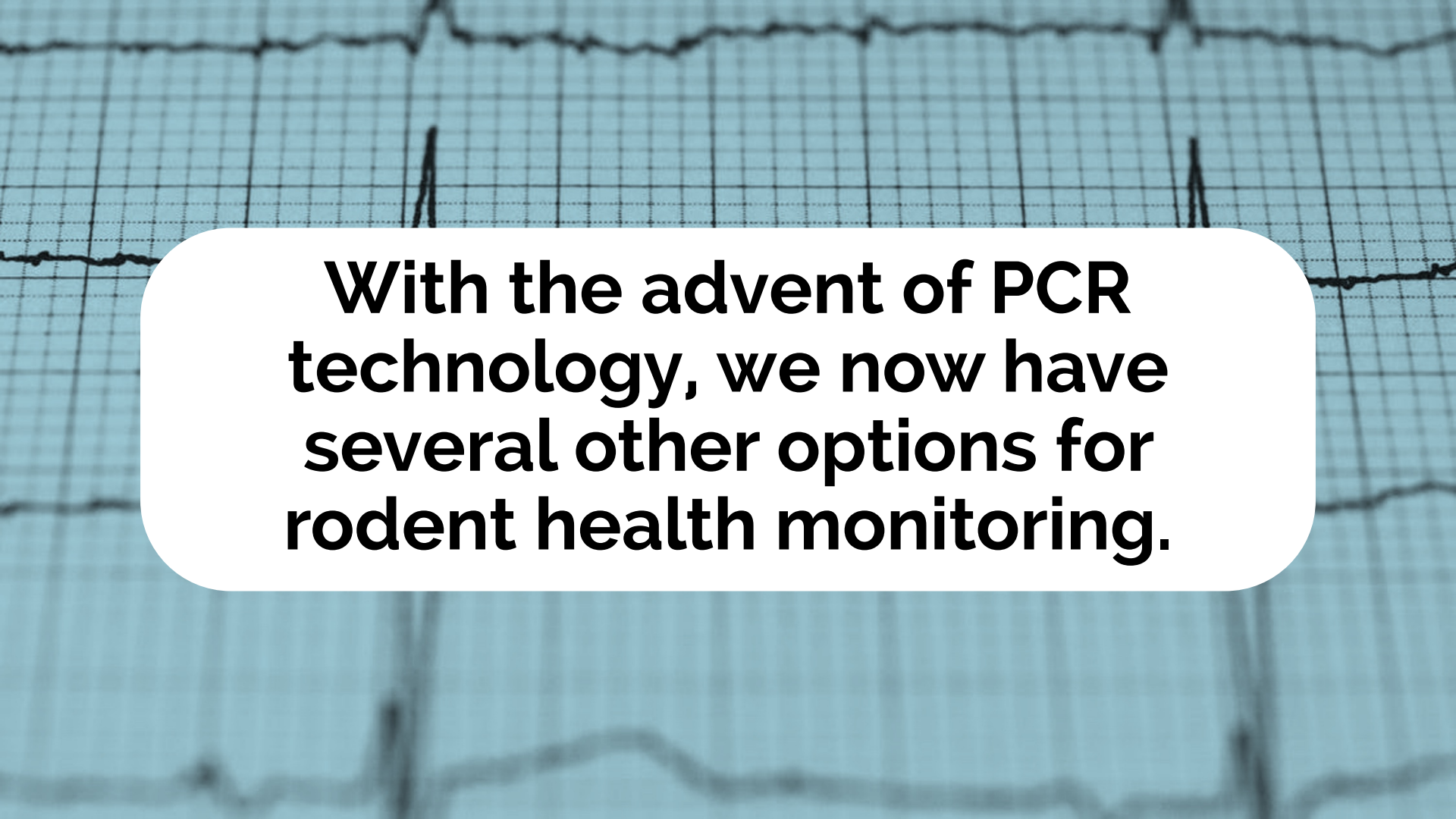
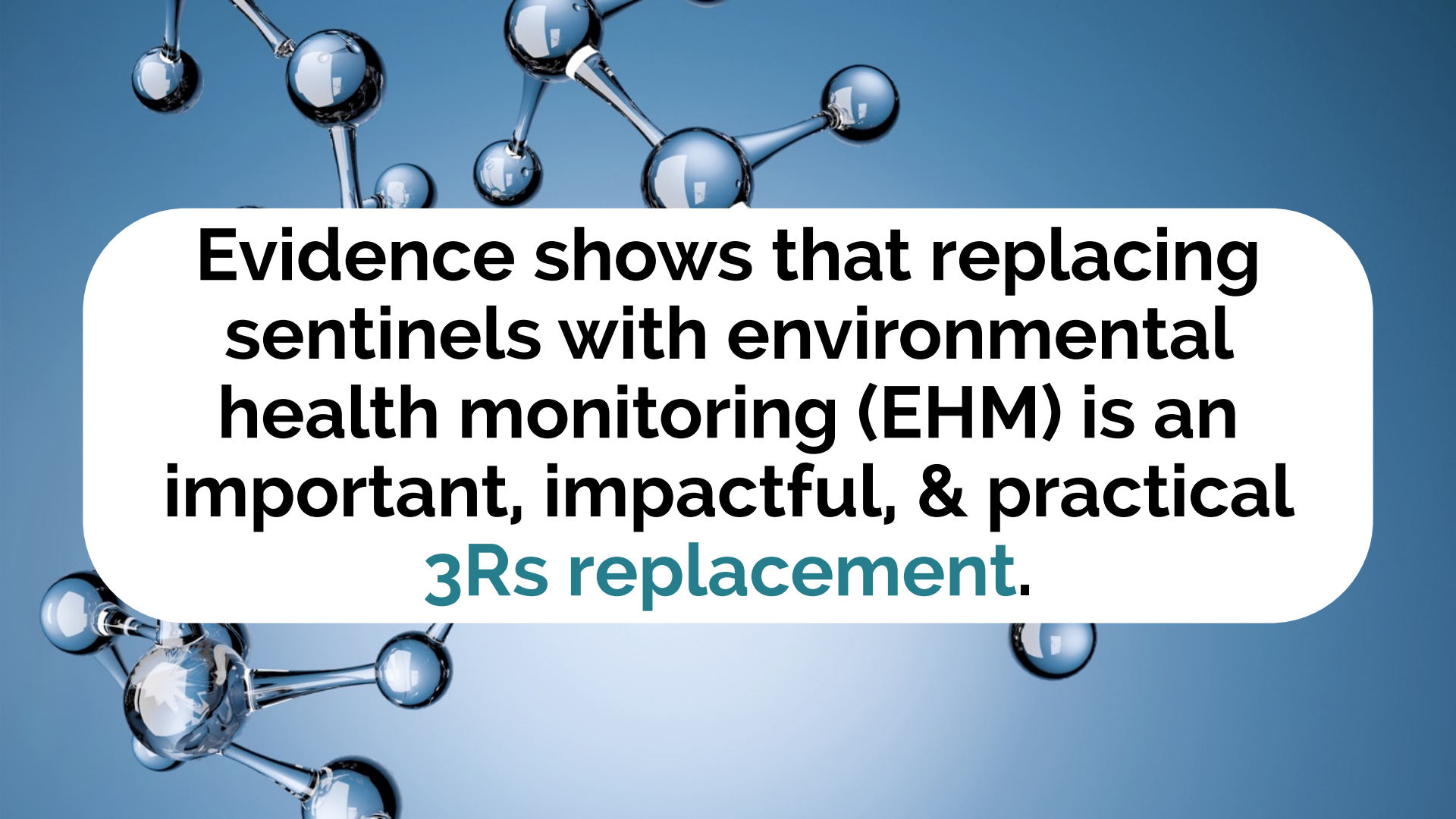
Making the Switch to Environmental Health Monitoring: Evidence, Data, & Practicalities

The North American 3Rs Collaborative

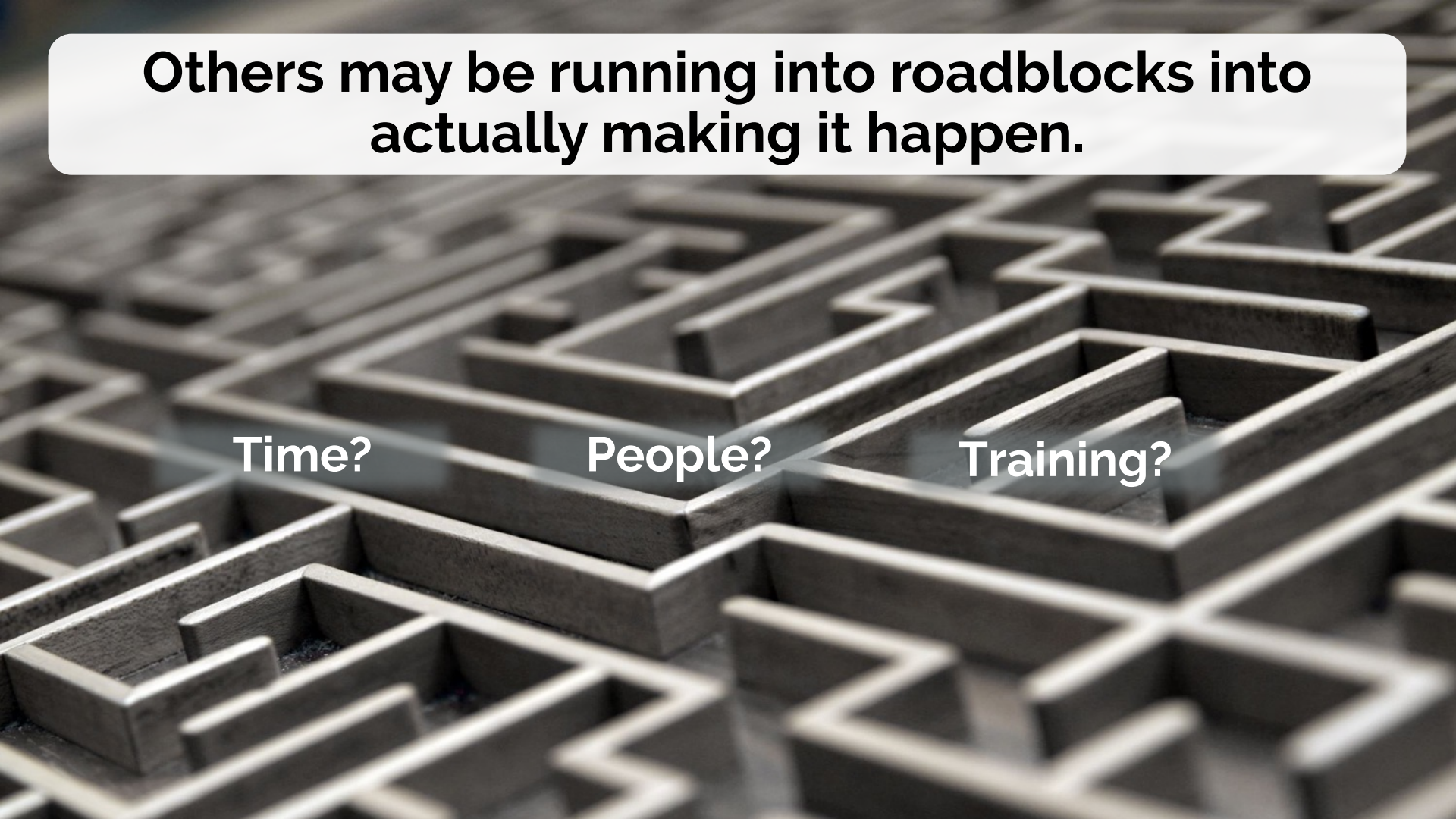
Traditionally, soiled bedding sentinel rodents are used to ensure colony health status.


With the advent of PCR technology, we now have several other options for rodent health monitoring.


Evidence shows that replacing sentinels with environmental health monitoring (EHM) is an important, impactful, & practical 3Rs replacement.


However, we know that there are still barriers to change.


Some may not be convinced that this is the RIGHT thing to do.
Evidence?
Impact?
Practicality?


Others may be running into roadblocks into actually making it happen.
Time?
People?
Training?

The North American 3Rs Collaborative was created by professionals who fully understand these experiences.

The North American 3Rs Collaborative

Refine. Reduce. Replace.

Collaborating to advance better science – for both people & animals

www.na3rsc.org | contactus@na3rsc.org

The 3RsC partners with you across the field.

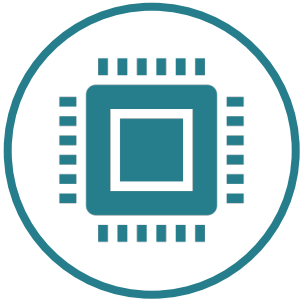
Lilly
scientist.com
USDA
Stanford University
3 R C C
zoetis
Roper Toxicology Consulting Limited
abbvie
EMORY
W
NC 3Rs

Our strategy is to identify initiatives with

- Strong Evidence
- Big Impact
- Real-World Practicality

NA3RsC currently has six key 3Rs initiatives.

Rodent Health Monitoring



Microphysiological Systems

Translational Digital Biomarkers

Refinement

3Rs Certification Course

Compassion Fatigue Resiliency

TODAY'S AGENDA

Megan LaFollette
Introduction & Systematic Review Results

Kerith Luchins
2022 Benchmarking Survey Results

Wai Hanson
Sentinel-Free Soiled Bedding Sampling

Chris Manuel
Academic Perspectives

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Our goal was to establish the evidence base
behind environmental health monitoring.

In 2016, there was a systematic review that evaluated the efficacy of soiled bedding sentinels.

