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Replacing Sentinel Rodents with Environmental Health Monitoring

Training Session for all IVS Staff

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Excluded Agents at WSU

Mice

Viruses
- MPV 1-5
- MVM
- MHV
- EDIM
- TMEV
- *MNV
- Sendai
- Ectromelia
- REO3
- PVM
- LCMV

Bacteria
- C. bovis
- *Helicobacter sp.*
- *M. pulmonis*

Protozoal
- *Entamoeba muris
- *Spironucleus muris

Parasites Internal/External
- Fur mites/Pinworms

Rats

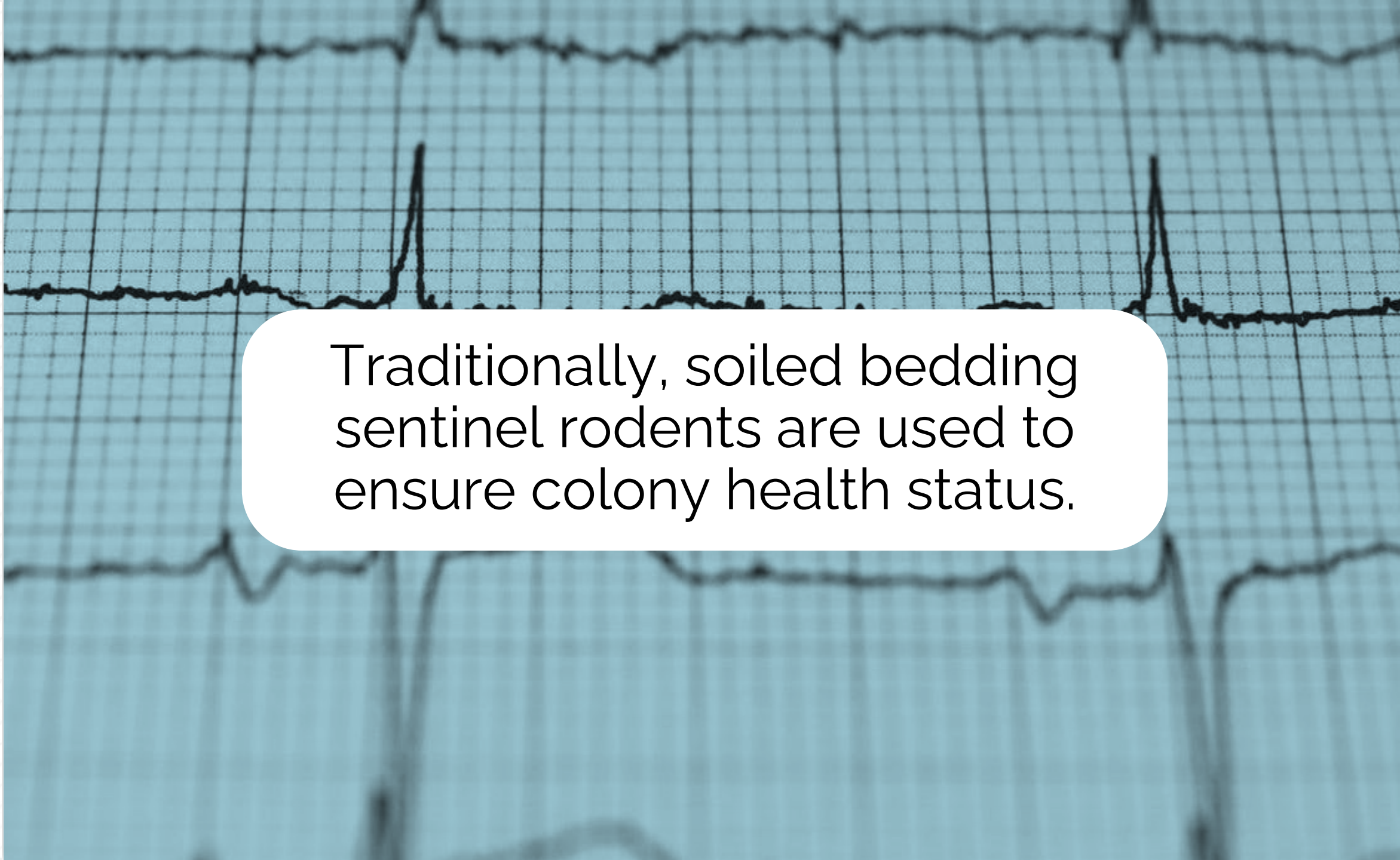
Viruses
- RCV/SDAV
- NS1
- RPV
- RMV
- KRV
- H-1
- RTV
- Sendai
- PVM
- REO3
- LCMV

Bacteria
- **Helicobacter sp.*
- *M. pulmonis*

Parasites Internal/External
- Fur mites/Pinworms

Fungus
- **P. carinii*

*Agents allowed in specific areas only with approval



Traditionally, soiled bedding sentinel rodents are used to ensure colony health status.

