

Introduction

Experimental studies on germ free animals in most institutions are typically performed in sterile isolators. These studies are often short term (2 – 12 weeks) and usually only utilize a small number of cages (2 – 6). For the facilities this means breaking down and recycling several isolators a month resulting in increased labor for the facility staff and ‘down time’ for investigators. The process of breaking down, recycling and testing is time consuming and can take up to 3 weeks. Not only does this process affect investigator’s ability to start their next experiment, it requires a good deal of a technician’s time, which could be spent in other ways. With so many negatives associated with this process, we decided to see if we could maintain germ free animals on a conventional mouse rack.

Objective

Could we maintain germ free animals on a Opti Mouse® caging system rack.

Opti Mouse Rack®



Methods

All cages, bottles with stoppers and animal drinking water were autoclaved before being used. Feed and bedding were double irradiated and tested to ensure sterility prior to being used in the experiment. Sterile gloves and Clidox-S® were used to spray everything into the hood. Finally, there was a dedicated technician for each cage.

Irradiated Feed and Bedding



Autoclaved Setup



Methods

Supplies/Cage Change:

- * Cages were autoclaved, empty and with an empty water bottle and stopper.
- A bottle of autoclaved animal water was dedicated to each experiment.
- In addition, 2 empty autoclaved cages were used to store irradiated feed and bedding and maintained on the rack a long with the experimental cage.
- Sterile gloves were used for changing cages and were changed between cages, within a Horizontal Laminar Flow Workbench.
- The hood was sprayed and wiped with Clidox-S® 1:3:1 prior to any work commencing.
- All materials were sprayed into the hood using 1:3:1 Clidox-S®.
- Forceps were kept in a cup filled with 1:3:1 Clidox-S® solution.
- Once a cage was changed, it was put back into its original location. The hood was then sprayed down before introducing the next experimental cage.
- Each cage took approximately 15 minutes to change.

Horizontal Laminar Flow Workbench.



Germ Free Dedicated Supplies and Cage



Methods continued

Testing:

- On a weekly basis, two fecal samples were taken from each cage and cultured using agar plates under anaerobic conditions.
- Plates were checked at 24, 48 and 72 hour for any visible bacteria growth.

Setup For Doing Fecal Sampling



Technician Changing A Germ Free Cage



Discussion/Conclusion

Original study cages were maintained germ free for 16 weeks before the study ended and animals were transferred into an isolator. Additional staff members have been trained and further evaluation is ongoing. Currently, we have one study that has been germ-free for 22 weeks and another for 8 weeks. We have also had study cages that became contaminated after only a short period of time. The probable reason being that strict aseptic technique was not adhered to.

To date, investigators have not been trained to work with this system. However, we are expecting an investigator to start by the end of the year. At that time, the investigator will first be trained by our staff and then must practice and maintain sterility equal to the length of the time of their study. Once they have achieved this, they can begin their studies using caging instead of an isolator.

In conclusion, maintaining germ-free animals at the cage level is possible as long as all items are processed and introduced into the hood the same as if they were going into an isolator. It’s imperative that aseptic technique is adhered to at all times otherwise you increase your chances of a break.

The process of breaking down, recycling and testing is time consuming and can take up to several weeks. Performing experiments at the cage level will not only reduce labor for the facility staff but also ‘down time’ for investigators. At the cage level there is no loss of experimental time for investigators. Once their experiment is complete they just need to request new cages and materials which can occur the same day if needed. Processing cages is a lot less time consuming for the technicians which in return saves on labor and technician costs. Technicians can spend that time performing other responsibilities..

Acknowledgements

- Animal Care Systems, Inc. Centennial, Colorado 80112
- Scotts Distributing Inc. Hudson, NH. 03051
- Pharmical Research Laboratories, Inc. Clidox-S®
- Naugatuck, CT 06770 EPA Registration #8714-8th
- **Special Thanks To:**
 - George Isaacs
 - Michael Marcotrigiano
 - Terrance Washington
 - Trang Dang