PLOS ONE

RESEARCH ARTICLE

Efficacy of Soiled Bedding Transfer for Transmission of Mouse and Rat Infections to Sentinels: A Systematic Review

W. C. C. de Bruin[1]*, E. M. E. van de Ven[1], C. R. Hooijmans[2]

1 QM Diagnostics BV, Nijmegen, The Netherlands, **2** Radboud University Medical Centre, SYRCLE at Central Animal Laboratory, Nijmegen, The Netherlands

* Wieke.debruin@qmdiagnostics.org

de Bruin 2016

As of 2016, only 15 articles, conference presentations, or posters had been published on the efficacy of soiled bedding sentinels.

de Bruin 2016

This article evaluated the evidence supporting the use of SBS on a per pathogen basis,

Table 2. Efficacy of transfer of pathogens via soiled bedding and accordance of results between the included studies.

Pathogen	Author	Conclusion: Bedding transfer effective per experiment[1)]	Meta conclusion:Effective bedding transfer [2)]
Mouse			
EDIM	Compton, 2004 [10]	no	n.a.
Endoparasites other than pinworm	Brielmeijer, 2006 [8]	yes	n.a.
Helicobacter spp.	Compton, 2004 [10]	yes	yes (100%)
	Henderson, 2013 [20]	yes	
	Livingston, 1998 [15]	yes	
	Myers, 2003 [18]	yes	
Fur mites	Arbona, 2010 [7]	yes	yes (100%)
	Henderson, 2013 [20]	yes	
	Lindstrom, 2011 [14]	yes	
	Thigpen, 1989 [19]	yes	
MAdV	Henderson, 2013 [20]	no	n.a.
MPV	Brielmeijer, 2006 [8]	no	yes (75%)
	Compton, 2004 [10]	yes	
	Compton, 2012 [21]	yes	
	Henderson, 2013 [20]	yes	
MNV	Manuel, 2008 [16]	yes	n.a.
MVM	Henderson, 2013 [20]	yes	n.a.
Pasteurella pneumotropica	Henderson, 2013 [20]	no	n.a.
	Myers, 2003 [18]	yes	
Pinworms	Henderson, 2013 [20]	yes	n.a.
Pneumocystis murina	Myers, 2003 [18]	yes	n.a.
Pseudomonas aeruginosa	Henderson, 2013 [20]	yes	n.a.
Sendai	Compton, 2004 [10]	no	no (100%)
	Dillehay, 1990 [12]	no	
SDAV	La Regina, 1992 [13]	no	n.a.
TMEV	Brownstein, 1981 [9]	yes	yes (100%)
	Henderson, 2013 [20]	yes	
Rat			
CAR bacillus	Cundiff, 1995 [11]	no	n.a.
Clostridium piliforme	Motzel, 1992 [17]	yes	n.a.
SDAV	La Regina, 1992 [13]	yes	n.a.

Sufficient Evidence

If 2 or more publications reported at least 1 sentinel infected.

(max of 4 articles)

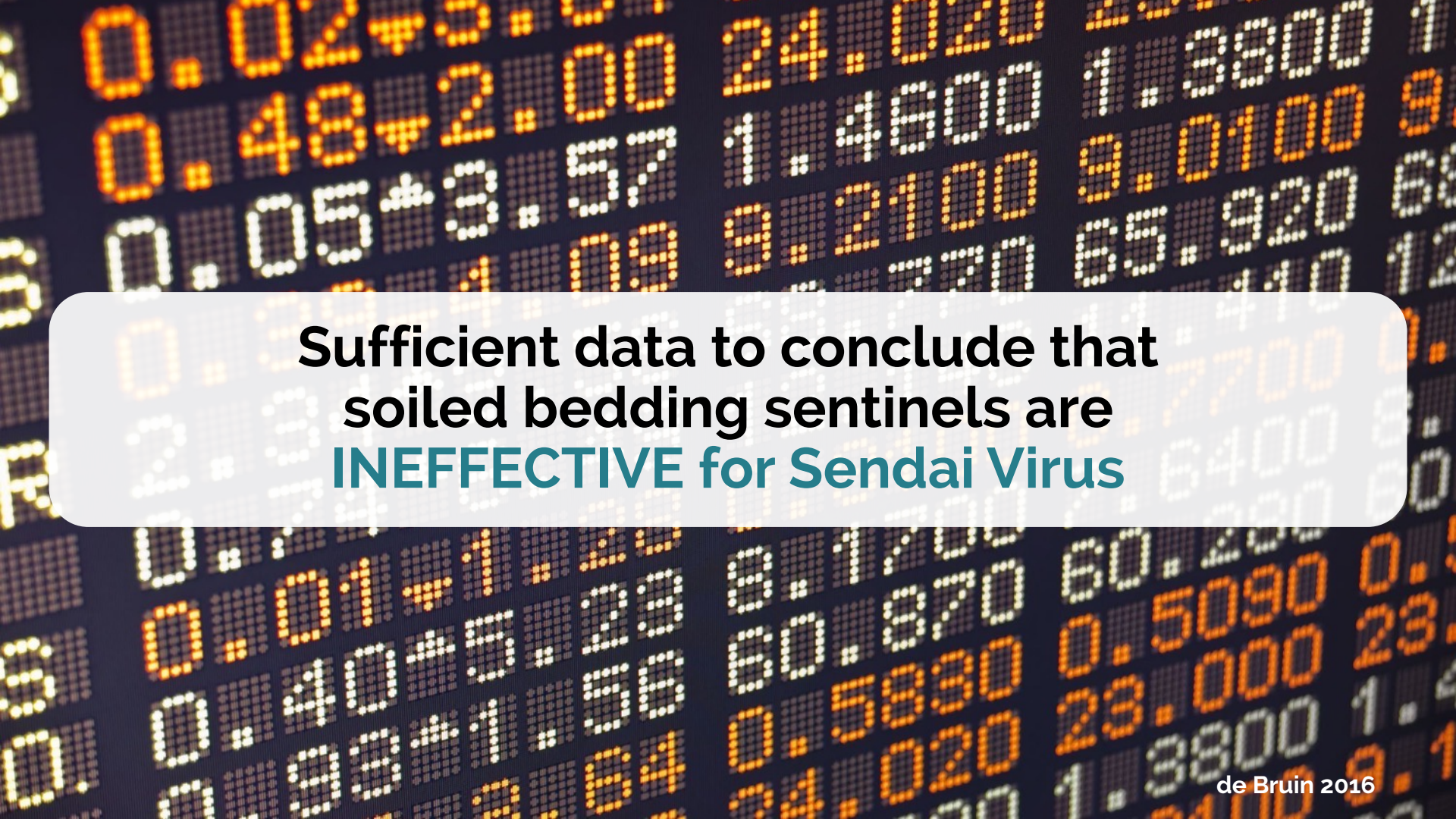
de Bruin 2016

Sufficient data to conclude soiled bedding sentinels are effective for ONLY 5 pathogens.

MHV, MPV, TMEV
***Helicobacter* spp.**
Fur Mites*

Note subsequent research has found sentinels do NOT consistently detect fur mites

de Bruin 2016



Sufficient data to conclude that soiled bedding sentinels are INEFFECTIVE for Sendai Virus

de Bruin 2016

Insufficient data exists to conclude whether soiled bedding sentinels are effective in detecting 11 additional agents:

- MNV
- EDIM
- MVM
- SDAV
- MAdV
- *Clostridium piliforme*
- Pinworms
- *Pseudomonas aeruginosa*
- *Filobacterium rodentium*
- *Rodentibacter spp.*
- *Pneumocystis murina*

(de Bruin 2016)

This article was a helpful template & comparison for our systematic review.

We wanted to answer the following questions:

Is there evidence to show that environmental health monitoring works?

For what agents?

For what types of EHM?

Is EHM better than SBS?

Objectives & Specific Aims

A comprehensive overview of empirical EHM research to provide direction for future application & investigation.

Methods

Outcomes

Moderators

Our review has high rigor & objectivity.

PRISMA Guidelines

Preferred **R**eporting **I**tems for
Systematic Reviews and **M**eta-**A**nalyses

SYRCLE Guidelines

Systematic **R**eview **C**enter for
Laboratory Animal **E**xperimentation

Liberati 2009; Moher 2009; Hooijmans 2014

We searched 3 databases to identify articles

Scopus®

*Conference abstracts were NOT included

- Peer-reviewed
- In English
- Rats or Mice
- Biological Monitoring OR Environmental/Health/Hygenic/Microbiological/Routine Sampling/Monitoring/Surveillance OR Exhaust Air/dust/debris

20% of extraction was replicated by a second reviewer

Thank you to
- Caroline Starla Clement for data collection
- Joe Garner for supervision

Data were analyzed via descriptive statistics & mixed linear regression (in progress).

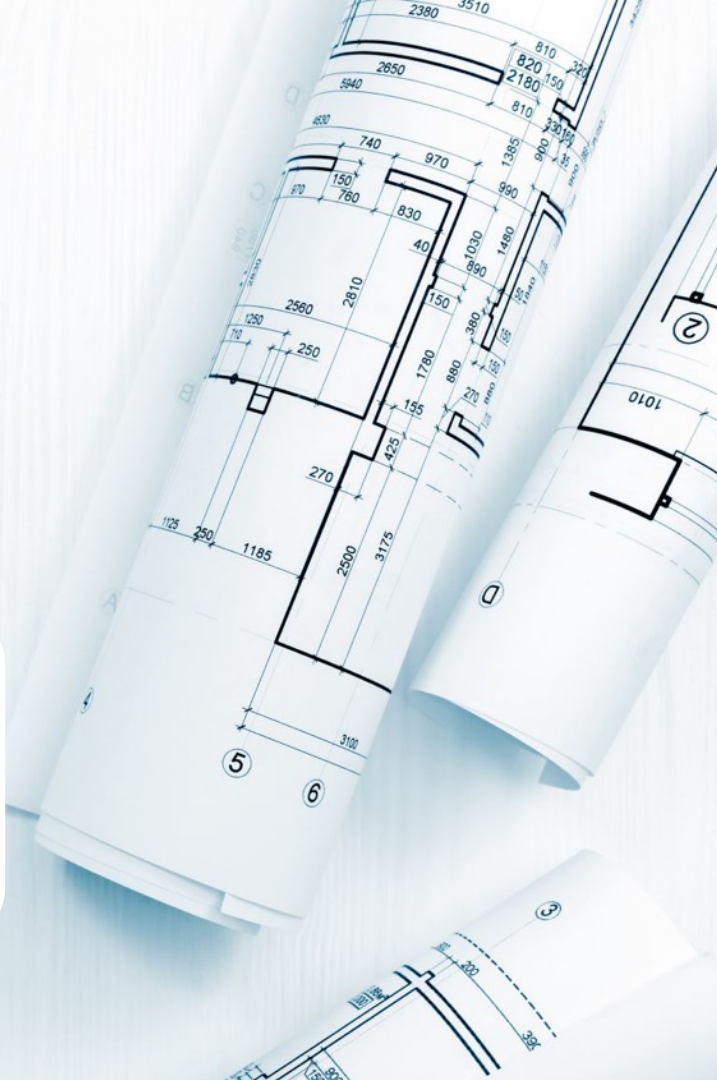
Dependent Variables
Detection (Yes/No)
% Detection

Independent Variables
Sampling Type
Pathogen Type
Sampling Type*Pathogen

RESULTS

(preliminary)

A total of 31 papers were included.



Many different terms were used for environmental health monitoring.

We've developed expert consensus on terminology.

We've developed expert consensus on terminology.

- **Soiled Bedding Sentinels (SBS)** = traditional rodent health monitoring that involves transferring soiled bedding to a cage with live rodents which are periodically sampled/euthanized to determine colony health status. (Sometimes referred to as "sentinels")
- **Environmental Health Monitoring (EHM)** = any type of health monitoring that does not require use of live animal sentinels

We've developed expert consensus on terminology for Exhaust Dust Testing.

- **Exhaust Dust Testing (EDT) = EHM via swabbing plenums or using in-line media for cages that filter at the rack level. This terminology is vendor independent.**
  - **Exhaust Air Dust (EAD®) – Charles River**
  - **Environmental Diagnostics (Edx) - IDEXX**
  - **EnviroRax – VRL Laboratories**
  - **Sentinel™ EAD® or Sentinel2™ - Allentown**
  - **Intercepter EAD® - Tecniplast**

We've developed expert consensus on terminology for Sentinel-Free Soiled Bedding (SFSB) Testing.

- **Sentinel-Free Soiled Bedding (SFSB)** = **EHM via transferring soiled bedding & testing without live sentinel animals. This includes single event exposure or indwelling media/swabs.**
  - **"Shake and Bake" – Patricia Foley**
  - **PathogenBinder™ - Charles River**

We've developed expert consensus on terminology.

- **Room & Equipment Monitoring (REM)** = EHM via testing bedding dump stations, IVC rack pre-filters, cage change stations, BSC, floors, etc.
- **Direct Colony Sampling (DCS)** = EHM via testing the research colony directly such as via feces, cage swabs, etc.

