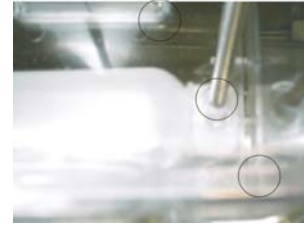


## Prevent Release of Allergens, Odors, and Infectious Agents

The M.I.C.E.<sup>®</sup> system consists of negative-pressure, vented isolation containers with enrichment structures (M.I.C.E.). It has adequate and filtered ventilation that assures microenvironmental comfort, isolation, containment, and enrichment at cage level. The cage and rack closed-system uses barrier seals and filtered passive ventilation by convection, HVAC-assist, and direct exhaust venting airflow under negative pressure differential to protect laboratory personnel and others who come in contact with or transport animals from animal-related allergens and infectious agents. Barrier seals are at cages top, bottle, and feeder-lid junctions.

Figure 1: Barrier Seals. Contrary to a ‘petri-dish’ design, the closed-system design of the M.I.C.E. top has a U-shape groove that rests and surrounds an inversed L-shape rim at the M.I.C.E. bottom. The bottle has a cap with a specially designed outer ring that seals into a conical rim on top. The feeder-lid has a press-fit closure onto an outer rim of the cage’s top. The particulates tight construction and passive filtered vent ports prevent contaminants from escaping the microenvironment, providing protection from occupational health hazards and minimizing workers’ compensation claims associated with allergies.



Due to the closed-system design, everyday users of the vented M.I.C.E. caging system fail to detect odors emanating from both cages and racks populated with rodents. Also, rodent users who suffer from allergies testified on their ability to work again in animal facilities that use the vented M.I.C.E. caging system.

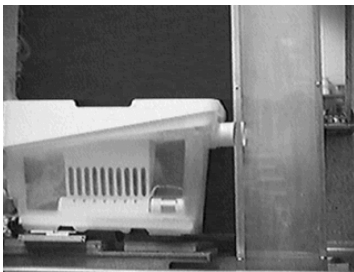


Figure 2: Smoke tests for air leakage. Visible smoke (0.3 micron) was introduced directly in the occupied cage from a smoke match at the inlet vent port. A digital camera was used to make time-lapse photographs of the smoke plume as it flowed inside and out of the cage, through its exhaust vent port, and into the chimney attached to the exhaust vent port. Smoke velocity and leakage was estimated by smoke plume observation on photographs at two frames per second. Smoke did not leak from any junctions; it was contained inside the cage and the rack. Also, low-pressure draft prevents airborne particulates from escaping the rack plenums. The exhaust plenums draw effluent evenly from each cage, anywhere on the rack.

Figure 3: Barrier filters. Filtered passive ventilation uses thermodynamics of convection, air displacement under HVAC-assist, and exhaust ventilation system under negative pressure differential at low velocity. To filter large volumes of air and maintain low velocity requires a large surface area of filter media per unit volume of air, as it is accomplished by deep pleating the media in a HEPA filter. Instead, we use a combination of stainless steel screens (charged +) and polyester media (charged -) to achieve high efficiency filtration. The filter media is a Reemay<sup>®</sup> #2024 with a proven track record. It is the same filter that has been used on filter bonnets, isolation filter-top cages and transport boxes since 1964. The filter’s combination of steel and polyester uses direct capture and inertial impaction in the filter matrix as well as gravitational settling, electrostatic attraction, and Brownian movement to optimize particle capture. As the air passes through the filter unit, inertial forces bring larger particles into contact with filter fibers and Brownian motion brings the very small particles in contact with the filter fibers. The filter unit efficiently arrests airborne particles commonly found in laboratory animal facilities. A dual filter combination at both inlet and exhaust vent ports could provide a containment protection up to biosafety level 3.



Figure 6: Biological Challenge. We conducted two biological challenges using Mouse Hepatitis Virus (MHV) contamination and exposure. The first one compared open, static isolation, and M.I.C.E. cages. Mice (Balb/c) in those three different caging systems were exposed to MHV via aerosolization. Each cages were placed in a closed chamber and sprayed with MHV. Several M.I.C.E. filters configurations were tested to find out that a single stainless steel screen of 40x40 mesh size is necessary to prevent MHV from spreading from cage-to-cage on the same rack and in the room



	+/+	0/0	+/-	
	s/s	-/-	c/c	
+/-	0/0	(SB)	-/-	+/+
	s/s	-/-	c/c	
	+/+	0/0	+/-	
(SB)	c/c	(SB)	s/s	(SB)

Figure 7: Biological Challenge. A second experiment was conducted to evaluate cross-contamination of mouse hepatitis virus (MHV) on a vented M.I.C.E. rack. Naive mice (n=72) of different MHV-susceptible strains (+/+, +/-, -/-) were inoculated with a virulent strain of MHV. Pathogen-free mice (n=60) were used as sentinels in three sealed M.I.C.E. cages (s/s), three no filters M.I.C.E. cages (0/0), three no filters with MHV-inoculated mice M.I.C.E. cages (c/c), and four filter-top shoebox cages (SB). MHV-inoculated mice had macroscopic lesions but failed to show clinical symptoms. We think the mice did not get or look as sick as they should have because of their clean environment. We sample the exhaust filters for MHV contamination and the mice for antibodies against MHV. We failed to recover any virus or antibodies. No sentinels have been contaminated except sentinels housed in filter-top shoebox cages. The dual filters combination is necessary to prevent MHV contamination from cage-to-cage on the same rack and in the room.

Also, an experiment was conducted to verify prevention of cross-contamination and to determine if germ-free mice could be raised outside of a gnotobiotic isolator utilizing M.I.C.E. system equipment in a bioBubble clean room. Pairs of germ-free Swiss mice were housed in the vented M.I.C.E. caging system. ‘Dirty’ feeder mice were housed on the same rack and adjacent to germ-free mice. Bedding was changed every two weeks in a changing station and cages monthly. Cages and bedding were disinfected prior to use. The animals were provided with irradiated feed and ‘structured matrix’ purified water. The test results demonstrated that the animals maintained in M.I.C.E. cages were free of parasites, viruses and aerobic bacteria. Also, the presence of contaminated feeder mice on the same rack proves that contamination from cage-to-cage on the same rack and in the room is prevented. Published as an abstract: Maintenance of Parasite, Virus and Aerobic Bacteria Free Mice without the Use of a Gnotobiotic Isolator.

The induced negative pressure through the M.I.C.E. cage is maintained in a plenum and a venting hose. The hose is connected to the building exhaust to providing complete evacuation of contaminants and allergens directly to outside. This type of ventilation provides a Contamination and Allergen-Free environment for the users. The closed-system design, passive filtered ventilation, HVAC-assist, and direct exhaust venting of the M.I.C.E. caging system assures in/out biological barriers while safeguarding animal and occupational health.

We are currently rating different filters combination efficiencies for particles of 0.1, 0.3, 0.5, and 1micron at the Lovelace Respiratory Research Institute, Albuquerque, NM. Please, call for preliminary data information.