

## **Maintenance of Parasite, Virus and Aerobic Bacteria Free Mice without the Use of a Gnotobiotic Isolator**

**ABSTRACT:** An experiment was conducted to determine if germ-free mice could be unknowingly raised outside of a gnotobiotic isolator utilizing commercially available equipment in a bioBubble clean room. Eight pairs of germ-free Swiss mice were shipped in Taconic Transit Containers to Dr. Rivard in Colorado. Dr. Rivard, attempting to demonstrate his systems effectiveness, was not made aware of the animals' germ-free status until 2 months into the project. Four pairs were housed in the vented M.I.C.E.<sup>TM</sup> caging system with solid bottoms and the other four pairs in cages with a perforated floor and nest box. Bedding was changed every two weeks and cages monthly. Cages were sprayed with Clidox prior to use and bedding was microwaved. The animals were provided with irradiated feed ad libitum and 'structured matrix' purified water. All procedures were performed in a changing station. The six-month study required health monitoring at two month intervals. The test results demonstrated that the animals maintained in solid bottom cages were free of parasites, viruses and aerobic bacteria.

**INTRODUCTION:** This shipment group comprises an evaluative sample of the 2<sup>nd</sup> lot of Taconic mice held for microbial surveillance within vented racks at Animal Care Systems (ACS), Littleton, CO. As with the 1<sup>st</sup> group, Germfree Swiss Webster mice were sent from the breeder as a trial for their maintenance in the vented rack system manufactured by ACS.

One each albino male and female adult and a large number of albino weanling mice were received for microbiologic evaluation as an assessment of the range and variety of organisms they may have acquired while maintained at ACS. Of this group, one adult mouse and three weanlings were subjected to the examinations indicated below. The remainder of the mice were euthanized and discarded.

The examined animals were euthanized and blood samples collected (for serum). The mice were then subjected to necropsy dissection and gross observation. Key tissues were collected in formalin. The serum and tissues were collected and stored, but not further processed. Depending on the interest level of the results below, these latter specimens could either be discarded or processed for viral antibody status and microscopic lesions, respectively.

**MICROBIOLOGIC EVALUATION:** Serology results were negative for the following viruses: MVM, MPV, PVM, REO3, MHV, KV, GD7, SEN, LCM, MAV, ECTR, POLY, MYCO, EDIM, MCMV, CARB, HAN, ECUN, CPIL (ELISA); LDHV (CHEM), and MTV, CKUT (IFA).

Serology results detected no aerobic microbial forms from nasopharyngeal, fecal, and oropharyngeal cultures. No helminths and protozoa were detected in the fecal samples.

**RESULTS:** The mice were clinically normal in appearance, posture, locomotion and disposition and were without discharges from the conjunctiva, nares or rectum. The adult male (No. 1 below) weighed 49.7 g, and the weanling females (Nos. 2-4 below) had a mean weight of 15.0 g. Necropsy dissection revealed all organ systems to be normative in appearance, the tympanic bullae were without exudates and there were no otherwise noteworthy findings. Enteric parasitology revealed the mice to be free of protozoan and helminth contaminants. Standard cultures were made of nasopharyngeal washes, the oropharynx and colonic feces.

**INTERPRETATION:** The animals were in good apparent health and no aerobic bacterial forms were detected, indicating continuance of the germfree status.