

## Prevent environmental and biological cross-contamination

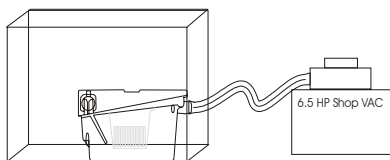
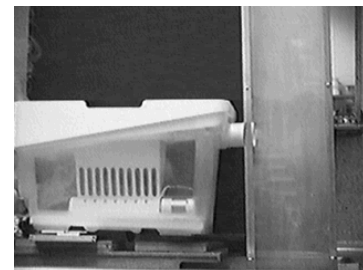
The M.I.C.E.<sup>®</sup> system uses negative pressure, vented isolation cages with integral enrichment structures. It has adequate and filtered ventilation that assures microenvironmental comfort, isolation, containment, and enrichment at the cage level. The cage and rack closed-system uses barrier seals and filtered passive ventilation by natural convection with HVAC-assisted direct exhaust venting to prevent environmental and biological cross-contamination. Barrier seals are located at cage tops, water bottle, and feeder-lid junctions (Fig. 1).

**Figure 1:** Barrier Seals. In contrast to a ‘petri-dish’ design, the closed-system design of the M.I.C.E. cage top uses a U-shaped groove that rests and surrounds the inverted L-shape rim of the cage bottom. The bottle cap contains a specially designed outer ring that seals into a conical rim in the cage top. The feeder-lid also seals to the cage top with a labyrinth U-shaped groove.



**Figure 2:** Smoke tests for air leakage. Smoke tests were used to characterize the air leakage in and out of the cage. Visible smoke is 0.3 micron in size. Air leakage from the filtered cage is determined qualitatively by visual observation. Junctions were monitored and no smoke was observed leaking from any junction.

**Figure 3:** Smoke tests for air leakage. The smoke was introduced directly into 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 through the cage, out the exhaust vent port, and into the plenum fixed to the exhaust vent port. Smoke velocity and leakage was estimated by smoke plume observation on photographs a two frames per second. Again, smoke did not leak from any junctions.



**Figure 4:** Smoke tests for air leakage. A fully assembled M.I.C.E. cage including feed, water bottle, and exhaust nozzle was placed in a sealed chamber with a volume five times the cage volume to evaluate the leakage rate under excessive negative pressure conditions. The cage exhaust nozzle was passed through the chamber walls and connected to a 6.5 HP Craftsman Shop-Vac to simulate high negative pressure conditions in the cage. Non-toxic cooled smoke (Bjornax AB, Sweden) from five smoke matches was introduced to the chamber near the cage inlet port. Observations were made over a 10-minute period of high induced negative pressure. All visible smoke (0.3 micron) was captured by the inlet filter (Reemay<sup>R</sup> 2024) and the perimeter seal of the cage top. No smoke leaked inside the cage from the top, feeder lid, water bottle, or the inlet port.

**Figure 5: Barrier filters.** Filtered ventilation uses convection thermodynamics and HVAC-assisted exhaust venting to achieve negative cage pressure at low velocity. Animal Care Systems uses a combination of stainless steel screens and polyester media (Reemay® 2024) to achieve high efficiency filtration. This media is commonly used on filter bonnets, filter-top cages, and transport boxes, and has a proven track record. Our filter assembly uses direct capture in the filter matrix as well as gravitational settling, electrostatic attraction, and Brownian movement to optimize particle capture. The filter unit efficiently arrests airborne particles commonly found in laboratory animal facilities.



Robert Matyas, DVM, of IGBMC, Strasbourg, France, conducted similar studies at an independent site. Smoke tests revealed a one-pass airflow without high velocity and dead air spaces or leaks. Particulate counts in the cage suggested that the filter unit is highly effective for particles larger than 1 micron.



**Figure 6: Biological Challenge.** Two biological challenges were conducted using Mouse Hepatitis Virus (MHV) contamination and exposure. The first compared open, static isolation and M.I.C.E. cages. Mice (Balb/c) in these three caging systems were exposed to MHV via aerosolization. Each type of cage was placed in a closed chamber and sprayed with MHV. Several M.I.C.E. filter configurations were tested, and it was noted that a single stainless steel screen of 40x40 mesh size was necessary to prevent MHV contamination in the cage.

**Figure 7: Biological Challenge.** A second experiment was conducted to evaluate cross-contamination of mouse hepatitis virus (MHV) in a vented M.I.C.E. rack. Naïve 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. Speculation was that mice showed no symptoms due to the clean environment. Exhaust filters were examined for MHV contamination. Mice were examined for MHV antibodies. None were found. Only sentinels housed in filter-top shoebox cages were contaminated.

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

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

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. Bedding was changed every two weeks in a changing station and cages were changed monthly. Cages and bedding were disinfected prior to use. Animals were provided with irradiated feed and 'structured matrix' purified water. Test results demonstrated that the animals maintained in M.I.C.E. cages were free of parasites, viruses and aerobic bacteria.

The closed-system design and passive filtered venting of the M.I.C.E. caging system assures in/out biological barriers and adequate, draft-free ventilation.