IDEXX **BioAnalytics**

Exhaust Dust Testing
with REPLACE™

The IDEXX BioAnalytics **REPLACE™** matrix is a pathogen collection material that consistently detects higher copy numbers than other commercially available environmental collection media, for both sentinel-free soiled bedding (SFSB) and exhaust dust testing (EDT). REPLACE™ matrices are validated to ensure the absence of pathogenic nucleic acids and rigorously tested to verify pathogen capture and detection. Environmental rodent health monitoring with REPLACE™ is the most robust PCR-based surveillance method available.

Implementing REPLACE™ for exhaust dust testing

- **REPLACE™** detected higher copy numbers for viruses, bacteria and parasites than other collection materials evaluated.

Background | Exhaust dust testing (EDT) and sentinel free soiled bedding (SFSB) are PCR-based environmental health monitoring (EHM) approaches that allow superior monitoring of rodent colony health while reducing or eliminating the use of sentinel animals. Both methods utilize specialized matrices to capture microbial nucleic acids shed from infectious agents by colony animals. We have previously reported on the use of REPLACE™ for SFSB testing and here we describe its use for EDT.

EDT can be employed with individually ventilated cage (IVC) systems that have open airflow without cage level exhaust air filtration. The premise of EDT is that dust particles generated during normal movement of colony rodents contain microbes or their nucleic acids. With open airflow IVC racks, this microbe/nucleic acid-containing dust exits cages and accumulates within the exhaust air manifolds. To perform regularly scheduled colony health monitoring, exhaust dust representing the health monitoring period (often 3 months) is collected and tested by real-time PCR. Collection material

is commonly placed within the exhaust system in open air flow racks, allowing accumulation of pathogen-containing dust over the course of the health monitoring period. In this report we compare the analytical and diagnostic sensitivity of exhaust dust collected using the REPLACE™ matrix positioned in front of the exhaust air prefilter to manufacturers' collection media placed in the manufacturers' designated holders.

Methods | We evaluated REPLACE™ matrices for EDT in two different manufacturers' open airflow IVC rack systems. The REPLACE™ matrices were positioned on the IVC rack prefilters oriented with the fleece side facing the oncoming exhaust air, attached by tucking one end under the prefilter perimeter frame and securing the other end with a flat metal clip (Figure 1). For each of the two IVC rack systems, the corresponding rack manufacturers' EDT filters were placed in the recommended holders and positioned on the rack. For rack style A, four REPLACE™ matrices and one manufacturer filter, the maximum allowed by holder design, were evaluated (Figure 2A). For rack style B, three REPLACE™ matrices and two manufacturer filters, the maximum allowed by holder design, were evaluated (Figure 2B). The racks were populated by naturally infected mice that were confirmed upon intake to be positive for viruses, bacteria, parasites, and between bacteria and parasites. Prevalence and shedding levels for each pathogen were determined by real-time PCR testing of feces before mice were placed in the racks. Fifteen cages containing one mouse per cage were placed on each of the two racks and housed for the 3-month testing period. At the conclusion of the testing period, commercial filters for each rack and REPLACE™ matrices were tested for pathogens by real-time PCR.

Figure 1. REPLACE™ matrix secured on prefilter with flat metal clip.

Results | In IVC rack style A, 16 viral, bacterial, parasitic, or fungal pathogens were detected using the manufacturer's EDT device, whereas 22 pathogens were detected in all four REPLACE™ samples placed at the exhaust prefilter (Figure 2; Rack A). Of the pathogens detected in both sample types, the mean copy number per PCR reaction of the four REPLACE™ exhaust prefilter samples ranged from 4- to 71-fold more template copies than that of the manufacturer's EDT sample (Figure 3A). For the 2 agents where REPLACE™ had less than 4 matrices positive, the percent prevalence of the pathogen in donor mice was notably lower (Figure 2; Rack A).

COMPARISON OF COLONY MOUSE PATHOGEN SHEDDING AND COLLECTION MATERIAL DETECTION RATES.

