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The Sentinel™ EAD® program can detect more microorganisms than bedding sentinel animals

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Abstract

Bedding sentinel programs have been the standard method for monitoring the health status of rodents housed in individually ventilated cages. However, it has been reported that some infectious microorganisms cannot be detected by bedding sentinels. Thus, more sensitive microbiological monitoring methods are needed. In the present study, we assessed the sensitivity of the Sentinel™ EAD® program, developed by Allentown Inc. and Charles River Laboratories Inc., which involves a combination of exhaust air dust and environmental PCR testing. We compared the sensitivity of Sentinel™ EAD® to that of bedding sentinels and fecal samples collected from mice housed in rooms. In conclusion, Sentinel™ EAD® was more sensitive than the bedding sentinel method.

Key Words: exhaust air dust (EAD), monitoring

Bedding sentinel health surveillance programs have been the standard practice for monitoring the health of research animal colonies housed in individually ventilated cages (IVCs). These types of programs rely on naïve animals that are introduced into a resident animal population and exposed to the resident animals or soiled bedding. Samples are then taken from the naïve animals, instead of the principal animals, for testing via health surveillance programs. Sentinel animals are usually exposed to infectious agents indirectly; this is a disadvantage of using bedding sentinel animals. Certain infectious agents are not effectively detected by bedding sentinel animals[1,3,6,7]; as such, bedding sentinel health surveillance programs can be a poor indicator of the actual infection status of animals housed in IVCs. Therefore, more sensitive microbiological monitoring methods are needed. Recently, exhaust air dust (EAD) monitoring was reported to be superior to bedding sentinels for the detection of

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bacteria such as *Pasteurella pneumotropica* and *Helicobacter hepaticus*[4,5]. Sentinel™ EAD®, which involves a combination of EAD and environmental polymerase chain reaction (PCR) testing, was developed by Allentown Inc. and Charles River Laboratories Inc. This health surveillance program makes it possible to assess the health of resident animal colonies accurately and specifically, with limited or no use of sentinel animals. In the present study, we assessed the sensitivity of this program compared to the standard bedding sentinel method in our animal facility. This study was approved and overseen by the Animal Experiments Committee of RIKEN (Saitama, Japan), and was conducted in accordance with the Institutional Guidelines for Experiments using Animals.

A Sentinel™ EAD® sample capture unit was placed on one out of four IVC racks (Micro-VENT Mouse; Allentown Inc., Allentown, NJ) per animal holding room according to the manufacturer's instructions (Fig. 1). Seven rooms in our specific pathogen-free area were tested. After 3 months of exposure to the EAD, each unit was sent to the monitoring center at Charles River Laboratories Japan, Inc. (Ishioka, Ibaraki, Japan) and tested for 33 agents (Table 1). In parallel, live sentinel animals (Crlj:CD1-Foxn1nu/+) that had been exposed to soiled bedding in each room for more than 3 months were also tested for the same agents. Sample collection for PCR test from sentinel animals were conducted as reported[2]. All mice were housed in IVCs (Micro-Barrier cage MBS7115RH; Allentown Inc.) in the IVC racks described above with a maximum cage density of five adult mice per cage. The mice were exposed to a 12-h/12-h light/dark cycle and the rooms were maintained at 21–25°C and 45–65% humidity. Routine health monitoring surveillance using bedding sentinel animals was performed semi-annually for the agents listed in Table 1. Routine health monitoring surveillance included a visual examination for parasites and fungi, serological tests to detect antibodies against all 11 viruses and 5 out of 12 bacteria

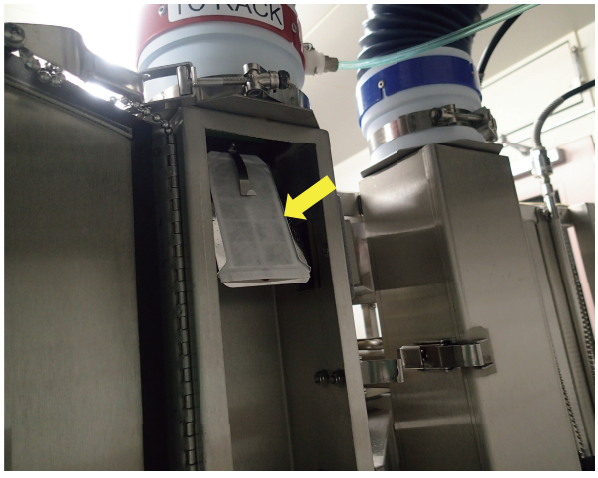

Fig. 1. A Sentinel™ EAD® sample capture unit. It was placed above the vertical plenum according to the manufacturer's instructions. The capture unit is indicated by a yellow arrow.