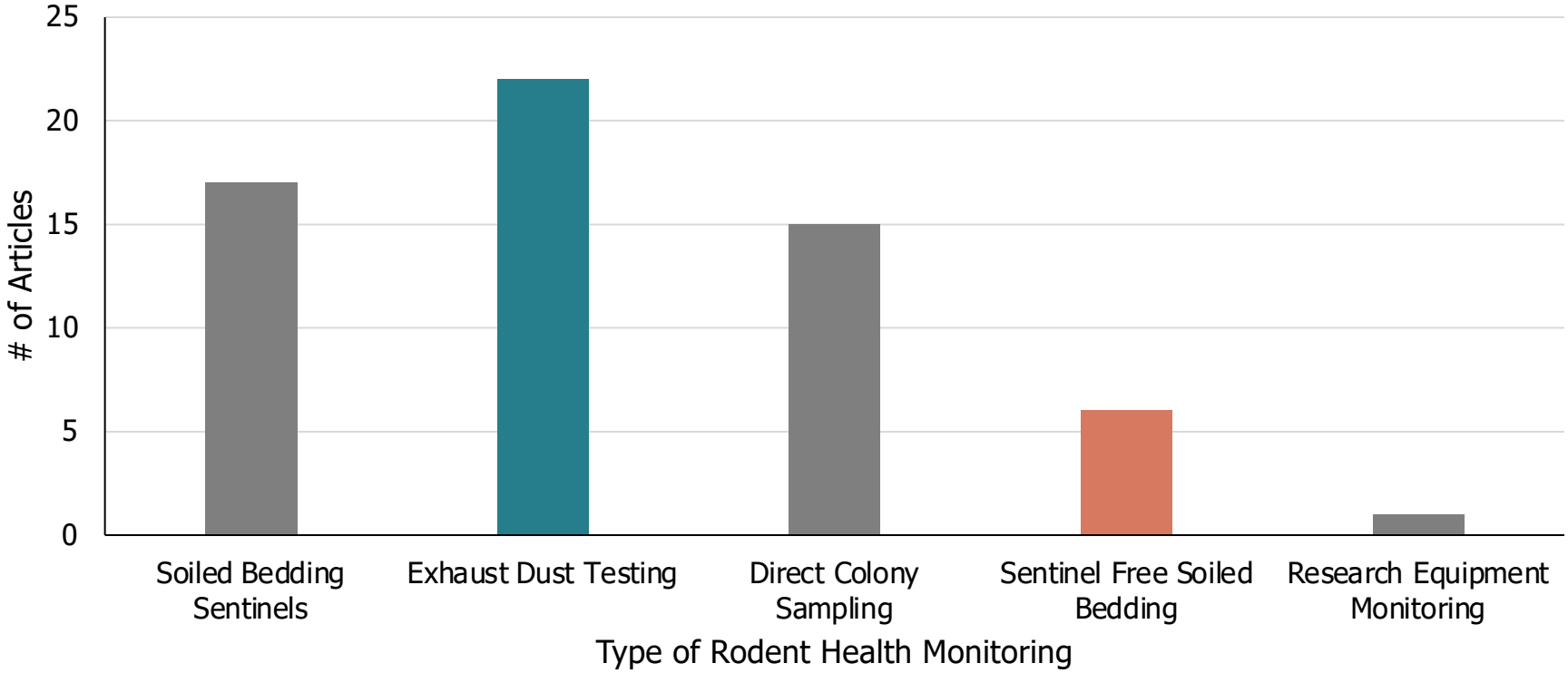
Articles used a variety of caging types, mouse strain, & mouse sex

Caging Type
13 Allentown
10 Tecniplast
3 BioZone
2 Lab Products
1 Thoren
2 Unclear

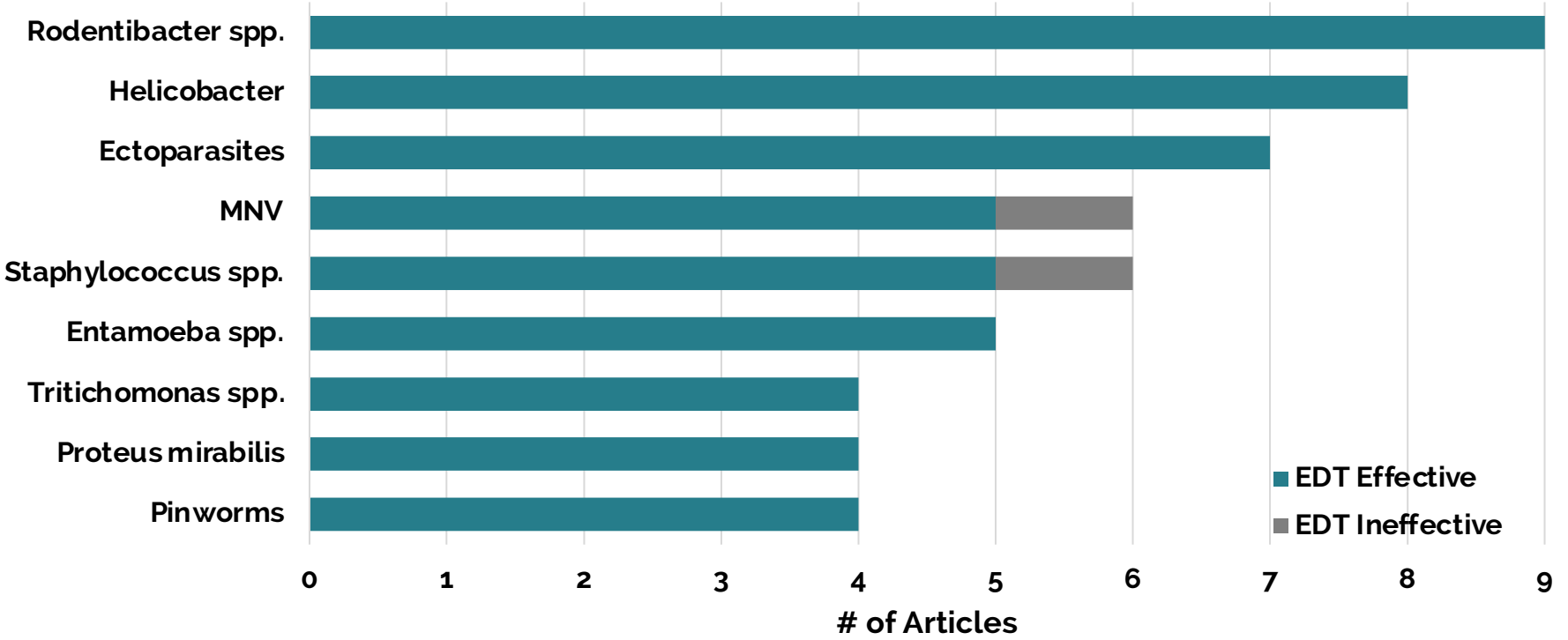
Mouse Strain
14 SW/CD1/ICR
2 C57BL/6
3 Nude
12 Other

Mouse Sex
15 F
8 MF
3 M
5 Unclear

31 Articles investigated types of environmental health monitoring, some in direct comparison to SBS

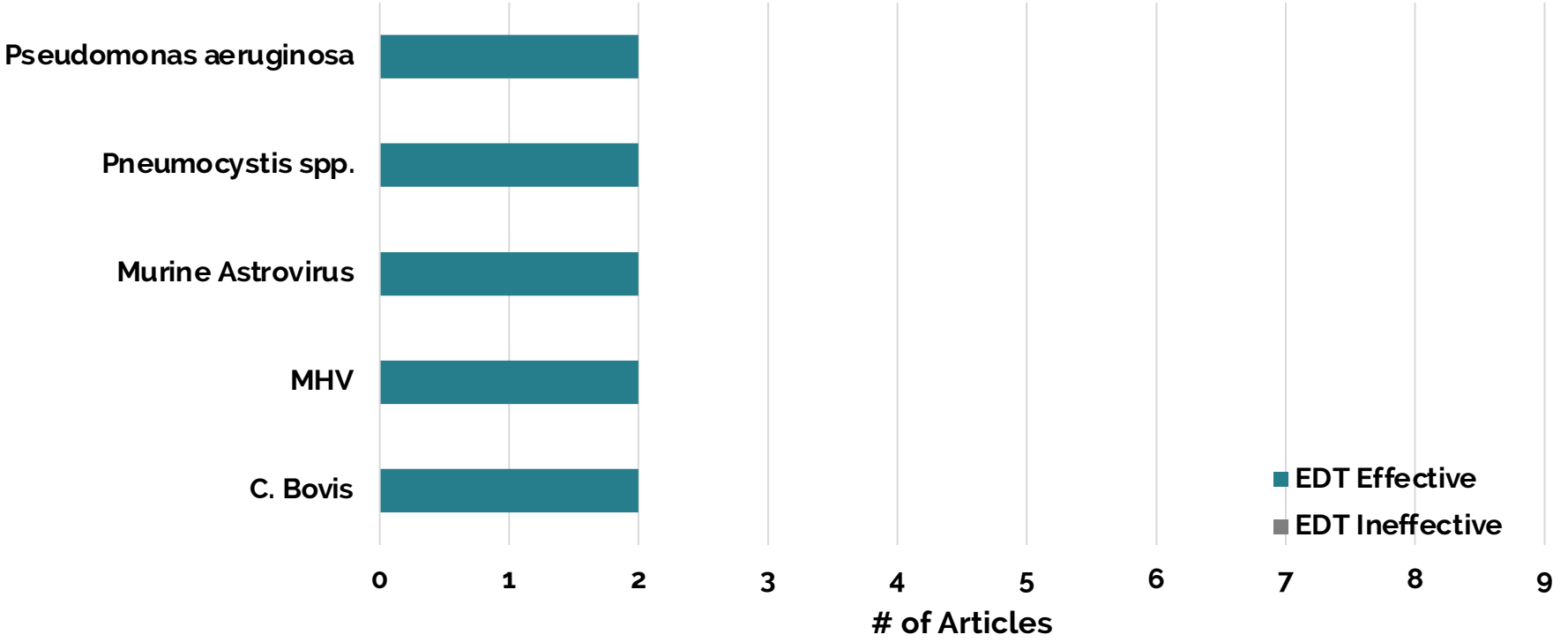


Strong evidence supports using Exhaust Dust Testing for 9 pathogens:



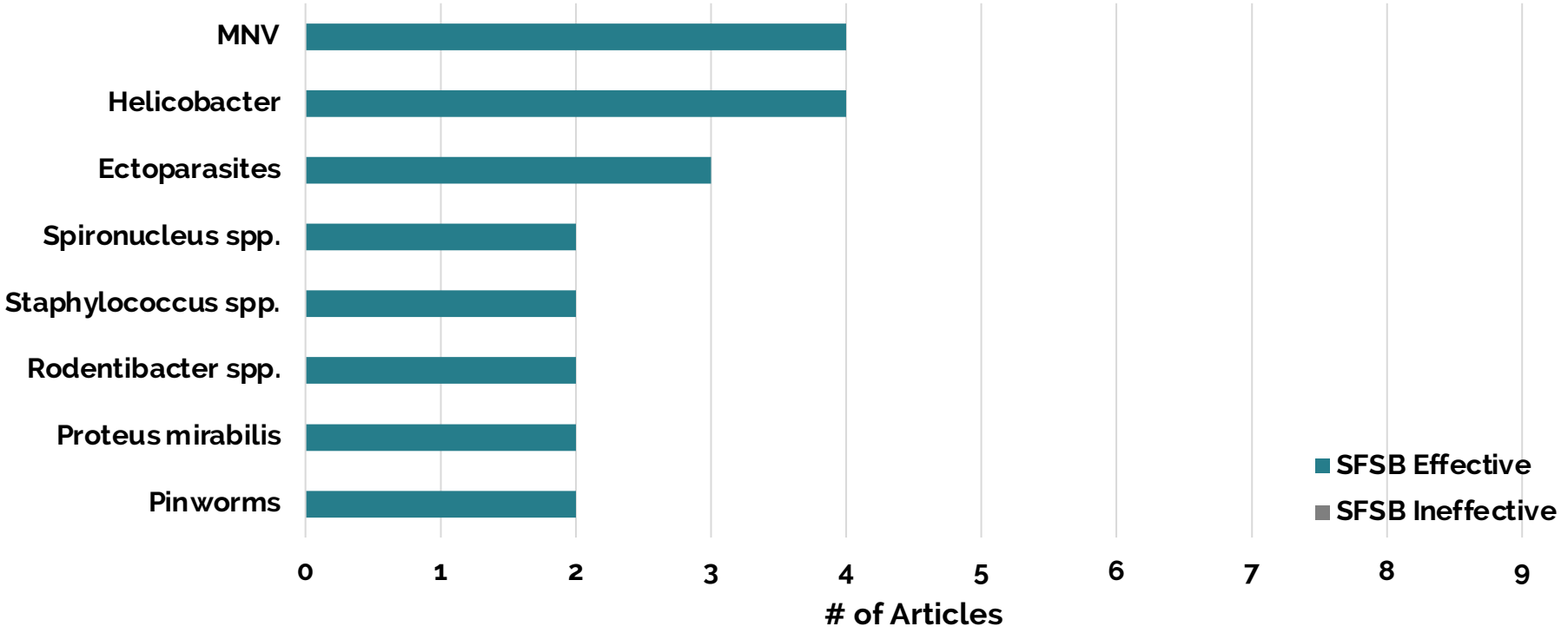
(Bauer 2016, Buchheister 2020, Compton 2004, Jensen 2013, Korner 2019, Mahabir 2019, Mailhiot 2020, Miller 2016, Miller 2018, Niimi 2018, Pettan-Brewer 2020, Ragland 2019, Schlapp 2018,Varela 2022, Zorn 2016)

Good evidence supports using Exhaust Dust Testing for 5 more pathogens:



(Compton 2004, Compton 2015, Manuel 2016, Manuel 2017, Korner 2019, Miller 2018, Niimi 2018, Ragland, 2019)

Good evidence supports using Sentinel-Free Soiled Bedding for 8 pathogens:



(Dubelko 2018, Hanson 2021, Gerwin 2017, O'Connell 2021, Varla 2022, Winn 2022)

10 pathogens were only evaluated by a single article. All but one were detected

1. Astrovirus – 1 (detected by SFSB & DCS)
2. Lactate Dehydrogenase Elevating Virus (Detected by EDT)
3. LCMV (False positive from EDT)
4. Mouse kidney parvovirus (detected by EDT, SFSB, & DCS)
5. MVM (detected with SFSB)
6. *Mycoplasma* spp. (detected by EDT & DCS)
7. TMEV (detected by SFSB)
8. *Chlamydia muridarum* (Detected by DCS)
9. *Citrobacter rodentium* (Detected by DCS)
10. Beta Grp B Strep (not detected by SFSB or DCS)

Exhaust Dust Testing or Sentinel-Free Soiled Bedding were able to detect 13 pathogens, when SBS failed.

