EMMA SOPs - Production of Germ-Free Mice

SOP for Generating Germ Free (Axenic) Mice Using Caesarean Section Rederivation

1. Purpose

To describe the process used to generate germ free mice using caesarean section rederivation.

2. Scope

All personnel working with germ free conversion.

3. Content:

Equipment

Sterile isolator and set up for rearing germ-free mice.
Transfer chamber compatible with the isolator.
Autoclaved water inside the transfer chamber.
Surgical equipment.
Medroxyprogesterone acetate, 150 mg/ml, Pfizer
Virkon S, 1% solution, Room temperature, Antec Int. Ltd.

Method

Ensure availability of isolator reared, germ free surrogate mother with newborn pups (<5 days old) at day **19** of procedure (see below).

Day -2 - Set up the relevant mating of foster strain inside the recipient isolator. (Usually on Sundays)

Day -1 - Check for mating plugs inside the isolator and identify the foster females.

Day 0 - Check for mating plugs inside the isolator and identify the foster females. If more than two plugs between day -1 and day 0, set up the relevant mating of mouse strain to be converted to germ free status. (Usually on Wednesdays)

Day 1 - Check for mating plugs inside and outside the isolator. Identify the foster and

donor female(s) for the experiment.

Day 2 - Check for mating plugs inside and outside the isolator. Identify the foster and donor female(s) for the experiment. Separate females from males (if some remain without plug), inside the isolator.

Day 3 - Check for mating plugs outside the isolator. Identify the donor female(s) for the experiment. Separate females from males (if some remain without plug), from the strain to be converted to germ free.

Day 18 - Check pregnancies inside and outside the isolators. Give pregnant donor female(s) from day 1, at 17.5 days post coitus (dpc), a subcutaneous injection of medroxyprogesterone acetate (5 mg/0.1 ml).

Day 19 - Carefully following the SOP for isolator entry procedures, transfer the sterile instruments and supplies required for surgery into the isolator in which the surrogate female(s) are housed.

Prepare the hysterectomy suite/surgical transfer chamber: fill up the reservoir with 1% Virkon S, sterilize the surgical compartment and ventilate it overnight.

Give pregnant donor female(s) from day 2, at 17.5dpc, a subcutaneous injection of medroxyprogesterone acetate (5 mg/0.1 ml).

Day 20 - Give pregnant donor female(s) from day 3, at 17.5dpc, a subcutaneous injection of medroxyprogesterone acetate (5 mg/0.1 ml)

Transfer water, paper towels and surgical instruments from the isolator to the sterilized compartment of the transfer chamber.

Working in the non-sterile compartment of the surgical transfer chamber or the place where the animals are allocated, sacrifice the donor female by cervical dislocation and submerge the whole animal in the 1% Virkon S solution for **1 minute**. Use sterile scissors to open the abdomen. Clamp the top of each uterine horn and the base of the uterus close to the cervix, with Mosquito scissors. Cut out the ‘uterine package’ and place it in the transfer chamber reservoir filled with 1% Virkon S for **1 minute**. This procedure can be performed for a max of 2 females at the same time.

Inside the sterile compartment of the transfer chamber rinse the ‘uterine package’ with sterile water to remove the Virkon S (200ml minimal volume of water). On top of a heating pad at 37ºC, open the ‘uterine package’ with scissors and take out the pups. Do not cut the umbilical cord!!! After removing the pup from the placenta

gently pull the umbilical cord with your forceps. Stimulate breathing of the pups while cleaning them with dry paper towel. When pups are breathing normally and have gained a 'healthy' skin color, transfer them to the isolator housing the foster mother. Gently rub the pups with bedding material from the foster mother's cage. Leave them mixed with the bedding 1 or 2 minutes. Remove some of the original pups so that the foster mother has the same number of pups to feed. If some pups from the foster mother remain in the cage, mix the adopted ones with them (clean the bedding). Check for adoption not earlier than 24 hours after transfer.

Monitor a microbiological status of the isolator and the animals it houses 3 weeks after transfer.

Day 21/22 - repeat step 20 for pregnant donor females of day 2 and 3, if necessary.

NOTES:

- All strains re-derived and maintained in SPF (FELASA recommendations) before transfer to isolator.
- Germ-free foster females (C3H/HeN) usually deliver the pups at 20,5dpc.
- Our SPF strains deliver the pups at 19,5dpc.
- After removal of the uterine package from donor females, pups survive inside the uterus for a few minutes.
- Not removing the foster mother from her cage decreases the stress and increases the success of adoption.
- Removal from original litter of the same number of pups that will be introduced, except if the same color coat (remove all). If necessary, they could adopt 2 or 3 pups more than the number of the original litter.
- Best adoption efficiency when two foster mothers are kept together and when performed up to three days after delivery of foster mother.