	Agent	Rack A				Rack B			
		Percent Positive Donor Mice (n=15)	Average Genomic Copy Number	Media A (n=1)	REPLACE (n=4)	Percent Positive Donor Mice (n=15)	Average Genomic Copy Number	Media B (n=2)	REPLACE (n=3)
Viruses	MHV	87	1684	0	4	93	3130	0	3
	MAV	93	6343	1	4	93	33030	2	3
	MNV	27	26	1	4	20	22	0	2
	MKPV	33	33	1	4	20	144	0	3
	TMEV	100	11670	0	4	93	36062	0	1
	MPV	100	49232	1	4	100	20457	1	3
	MVM	100	1321	1	4	73	4386	2	3
Bacteria	Campylobacter jejuni	33	87	0	3	47	1205	0	3
	Pseudomonas aeruginosa	7	2	0	2	7	2	0	1
	Mycoplasma pulmonis	53	4	1	4	33	5	2	3
	Proteus mirabilis	20	15	0	4	13	9	0	2
	Rodentibacter pneumotropicus	100	71	1	4	80	20	0	3
	Rodentibacter heylii	87	14	1	4	73	7	0	3
	Helicobacter spp.	100	290171	1	4	100	250848	2	3
Parasites	Giardia muris	87	2375	1	4	93	2657	1	3
	Entamoeba muris	20	4	1	4	33	1058	0	2
	Spironucleus muris	100	2964	1	4	93	12330	1	3
	Tritrichomonas muris	80	667	1	4	73	2103	2	3
	Aspiculuris tetraptera	13	37	1	4	7	7	1	3
	Syphacia obvelata	13	293	1	4	27	75	1	3
	Rodentolepis nana	7	306	0	4	7	45	0	1
	Myocoptes musculinis	40	13	0	4	40	23	0	3
	Radfordia/Myobia	20	44	1	4	47	274	2	3
Fungi	Pnemocystis murina	NT	NA	0	4	NT	NA	0	3

Figure 2. Naturally infected mice were individually housed on each of two IVC racks and tested by fecal PCR at the beginning of the experiment. Percent pathogen prevalence and average pathogen genomic copy number for colony mice on each rack are shown in reference to the number of Media A or B filters and REPLACE™ matrices positive for each corresponding pathogen. NT= not tested; NA = not applicable

In IVC rack B, 6 viral, bacterial, parasitic, or fungal pathogens were detected in both manufacturer's EDT device samples, whereas 18 pathogens were detected in three out of three REPLACE™ matrices placed at the exhaust prefilter (Figure 2; Rack B). Of the pathogens detected in two of two manufacturers EDT samples and three of three REPLACE™ samples, the mean copy number per PCR reaction of the REPLACE™ exhaust prefilter samples contained 22- to 96-fold more template copies per PCR reaction than the mean copies per PCR reaction of the manufacturer's EDT samples (Figure 3B). For most agents where REPLACE™ had less than 2 matrices positive, the percent prevalence was found to be low in donor mice (Figure 2; Rack B).

THREE-MONTH EXHAUST DUST PCR TESTING PERFORMANCE OF REPLACETM MATRIX ON PREFILTER COMPARED WITH MANUFACTURER'S RECOMMENDED MEDIA A OR MEDIA B PLACED IN DESIGNATED MEDIA HOLDER.