- Ectoparasites (Korner 2019, Miller 2018, Varela 2022, Hanson 2021)
- Helicobacter (Compton 2004, Jacobsen 2005, O'Connell 2021)
- Rodentibacter spp. (Miller 2016, Miller 2018, Niimi 2018)
- Tritichomonas spp. (Niimi 2018, Miller 2018)
- Entamoeba (Miller 2018, Dubelko 2018)
- Pneumocystis spp. (Milelr 2018, Niimi 2018)
- Proteus mirabilis (Miller 2018, Schlapp 2018)
- Sendai Virus (Compton 2004)
- Pinworms (Miller 2018)
- MNV (Zorn 2016)
- Klebsiella spp. (Miller 2018)
- Pseudomonas Aeruginosa (Miller 2018)
- Staphylococcus spp. (Schlapp 2018)

Environmental Health Monitoring helps replace rodents & save costs

- Across articles, 6876 rodents from 4 institutions are being replaced annually with environmental health monitoring.
- 9 Articles mentioned that cost-savings were identified from switching to environmental health monitoring

Limitations & Cautions from the Current Research

- Environmental Health Monitoring is so effective at detection that false positives are possible.
  - Any unexpected positives should be discussed with the diagnostic lab
  - Proper cage washing is important to remove residual nucleic acid
- Some pathogens may need more data although consider prevalence & exclusion lists
- EHM may not be perfect, but neither are sentinels

Takeaway: environmental health monitoring is advantageous.

3Rs
Replaces Sentinel Rodents

Science
Increases result sensitivity & accuracy

Operations
Reduces labor & cost

Staff
Reduces emotional fatigue

NA3RsC has created a resource hub to help institutions make the switch

Overview Presentations Publications Editable Slide Deck SOPs Cost Analysis FAQs

NA3RsC.org/health-monitoring

NA3RsC has created a resource hub to help institutions make the switch

Many institutions have replaced their sentinels.

Many institutions have replaced their soiled bedding sentinel health monitoring programs with EHM programs. This includes institutions such as University of Washington, Pfizer Comparative Medicine sites (Kendall Square, La Jolla, Pearl River, Groton), University of Florida, University of Colorado Anschutz Medical Campus, Emory University, University of Chicago, Emory National Primate Research Center, Research Institute of the McGill University Health Centre & McGill University comparative medicine & Animal Resources Center, Medical College of Wisconsin, Northwestern University, Benaroya Research Institute, UT Southwestern Medical Center, University of Alabama at Birningham, and many more.

THE UNIVERSITY OF CHICAGO

Pfizer

How to implement environmental health monitoring?

The first step to implementing EHM is to determine your type of caging. Multiple EHM sampling methods can be used, and the best method(s) are dictated by the type of caging that you have. Ultimately, an **environmental sample is collected and analyzed for rodent pathogens by PCR testing**.

1. For IVC racks that exhaust at the rack level (i.e., Allentown Inc., Tecniplast™): **Exhaust Dust Testing (EDT)**
2. For static cages or IVC racks that exhaust at the cage level (i.e., Animal Care Systems, Inc., Thoren, Inc., Innovive, Inc., Lab Products, LLC): **Sentinel-Free Soiled Bedding (SFSB)**
3. All cages and rack types: **Direct Colony Sampling (DCS)**
4. All cages and rack types: **Room & Equipment Monitoring (REM)**

Below we briefly describe each environmental health monitoring method.

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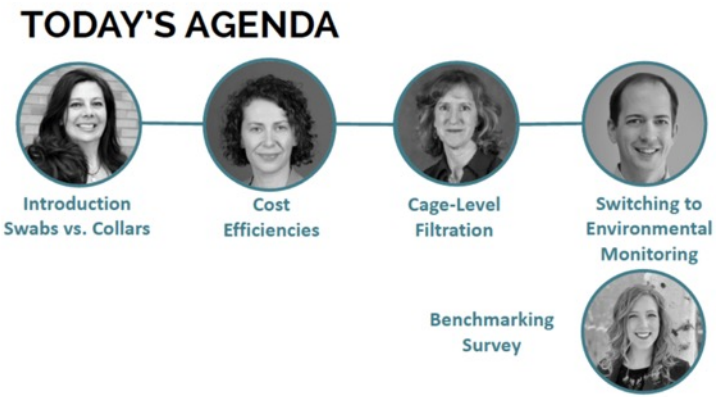
NA3RsC has created a resource hub to help institutions make the switch

AALAS 2021

At the 2021 National Meeting of the American Association for Laboratory Animal Science the NA3RsC organized a panel session titled "Switching to Environmental Health Monitoring for Rodents; How, Why & Will It Cost More." Click on the image below to see the PDF of this 2.5 hour panel presentation.



List of Publications

1. Bauer, B. A., Besch-Williford, C., Livingston, R. S., Crim, M. J., Riley, L. K., & Myles, M. H. (2016). Influence of rack design and disease prevalence on detection of rodent pathogens in exhaust debris samples from individually ventilated caging systems. *Journal of the American Association for Laboratory Animal Science*, *55*(6), 782-788.
2. Besselsen, D. G., Wagner, A. M., & Loganbill, J. K. (2000). Effect of mouse strain and age on detection of mouse parvovirus 1 by use of serologic testing and polymerase chain reaction analysis. *Comparative medicine*, *50*(5), 498-502.
3. Brielmeier, M., Mahabir, E., Needham, J. R., Lengger, C., Wilhelm, P., & Schmidt, J. (2006). Microbiological monitoring of laboratory mice and biocontainment in individually ventilated cages: a field study. *Laboratory animals*, *40*(3), 247-260.
4. Clancy, B. M., Theriault, B. R., Schoenberger, J. M., Bowers, C. J., Mitchell, C. M., Langan, G. P., Ostdiek, A.M.,, & Luchins, K. R. (2022). Identification and Control of an Ornithonyssus bacoti Infestation in a Rodent Vivarium by Using Molecular Diagnostic Techniques. *Comparative Medicine*.
5. Compton, S. R., Homberger, F. R., Paturzo, F. X., & Clark, J. M. (2004). Efficacy of three microbiological monitoring methods in a ventilated cage rack. *Comparative medicine*, *54*(4), 382-392.

NA3RsC.org/health-monitoring

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Are you interested in having your facility switch to environmental health monitoring? We recommend starting by giving an introductory presentation to management and other stakeholders about the evidence, advantages, and practicalities of replacing sentinel rodents with environmental health monitoring. We have a 10-15 min slide deck that you are free to use and modify for your facility that you can download by clicking the image below.

Click on the links below to download copies of the relevant SOPs for your institution. Please edit SOPs as needed to reflect the equipment and procedures chosen for use in your facility. Users should also ensure they contact diagnostic laboratories prior to submitting sample to ensure materials and procedures meet current acceptance criteria.

NA3RsC Standard Operating Procedures for Environmental Health Monitoring

1. Exhaust Dust Testing with Allentown Racks
2. Exhaust Dust Testing with Tecniplast Racks
3. Sentinel Free Soiled Bedding Sampling (for static, open top, or racks filtering at the cage level)
4. Direct Colony Sampling
5. Room and Equipment Monitoring

NA3RsC.org/health-monitoring

NA3RsC has created a resource hub to help institutions make the switch

be used as a guide for that assessment. Alternatively, institutions can use the EAD Cost Calculator from Charles River.

In addition, Luchins, et al. found that moving to exhaust dust testing reduced the amount of time the veterinary technician spent on the health monitoring program. For every veterinary technician, this amounted to ~1.5 hrs each week per 10,000 rodent cages. This extra time would be appreciated in any animal care and use program.

Below is an example cost analysis from one large institution that found that environmental health monitoring (EHM) was 26% less expensive than soiled bedding sentinels (SBS).

- Animal Ordering: **$0 for EHM** vs $415,084 for SBS
- Animal Shipping: **$0 for EHM** vs $3,876 for SBS
- Animal Maintenance: **$0 for EHM** vs $137,642 for SBS
- Technician Time: **$1,683 for EHM** vs $7190 for SBS
- Diagnostic Testing: $450,938 for EHM vs $449,629 for SBS

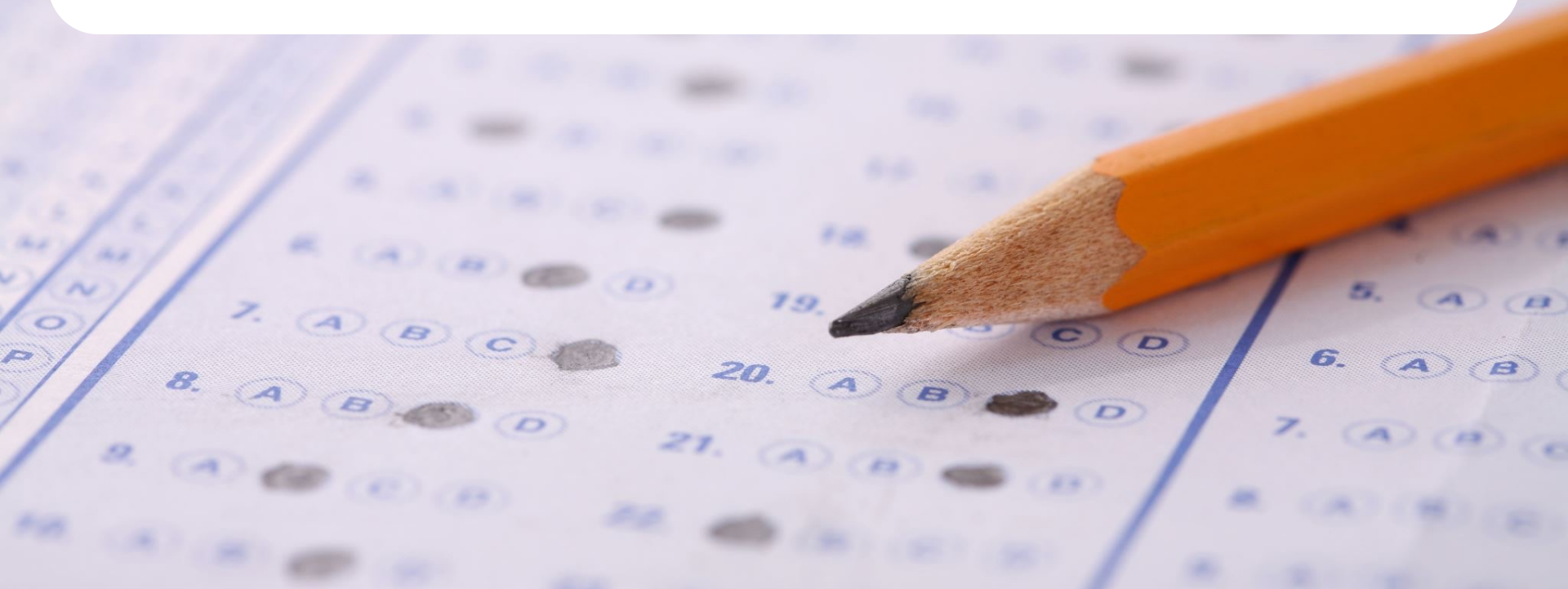
Total Annual Cost = $452,621 for EHM vs $613,421 for SBS

- Will this method of environmental health monitoring work for various types of IVC racks with different filtration levels (cage level vs. rack level)?
- In our facility, there are some rooms with static cages, can this method be used to monitor animals housed in static cages?
- Our type of IVC rack filters at the cage level and this EHM will still require dirty bedding transfer to monitor colony health status. So why switch to this type of monitoring?
- Is there really enough data to support Environmental Health monitoring?
- Will switching cost more money?
- Will other institutions accept our rodents if we switch?

NA3RsC.org/health-monitoring

If you participated in our survey this April, please make sure to participate next April!

New institutions are also welcome!



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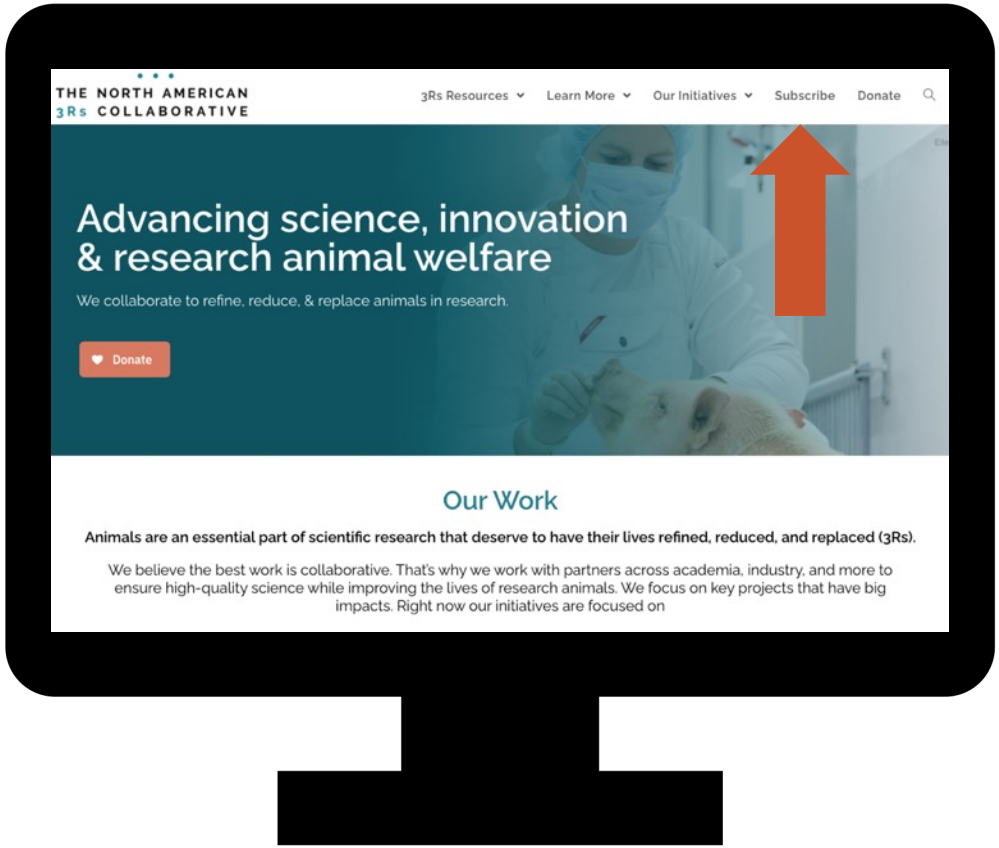

NA3RsC's AALAS Events

- **Booth #1150**
- ~~**3Rs Reception:** Monday, 5-7pm at Hyatt Pimlico Room~~
- ~~**Translational Digital Biomarkers Roundtable:** Tuesday, 12:30-2pm~~
- ~~**Environmental Health Monitoring Session:** Tuesday, 2:45-5pm~~
- **Compassion Fatigue Resiliency Session:** Wednesday, 2:45-5pm
- **Refined Mouse Handling Roundtable:** Thursday, 12:30-2pm

Acknowledgements: NA3RsC's sponsors

Princeton

Acknowledgments

- Aurore Dodelet-Devillers, McGill U.
- Barbara Stone, ParaTechs Corporations
- Beth Bennett, Pfizer
- Brian Bilecki, Allentown
- Bob Livingston, IDEXX
- Caroline Winn, Pfizer
- Chris Manuel, U. of Colorado Anschutz
- Christina Pettan-Brewer, U. of Washington
- Cris Torres, UCLA
- Joseph Garner, Stanford University
- John Hansenau, Tecniplast Consultant
- Kate Gates, Stanford University
- Ken Henderson, Charles River Laboratories
- Kerith Luchins, U. of Chicago
- Lise Phaneuf, Centre for Phenogenomics
- Massimo Foa, IDEXX
- Megan LaFollette, NA3RsC
- Norman Peterson, Seagen
- Ovidiu Jumanca, ICRM
- Patricia Foley, Georgetown U.
- Robert (Bob) Livingston, IDEXX
- Theresa Faughnan, Long Island U.
- Wai Hanson, Emory U.