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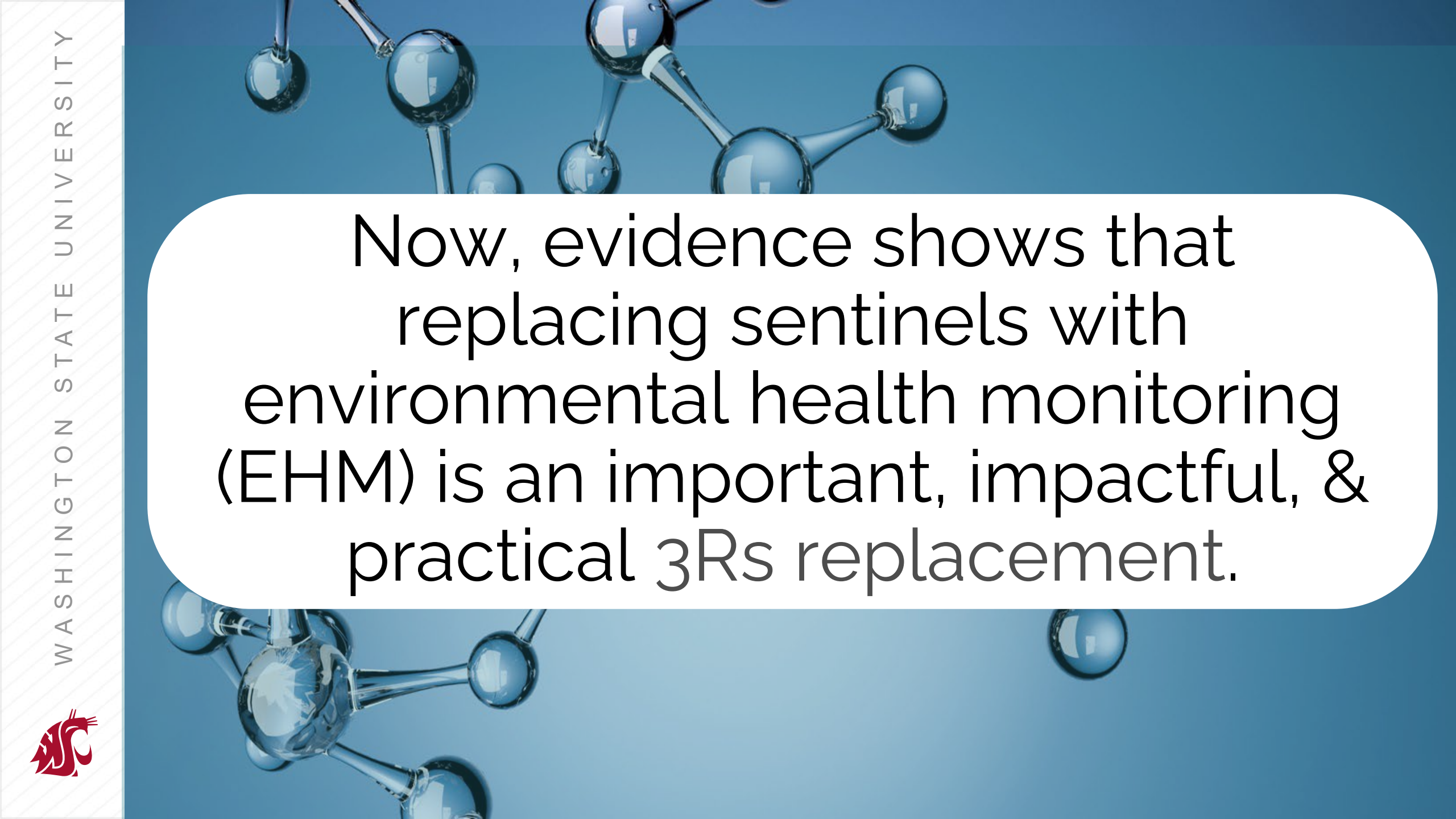


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Strong evidence indicates that environmental health monitoring is advantageous.

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Environmental health monitoring is supported by >25 peer-reviewed publications.

Journal of the American Association for Laboratory Animal Science
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Vol 59, No 2
March 2020
Pages 156–162

Adoption of Exhaust Air Dust Testing in SPF Rodent Facilities

Christina Pettan-Brewer,* Riley J Trost, Lillian Maggio-Price, Audrey Seamons, and Susan C Dowling

Reliable detection of unwanted microbial agents is essential for meaningful health monitoring in laboratory animal fa
ties. Most rodents at our institution are housed in IVC rack systems to minimize aerogenic transmission of infectious ag
between cages. The most commonly used rodent health monitoring systems expose live sentinel rodents to soiled bed
collected from other rodent cages on IVC racks and subsequently test these soiled-bedding sentinels for evidence of infec
with excluded agents. However, infectious agents might go undetected when using this health surveillance method, d
inefficient organism shedding or transmission failure. In 2016, our institution switched the health monitoring methodo
for the majority of our SPF rodent colonies to real-time PCR testing of environmental samples collected from the exh
plenums of IVC racks. Here we describe our rationale for this conversion, describe some interesting health monito

Journal of the American Association for Laboratory Animal Science
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Vol 60, No 3
May 2021
Pages 306–310

PCR Testing of Media Placed in Soiled Bedding as a Method for Mouse Colony Health Surveillance

Wai H Hanson,* Kelli Taylor, and Douglas K Taylor

Rodent colony health surveillance has traditionally been accomplished by testing sentinel animals that have been exposed to soiled bedding from colony animals. Collecting samples from exhaust plenums on ventilated caging systems, followed by PCR analysis, has emerged as another promising method for health surveillance. However, environmental testing at the rack level is not effective for all ventilated rack designs. In this study, we tested whether media placed in soiled bedding is