(*Mycoplasma pulmonis*, *Clostridium piliforme*, CAR bacillus, *Corynebacterium kutscheri*, and *Salmonella typhimurium*), and cultures for the other bacteria. The IVC racks were run with 60 air changes per hour in positive pressure relative to the holding room. Autoclaved wood fiber (ARBOCEL Comfort Natural; J. Rettenmeier & Söhne, Rosenberg, Germany) was used as bedding. Water filtered by reverse osmosis was supplied through an automated animal watering system (Edstrom Industries Inc., Waterford, WI). Irradiated basic and well-balanced food (CRF-1; Oriental Yeast Co. Ltd., Tokyo, Japan) was available *ad libitum*. Cage changes were performed biweekly without a HEPA-filtered cage changing station. In the animal holding rooms, breeding was performed and no immunodeficient mouse other than sentinel animals (Crlj:CD1-Foxn1nu/+) was housed.

The positive results from the Sentinel™ EAD® and sentinel animals are shown in Table 2. All 11 viruses and *C. kutscheri*, *M. pulmonis*, *Salmonella* spp., *Citrobacter rodentium*, *Pseudomonas aeruginosa*, *C. pilliforme*, CAR bacillus, *H. hepaticus*, *Syphacia obvelata*, *Aspiculuris tetraptera*, *Giardia* spp., *Spironucleus muris*, *Myocoptes musculinus* and *Radfordia affinis* were not detected in any samples.

Table 1. Agents investigated by PCR

	Agents
Viruses	Sendai virus*
	Mouse hepatitis virus
	Lymphocytic choriomeningitis virus
	Ectromelia virus
	Epizootic diarrhea of infant mice virus
	Minute virus of mice
	Mouse adenovirus type 1, 2
	Mouse cytomegalovirus
	Theiler's murine encephalomyelitis virus
	Pneumotia virus of mice*
	Reovirus
Bacteria	*Corynebacterium kutscheri*
	Mycoplasma pulmonis
	Mycoplasma spp.
	Salmonella spp.
	Citrobacter rodentium
	Pasteurella pneumotropica (Heyl)
	Pasteurella pneumotropica (Jawetz)
	Pseudomonas aeruginosa
	Staphylococcus aureus
	Clostridium piliforme
	CAR bacillus
	Helicobacter hepaticus
Parasites	*Syphacia obvelata*
	Aspiculuris tetraptera
	Giardia spp.
	Entamoeba spp.
	Tritrichomonas spp.
	Spironucleus muris
	Myocoptes musculinus (fur mites)*
	Myobia musculi (fur mites)*
	Radfordia affinis (fur mites)*
Fungi	*Pneumocystis* spp.*

*PCR testing of the fecal samples from C3 and C4 were not conducted because the fecal samples were not suitable to detect those organisms.

Furthermore, these agents were never detected by routine health monitoring surveillance.

Mycoplasma spp., *Staphylococcus aureus*, *Entamoeba* spp. and *Myobia musculi* were detected in sentinel animals, whereas *Mycoplasma* spp., *P. pneumotropica* (Heyl and Jawetz), *S. aureus*, *Entamoeba* spp., *Tritrichomonas* spp., *M.*

Table 2. The positive results are represented as number of positive sample(s) / number of total sample(s)

Room No.		C1		C2		C3			C4			C5		C6		C7	
	Agents	Sentinel animal(s)	Sentinel™ EAD®	Sentinel animal(s)	Sentinel™ EAD®	Sentinel animal(s)	Sentinel™ EAD®	Feces	Sentinel animal(s)	Sentinel™ EAD®	Feces	Sentinel animal(s)	Sentinel™ EAD®	Sentinel animal(s)	Sentinel™ EAD®	Sentinel animal(s)	Sentinel™ EAD®
Bacteria	*Mycoplasma* spp.	2/2	1/1	1/1	1/1	1/1	1/1	38/38	1/1	1/1	34/34	1/1	1/1	1/1	1/1	1/1	1/1
	Pasteurella neumotropica (Heyl)	0/2	0/1	0/1	0/1	0/1	0/1	0/38	0/1	1/1	34/34	0/1	1/1	0/1	0/1	0/1	1/1
	Pasteurella neumotropica (Jawetz)	0/2	0/1	0/1	1/1	0/1	1/1	21/38	0/1	0/1	0/34	0/1	1/1	0/1	0/1	0/1	1/1
	Staphylococcus aureus	0/2	1/1	0/1	1/1	0/1	1/1	38/38	1/1	1/1	16/34	0/1	0/1	0/1	1/1	0/1	1/1
Parasites	*Entamoeba* spp.	1/2	1/1	1/1	1/1	0/1	1/1	15/38	0/1	1/1	34/34	1/1	1/1	0/1	0/1	0/1	1/1
	Tritrichomonas spp.	0/2	1/1	0/1	1/1	0/1	0/1	0/38	0/1	1/1	34/34	0/1	0/1	0/1	0/1	0/1	0/1
	Myobia musculi (fur mites)	0/2	1/1	1/1	1/1	1/1	1/1	–*	0/1	1/1	–*	1/1	1/1	0/1	0/1	1/1	1/1
Fungi	*Pneumocystis* spp.	0/2	0/1	0/1	0/1	0/1	0/1	–*	0/1	1/1	–*	0/1	0/1	0/1	0/1	0/1	0/1