Visit NA3RsC.org to learn more & join us to further the 3Rs.

Email me: meglafollette@na3rsc.org

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Academic Perspectives

Beth Bennet
Industry Perspectives

Benchmarking, Barriers, & Solutions for Environmental Health Monitoring

Kerith Luchins, DVM, DACLAM
Director, Rodent Clinical Services

THE UNIVERSITY OF CHICAGO BIOLOGICAL SCIENCES

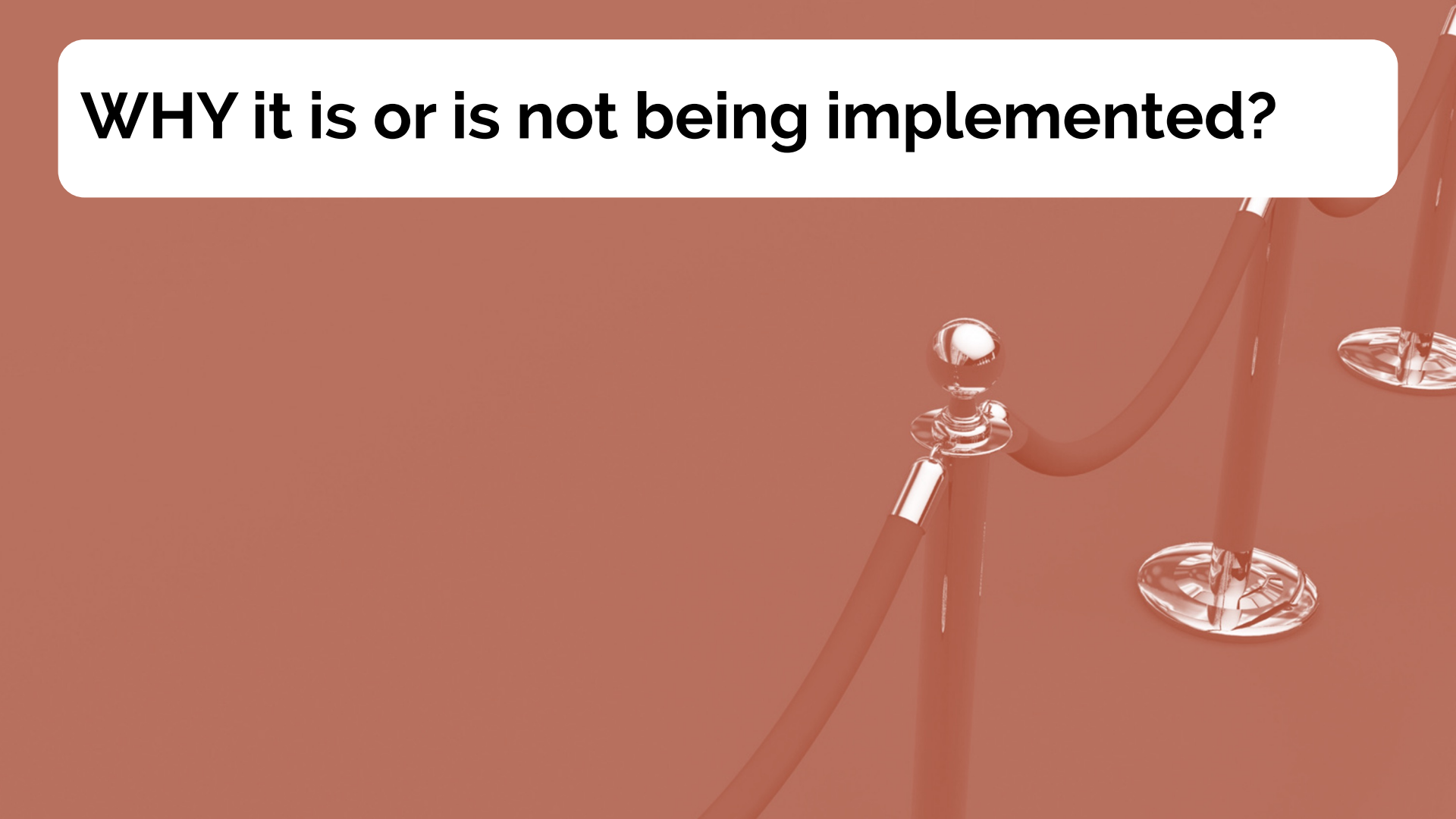
Rodent Health Monitoring Initiative

We promote replacing soiled bedding sentinel rodents with environmental health monitoring.

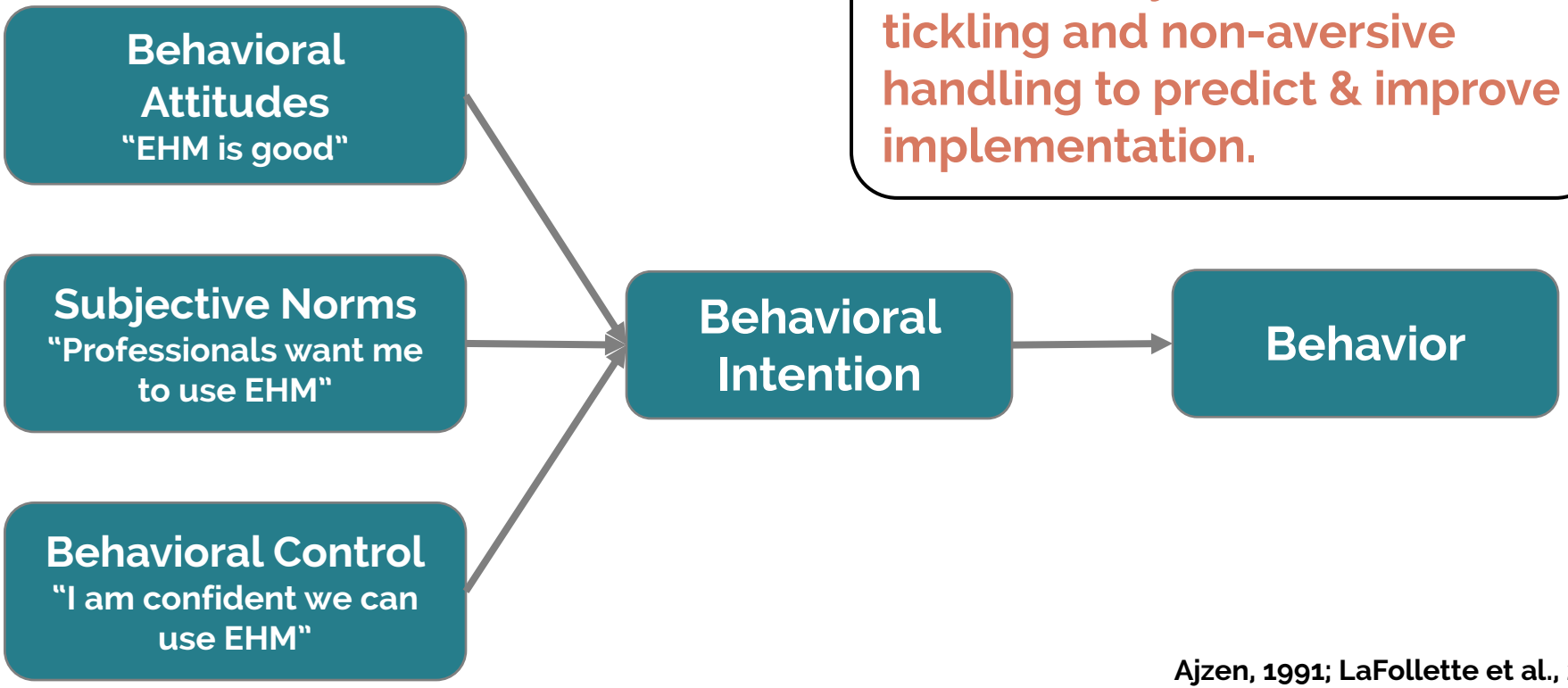
Benchmarking Survey

How common is environmental health monitoring (EHM)?

WHY it is or is not being implemented?



Theoretical basis for this survey is theory of planned behavior.



Successfully used with rat tickling and non-aversive handling to predict & improve implementation.

Ajzen, 1991; LaFollette et al., 2021

We were also interested in other possible factors.

- Knowledge
- Country
- Rack Design
- Familiarity
- Institution
- Caging Type

We hypothesized that current prevalence is moderated and affected by institutional beliefs.

More positive attitudes, norms, & control beliefs are associated with current implementation & higher intention to implement.

Experimental design was a longitudinal cross-sectional survey performed in 2021 and 2022.

We used a mixed methods online survey.

Mixed Methods

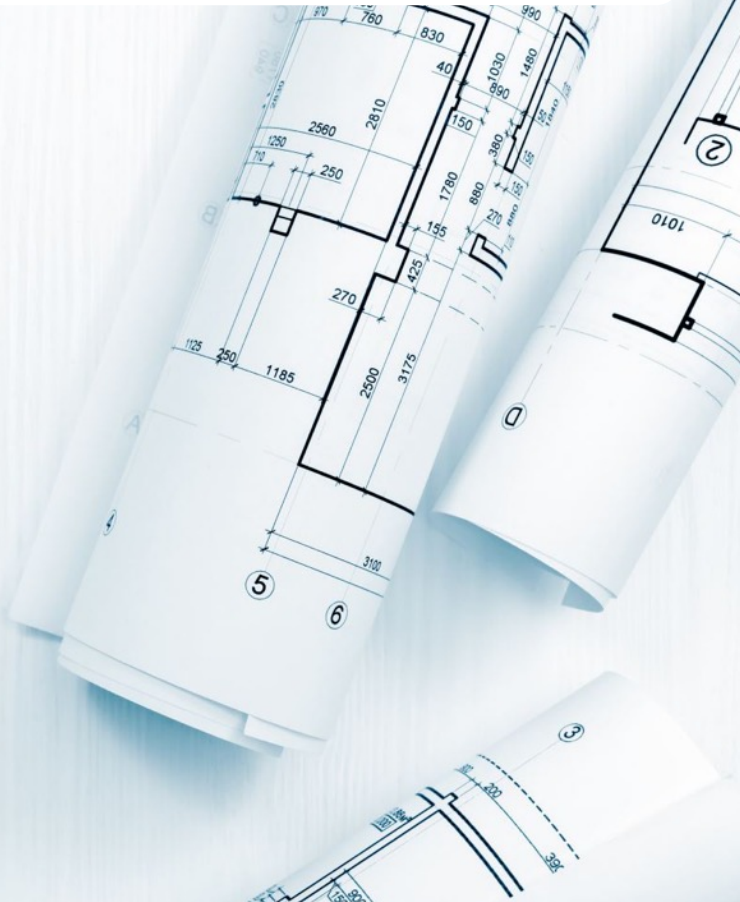
Qualitative: open-ended questions

Quantitative: close-ended questions

Data quality

Replicated questionnaires from validated instruments

Survey reviewed by experts & extensively piloted



We asked in detail about rodent health monitoring practices.

1. Demographics
2. Methods for routine health monitoring
3. Intention & beliefs about EHM
   - Theory of Planned Behavior (Francis et al., 2004 & Ajzen, 1991)
4. Familiarity & knowledge of EHM
5. Caging & rack design
6. Acceptance of EHM imports

Data were analyzed via thematic analysis, descriptive statistics, & linear regression.

