UiO : University of Oslo
Institute of Basic Medical Sciences, Section of Comparative Medicine

Standard operation procedure: Rederivation of rats by caesarean section
SOP nbr: 15-03
Created: 10.09.2014 Created by: Mikael Vestberg
Revised: 08.07.2021 Revised by: Frøydis Kilmer, Katarzyna Joanna Zelewska
Valid to: 08.07.2023 Approved by: Espen Engh

REDERIVATION OF RATS BY CAESAREAN SECTION

1.0 PURPOSE

1.1 Rederivation by caesarean section is used to purify one or several unique lines shown to contain undesirable pathogens.
1.2 To ensure that all phases of the procedure are well planned.

2.0 DIVISION OF RESPONSIBILITY

2.1 The Head of Department is responsible for the procedure, while coordination can either be carried out by the Head of Department or by another person appointed by the Head.
2.2 The person in charge of coordination must, together with all other involved parties, draw up a written plan in good time before the caesarean is to be performed.
2.3 All those involved must be familiar with their role in the procedure and must make sure that each stage is carried out according to the plan.

3.0 PROCEDURE

Preparations prior to performing the caesarean section

3.1 See “SOP 15-02 Preparing for rederivation of rats by caesarean section».
3.2 One or more caesarean sections are performed on the same day.
3.3 Three days before the scheduled birth date, the donor female is injected with Depo-Provera s.c. in order to postpone the birth. If a donor female gives birth unexpectedly, a new rederivation plan must be drawn up.
3.4 The foster mother should give birth on day 19 or on one of the immediately following days. Daily monitoring must be carried out to check for births and that everything is in order. Register the birth(s) in Science Linker (SL) and inform all those involved.
3.5 A caesarean section is performed on the donor female on the day after the foster mother has given birth.
3.6 In the dirty housing room: update the form listing the females to be rederivated (see appendix 1) and then inform all involved parties that this has been done. Make sure that Virkon and extra 900-cages are available.

3.7 In the corridor: make sure that a trolley with lab coats and Virkon are available.

3.8 In the lab: make sure that the CO_2-apparatus is in working order. All autoclaved equipment must be prepared no later than one day before the caesarean section is to be performed. Prepare three workstations. Station 1: bench with CO_2- apparatus, a lab coat used only for the handling of dirty animals, gloves and the bottom of empty 900-cages for disinfecting the females. Station 2: one set of sterile surgical instruments for skin and peritoneum, two pairs of scissors (one large and one small/two small) and two pairs of forceps (one surgical and one anatomical). Station 3: one set of surgical instruments for the womb, a small pair of scissors and two pairs of forceps (one surgical and one anatomical). Mandatory equipment: Virkon, nitrile gloves, the bottom of an empty 900-cage, two sets of surgical instruments, sterile gloves, sterile aprons/lab coats, sterile matting, heat pad, sterile water, glass bead sterilizer with glass beads, the bottom of a 500-cage for transporting pups. A disinfected heat pad must be placed under sterile autoclave paper. Print out the list of females to be rederivated (see appendix 1) and place the list in the lab.

3.9 Clean housing room: print out the list of females to be rederivated (see appendix 1).

CAESAREAN SECTION

Dirty housing room

3.10 Follow the order of females to be rederivated (see appendix 1). Label the cage where the donor female is to be housed with the number and line of the donor female in question. Place the female in the cage and disinfect the outside of the cage with Virkon. Inform person 2 when the female is ready for despatch.

Lab

3.11 Label the 500-cage with the number and line of the female on which the caesarean section is to be performed - see appendix 1.

3.12 Prepare hot/lukewarm Virkon (ca. 40 degrees) in the bottom of an empty 900-cage (for disinfecting the donor female) and in the bottom of an empty 500-cage (for disinfecting the womb). One tablet of Virkon to be dissolved in 500 ml of water.

3.13 Person 3 must be correctly clothed to receive the donor female: a separate lab coat for handling dirty animals, long inner gloves and nitrile outer gloves.