Comparative Medicine
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Vol 54, No 4
August 2004
Pages 382-392

Efficacy of Three Microbiological Monitoring Methods in a Ventilated Cage Rack

Susan R. Compton, PhD,[1,*] Felix R. Homberger, DVM, PhD,[2] Frank X. Paturzo,[1] and Judy MacArthur Clark, DVMS[3]

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Vol 56, No 2
March 2017
Pages 1–8

Detection and Elimination of *Corynebacterium bovis* from Barrier Rooms by Using an Environmental Sampling Surveillance Program

Christopher A Manuel,[1-3,*] Umarani Pugazhenthi,[4] Shannon P Spiegel,[1] and Jori K Leszczynski[1,2]

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Vol 57, No 5
September 2018
Pages 477–482

PCR Testing of Filter Material from IVC Lids for Microbial Monitoring of Mouse Colonies

Ariana R Dubelko,[1] Metanuj Zuwannin,[1] Samantha C McIntee,[1] Robert S Livingston,[2] and Patricia L Foley[1,*]

> J Am Assoc Lab Anim Sci. 2021 Mar 1;60(2):160-167. doi: 10.30802/AALAS-JAALAS-20-000086. Epub 2021 Feb 24.

Evaluation of In-cage Filter Paper as a Replacement for Sentinel Mice in the Detection of Murine Pathogens

Kathryn A O'Connell [1], Gabor J Tigyi [2], Robert S Livingston [3], Daniel L Johnson [4], David J Hamilton [5]

Affiliations + expand

PMID: 33629939 PMCID: PMC7974814 (available on 2021-09-01)
DOI: 10.30802/AALAS-JAALAS-20-000086

Original Article

Laboratory Animals
0(0) 1–7
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DOI: 10.1177/0023677217739329
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Environmental samples make soiled bedding sentinels dispensable for hygienic monitoring of IVC-reared mouse colonies

Manuel Miller and Markus Brielmeier

Journal of the American Association for Laboratory Animal Science
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Vol 59, No 1
January 2020
Pages 58–66

Comparing Mouse Health Monitoring Between Soiled-bedding Sentinel and Exhaust Air Dust Surveillance Programs

Darya Mailhiot,[1,2,*] Allison M Ostdiek,[1,2] Kerith R Luchins,[1,2] Chago J Bowers,[1]
Betty R Theriault,[1,2] and George P Langan[1,2]

Original Article

Laboratory Animals
0(0) 1–9
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DOI: 10.1177/0023677216661586
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SAGE

Murine norovirus detection in the exhaust air of IVCs is more sensitive than serological analysis of soiled bedding sentinels

Julia Zorn[1], Bärbel Ritter[1], Manuel Miller[1], Monika Kraus[1,2],
Emily Northrup[1] and Markus Brielmeier[1]

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EHM works well for agents that don't transfer well to soiled bedding sentinels (e.g. respiratory agents & fur mites).

De Bruin et al. 2016, Thigpen et al. 1989

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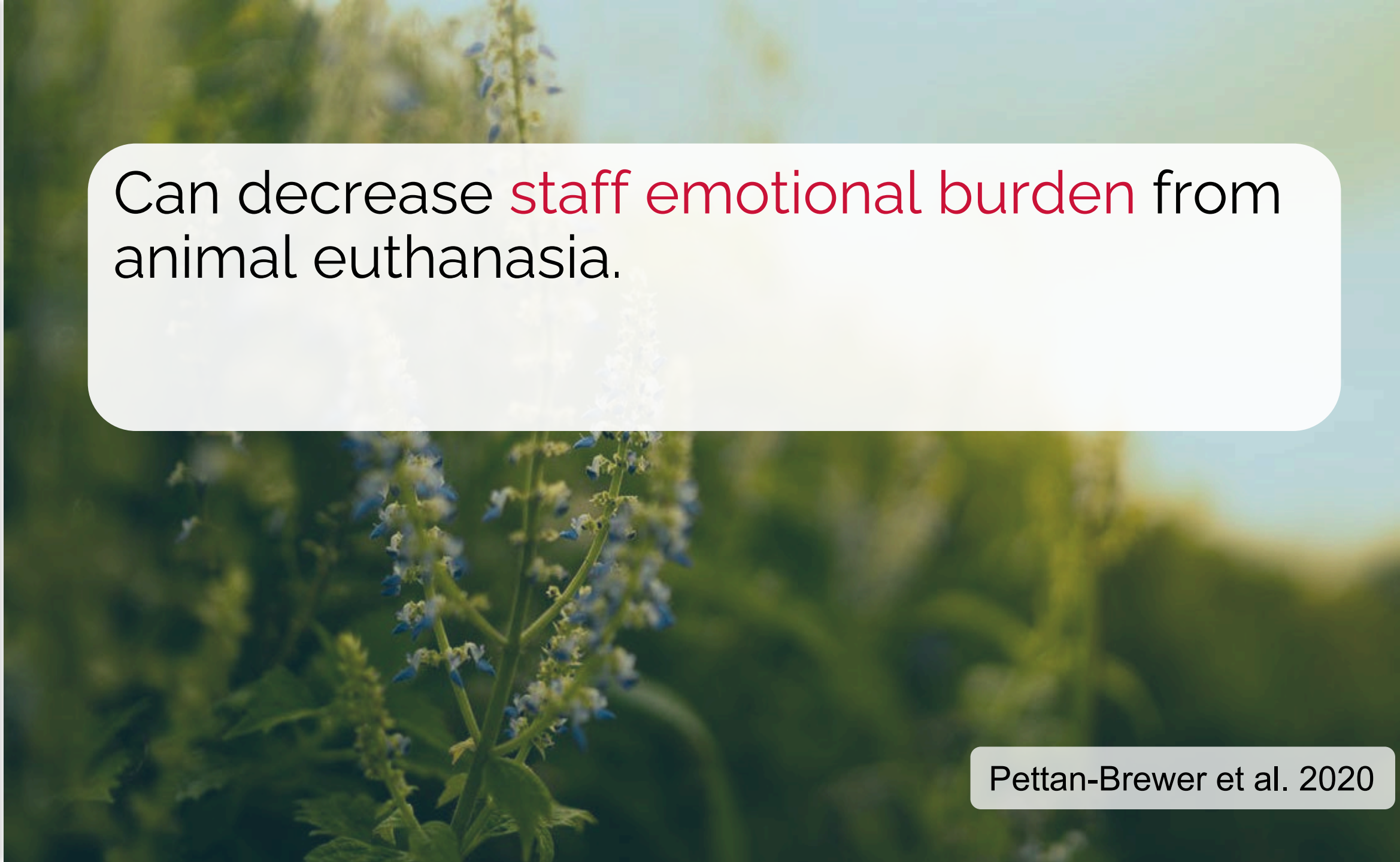
Publications show that environmental health monitoring successfully detects common pathogens of concern.

