Validation of Exhaust Air Dust Testing Using a Prototype Plenum Attachment and Interceptor in an IVC System with Centralized Ventilation

Lise Phaneuf DVM, DVSc, DACLAM & Brianna Attanasio, BSc(Hons), RLAT
The Centre for Phenogenomics, Toronto, ON

Abstract

Exhaust air dust (EAD) testing is a relatively new technique that can be used to perform rodent health monitoring by sampling the exhaust air from ventilated rack systems. Exhaust air is sampled through swabbing plenums or using a rack sampling device (filter), and samples are tested via PCR for microbial analysis. To perform EAD testing with our IVC rack system that has centralized ventilation, a prototype plenum attachment was developed for use with the rack sampling device Interceptor (Tecniplast). Results from EAD testing were compared to our regular health monitoring program which uses both sentinel mice (serology) and direct animal sampling (PCR). Two IVC racks (80 cage maximum capacity) were fitted with the prototype plenum attachment and Interceptor to perform the EAD testing. Racks and plenum attachments were washed and autoclaved prior to use and confirmed sterile by PCR swab analysis. Racks were initially populated with cages that were changed as they were placed onto the racks. To confirm the health status of the mice on the racks, pooled samples of feces and oral and body swabs (10 random cages per rack; 1 mouse sampled per cage) were submitted for PCR analysis at the beginning of the study. Interceptor was placed into the plenum attachment on Day 0. A sentinel cage containing 2 mice was placed on each rack and exposed to 1 tablespoon of soiled bedding from each cage at the time of cage change (every 14 days). EAD testing was performed at various intervals through a 90 day period and the results were compared to our regular health monitoring program. EAD testing using Interceptor reliably identified MNV, *Helicobacter*, *Pasteurella pneumotropica* (Heyl and Jawetz), and *Tritrichomonas* as early as 7 days after exposure to colony cages. EAD testing after 90 days of exposure to colony cages was consistent with our regular health monitoring program which uses a combination of sentinel mouse serology and direct animal sampling PCR analysis. EAD testing after 90 days of exposure to colony cages appeared to be slightly more sensitive than our regular program in detecting *Pasteurella pneumotropica* (Jawetz) and *Entamoeba*.

Materials and Methods

Mice. All mice used were obtained from breeding and research colonies at TCP with a known history for MNV, *Helicobacter*, and *Pasteurella pneumotropica* (Heyl & Jawetz). Sentinel mice (female, 5 week old ELITE health status) were obtained from Charles River Laboratories. For the duration of the study, cage numbers on each of the 2 racks varied from 46 to 73 per month (Figure 2).

Animal Housing. All mice were housed in individually ventilated cages (Tecniplast GM500) on racks connected to a central HEPA filtered air supply via a delivery plenum. Ventilation was maintained at 65 to 75 air changes per hour in positive pressure mode. All cages, bedding, enrichment, wire-bar lids, and filter tops were sanitized and autoclaved prior to use. Mice were housed with a maximum density of 5 adult mice per cage under standard environmental conditions (12:12 hour light:dark cycle, 20-24˚C, 40% to 60% humidity). Bedding material consisted of ¼ inch corncob and shredded paper nesting material was provided for enrichment. Mice were fed a commercial irradiated diet ad libitum and given UV irradiated, reverse-osmosis-filtered and acidified water via an automatic system. Cages were changed every 14 days. All personnel entering the animal holding room wore dedicated scrubs and clogs, an isolation gown, gloves, head cap and surgical mask. All cage manipulations were performed in a Biological Safety Cabinet or Animal Transfer Station.

Figure 1. Two racks were fitted with a prototype plenum attachment to perform EAD testing (red arrow).

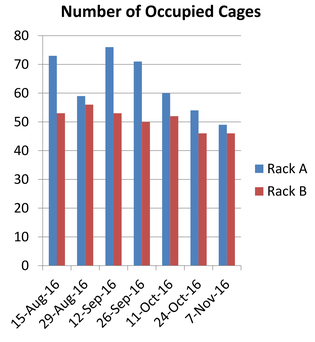

Figure 2. Number of occupied cages for each of the two racks (Rack A and Rack B) over 90 day study period.

Study Design

To compare EAD analysis with traditional health monitoring techniques, two IVC racks with 80 cage maximum capacity (Figure 1) were fitted with a prototype plenum attachment (Figure 3) to perform exhaust air dust testing using Interceptor (Figure 4). Racks and plenum attachments were sanitized and autoclaved prior to use and confirmed sterile by PCR swab analysis (Figure 5).

Figure 3. Prototype plenum attachment connected to the exhaust plenum.

Figure 4. Interceptor being placed into the plenum attachment.

Figure 5. Swabbing of rack plenum for PCR analysis to confirm sterility at beginning of study.