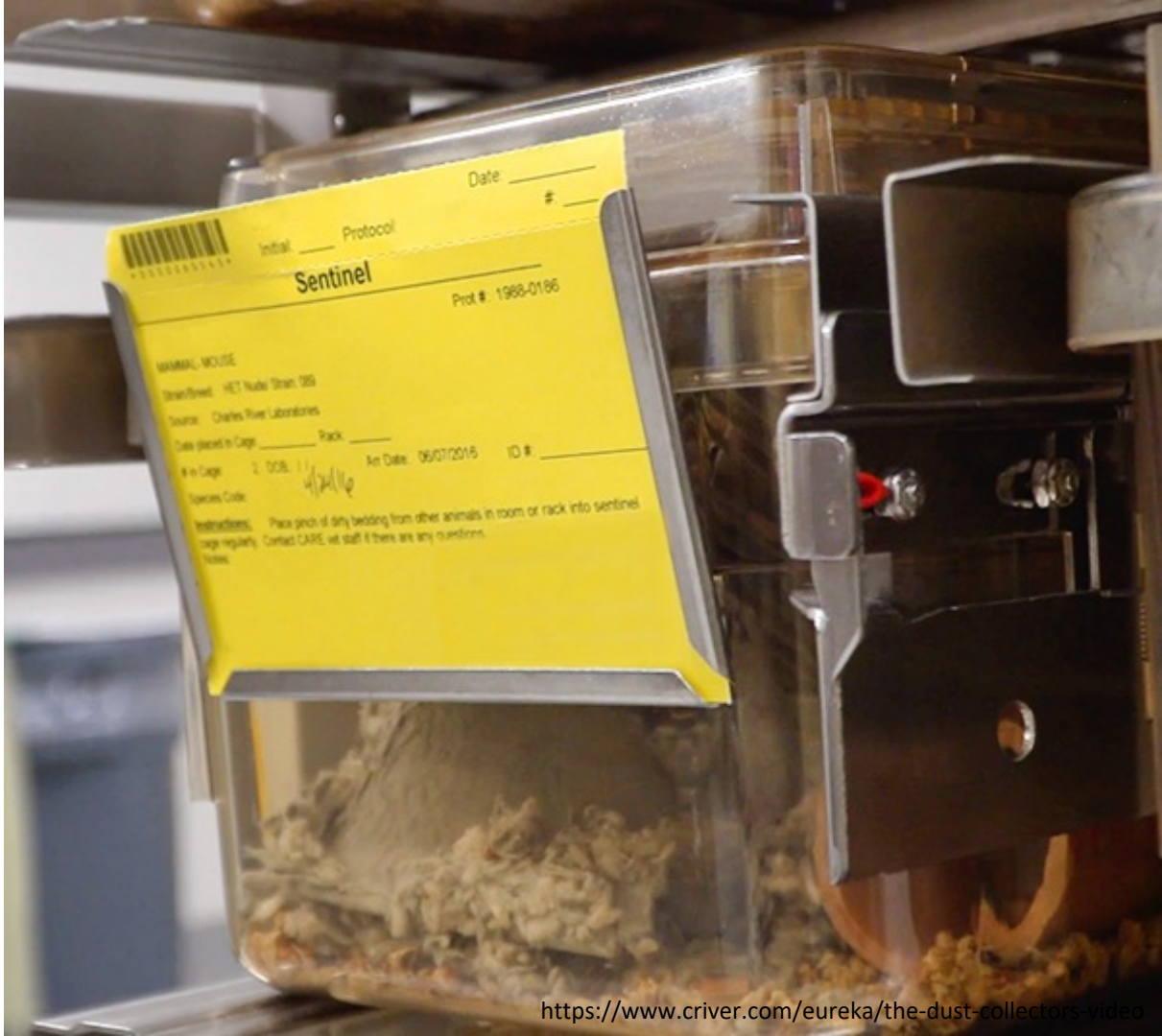
Dependent Variables
Intention

Main Factors
Theory of planned behavior
- Attitudes
- Norms
- Control Beliefs

Familiarity
Knowledge
Caging type
Rack design

Control Factors
Institution Type
Country

Results



https://www.criver.com/eureka/the-dust-collectors-video

Representatives from 52 unique institutions replied in both Year 1 and 2.

Country
77% USA
23% Other

Institution
77% Academic
17% Industry
6% Other (CRO, Government, etc.)

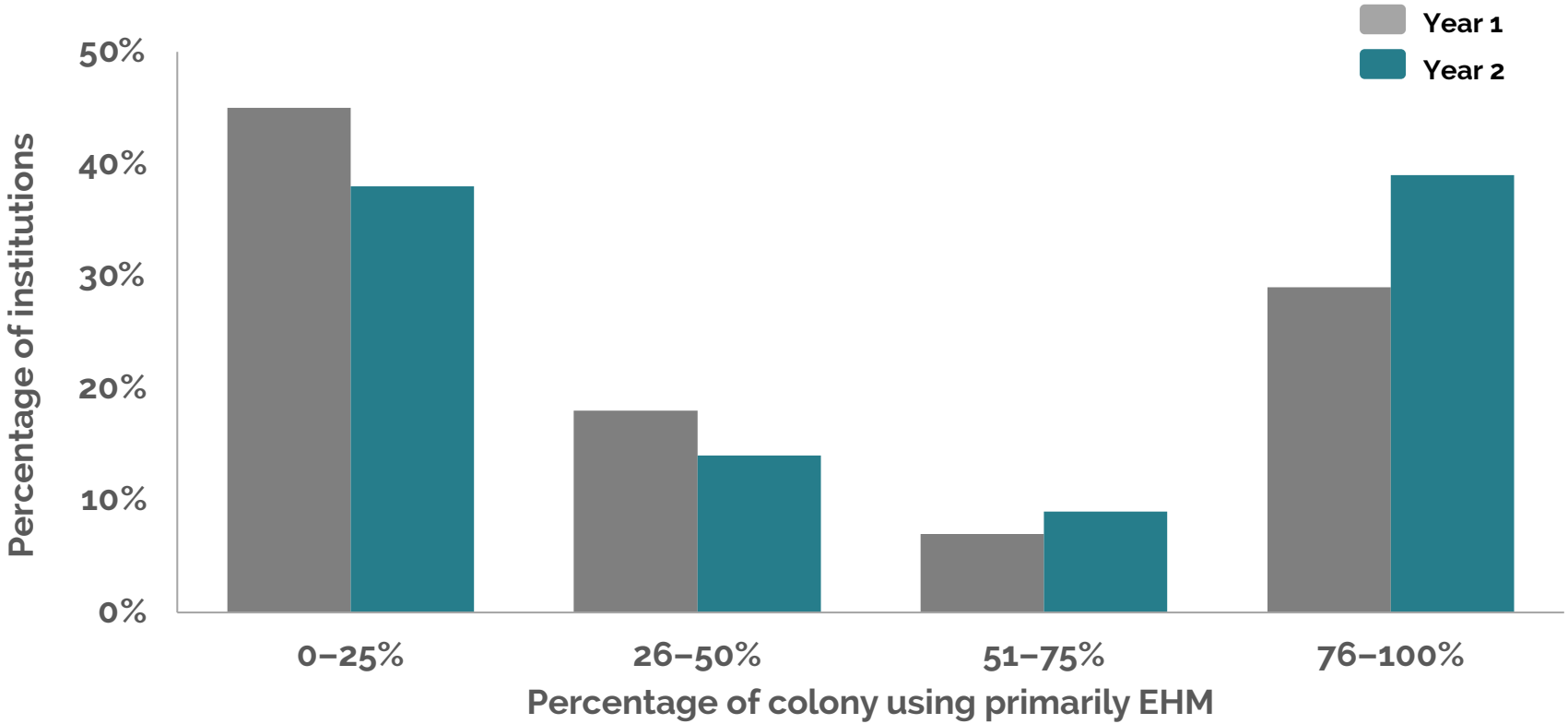
Roles of Primary Response
63% Vets
12% Managers
10% Techs
15% Other (researchers, etc.)

Descriptive statistics of EHM

Results taken from 1 individual institution

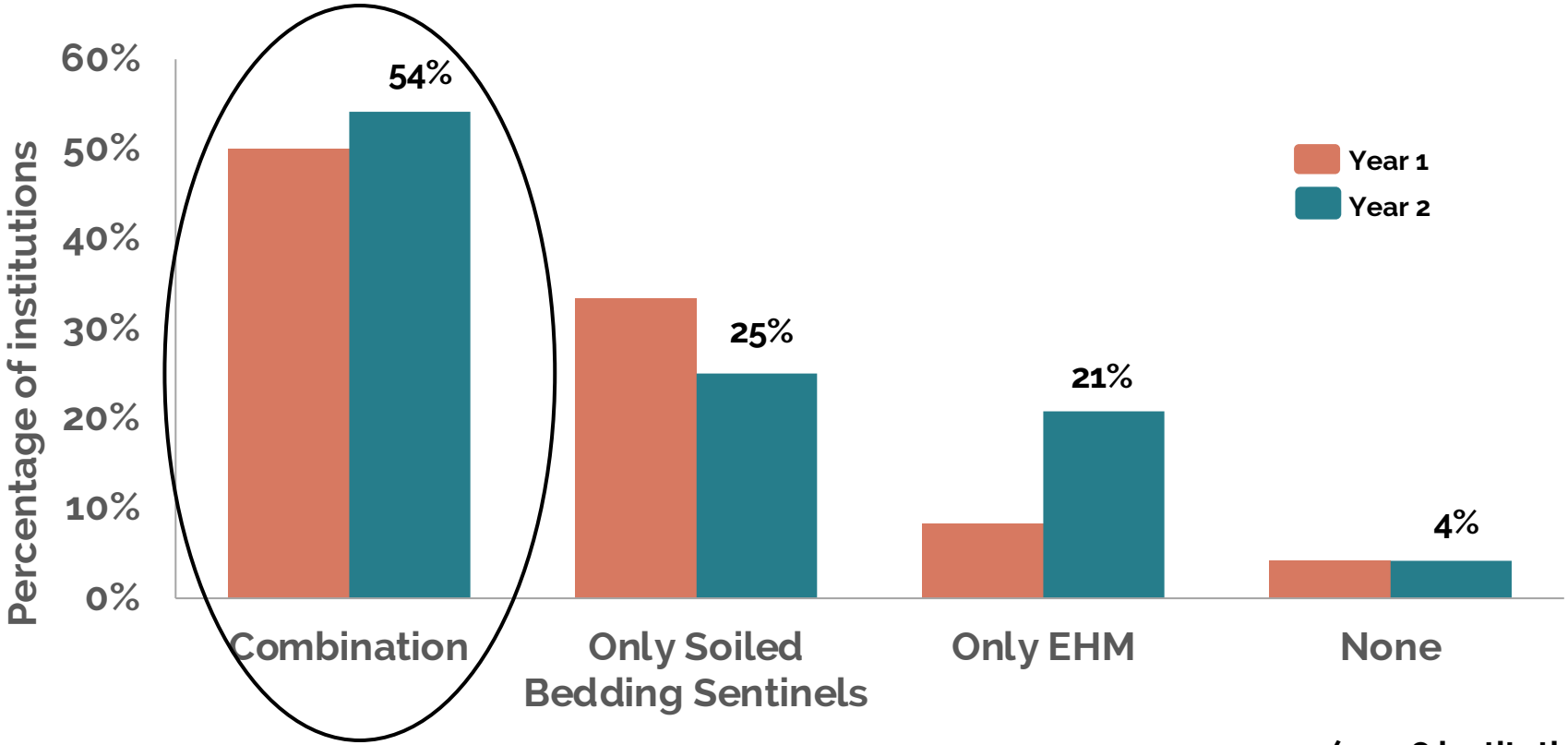
(vet or manager, most familiar with institutional policies)

From Year 1 to 2, more institutions are using primarily EHM for a larger percentage of their colony.



(n = 55 institutions)

In Year 2, 2 more institutions use a Combination of methods, which is used by majority of respondents.



(n = 48 institutions)

Even if you cannot shift 100% to EHM, replacing some sentinels with a combination of HM methods is good.

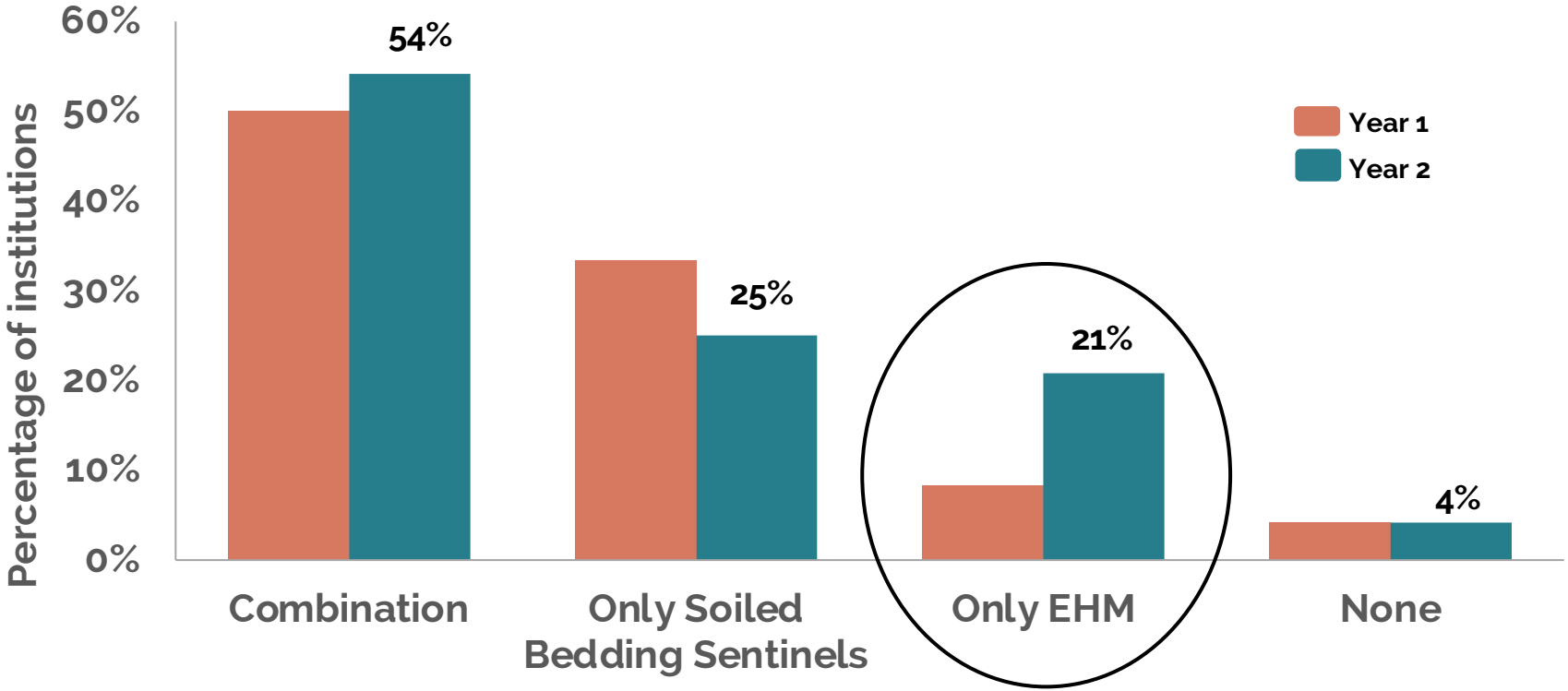
Hybrid programs: reduce animal use

Option 1
Alternate EHM and soiled bedding sentinel methods every quarter

Option 2
Simultaneously utilize EHM and soiled bedding sentinel methods each quarter

Perform blood micro sampling at 3 months & diagnostics/necropsy at 6 months

In Year 2, 6 more institutions use Only EHM. However, just 21% of institutions use Only EHM.



