Validation of the use of Interceptors in ScanClime

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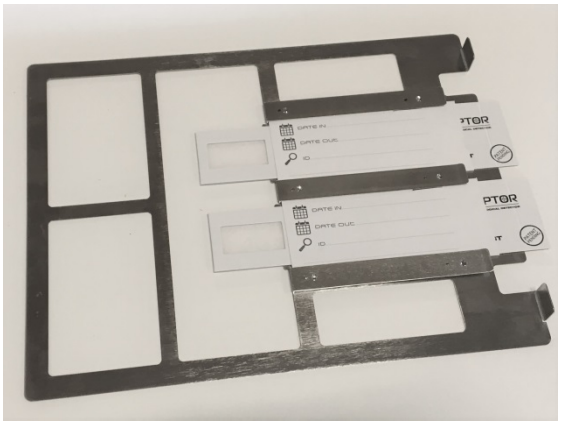
Aim:

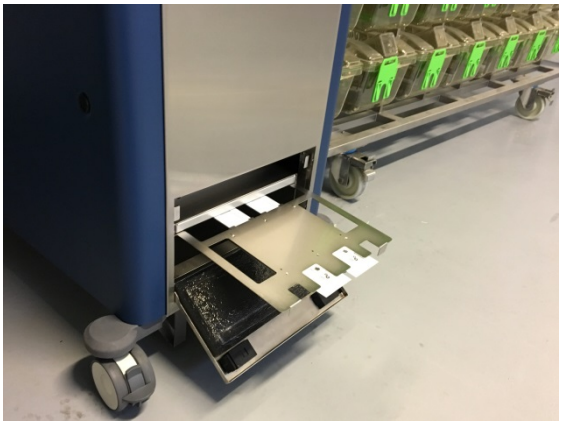
The Interceptor (Tecniplast, Italy) is an irradiated cardboard folder containing a sliding filter for exhaust air dust testing in laboratory animal facilities (see picture 1). Together with a specially designed metal frame the Interceptor can be allocated underneath the exhaust air prefilter in an air handling unit for individually ventilated cages (IVC). Particles moving from cages to the exhaust filtration area will be collected by the filter in the Interceptor. PCR testing is performed on the Interceptor filters to determine the presence of nucleic acids associated with the particles it collects and thus it functions as a microbiological monitoring. The system is originally validated for Tecniplast air handling units and this study was set up to validate the use of the Interceptor in the ScanClime air handling unit (SCANBUR, Denmark) for IVC systems as well. The ScanClime accurately controls relative humidity in the cages (±3%). The aim is to validate the use for a three month interval, as this is the FELASA recommended interval between health surveillance[1]. The test panel of infectious agents for the health monitoring were the full annual FELASA recommended list plus additional, especially bacterial, agents[1]. Astrovirus were included in the screening after three months, as this virus has shown to be widely spread in research facilities[2, 3]. A test of the Interceptor after three weeks were included as well to underline to what extend a short interval for particle collection is sufficient.

A

B



C



Picture 1. A. Interceptor package and Interceptor. B. Interceptor in frame for ScanClime. C. Interceptor in the ScanClime air handling unit.

Study design:

The ScanClime ventilated two IVC racks (Greenline GM500, Tecniplast, Italy) with a total of 160 cages (see picture 2). In average 130 cages contained animals over the 3 months, and the empty cages contained bedding. 4-5 mice were housed in each of the 130 cages. The ScanClime was set to a relative humidity of 55% and at 75 air changes pr. hour. Prior to the insertion of Interceptors to the ScanClime a baseline test of environment and animals was performed (see table 1). A Mouse Surveillance Plus PRIA test panel was used for all the tests of this experiment (see table 2 for list of pathogens tested) and samples were tested at Charles River Research Animal Diagnostic Services, USA. After this test the prefilter was changed to a new clean prefilter and the Interceptor frame was inserted. Afterwards two Interceptors were placed in the ScanClime. Three weeks after the insertion of the Interceptors the first Interceptor was sent off for testing and after three months the second plus fecal samples were sent the Charles River for testing (see table 1). For the three month test a test for Astrovirus was added.

Picture 2. ScanClime and IVC-racks in the study setup.

Table 1. Overview of PCR testings performed.

Test panel: Surveillance Plus PRIA + Astrovirus at the 3 months testing	
Baseline:	**Environmental:** 3x10 swabs pooled from each rack (6 tubes in total) **Oral + fur:** 10 oral + 10 fur swabs pooled in their own tubes from each rack (4 tubes in total, analysed together oral and fur from each rack) **Fecal:** 10 fecal samples pooled from each rack (2 tubes in total)
3 wks:	**Environmental:** 1 Interceptor tested after three weeks in the ScanClime
3 mos:	**Fecal:** 10 fecal samples pooled from each rack (2 tubes in total) **Environmental:** 1 Interceptor tested after 3 months in the ScanClime

mos=months, wks=weeks. All tests were performed by Charles River Research Animal Diagnostic Services, USA.