3.14 Person 2 receives the donor female from person 1 and delivers her to person 3.

3.15 Person 3 places the donor female in the CO_2 chamber. Terminate the female with a neck pull immediately after she has stopped breathing. The female is then placed on the bottom of a 900-cage which has been prepared with Virkon. The female must be completely covered with fluid. Allow to the Virkon to work for at least 30 seconds. Clear away the dirty cage. Remove the lab coat and both layers of gloves.

3.16 Person 3 and person 4 must then be ready and correctly clothed (autoclaved lab coat and sterile gloves) for the clean phase of the procedure.

3.17 Person 3 takes the donor female out of the bottom of the 900-cage and places her on station 1. Cut and pull aside as much of the skin as possible. Cut the peritoneum with the next pair of scissors and

remove the uterus by hand. Extract and place the uterus on the bottom of a 500-cage which has been prepared with Virkon.

3.18 Person 4: use a fresh set of surgical instruments. Pick up the uterus, make an incision between two fetuses and push the fetuses out of the uterus. Squeeze the fetuses out of the placenta and remove the placenta. Roll the fetuses in paper and dry their nose and mouth with a Q-tip.

3.19 Maintain room temperature by keeping the fetuses in your hand. Massage all the pups until they are breathing well and are pink in colour (may take up to 15 min). Any pups that are not breathing can be given a little drop of Dopram in the mouth (preferably placed under the tongue). Place the pups in the labelled 500-cage lined with paper.

3.20 Person 2, correctly attired in clean clothes/lab coat and disinfected gloves, stands ready outside the lab to receive the pups. Person 2 gives the cage containing the pups to person 5 in the clean housing room.

3.21 The workbench and equipment must be cleaned and disinfected between operating on each donor female. Surgical equipment must be sterilized in the glass bead sterilizer.

3.22 The lab must be tidied and cleaned after the last caesarean section has been performed. Remove the labelling on the 900-cages with 70% ethanol before placing the cages in the washroom.

Clean housing room

NB! Some of the procedures described below can be carried out shortly before the young arrive.

3.23 All the procedures performed in the clean housing room must be carried out by person 5.

3.24 Position the cage containing the foster mother.

3.25 Remove the fetuses from the foster mother so that there are a maximum of three left. Put the remaining pups aside in a cage with litter/paper. Do not terminate these until the procedure has been completed (no blood odour during the procedure!)

3.26 Person 2 moves the pups from the donor female from the lab to person 5 in the clean housing room.

3.27 Person 5 places the young on the LAF bench. Extract the three remaining young from the foster mother and place them in the cage with the rederivated young. Make sure that they are well mixed with dirty litter and paper soiled with urine and faeces from the foster mother's cage.

3.28 Put all the young back in the cage (apart from those set aside). Do not give the animals a new cage since this will stress the foster mother! Cut the tip of the tail of the foster mother's three young if the latter and the donor female's young have the same colour.

3.29 Place the cage in the rack, label the cage with the number and line and cover with blue paper. Register the colour of the foster mother's own young and the donor female's young in SL.

3.30 Terminate the remaining pups from the foster mother once the whole procedure has been completed.

3.31 Update the form (see appendix 1). Register the litter from the donor female and transfer the litter to the foster mother in SL. Register the donor female as terminated in SL.

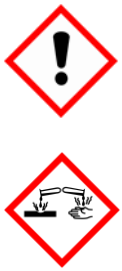
3.32 Remove the labels on the 500-cages with 70% ethanol before putting them in the washroom.

Follow-up and health monitoring

3.33 Leave the cage containing the rederivated pups undisturbed for three days.
3.34 Check the number and gender of the rederivated pups and register this under «Notes» in SL.
3.35 The foster mother's own pups can be terminated after a week if this is considered necessary due to a lack of space.
3.36 Monitor the health of the animals according to the plan drawn up by the PMSK. Do not start monitoring the health of the young until at least two weeks after the birth.
3.37 Take biopsies of the pups after separation and send these to the user.
3.38 The foster mother and the foster mother's own pups are terminated after separation of the rederivated pups.
3.39 Inform the users of the results of the health monitoring.
3.40 Clean animals can be returned to the housing room for rats once the room has been emptied and sanitized.