(n = 48 institutions)

From these institutions, sentinel rodent use decreased by ~3000 yearly.

(n = 52 institutions)


Clearly, more work needs to be done to increase EHM & decrease number of sentinels used.

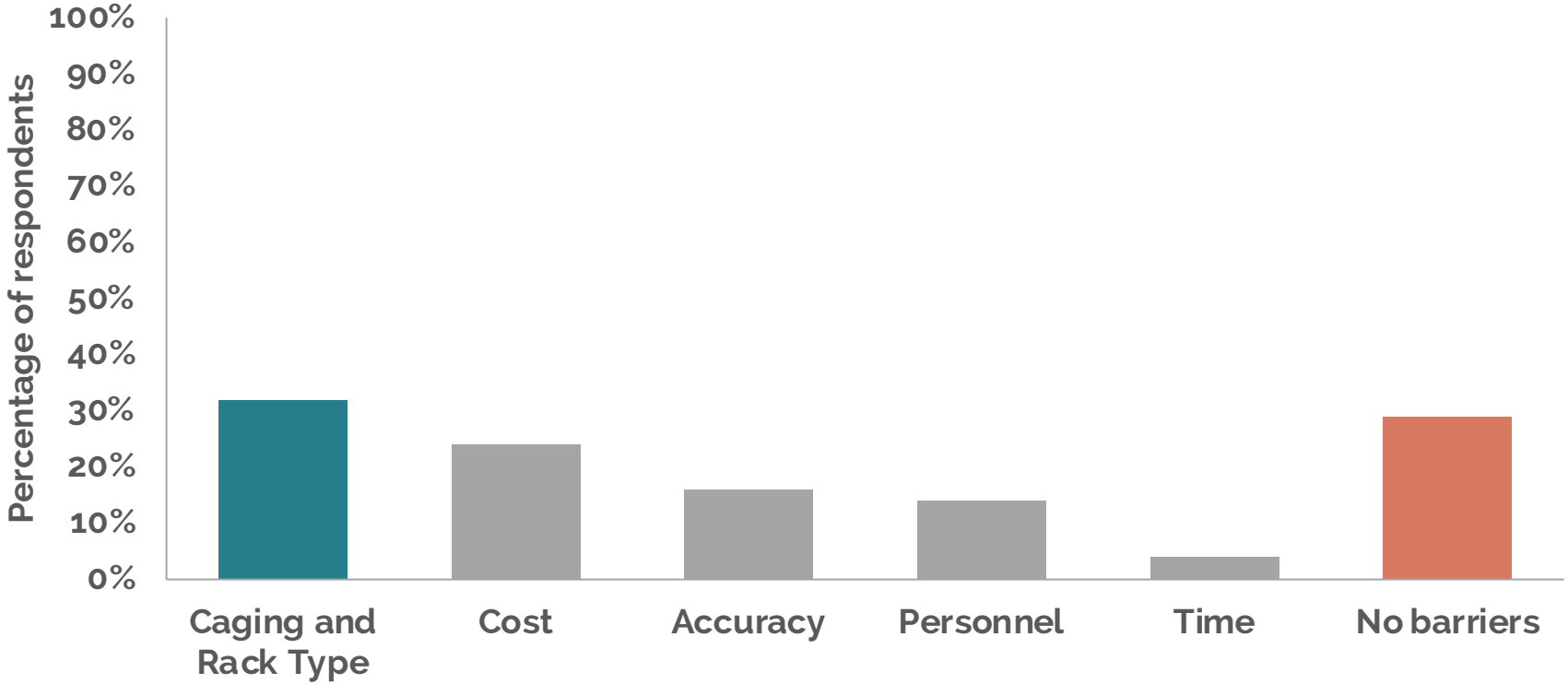
So what are the barriers to switching to only EHM?

Descriptive statistics of barriers and advantages

Descriptive statistics for Year 2

Asked of all survey participants

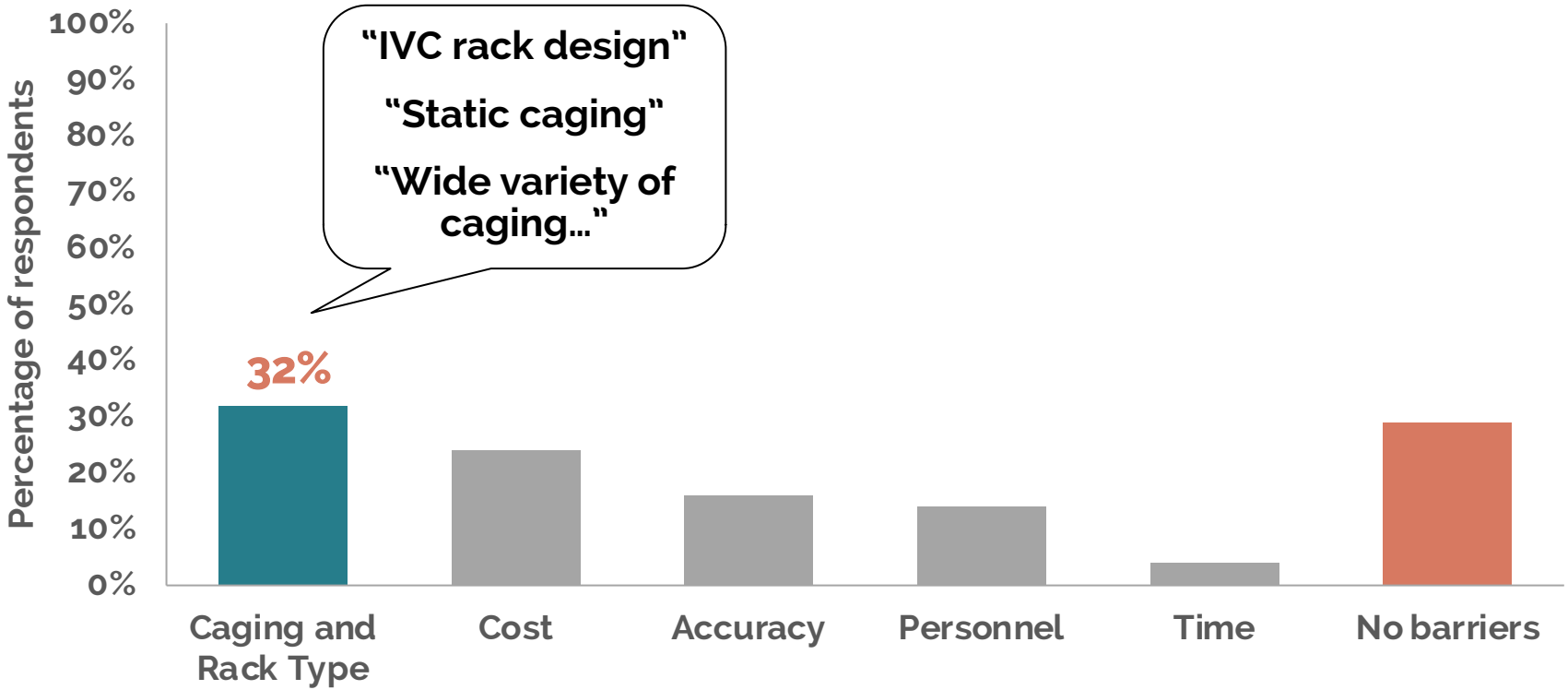
What, if anything, makes it difficult or impossible for your institution to use EHM?



(n = 113 participants)

When comparing barriers to advantages, there is one very obvious advantage: Replacement.

Caging and rack type were the most common perceived barriers to EHM.



(n = 113 participants)

Early publications on EHM focused on exhaust dust testing (EDT).

Journal of the American Association for Laboratory Animal Science
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Vol 52, No 1
January 2013
Pages 28–33

PCR Testing of a Ventilated Caging System to Detect Murine Fur Mites

Eric S Jensen,[1,3,*] Kenneth P Allen,[1,4] Kenneth S Henderson,[6] Aniko Szabo,[2] and Joseph D Thulin[1,5]

Microbiological monitoring of laboratory mice and biocontainment in individually ventilated cages: a field study

M Brielmeier[1], E Mahabir[1], J R Needham[2], C Lengger[3], P Wilhelm[1] and J Schmidt[1]

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[2]

Journal of the American Association for Laboratory Animal Science
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Vol 55, No 1
January 2016
Pages 58–65

Surveillance of a Ventilated Rack System for *Corynebacterium bovis* by Sampling Exhaust-Air Manifolds

Christopher A Manuel,[1,2,*] Umarani Pugazhenthi,[3] and Jori K Leszczynski[1,2]

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]

Journal of the American Association for Laboratory Animal Science
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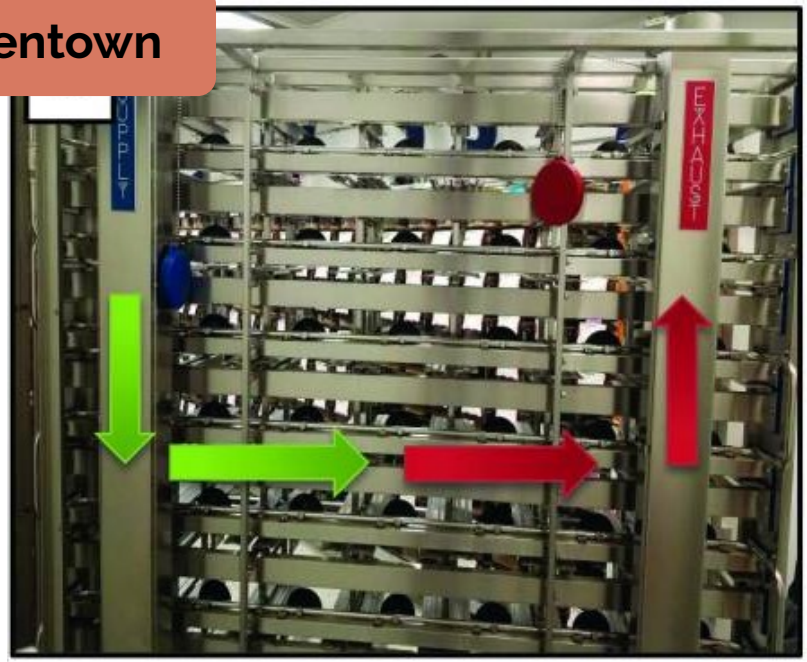
Vol 55, No 6
November 2016
Pages 775–781

Exhaust Air Dust Monitoring is Superior to Soiled Bedding Sentinels for the Detection of *Pasteurella pneumotropica* in Individually Ventilated Cage Systems

Manuel Miller,* Bärbel Ritter, Julia Zorn, and Markus Brielmeier

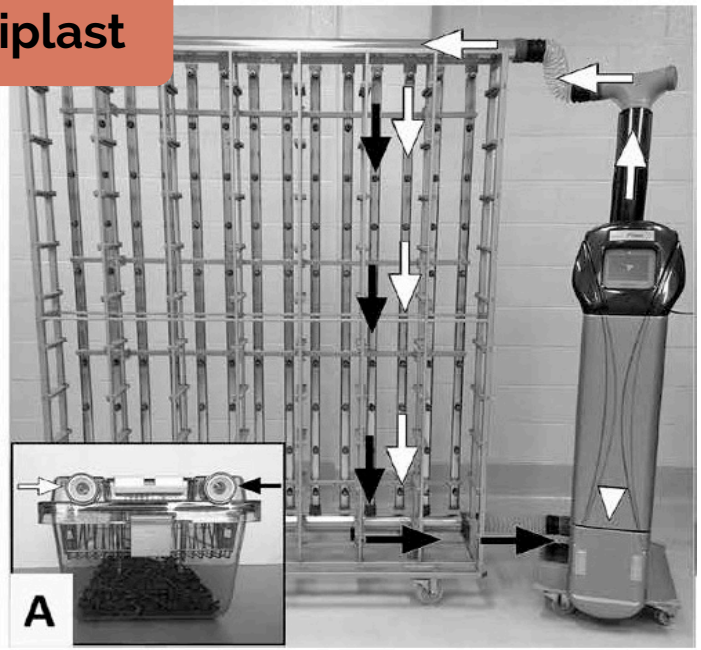
EDT is conducive for IVCs with rack-level filtration, but not all rack systems.

