SANGER INSTITUTE
STANDARD OPERATING PROCEDURE

SUBJECT: Neonate Daily Checks P0-P9 -V1

SOP Number: SOP0187	To be reviewed:
Author: Signed: Date :	
Editor: Signed: Date:	
Risk Assessor: Signed: Date: **Risk Approver:** Signed: Date:	
Date of Amendment:	

INTRODUCTION:

The purpose of this procedure is to perform daily welfare checks on P0 to P9 pups, plus expectant and littered-down dams. It should be performed twice daily (~9am and ~2pm) from Monday to Friday by members of our team. Weekend welfare checks will be performed by the RSF team.

Staff requirements:	One staff member is required to perform this task. Staff member must have a PIL and be fully competent.
This is a regulated procedure:	Neonate Daily Checks P0-P9 Severity: Mild

QUALITY CONTROL (QC) DURING PROCEDURE:

Problem / Issue	Comment on DCF / action to be taken
A welfare issue makes it impossible to perform the procedure	Do not perform procedure. If necessary, cull distressed mice (see section E). If possible, take genotyping samples, and fix neonate in formalin (see section D). Record the welfare issue on DCF.
Procedure affected due to fire alarms	Stop procedure, and return mice to the home cage on a holding rack. Return and complete procedure when/if it safe to do so.

HEALTH & SAFETY:

This procedure is covered by a risk assessment.

- Entry procedure to the RSF should be followed (See SOP IC1).
- Appropriate PPE is to be worn at all times when handling animals. This includes:
    - Overshoes
    - Gown

- Gloves
- Access to a <u>functional down-flow table</u> is required.
- <u>New Workers</u> are to be supervised until deemed competent to perform this assay.
- When <u>sources for LAA</u> (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.
- <u>Lone worker alarms</u> 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.

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 License (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 License (PIL).

RESOURCES:

Equipment:
1. Appropriate PPE
2. Functional change station

Additional:
3. Dissection tools
4. Genotyping plates, seals, 96 well cooler block
5. Ethanol wipes
6. Weighing scales
7. Dysmorphology camera
8. 60ml Formalin pots
9. Pentobarbital
10. 0.5ml syringes

Associated SOPs/Documentation:
- SOP0188 Neonate Assessment P0-P3
- SOP0189 Neonate Assessment P4-P9
- SOP0192 Neonate Terminal Collection for Drop Fixation P0-P1
- SOP0193 Neonate Terminal Collection for Histopathology P0-P9
- SOP IC1 Entry and Exit Procedures for the RSF Barrier Areas
- SOP AH19 Euthanasia of Mice by Dislocation of the Neck
- SOP0174 Confirmation of death by heart removal
- SOP AH23 Disposal of Mouse Cadavers and Carcasses
- SOP0118 Label printing module

SOP Procedure Contents:

PROCEDURE:

Note: **Keep pup handling at a minimum. If to be performed on multiple litters, gloves must be sprayed with Hydrex Pink hand spray and the working area must be cleaned with Hydrex Hard surface spray between litters, so as to reduce the transfer of smell and reduce the chance of rejection.**

A)   **Expectant Dams and New Litters:**

1.   Locate cages with 'confirmed pregnant' dams in the relevant animal holding room.
      Checks should be performed on all expectant dams from 18.5 days of gestation.

2.   Transfer first cage to a change station, open cage, and check if dam has littered down.

3.   **If dam has not littered down:**
      i.   Return cage to rack.
      ii.   Email relevant staff to update.
      iii.   Check dam again once every 2 hours.

4.   **If dam is in the process of littering down, but not finished:**
      i.   See **Section D) 1. & 2.**

5.   **If dam is struggling to litter down:**
      i.   See **Section D) 3.**

6.   **If dam has littered down:**
      i.   Move bedding, minimal disturbance, to see the full nest.
      ii.   Count the number of pups.
      iii.   Perform a quick, visual assessment of pup welfare (see **Section E) 1.**).
      iv.   **If any pups are dead, or in clear distress, these should be removed see Section C) 3. & 4. for details.**

      o   Add the litter to the database:
           i.   Enter Birth Date.
           ii.   Enter number of pups under '#Born'.
           iii.   Edit the fate for any pups found dead or culled sick.

      o   Create a cohort for the litter.

7.   **If litter is to be phenotyped and culled at P0:**

      o   Add '**Neonate Daily Checks P0-P9**' procedure to litter on the database.

      o   Print a new cage card and return cage to the rack.
           Attach a blue tag to the cage card (small side up) to indicate there are pups in the cage.

      o   Email relevant staff to update.

8.   **If litter is to be phenotyped at P0 and returned to Dam:**

- o Create a '**Neonatal Screen Daily Checks**' DCF.