Viral	Bacterial	Parasitic
•Mouse hepatitis virus (MHV) •Murine norovirus (MNV) •Minute virus of mice (MVM) •Mouse parvovirus (MPV) •Theiler murine encephalomyelitis virus (TMEV) •Lactate dehydrogenase virus (LDV) •Lymphocytic choriomeningitis virus (LCMV) •Astrovirus •Sendai virus	•*Helicobacter* spp. •*Corynebacterium bovis* •*Rodentibacter* spp. •*Klebsiella oxytoca*	•Pinworms •Fur mites •*Entamoeba muris* •*Spironucleus* spp. •*Demodex* spp.


Environmental health monitoring can cost less including materials, testing, and staff labor.
Luchins et al. 2020



Can decrease staff emotional burden from animal euthanasia.

Pettan-Brewer et al. 2020

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How to switch to environmental health monitoring?

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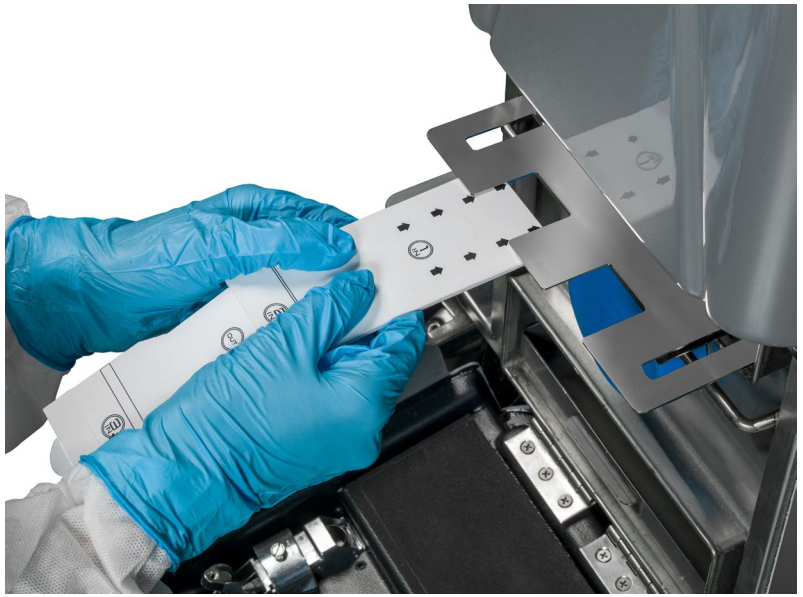
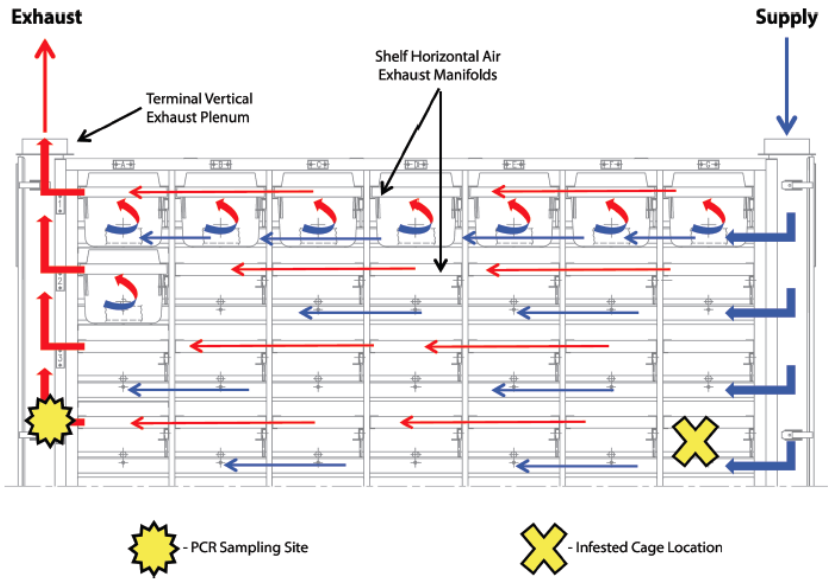
There are 2 options if you have IVCs filtered at rack level (e.g. Allentown or Tecniplast).