*PCR testing was not conducted because the sample was not suitable for detecting this organism.

musculi and *Pneumocystis* spp. were detected using Sentinel™ EAD® (Table 2). Many more microorganisms were detected using Sentinel™ EAD® than sentinel animals.

Next, we confirmed the existence of the microorganisms detected by Sentinel™ EAD® by analyzing the feces from not only mice held in the rack, but also from all mice kept in the room because there was a possibility that microorganisms could have been sucked into the exhaust plenum through the inlet port of vacant cage spaces. As it would have been too time and labor intensive to collect feces from the animals in all seven rooms, we collected samples from only two rooms, C3 and C4. Every ten fecal samples were pooled and PCR tests were conducted. In total, 38 and 34 pooled samples from C3 and C4, respectively, were tested for the same microorganisms as Sentinel™ EAD®, except for Sendai virus, Pneumonia virus of mice, *Pneumocystis* spp. and ectoparasites (*M. musculinus*, *M. musculi* and *R. affinis*) because these agents are difficult to detect in fecal samples. PCR was conducted as described previously[2] with a slight modification. The PCR test results from the fecal samples are also presented in Table 2. *Mycoplasma* spp., *P. pneumotropica* (Heyl or Jawetz), *S. aureus* and *Entamoeba* spp. were detected in both rooms, and *Tritrichomonas* spp. was detected in C4. These same results were obtained using Sentinel™ EAD®, with the exception of Sendai virus, Pneumonia virus of mice, *Pneumocystis* spp. and ectoparasites.

In the present study, *P. pneumotropica*, *S. aureus* and *M. musculi* were frequently detected using Sentinel™ EAD®. However, no sentinel animals were positive for *P. pneumotropica*. *S. aureus* was detected from only one sentinel animal, and *M. musculi* was not always detected. It is known that soiled bedding sentinels are not suitable for detecting *P. pneumotropica*[6,7]. *S. aureus* has been reported to be a low-copy bacterium that is not readily detected in sentinel mice[2]. Fur mites, including *M. musculi*, was also reported, indicating that detection from soiled bedding sentinels is unreliable[3]. This presumably explains why *P. pneumotropica* was never detected, and *S. aureus* and *M. musculi* were not always detected from sentinel animals in the present study. *Mycoplasma* spp. was also highly detected using Sentinel™ EAD® and this agent seemed to be readily detectable from soiled bedding sentinels.

Entamoeba spp. was not always detected from sentinel animals, but was frequently detected using Sentinel™ EAD®. *Tritrichomonas* spp. was never detected from sentinel animals, although three rooms were positive for this agent when analyzed using Sentinel™ EAD®. In fact, these microorganisms were highly prevalent in fecal samples. The high air change rate in the IVC racks might have negatively affected the survival rate of these agents in soiled bedding, which could explain why this agent was not transmitted to sentinel animals. The life cycle of *Entamoeba* might also have affected the transmission efficiency.

Pneumocystis spp. was not detected from sentinel animals in C4, but was detected using Sentinel™ EAD®. It has been reported that *Pneumocystis carinii* is rarely transmitted to mice exposed to contaminated bedding[6]. This may explain why *Pneumocystis* spp. was not transmitted to the soiled bedding sentinel of C4.

Our results suggest that Sentinel™ EAD® is more sensitive than the standard bedding sentinel method. An advantage of Sentinel™ EAD® is that it can reduce the need for sentinel animals, which is in keeping with the “3 Rs” (replacement, reduction and refinement).

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