



EMMA protocol - Production of Germ-Free Mice

Protocol 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
- Autoclave with validated programs for the sterilization of supplies and drinking water inside the transfer chamber
- Surgical equipment
- Luteolil Vet, 50 mg/ml, 0.1 ml, room temperature, CeVa, Holland
- VirkonS, 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 0

Set up the relevant mating of mouse strain to be converted to germ-free status.

Day 1

Check for mating plug and identify the donor female(s) for the experiment.

Day 17

Give pregnant donor female(s) a subcutaneous injection of Promon (5 mg/0.1



ml).

Day 18

Carefully following the protocol 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% VirkonS, sterilize the surgical compartment and ventilate it overnight.

Day 20

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; sacrifice the donor female by cervical dislocation and submerge the whole animal in the 1% VirkonS solution for 1 minute. Use sterile scissors to open the abdomen, wear sterile gloves. Clamp the top of each uterine horn and the base of the uterus close to the cervix. Cut out the 'uterine package' and place it in the transfer chamber reservoir filled with 1% VirkonS for 1 min.

Inside the sterile compartment of the transfer chamber rinse the 'uterine package' with sterile water to remove the VirkonS. Open the 'uterine package' with scissors and take out the pups. 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 surrogate mother. Take out her own litter and mix pups from both litters outside the cage. Remove some of the original pups so that the surrogate mother has the same number of pups to feed. Gently rub the pups with bedding material from the surrogate mother's cage soaked in water. Put the mixed litter together with the foster mother. 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.