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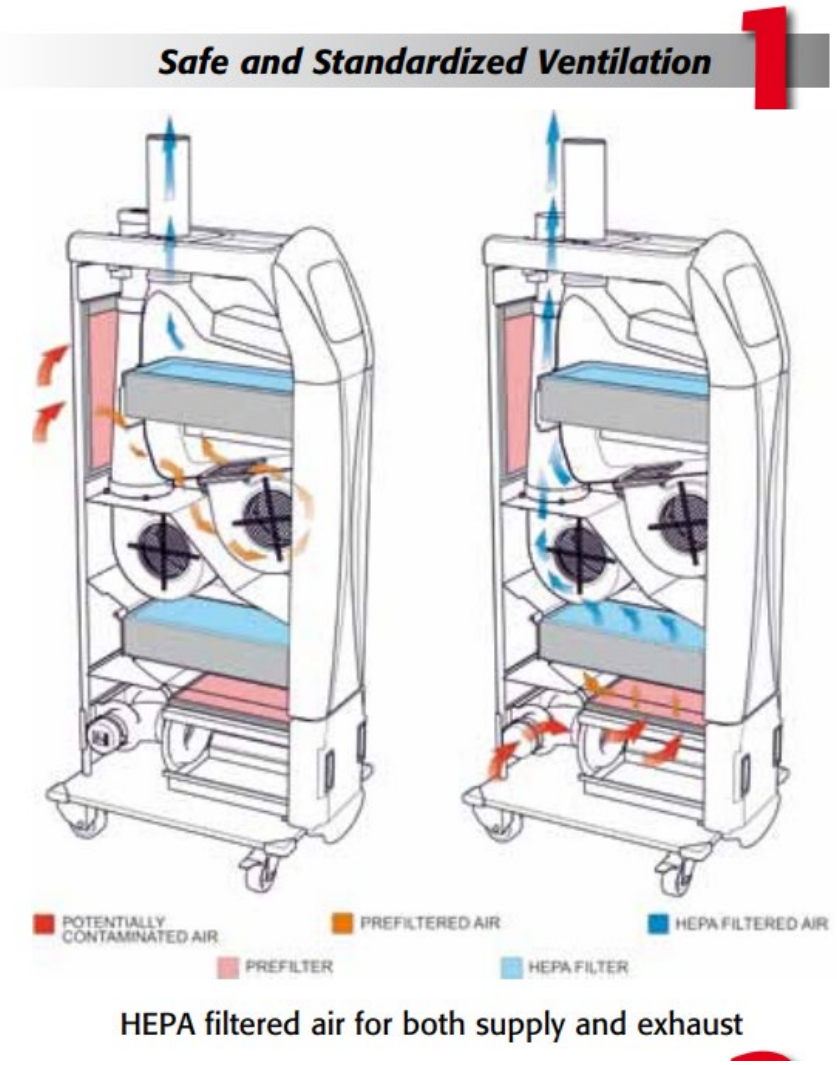
Smart Flow Air Handler

- Air Handlers need to be cleaned at least every 6 months
- Prefilters cleaned monthly
  - We will be looking into have extras on hand for wash days
- Important that the media filter does not get wet



INTERCEPTOR

- https://www.youtube.com/watch?v=u9XP5J9nhC0
- https://www.criver.com/sites/default/files/resources/TechniplastInterceptorFrequentlyAskedQuestions.pdf

SENTINEL EAD

- Pros: Specialized filter placed in ideal location to trap as much dust as possible. Minimized operator variability and requires minimal handling. Less time consuming.
- Cons: May be costly to adapt older racks