4.0 HEALTH, ENVIRONMENT AND SAFETY (HSE)

4.1 Everyone must have undergone sufficient training to ensure the correct use of clothing and protective equipment.
4.2 Everyone involved must have undergone sufficient training and practice to ensure that animals and equipment are handled correctly.
4.3 The work must be carried out on a ventilated worktop or LAF-bench in order to minimise exposure to allergens and agents and to limit the spread of such to the surroundings.

Rely+On Virkon Tablets	**CAS no.**	**Pictogram**	**Hazard statements**	**Precautionary statements**
Pentakalium-bis(peroksymonosulphate)-bis(sulphate) Malic acid Sulphamidic acid Sodium dodecylbenzensulphonate Dipotassium peroxodisulphate	70693-62-8 6915-15-7 5329-14-6 25155-30-0 7727-21-1		H315: Skin irritant. H318: Causes serious eye damage. H335: May cause respiratory irritation. H412: Harmful to aquatic life with long-lasting effects. EUH 208: Contains Dipotassium peroxodisulphate, which may produce an	P102: Keep out of the reach of children. P273: Avoid escape into the environment. P280: Use protective gloves/clothing/eye and face protection. P305+P351+P338: IF IN CONTACT WITH EYES: Rinse carefully with water for several minutes. Remove contact lenses if used

			allergic reaction.	and easy to do. Continue rinsing. P310: Contact a POISON CENTRE immediately or a doctor. P501: Dispose of contents/container in accordance with local, regional and national regulations.

Ethanol	**CAS no.**	**Pictogram**	**Hazard statements**	**Precautionary statements**
Ethanol	64-17-5		H225: Highly flammable liquid and vapour. H319: Causes severe eye irritation.	P210: Keep away from heat sources, hot surfaces, sparks, open flames and other sources of ignition. No smoking. P280: Use protective gloves/clothing/eye and face protection. P305+P351+P338: IF IN CONTACT WITH EYES: Rinse carefully with water for several minutes. Remove contact lenses if used and easy to do. Continue rinsing. P370 + P378 In case of fire: Extinguish with carbon dioxide (CO2), foam, powder or water. P403+P235: Store in a cool, well-ventilated place. P501: Dispose of contents/container in accordance with local, regional and national regulations.

5.0 EQUIPMENT AND MAINTENANCE

5.1 Dirty housing room

- Depo-Provera for injecting 3 days before the expected birth date
- Virkon
- Extra number of 900-cages

5.2 Lab

- CO_2- apparatus
- Nitril gloves
- Bottom of an empty 900-cage
- Instrument set 1: 2 pairs of scissors (one small and one large/two small pairs) and 2 forceps (one surgical and one anatomical)
- Instrument set 2: one small pair of scissors and 2 forceps (one surgical and one anatomical)
- Sterile aprons/lab coats
- Sterile gloves
- Disinfected heat pad
- Sterile matting/autoclaved paper for covering the bench top and heat pad
- Sterile water
- Glass bead sterilizer with glass beads
- Virkon
- Bottom of an empty GM500-cage
- Sterile paper towelling (autoclaved blue paper)
- Sterile Q-tips
- Dopram in pipettes
- 500-cage for transporting pups

5.3 Clean housing room

- Paper for covering cages

6.0 HISTORY AND EDITING

6.1 10.09.2014: Written by Mikael Vestberg

6.2 27.05.2021: Frøydis Kilmer, Katarzyna Joanna Zelewska

7.0 REFERENCES

7.1 Regulations governing the use of animals in scientific experiments, last amended FOR-2010-08-06-1147

7.2 APPENDIX A to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. Guidelines for accommodation and care of animals

7.3 DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2010 on the protection of animals used for scientific purposes

Appendix: List of lines for rederivation

Number	Line	Cage ID, donor female	Position of cage, donor female	Caesarean performed (date)	Number of pups	Cage ID, foster mother	Position of cage, foster mother
1	Example 1						
2	Example 1						
3	Example 1						
4	Example 2						
5	Example 2						
6	Example 2						
7	Example 3						
8	Example 3						
9	Example 3						
11	Example 4						
12	Example 4						
13	Example 4						