) core hours (7:30am-7:30pm).
- All electrical equipment is to be inspected for damage before use.

RESPONSIBILITIES:

All staff performing this procedure are responsible for ensuring that this Standard Operating Procedure (SOP) and accompanying RA have been read, understood and where applicable is followed in accordance with the relevant Procedure Project Licence (PPL). All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure with a valid Procedure Individual Licence (PIL).

For secondary phenotyping, seek confirmation with project manager for deviations from this SOP. Any deviation will be detailed in the Project Authorisation Form (PAF).

RESOURCES:

Equipment:

1. Biological Safety Cabinet
2. 70% Ethanol **- Hazardous substance: highly flammable**
3. 250ml Duran laboratory bottle with lid (autoclaved)
4. 100ml Measuring cylinder (autoclaved)
5. 100ml 40% glucose solution *(Supplier name; VWR International Ltd. Supplier product code; AMREE701-100)*
6. 6.16ml 5M Sodium Chloride (NaCl) stock *(Supplier name; Sigma Aldrich Co.Ltd. Supplier product code; S5150-1L)*
7. 94ml Embryo transfer water from unopened bottle (*Supplier name; Sigma Aldrich Co.Ltd. Supplier product code; W1503-100ML)*
8. 15x 15ml Sterile falcon tubes
9. 200µl Pipette
10. 200µl Sterile pipette tips
11. Pipette gun
12. 2x 5ml Sterile stripettes
13. 25ml Sterile stripette
14. 2x Autoclave pouches

Staff: This procedure requires one member of staff.

NOTE: Solution **MUST** be prepared under sterile conditions

PROCEDURE:

1. Prepare the Biological Safety Cabinet for use.

2. Wipe all consumables and reagents with 70% Ethanol as they enter the cabinet to ensure sterile environment is maintained. Autoclaved equipment should be removed from their autoclave pouch straight into the hood with minimal exposure to the airspace outside of the hood.

3. Check that all reagents are clear and free of debris. Any which are not, should be disposed of and an alternative used for solution preparation.

4. Wearing the correct PPE, combine all reagents in a sterile 250ml Duran laboratory bottle, taking care to avoid any cross-contamination of stock reagent solutions:
   4.1. 100ml of 40% Glucose solution – using the measuring cylinder
   4.2. 94ml of Embryo Transfer Water – using the measuring cylinder and a 5ml stripette
   4.3. 6.16ml of 5M NaCl – using a **new** 5ml stripette and the 200µl pipette.

5. Mix thoroughly by closing the lid and shaking the bottle.

6. The final solution should also be clear and free of debris.
   6.1. If it isn't, check stock bottles and try again, making sure to properly autoclave the equipment first.
   6.2. If it still isn't clear, contact the primary phenotyper responsible or the Senior Leadership Team (SLT).

7. Each sterile falcon tube should be labelled with:
   '20% Glucose in NaCl', date of preparation, and your initials.

8. Transfer 13ml of the prepared solution into each of the first 3 falcon tubes using a 25ml stripette.

9. Close the lid of the Duran laboratory bottle and shake thoroughly to mix and redistribute the solution.

10. Repeat steps 8 and 9 until all falcon tubes have been filled.

11. Rinse equipment thoroughly in the sink and dry with tissue.

12. Bag up used equipment in two autoclave pouches, seal and place into the basket near the autoclave.

13. Clean all equipment, surfaces and the floor**. Transfer all waste to a yellow offensive waste bag or clearly labelled waste container.**

14. Switch off the Biological Safety Cabinet.

15. Store prepared falcon tubes at -20°C.