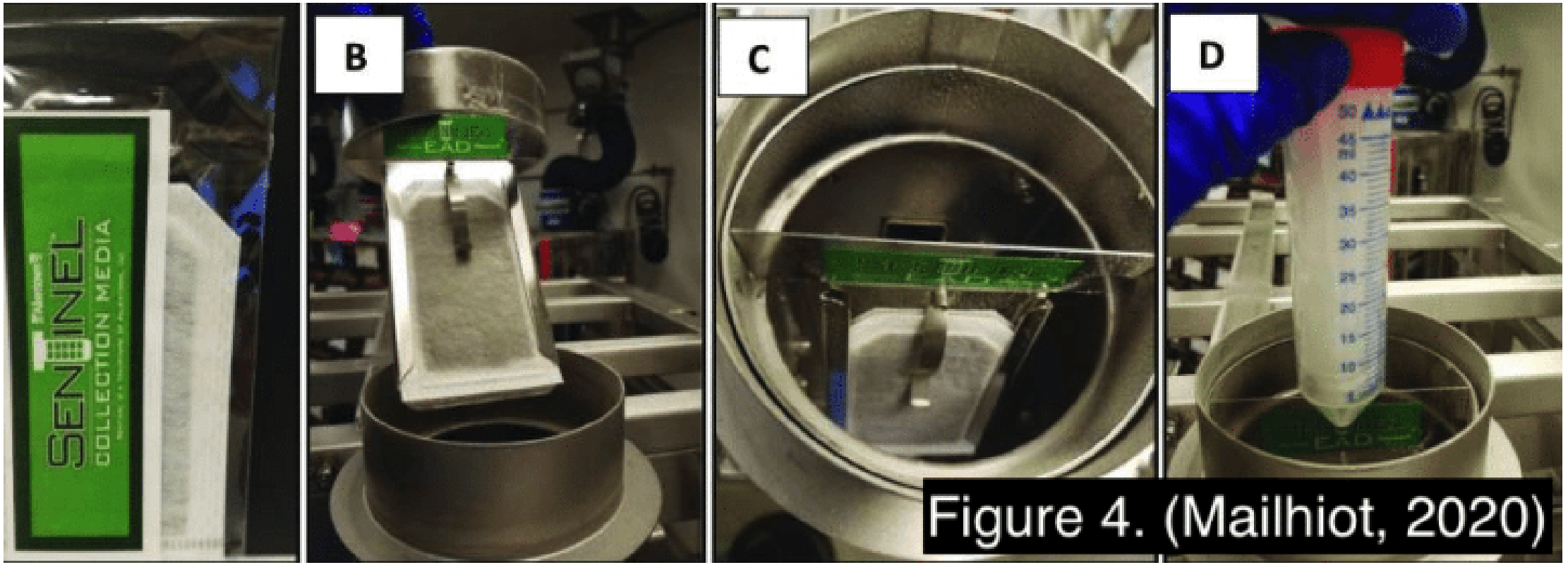


Figure 4. (Mailhiot, 2020)

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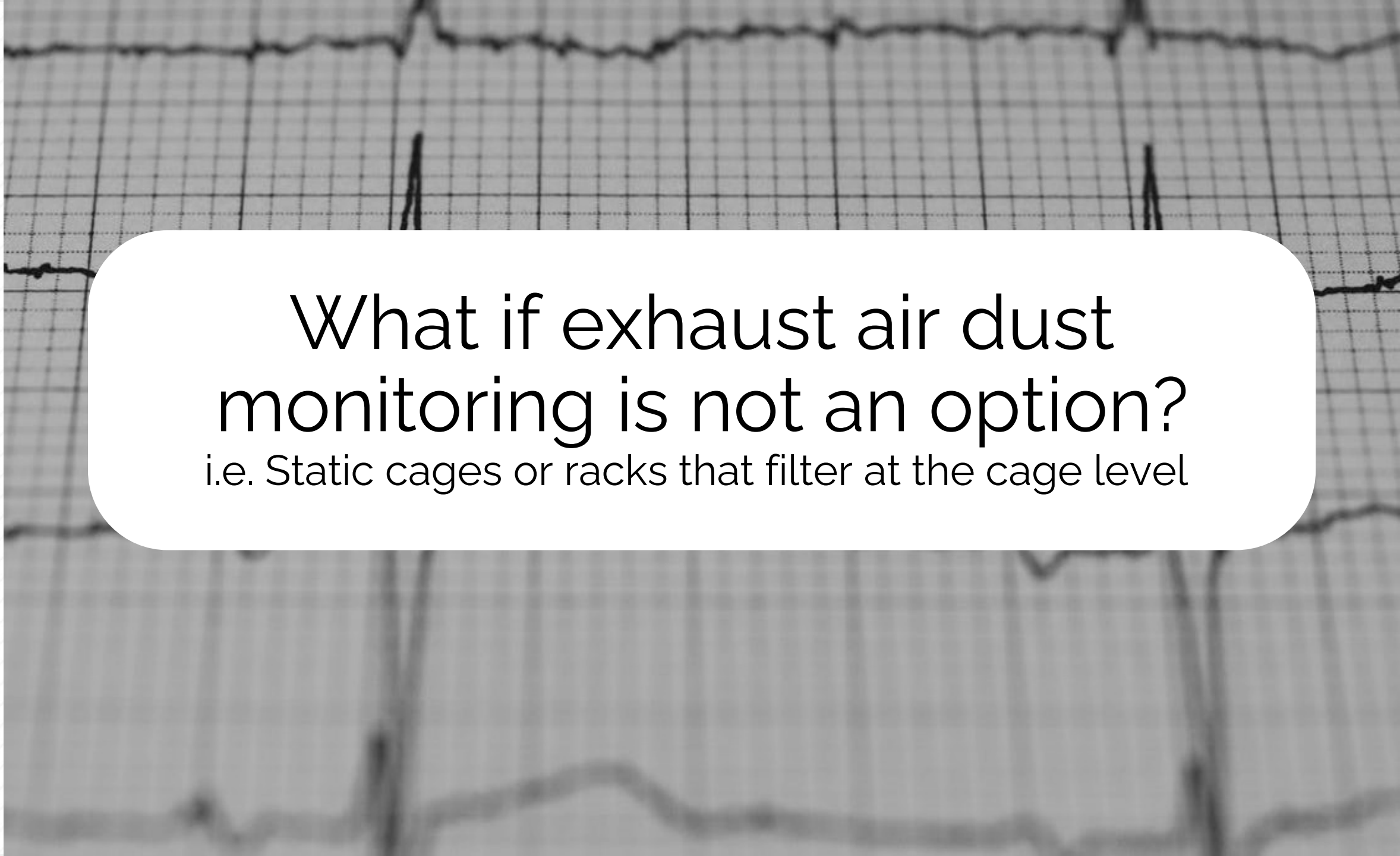
Plenum swabs for existing racks