- o Start and fill in the DCF for each mouse.
    - i. Starting the DCF adds a regulated procedure to the mice.
        The DCF must be filled in for all mice (including those found dead).
        Fill in 'Gestation Period' and 'P0 Morning'/'P0 Afternoon' as applicable.
        If litter born after 12pm, 'P0 Morning' = 'n/a'.
        Note any abnormalities in the 'Life Status Comment' field.
    - ii. When all mice are filled in, click 'Save' and review table.

- o Print a new cage card and return cage to the rack.
    Attach a blue tag to the cage card (small side up) to indicate there are pups in the cage.

- o Email relevant staff to update.

B) Existing Litters

1. Locate cages in correct animal holding room.

2. Transfer first cage to a change station, open cage, and check litter.
    - i. Aiming for minimal disturbance, move bedding to see the full nest.
    - ii. Count the number of pups.
    - iii. If there are fewer pups in the nest than expected, attempt to identify which pups are missing using the toe clips of the remaining littermates (see **Section C) 1.**).
    - iv. If there are more pups in the nest than expected, the new pups should be identified, cohorted, and be added to a DCF (see **Section C) 2.**).
    - v. Perform a quick, visual assessment of pup welfare (see **Section E) 1.**).
    - vi. **If any pups are dead, or in clear distress, these should be removed.**
        See Section C) 3. & 4. for details.

3. Update Relevant DCF
    - i. For each pup update the relevant time point field to 'Alive', 'Missing', or 'Dead'.
        Note any abnormalities in the 'Life Status Comment' field.
    - ii. On a Monday am check update the weekend time points as 'Not Done – Weekend'.

4. **For all morning checks on existing litters:**
    - i. Manually add '**Neonate Daily Checks P0-P9**' procedure to the litter.
    - ii. Print a new cage card and return cage to the rack.
    - iii. Ensure a blue tag is attached to the cage card (small side up).
    - iv. Email neonate_pregnant_update@sanger.ac.uk to update.

5. **For all afternoon checks on existing litters:**
    - i. Sign and date the cage card to indicate that an afternoon check has been performed.

6. **For Monday pm and Friday pm checks on existing litters:**

- o Weigh the pups
    - i. Set up a set of weighing scales in a change station.
    - ii. Seed weighing pot with bedding from the home cage (being careful not to disturb the nest too much).
    - iii. Remove one pup from the cage at a time.
        Identify the pup using its toe clip.
        Place pup onto weighing scales & record weight.
        Return pup to the nest.

    iv.   Repeat until all pups have been weighed.

    v.   Return bedding to cage and cage to home rack.

- o Add weights to the database
    - i.   Add weight measurements for each pup.
    - ii.   As each weight is entered, check the Latest Weight value for each pup to identify any pups whose weight has decreased.
        - **If a pup's weight has decreased from the last weight, this is a health concern.**
        Add health concern to the database & card to the cage.
        Email relevant staff to update.

C) Troubleshooting Section: Neonates

1. **If the number of pups is <u>less than expected</u>:**
    - i.   Identify missing pup using toe clips of remaining pups.
    - ii.   Cull pup on the database, recording 'Fate' as 'Missing'.
    - iii.   Update and complete **relevant** DCF.
    Record the pup's fate as 'Missing' in the relevant time-point field.
    - iv.   Do <u>not</u> add a '**Neonate Daily Checks P0-P9**' procedure to a missing pup.
    - v.   Print new cage card, and return cage to home rack.
    - vi.   Email relevant staff to update.

2. **If the number of pups is <u>more than expected</u>:**

    - o Identify new pup:
        - i.   Give the previously undiscovered pup an identification by taking a toe clip.
        - ii.   Place the toe clip sample in a genotyping plate and label the plate with the 'Mouse ID' and 'Toe'.
        - iii.   Seal the plate and store it in a freezer.
        - iv.   Create a genotyping list on the database for this plate.
        Print off the genotyping sheet and store it with the plate in the freezer.

    - o Create database record for new pup:
        - i.   'Edit Litter' and update.
        - ii.   Add pup to cohort.
        - iii.   Create new DCF for new pup:
        Fill in time points for when pup was not yet discovered as 'Not Done – Other'.
        Fill in current time point as 'Alive'.
        Record when the pup was discovered in the 'Life Status Comment' field.
        - iv.   Print new cage card, and return cage to home rack.
        - v.   Email relevant staff to update.

3. **Neonates Found <u>Dead</u>:**

    - o Remove dead pup from the cage.
    If pup is not whole, remove as much as possible.

