Evaluation of Exhaust Air Dust PCR Testing of the Bedding Sentinel Cage Filter

Nicole A. Monts de Oca[1], Latisha R. Gray[2], Angelina M. Williams[2], David W. Brammer[2]

[1]Charles River, Research Models, Houston, TX [2]Animal Care Operations, University of Houston, Houston, TX

1 Introduction

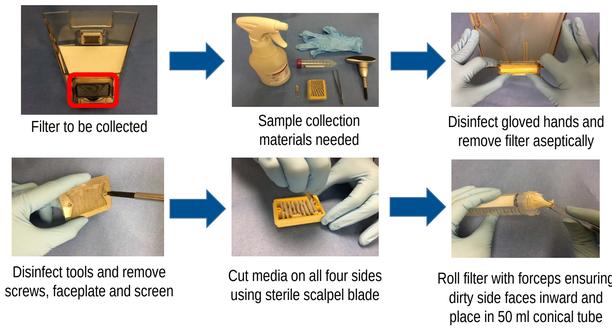
In biomedical research, rodents must remain free of adventitious pathogens in order to reduce interference with research variables or prevent the compromise of animal health. The soiled bedding sentinel method has been the gold standard for health monitoring of rodent colonies for many years. The availability of PCR technology for the testing of research animal pathogens, including bacteria, viruses and parasites, has significantly improved health monitoring programs and may reduce the limitations of live soiled bedding exposed sentinels. The high sensitivity and amplification of PCR assays allows for the detection of tiny quantities of pathogens, including environmental specimens. Based on previous reports which suggested sentinel cage filters could potentially be used to monitor an individually ventilated cage (IVC) rack, we evaluated the use of a filter sampling method and compared the test results with results obtained using traditional methods for soiled bedding sentinels. The Exhaust Air Dust (EAD) PCR testing is a newly designed, evolutionary monitoring solution that advances the standard in health monitoring. We hypothesized that this unique monitoring program would produce comparable results, reduces labor, and requires less sentinel animals than traditional rodent colony sentinel testing.

2 Materials & Methods

Design: Two bedding sentinels were placed in one sentinel cage on each of four racks. Each rack had between 70 and 95 cages and 10 cm^3 of dirty bedding from each cage on the rack were transferred weekly to the sentinel cage and to another cage on each rack containing no mice. A new filter was placed in both cages on each rack at the beginning of the study. The filters were transferred to new cages as cage cleaning and bedding transferred occurred. After 3 months, the sentinel mice were evaluated by traditional screening methods and the cage filters from the sentinel cage and from the cage with no mice were evaluated by EAD PCR testing to detect rodent pathogens. Traditional methods included bacteriology, serology and parasitology. Filters were collected using procedure described in Figure 1. All testing was performed at Research Animal Diagnostic Services, Charles River Laboratories.

Animals: Eight female (4-6 weeks old) CD-1 mice obtained from Charles River Laboratories were used as sentinels. Mouse colonies were maintained under a cage level barrier known to be positive for Murine Norovirus, Pasteurella pneumotropica-Heyl, Pasteurella pneumotropica-Jawetz, Entamoeba, Spironucleus muris and Helicobacter spp. were used as a source of infectious agents for the evaluation of transmission to bedding sentinels and to cage filter material. All mice were maintained in individually ventilated cages (OptiMice, Animal Care Systems) that were negative pressure to the room on irradiated cotton fiber bedding (Iso-PADS, Envigo); irradiated feed (LabDiet 5053) and RO water were provided ad libitum.

Figure 1: Filter sample collection method



3 Results

Figure 2: Organisms detected on IVC racks using three methods of detection

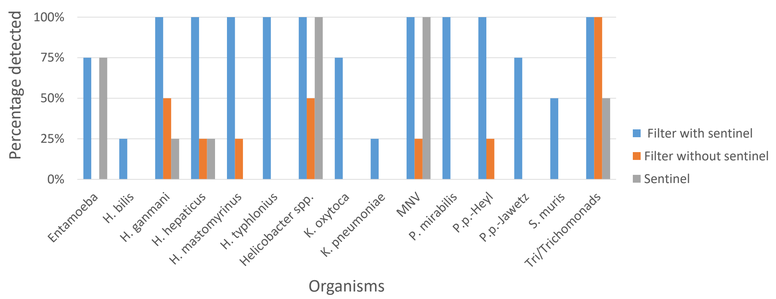
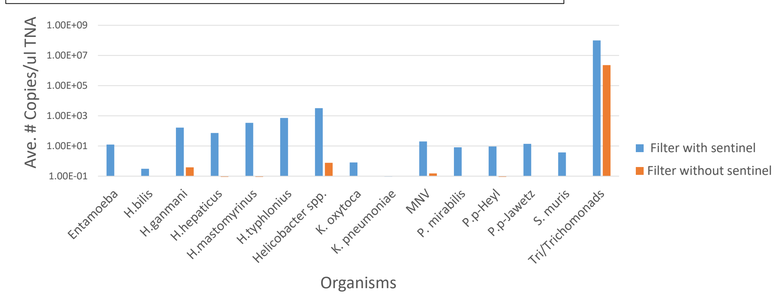


Figure 3: Average number of copies/uL TNA determined by real-time TaqMan PCR



4 Discussion

Our results demonstrated that EAD PCR on exhaust filters at the cage level was a reliable method for detecting the murine pathogens present in our colony when compared to traditional methods. MNV, Entamoeba and Helicobacter spp. were equivalently detected by both sentinel cage filter and in the sentinel mouse. Specific species of Helicobacter, Pasteurella pneumotropica, Proteus mirabilis, Klebsiella spp., Spironucleus muris, and Tritrichomonas were best detected using EAD PCR testing of the cage filter (Figure 2). However, the filter taken from the sentinel cage detected a substantially higher number of agents when compared to the filter from the cage that did not house sentinel mice. Even in the cases in which the organisms were detected in both filters, the number of copies/uL of TNA was significantly higher in the filter of the cage housing sentinel mice (Figure 3). This data supports the idea that the presence of the mice may be important to stir up material from dirty bedding.

In conclusion, as previous studies found in other IVC rack systems, testing of exhaust cage filters was shown to be a viable alternative to testing of sentinels. Further studies will have to be performed to evaluate the use of artificial methods of stirring the soiled bedding in an effort to perhaps replace the use of sentinel animals.

References

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