- Pro: Initially less costly than collar-mounted media
- Cons: Requires more handling = more time consuming & potential operatory variability



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What if exhaust air dust monitoring is not an option?

i.e. Static cages or racks that filter at the cage level

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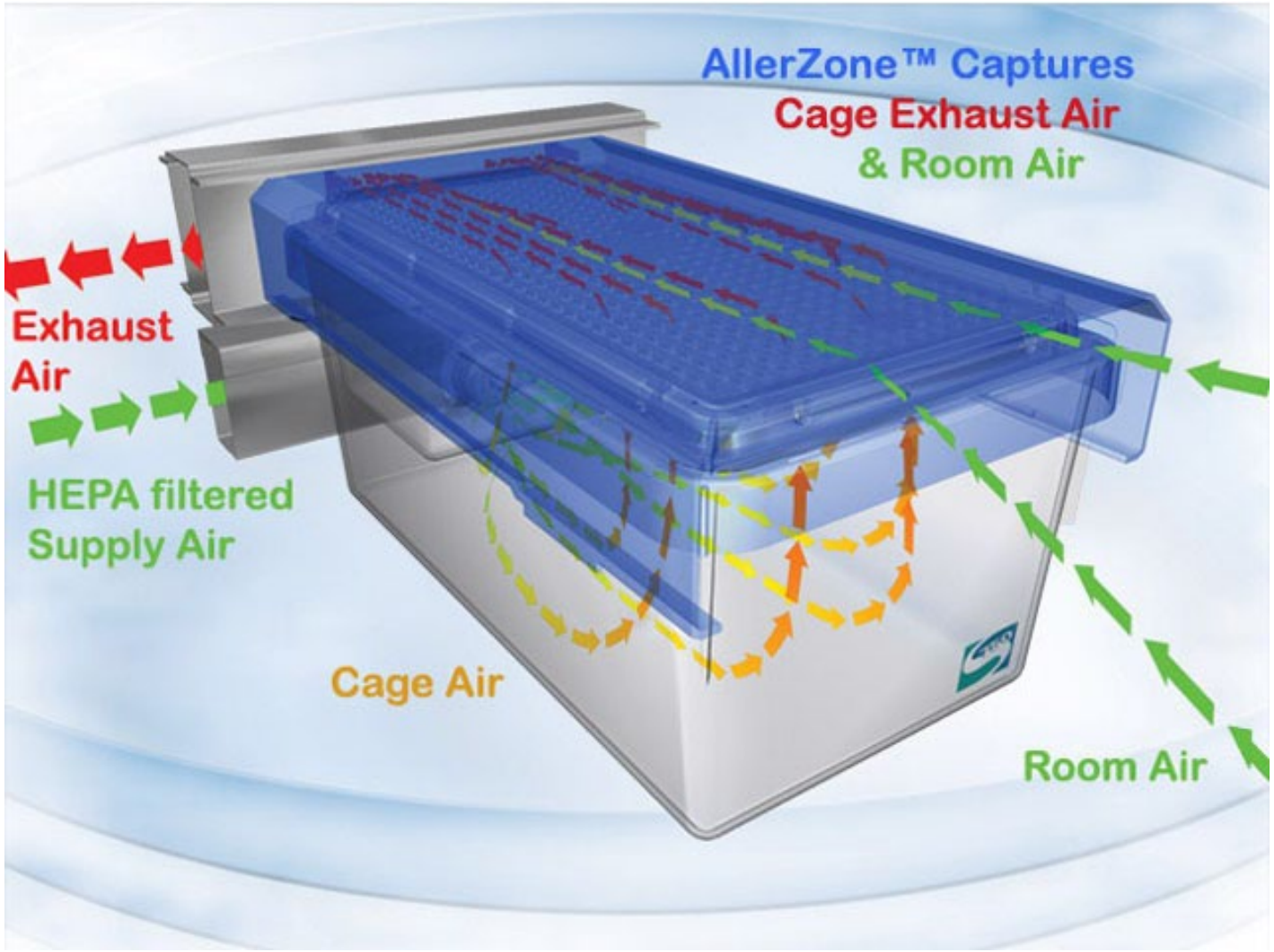
Options for Static Cages or IVCs filtered at the cage level (e.g., Animal Care Systems, Thoren, Innovive, & Lab Products.).



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Standard Operating Procedure

PathogenBinder®

Materials

1. A Collection Box with filter media will be provided for every 80-100 cages per room. The Collection Box will have an identifier sticker with rack and PI.
2. Proper PPE
    1. Change gloves between racks
    2. Clean gloves between different PI's