    - o **If from a <u>new litter</u>:**
        - i.   Give the pup a toe clip identification starting at 1.a).
        Keep the toe clip sample for genotyping.
        - ii.   Cull pup on the database, recording 'Fate' as 'Found Dead'.
        - iii.   If litter was due to be culled at P0 add '**Neonate Daily Checks P0-P9**' procedure.

iv. If litter was due to be culled after P0, create a **'Neonatal Screen Daily Checks'** DCF, recording the current time point as 'Found Dead (am)' or 'Found Dead (pm)'.

- o **If from an existing litter:**
  - i. Identify pup using toe clipping.
  - ii. Cull pup on the database, recording 'Fate' as 'Found Dead'.
  - iii. Update the **'Neonatal Screen Daily Checks'** DCF, recording current time point as 'Dead'.
  - iv. If pup is at P2 or older, take an ear sample for genotyping.

- o Perform a Neonate Assessment on the dead pup:
  - i. Ensure dead pups are listed as dead on the database before creating DCF.
  - ii. Create a '**Neonatal Screen P0-P3'** or '**Neonatal Screen P4-P7**' DCF for the pup (depending on pup age).
  - iii. Perform as much of a Neonate Assessment as possible – dysmorphology comments, weights, lengths – as per SOP0188 or SOP0189 (depending on pup age).
  - iv. If pup is not intact and this impairs a measurement then do not take the impaired measurement.
    For example: if the head is missing, do not measure Crown to Rump length or Weight.

- o Clearly label any genotyping plates with the litter ID and tissue type (toe and tail for P0 litters, ear for P4 and older).
  Store genotyping plates in a freezer.

- o Place dead pup in a 60ml formalin pot.
  Label the lid with the pup ID.
  Store the pot in a chemical cupboard.

- o Print a new cage card, and return cage to home rack.

- o Email relevant staff to update.

4. Neonates in Clear Distress:

If during visual assessment of the pups, there are any moderate or substantial signs of stress (**see Section E**) they should be culled as soon as possible. **Pups that are immobile, unresponsive, or cold to touch should be culled immediately**. If you are unsure, please contact the NACWO for advice.

For neonates in **Moderate** distress: Phenotype
  - i. Email relevant staff to update.
  - ii. Add relevant health concerns to the database.
  - iii. Phenotype as soon as possible as per SOP0188 or SOP0189 (depending on pup age).
  - iv. Check mouse every hour until phenotyping can be performed.
  - v. Cull after phenotyping (see below).

For neonates in **Substantial** distress: Cull
  - i. Prepare a 0.5ml syringe with 0.05ml Pentobarbital.
  - ii. Remove pup from the cage and administer Pentobarbital by IP injection.
  - iii. Wait for onset of rigor mortis, indicated by a drop in temperature, a change in skin colouration, complete lack of movement, and rigidity of tail and limbs.
  - iv. Add relevant health concerns to the database.
  - v. Cull pup on the database, recording 'Fate' as 'Culled Sick'.

vi.   Proceed from here as for a found dead pup (see **Section C) 3.**).

D)   Troubleshooting Section: Dams

If at any point pups are found dead, or in clear distress, these should be removed
See **Section C) 3.** for details.

P0 phenotyping will not be performed if Dam is still in the process of littering down.
If Dam has not littered down by 2.30pm, she will be left and phenotyping will occur the next day.
In this eventuality, neonates will be phenotyped as a mixture of P0 and P1 once the whole litter has been delivered.

1.   **Dam is <u>part-way</u> through littering down <u>during an am Check</u>:**
      i.   With minimal disturbance to the cage, attempt to spot any dead pups. If any are found, remove them and refer to **Section C) 3.**
      ii.   Leave the dam to continue littering.
      iii.   Attach a blue tag to the cage card (small side up).
      iv.   Email relevant staff to update.
      v.   Check cage again in 2 hours.
      vi.   Proceed with counting and recording pups only once the dam is finished littering.

2.   **Dam is <u>part-way</u> through littering down <u>during a pm Check</u>:**
      i.   With minimal disturbance to the cage, attempt to spot any dead pups.
           If any are found, remove them and refer to **Section C) 3.**
      ii.   Leave the dam to continue littering. Phenotyping will be rearranged to take place the following day.
      iii.   Attach a blue tag to the cage card (small side up).
      iv.   Email relevant staff to update.
      v.   Check cage again at the end of the day.
      vi.   Proceed with counting and recording pups only once the dam is finished littering.

3.   **Dam is <u>struggling</u> during littering down <u>during an am Check</u>:**
      i.   Ideally, let the Dam try to complete birthing.
      ii.   Perform a visual welfare assessment of dam.
           Add a health concern to the dam on the database & a card to the cage.
           Routinely check dam every hour.
      iii.   Email relevant staff to update progress.
      iv.   If **Dam exhibits any moderate signs of pain/distress over and above what is expected during littering difficulties** (see **Section E) 2.**), she should be **culled immediately**. If in doubt, check with a NACWO.
      v.   If dam needs to be culled see **Section D) 5.** below.