Table 2. List of pathogens tested by Mouse Surveillance Plus PRIA test panel.

Viruses	Bacteria	Parasites/Protozoa/Fungi
Mouse Parvoviruses (MVM/MPV)	Helicobacter	Fur mites (Myobia, Myocoptes, Radfordia)
Mouse norovirus	Ctirobacter rodentium	Pinworms (Aspiculuris, Syphacia)
Mouse coronavirus (MHV)	Mycoplasma pulmonis	Giardia
Mouse rotavirus (EDIM)	Streptobacillus moniliformis	Spironucleus muris
Mouse theilovirus (TMEV, GDVII)	Pasteurella pneumotropica (Heyl & Jawetz)	Cryptosporidium
Adenovirus type 1 (FL) & 2 (K87)	Clostridium piliforme	Entamoeba
Reovirus type 1,2 3, 4	Cilia-associated respiratory bacillus	Pneumocystis*
Pneumovirus of mice	Pseudomonas aeruginosa	
Sendai virus	Salmonella	
Ectromelia (mousepox)	Campylobacter	
Lymphocytic choriomeningitis virus	Bordetella bronchiseptica	
	Bordetella hinzii	
	Corynebacterium kutscheri	
	Corynebacterium bovis	
	Staphylococcus aureus	
	Strepococcus pneumoniae	
	Klebsiella pneumoniae	
	Klebsiella oxytoca	
	Beta-hemolytic Streptocccus group A	
	Beta-hemolytic Streptocccus group B	
	Beta-hemolytic Streptocccus group C, G	
	Proteus mirabilis	

***** P. murina can be detected in oral swabs, but for better detection lung tissue is required.

Results:

The test results are shown in table 3. At baseline Helicobacter ganmani, H. hepaticus, H. typhlonius, Pasteurella pneumotropica (Heyl + Jawetz) and Staphylococcus aureus were tested positive by environmental, oral and fur swabs plus fecal samples together. At the three week Interceptor test the results showed the presence of Helicobacter ganmani, H. hepaticus, H. typhlonius, Pasteurella pneumotropica (Heyl) and Tritrichomonas. After three months the fecal samples showed positive test results for H. hepaticus, H. typhlonius, Pasteurella pneumotropica (Heyl) and Tritrichomonas, while the Interceptor showed the same plus H. ganmani and S. aureus as well. The biotype Pasteurella pneumotropica Jawetz were only detected in the environmental swab at baseline. However, the biotype Heyl was detected in all samples throughout the test and thus, the detection of Pasteurella pneumotropica Jawetz is not seen as a unique finding.

Table 3. Positive findings of health monitoring. Positive PCR results for given pathogens are stated in the figure by plus symbols.

	Time 0 (E)	Time 0 (O+Fu)	Time 0 (Fe)	Time 3 wks. (I)	Time 3 mos. (Fe)	Time 3 mos. (I)
Astrovirus	Not tested	Not tested	Not tested	Not tested	+	+
Helicobacter genus			+	+	+	+
H. ganmani			+	+		+
H. hepaticus			+	+	+	+
H. typhlonius			+	+	+	+
P. pneumotropica-Heyl	+	+	+	+	+	+
P. pneumotropica-Jawetz	+					
S. aureus			+			+
Tritrichomonas genus				+	+	+

E=environmental swabs, Fe=fecal samples, Fu=fur swabs, I=Interceptor, mos.=months, O=oral swabs, wks.=weeks

Conclusion:

Our results show that using the Interceptor for exhaust air dust filter testing in IVC systems test positive for the same agents as traditional testing and more, reducing the workload for health monitoring procedures while leaving mice undisturbed in their cages. This study shows that the Interceptor can be used in the ScanClime in the same fashion as in Tecniplast air handling units. The Interceptor can be used for accurate health monitoring of animals housed in IVC systems ventilated by ScanClimes.

References:

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2. Ng TFF, Kondov NO, Hayashimoto N, Uchida R, Cha Y, Beyer AI, et al. Identification of an Astrovirus Commonly Infecting Laboratory Mice in the US and Japan. PLOS ONE. 2013;8(6):e66937.
3. Schmidt K, Butt J, Mauter P, Vogel K, Erles-Kemna A, Pawlita M, et al. Development of a multiplex serological assay reveals a worldwide distribution of murine astrovirus infections in laboratory mice. PLOS ONE. 2017;12(10):e0187174.