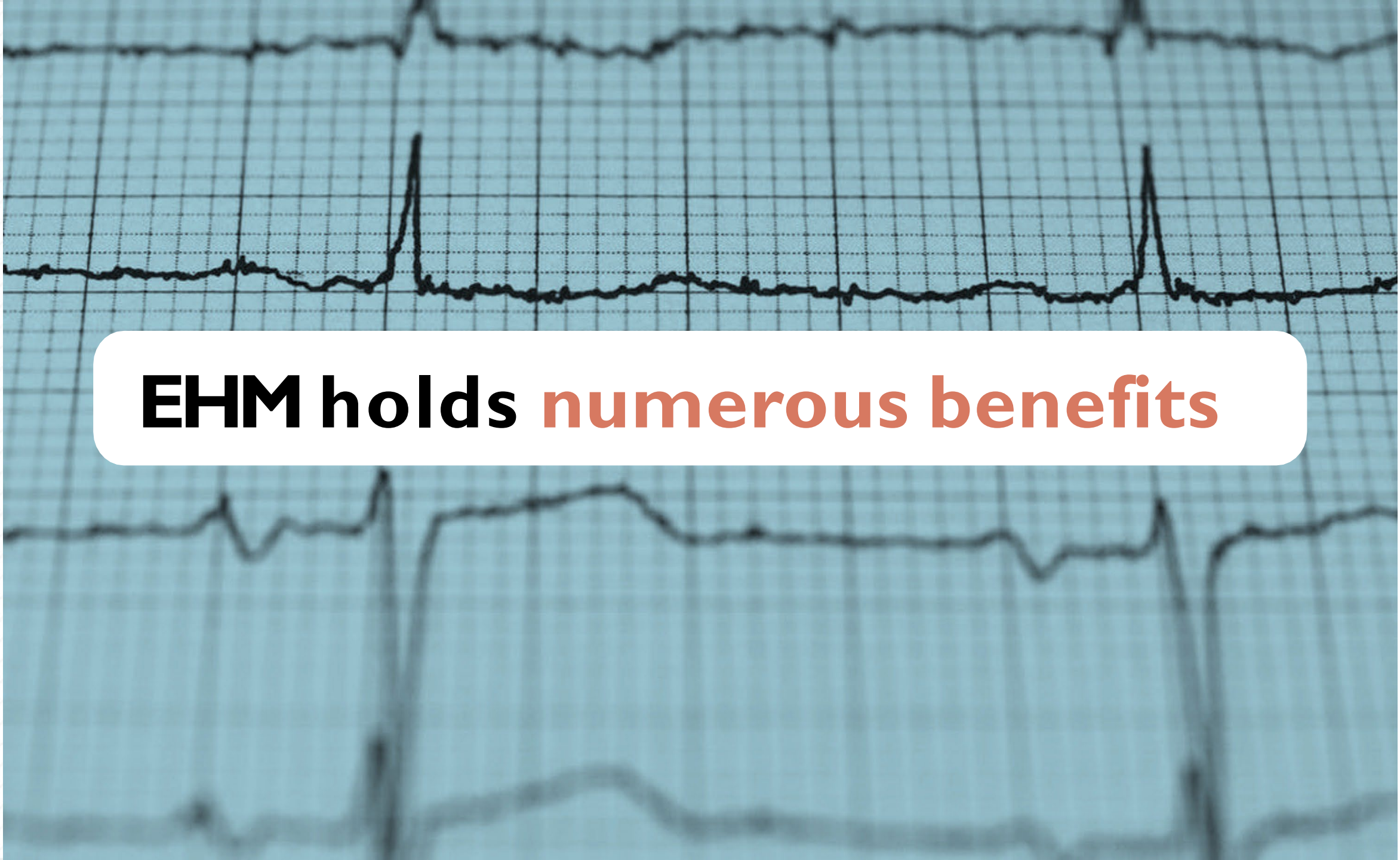
Placement of Filter/Weekly Cage Change

1. Do not open Collection Box when mice are exposed in the hood
2. Move mice to clean cage, close lid, then collect soiled bedding from resident cage
    1. Add approximately 1 Tablespoon (100 cages=6.25 cups of dirty bedding)
    2. Take sample from area used to urination and defecation
3. Collect soiled bedding from resident rodent cages, close lid on Collection box, shake well for 10-15 seconds.
4. Open the lid and place upside down in hood, pick up filter media with clean forceps and place on inverted lid, dump out bedding, return filter to box, seal and place box on tabletop in room.
5. At cage change, every 2 weeks, add soiled bedding, shake for 10-15 seconds, remove filter media as described above, discard dirty bedding, return filter media to empty box. KEEP same Collection box. Repeat every 2 weeks
6. CVS will collect the media end of trimester.

Sampling

PathogenBinder®

- **Racks**
  - It is important that our sampling is consistent for the ~12 weeks with the same cages
  - Ideally, a Pathogen Binder Collection Box (PBCB) will have ~80-100 cages and same PI animals
  - Lab Products racks hold 48 single cages per side.
    - Example: a double-sided full rack would have one PBCB
    - Example: two double sided ½ full racks would have one PBCB
  - All Racks must be labeled with a rack number for identification
  - If a rack is empty and there are no colony animals to sample into the PBCB, email or.ocv.alert@wsu.edu and they will coordinate with the PI and may come pick up the filter media.
  - If you note the filter media is wet or the PBCB has moisture, call OCV. We need to prevent molding of the filter media.



EHM holds numerous benefits

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EHM provides additional cage space.

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EHM replaces use of live animals

We estimate the reduction of ~120-150 animals annually

Environmental health monitoring is not without limitations.

PCR testing has potential for false positive results due to increased sensitivity.

Positive results not confirmed to be true outbreaks have been acceptable & manageable.

Takeaway: Environmental health monitoring is advantageous for science, animals, & people.

Replaces sentinel mice = 3Rs compliant

Increases result sensitivity & accuracy

Reduces labor & cost

Reduces emotional fatigue

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