4.   **Dam is <u>struggling</u> during littering down <u>during a pm Check</u>:**
      i.   Perform a visual welfare assessment of dam.
           Add a health concern to the dam on the database & a card to the cage.
      ii.   **If Dam exhibits 3 or more mild signs, or ANY moderate signs of pain/distress,** (see **Section E) 2.**), she should be **culled** rather than being left overnight. If in doubt, check with a NACWO.
      iii.   If dam needs to be culled see **Section D) 5.** below.

5.   **If <u>dam needs to be culled</u> before littering down completely*:***
      i.   Remove Dam from the cage, cull by cervical dislocation, followed by removal of the heart.
      ii.   Open up the dam, to identify if there any pups still in the uterus.
           Remove the uterus and dissect out pups.
      iii.   If pups are dead, process as for a found dead pup (see **Section C) 3.**).

  iv. If pups are alive, cull pups by overdose of 0.05ml Pentobarbital by IP injection, and process as for a found dead pup (see **Section C) 3.**).

  v. Cull Dam on the database, recording 'Fate' as 'Culled Sick'.
Make sure the relevant health concern is added on the database.

  vi. <u>Any pups that have already been born</u> can be phenotyped and then terminally collected, as in SOP0188.

  vii. Email relevant staff to update.

The animal carcass must be disposed of in a cadaver waste bag, sealed and put in a freezer. Clean dissection tools and board with antibacterial solution.

6. **If a Dam with an <u>existing litter</u> is in <u>clear distress</u>:**

  If Dam exhibits 3 or more mild signs, or ANY moderate signs of pain/distress (see **Section E) 2.**), **she should be culled immediately.** If in doubt, check with a NACWO.

  i. Cull Dam by cervical dislocation, followed by removal of the heart.
Cull Dam on the database as 'Culled Sick'.
Make sure the relevant health concerns are added on the database.

  ii. Phenotype and then terminally collect pups as stated in SOP0188 or SOP0189.

  iii. Email relevant staff to update.

The animal carcass must be disposed of in a cadaver waste bag, sealed and put in a freezer. Clean dissection tools and board with antibacterial solution.

E) **Welfare Assessment Criteria**

  If any dam exhibits 3 or more mild signs, or a moderate sign of pain/distress, she should be culled immediately.

  Pups that are immobile, unresponsive, or cold to touch should be culled immediately.

  If in doubt, please check with a NACWO.

1. **For Neonates:**

Mild (observe progress)	Moderate (phenotype and cull)	Substantial (cull immediately)
Growth retarded Reduced weight gain Consistent lack of milk spot (<P4)	Sustained weight loss Sustained signs of dehydration	Weight loss > 20%
No gross dsymorphologies	Kyphosis (abnormal body curvature) Abnormal craniofacial morphology	Abnormal brain morphology Omphalocele (Herniation of intestines)
Normal skin colouration Normal respiration Partial Piloerection (>P4)	Cyanosis, with response to stimulus Intermittent abnormal breathing Marked piloerection (>P4)	Cyanosis, without response to stimulus Laboured respiration Marked piloerection (>P4) with signs of dehydration

2. **For Dams: See table below**

Appendix 6: **Pain and Distress in Laboratory Rodents: Guidelines for determining humane endpoints in protocols**

Mild	Moderate	Substantial
• Reduced weight gain • Food and water consumption 40-75% of normal for 72 hours	• Weight loss of up to 20% • Food and water consumption less than 40% of normal for 72 hours	• Weight loss greater than 25% • Food and water consumption less than 40% for 7 days, or anorexia (total inappetence) for 72 hours
• Partial Piloerection	• Staring coat – marked piloerection	• Staring coat – marked piloerection with other signs of dehydration such as skin tenting
• Subdued but responsive, animal shows normal provoked patterns of behaviour • Interacts with Peers • Hunched transiently especially after dosing • Transient vocalization	• Subdued animal shows subdued behaviour patterns even when provoked. • Little peer interaction • Hunched intermittently • Intermittent – vocalization when provoked	• Unresponsive to extraneous activity and provocation • Hunched persistently • 'Distressed' – vocalization unprovoked
• Oculo-nasal discharge transient • (typically signs of chromorhino-dacryorrhoea in rodents) • Normal respiration	• Oculo-nasal discharge persistent • Intermittent abnormal breathing pattern	• Oculo-nasel discharge-persistent and copious • Laboured respiration
• Transient tremors • No convulsions • No prostration • No self mutilation	• Intermittent tremors • Intermittent convulsions • Transient prostration (less than 1 hour) • No self mutilation	• Persistent tremors • Persistent convulsions • Prolonged prostration (more than 1 hour) • Self mutilation