Moving away from soiled bedding sentinels – the (R) evolution in rodent health screening

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Introduction

Ensuring an adequate health status of laboratory rodents used in biomedical research has become a key priority for facilities everywhere.

Health screening boosts research by monitoring the presence of infectious agents which can compromise the animals' health and become a confounding factor.

The history of rodent pathogen control is linked with the advancements in rodent husbandry, laboratory animal science and the development of new diagnostic methods.¹



Traditional health monitoring methods

Traditional systems consisted of Soiled Bedding Sentinels (SBS (i.e. animals exposed to dirty bedding from colony animals)) tested using conventional diagnostic methods which require the euthanasia of SBS.

Numerous studies have demonstrated that SBS may not accurately represent a colony's health status. There are several pathogens (e.g. viruses, bacteria, parasites and protozoa) that are not easily transmitted to SBS, especially when in low prevalence, such as for rodents housed in Individually Ventilated Cages (IVCs).^{1,2,3}

Poorly transmitted agents include Mouse adenovirus, Sendi virus, PVM, *Rodentibacter spp, Mycoplasmas pulmonis, Giardia spp* and *Spironuleus spp*.

(R) evolutional health monitoring methods

During the last decade, researchers have proposed alternative sentinel free methods to monitor the health status of rodents housed mainly in IVCs, which include the collection of samples from (see figure 1 to 4).^{2,5}

- Colony animals (i.e. direct sampling via fresh faecal pellets, body swabs and oral swabs).
- Environment (e.g. swabbing of cages, work stations and biosafety cabinets).
- Exhaust air duct (EAD), (i.e. dust, debris, fur and microorganisms removed actively by IVC exhaust fans and collected using swabs or vendor in-line collection devices).
- Recently, soiled bedding agitation via contact media (e.g. PathogenBinder[™]) has been proposed as an alternative sampling method for IVC systems that cannot accommodate EAD.^{6,7} Agitation exposes the collection media to dust particles allowing the collection of associated infectious agent, nucleic acid.

In addition, the development of molecular diagnostic assays such as Polymerase Chain Reaction (PCR) using TaqMan chemistry provides extremely sensitive and specific methods to evaluate the current status of animals.^{1,2}

Direct Sampling





Advantages:

es: Disadvantages:

- Sentinel-free method possible.
- Increased capacity to detect low transmission agents compared to SBS.
- Requires manipulation of animals.
 - Can be disruptive to studies.
 - Single time point collection.
- Sample size.

Figure 1.

Environmental Sampling



Advantages:

- Reduced cost compared to SBS.
- Can be adapted to a wide range of caging systems.

Disadvantages:

- Prone to interuser variations if procedure not standardised.
- Requires manipulation of cages/air ducts.

Exhaust Air Dust



Advantages:

- Standardised sampling method.
- Time saving.
- Increased capacity of agent detection compared to SBS.

Disadvantages:

- Requires purchase of filter holders.
- Prone to false positives if ducts/ holders not cleaned properly between sampling points.

Figure 3.

Sentinel-Free Contact Media Agitation



Advantages:

- Can be adapted to all caging systems.
- Low cost.
- Non dependent on agent transmission.

Disadvantages:

- Requires manual agitation of bedding.
- Prone to interuser variations if procedure not standardised.

Figure 4.

Conclusions

A wide range of studies have demonstrated the effectiveness of alternative sentinel free methods to detect rodent pathogens. Such findings could have major implications for the 3Rs principles of animal research by reducing or replacing the use of animals as sentinels.

The combination of sentinel free methods with molecular diagnostic assays can be a (R)evolutionary solution for rodent health screening, an option worth exploring for facilities worldwide.

References

- ¹ **Buchheister, S.; Bleich, A.** Health Monitoring of Laboratory Rodent Colonies Talking about (R) evolution. Animals 11.5 (2021), 1410.
- ² Henderson, K.S., *et al.* Efficacy of direct detection of pathogens in naturally infected mice by using a high-density PCR array. Journal of the American Association for Laboratory Animal Science 52.6 (2013): 763-772.
- ³ **Mailhiot, D., et al.** Comparing mouse health monitoring between soiled-bedding sentinel and

exhaust air dust surveillance programs. Journal of the American Association for Laboratory Animal Science 59.1 (2020): 58-66.

- ⁴ Luchins, K.R., *et al.* Cost comparison of rodent soiled bedding sentinel and exhaust air dust healthmonitoring programs. Journal of the American Association for Laboratory Animal Science 59.5 (2020): 508-511.
- ⁵ Manuel, C.A., Pugazhenthi, U. and Leszczynski, J.K. Surveillance of a ventilated rack system for Corynebacterium bovis by sampling exhaust-air manifolds. Journal of the American Association for Laboratory Animal Science 55.1 (2016): 58-65.
- ⁶ **Winn, C.B.**, *et al.* Using filter media and soiled bedding in disposable individually ventilated cages as a refinement to specific pathogen- free mouse health monitoring programs. Journal of the American Association for Laboratory Animal Science 61.4 (2022): 361-369.
- ⁷ Momtsios P., Perkins C. and Henderson K.S. PathogenBinder™: A Refined and Standardized Soiled-Bedding Sampling Method for the Detection of Rodent Infectious Agents (2022). <u>https://www.criver.com/</u> resources/info-pi-rm-pathogenbinder-soiled-beddingsampling-method-detection-rodent-infectious



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