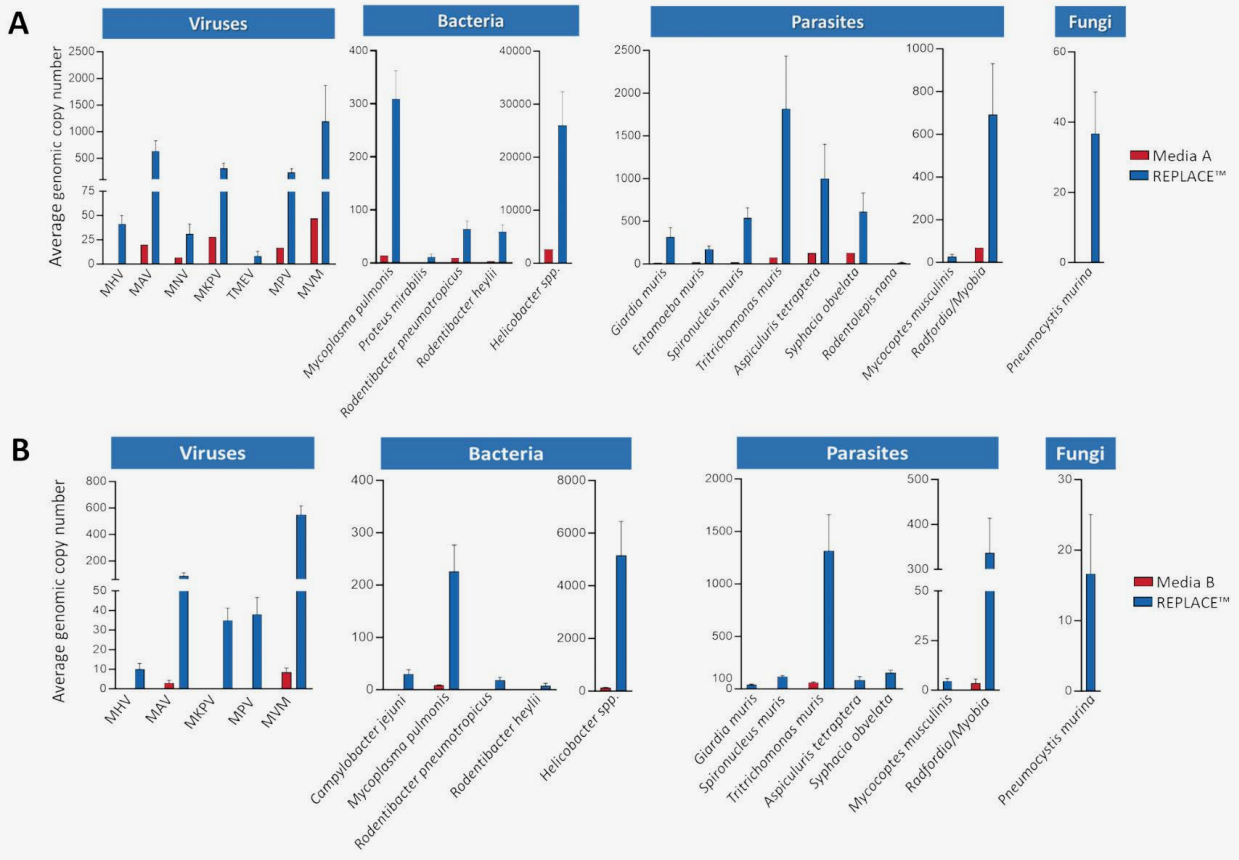

Figure 3. A) Pathogen genomic copy number of Media A (n=1) and average pathogen genomic copy number per PCR reaction oof REPLACE™ (n=4) are shown for agents where all four replicates were positive. B) Average pathogen genomic copy number per PCR reaction of Media B (n=2) and REPLACE™ matrices (n=4) are shown for agents where all two or all four replicates were positive, respectively.

Summary | Exhaust dust testing to perform PCR-based health monitoring of rodent colonies can be used in open airflow IVC racks. Central to this approach is collecting a high-quality sample of the IVC exhaust dust that is representative of the health monitoring period. In this report we show that REPLACE™ positioned in front of the exhaust air prefilter of provides an excellent sample for detecting pathogens by EDT. EDT samples were easily collected with REPLACE™ from the rack prefilter location, and showed the highest diagnostic sensitivity compared to two manufacturers' media placed in their respective designated holders. Using REPLACE™ provides an improved approach to detect rodent pathogens by EDT and does not require investment in specialized equipment.

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