Racks were populated with cages that were changed as they were placed onto the racks. To determine the health status of the mice on the racks, direct animal sampling was performed using pooled samples of feces and oral and body swabs (10 random cages per rack; 1 mouse sampled per cage) submitted for PCR analysis (Table 1). The prevalence of agents on each rack was not known. Interceptor was placed into the plenum attachment of each rack on Day 0. A sentinel cage containing 2 mice was placed on each rack and exposed to 1 tablespoon of soiled bedding from each cage on the rack at the time of cage change (every 14 days).

Sentinel mice were tested using serology after 60 days and 90 days of exposure to colony cages. Pooled samples (10:1) using direct animal sampling of feces and oral and body swabs (8 random cages from colony mice and 2 samples from sentinel cage per rack; 1 mouse sampled per cage) were submitted for PCR analysis after 60 days and 90 days of exposure to colony cages (Figures 6-8). Interceptor from each rack was submitted for EAD PCR analysis after 7 days and 90 days of exposure to colony cages. A control Interceptor (not exposed to a rack) tested negative after 7 days . All testing profiles were performed by Charles River Laboratories (Table 1).

Figure 6. Body swab from colony mice for PCR analysis. Body swabs were performed on the ventral abdomen and perianal region. Oral swabs were also obtained.

Figure 7. Fecal collection from colony mice for PCR analysis.

Figure 8. Pooled samples (10:1) of feces and oral and body swabs using direct animal sampling (10 random cages per rack; 1 mouse sampled per cage) PCR analysis.

Table 1. List of agents in testing profiles for direct animal sampling PCR analysis, EAD PCR analysis, and sentinel serology. Testing was performed by Charles River Laboratories.

Direct animal sampling PCR & EAD PCR profiles:	MVM/MPV, MNV, MHV, MRV/EDIM, TMEV/GDVII, MAV-1/2, Reovirus 1-4, PVM, Sendai virus, Ectromelia, LCMV *Helicobacter, Citrobacter rodentium, Mycoplasma pulmonis, Streptobacillus moniliformis, Pasteurella pneumotropica (*Heyl & Jawetz*), Clostridium piliforme, CAR Bacillus, Pseudomonas aeruginosa, Salmonella, Campylobacter, Bordetella bronchiseptica, Bordetella hinzii, Corynebacterium kutscheri, Corynebacterium bovis, Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Klebsiella oxytoca,* Beta hemolytic *Streptococcus* group *B, C, G, Proteus mirabilis* Fur mites, Pinworms, *Giardia, Spironucleus muris, Cryptosporidium, Entamoeba, Pneumocystis, Demodex, Tritrichomonas*
Sentinel serology profile:	MVM/MPV, Generic parvovirus NS-1, MNV, MHV, MRV/EDIM, TMEV/GDVII, MAV-1/2, Reovirus, PVM, Sendai virus, Ectromelia, LCMV, *Mycoplasma pulmonis*, Mouse Pneumonitis virus, Polyoma virus

Results

Table 2. Number of racks testing positive / number of racks tested by direct animal sampling PCR analysis, EAD PCR analysis, and sentinel serology from Day 0 to Day 90.

	Day 0	Day 7	Day 60		Day 90		
	Direct Animal Sampling PCR*	EAD PCR Rack Collection Device†	Serology‡	Direct Animal Sampling PCR	EAD PCR Rack Collection Device	Serology	Direct Animal Sampling PCR
MNV	2/2	1/2	2/2	2/2	2/2	2/2	2/2
Helicobacter	2/2	2/2	-	2/2	2/2	-	2/2
P. pneumotropica Heyl	2/2	2/2	-	2/2	2/2	-	2/2
P. pneumotropica Jawetz	2/2	1/2	-	1/2	2/2	-	1/2
Entamoeba	0/2	0/2	-	0/2	2/2	-	1/2
Tritrichomonas	2/2	2/2	-	2/2	2/2	-	2/2

*** Pooled samples (10:1) of feces and oral and body swabs using direct animal sampling PCR analysis (10 random cages per rack; 1 mouse sampled per cage)**
† EAD prototype plenum attachment and a rack sampling device (Interceptor) PCR analysis
‡ Serology performed on soiled-bedding sentinel mouse (1 cage per rack) using EZ Spot®

Conclusion

- We demonstrated that EAD testing can be performed on an IVC system that has centralized ventilation using a prototype plenum attachment and the rack sampling device Interceptor.
- EAD testing reliably identified MNV, *Helicobacter*, *Pasteurella pneumotropica* (Heyl and Jawetz), and *Tritrichomonas* as early as 7 days after exposure to colony cages.
- EAD testing after 90 days of exposure to colony cages was consistent with our regular health monitoring program which uses a combination of sentinel mouse serology and direct animal sampling PCR analysis.
- EAD testing after 90 days of exposure to colony cages appeared to be slightly more sensitive than our regular program in detecting *Pasteurella pneumotropica* (Jawetz) and *Entamoeba*.

Acknowledgements

The authors would like to thank Dr. Gianpaolo Milite and Massimo Aspesi (Tecniplast) for their guidance and assistance with this project. We would also like to thank Tanya Cini-Kirk, RLAT for technical assistance and Francesca Fabry for administrative assistance.