Allentown



Mailhiot, et al., 2020

Tecniplast

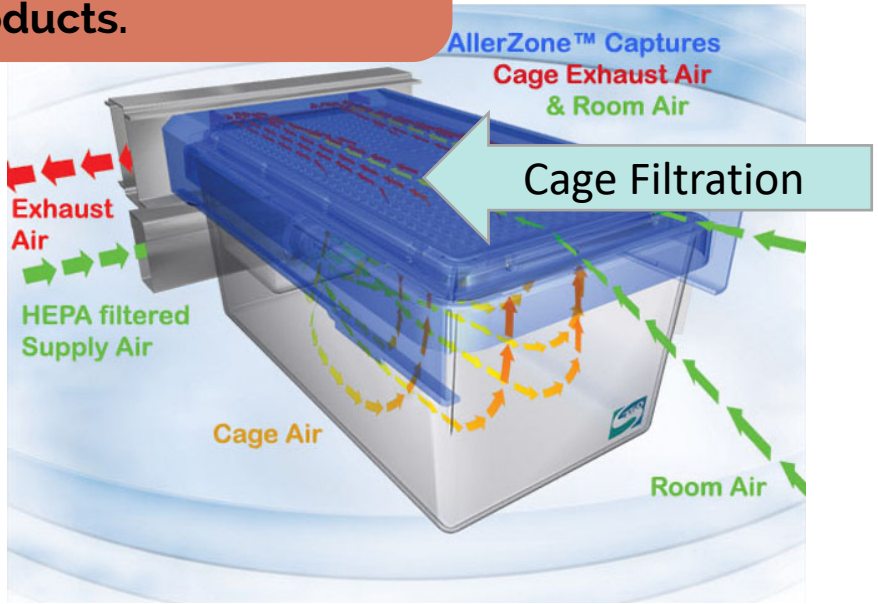


Bauer, et al., 2016

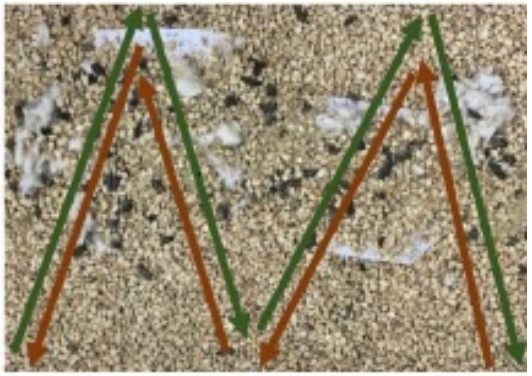
Recent data shows that it is possible to use EHM with any type of caging.

Static cages

Cage level filtration: Animal Care Systems, Thoren, Innovive, & Lab Products.



Most common barrier is a solvable problem by use of Sentinel-Free Soiled Bedding (SFSB) program.



O'Connell et al, 2021

Media (swabs or filter) in a cage with soiled bedding, but without sentinel animal.

Hanson et al., 2021

5 key publications to reference for SFSB.

- Dubelko, 2018. PCR Testing of Filter Material from IVC Lids for Microbial Monitoring of Mouse Colonies. (Georgetown)
- O'Connell, 2021. Evaluation of In-cage Filter Paper as a Replacement for Sentinel Mice in the Detection of Murine Pathogens. (University of Tennessee)
- Hanson, 2021. PCR Testing of Media Placed in Soiled Bedding as a Method for Mouse Colony Health Surveillance. (Emory)
- Winn, 2022. Using Filter Media and Soiled Bedding in Disposable Individually Ventilated Cages as a Refinement to Specific Pathogen-free Mouse Health Monitoring Programs. (Pfizer)
- Varela, 2022. Using Sterile Flocked Swabs as an Alternative Method for Rodent Health Monitoring. (Duke-NUS Medical School)

SOP for SFSB available on NA3RC website.

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Environmental Health Monitoring Standard Operating Procedures (SOPs)

Overview | Presentations | Publications | Editable Slide Deck | SOPs | Cost Analysis | FAQs

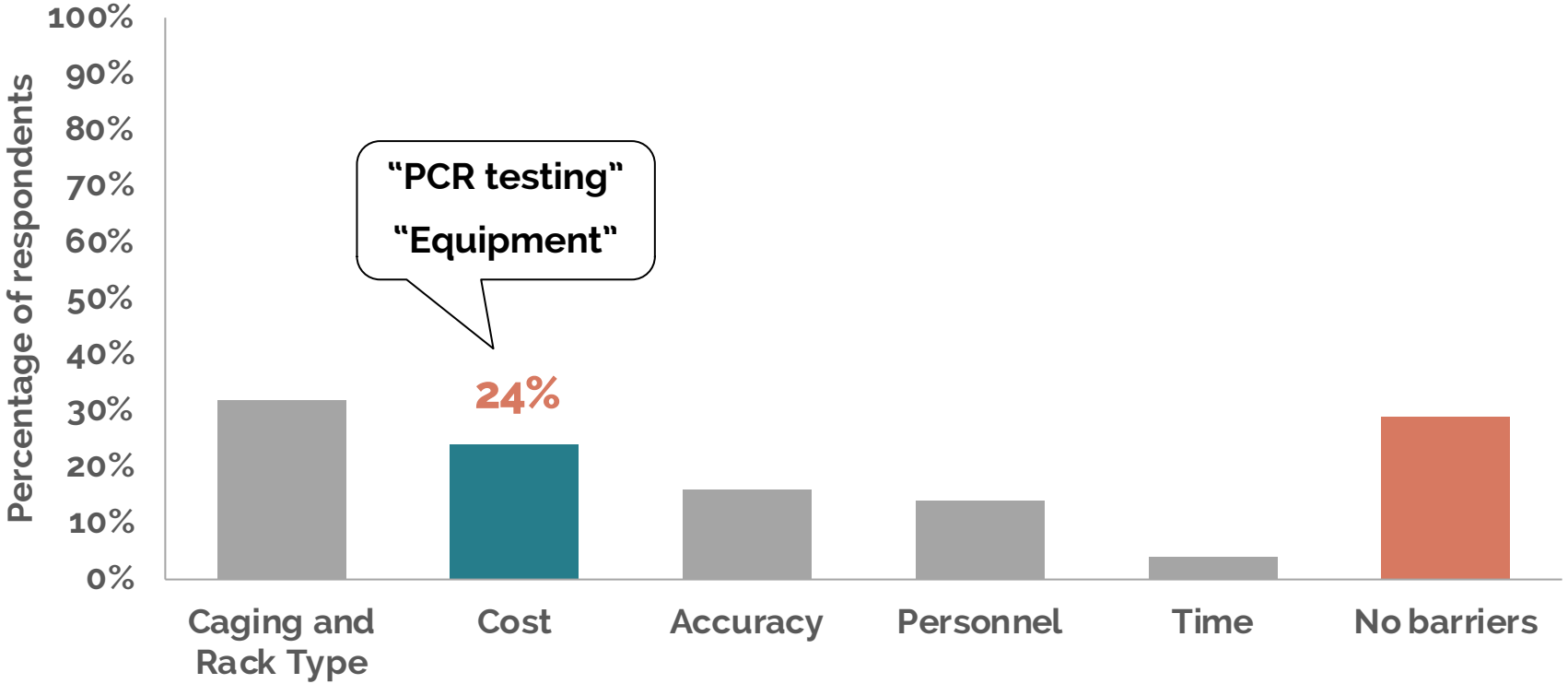
A key step in switching a facility to environmental health monitoring is developing standard operating procedures (SOPs). These detailed written instructions are critical for helping professionals perform procedures correctly, uniformly, and efficiently. However, writing SOPs and determining the most correct and efficient procedures can be challenging. Therefore experts on the NA3RsC's Rodent Health Monitoring initiative has developed a series of downloadable and editable SOPs for institutions.

Click on the links below to download copies of the relevant SOPs for your institution. Please edit SOPs as needed to reflect the equipment and procedures chosen for use in your facility. Users should also ensure they contact diagnostic laboratories prior to submitting sample to ensure materials and procedures meet current acceptance criteria.

NA3RsC Standard Operating Procedures for Environmental Health Monitoring

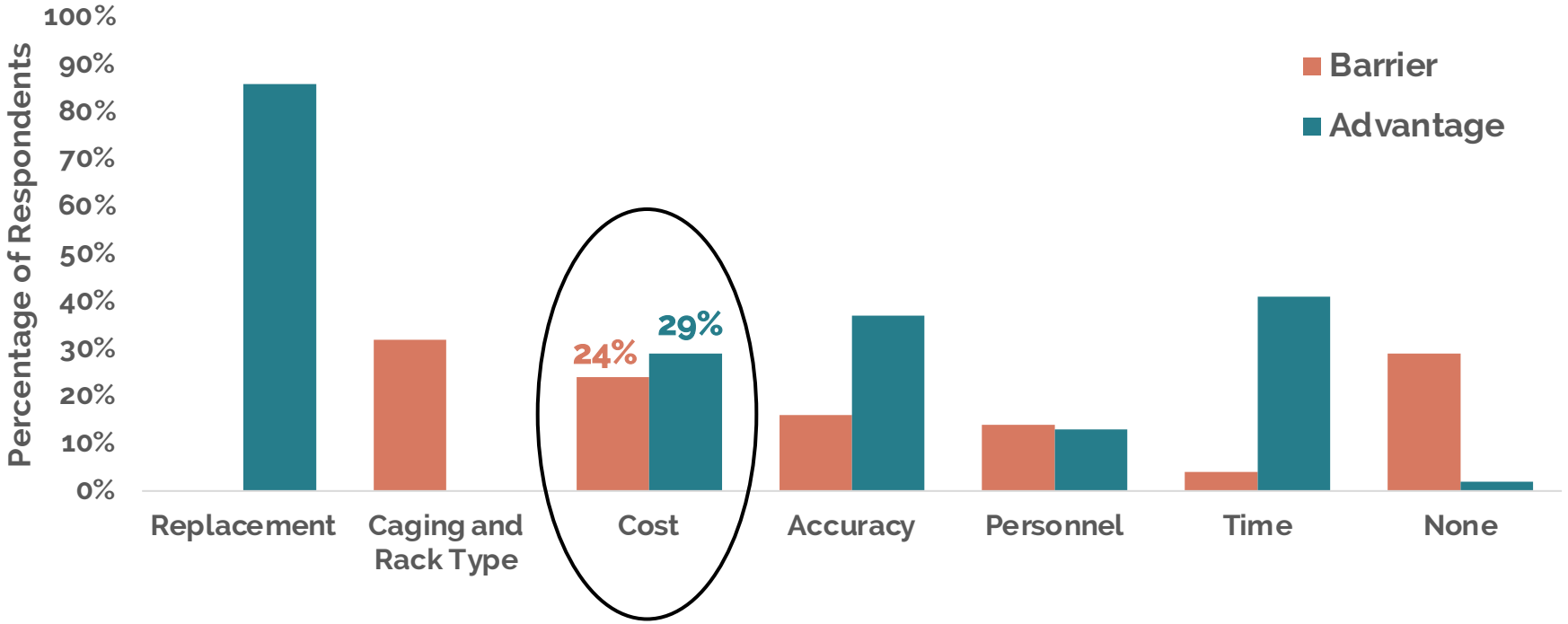
1. Exhaust Dust Testing with Allentown Racks
2. Exhaust Dust Testing with Tecniplast Racks
3. Sentinel Free Soiled Bedding Sampling (for static, open top, or racks filtering at the cage level)
4. Direct Colony Sampling
5. Room and Equipment Monitoring

Cost was the 2nd most common perceived barrier to EHM.



(n = 113 participants)

However, cost was considered an advantage to EHM more often than barrier.



Animal ordering, shipping, & maintenance costs are not necessary for EHM.

Sentinel Costs	EHM Costs
Animal ordering	~~Animal ordering~~
Animal shipping	~~Animal shipping~~
Animal maintenance	~~Animal maintenance~~
Veterinary technician	Veterinary technician
Diagnostic testing	Diagnostic testing

Luchins et al., 2020

Total annual cost was 26% lower for EHM using Exhaust Dust Testing (EDT).

Cost	Sentinel ($)	EDT ($)
Animal ordering	15,084	0
Animal shipping	3,876	0
Animal maintenance	137,642	0
Veterinary technician	7,190	1,683
Diagnostic testing	449,629	450,938
Total annual cost	**613,421**	**452,621**

Census = 21,000 cages

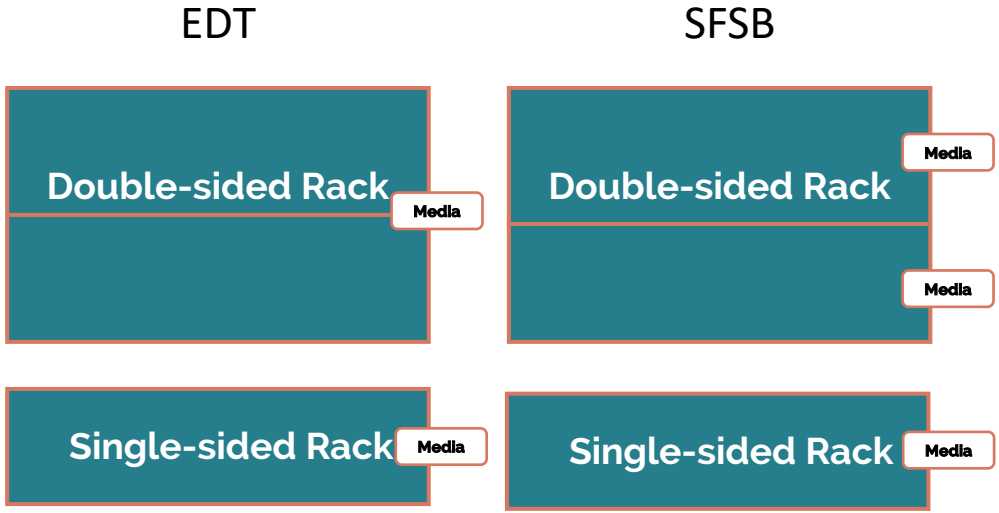
Luchins et al., 2020

Is there a cost savings when using a Sentinel-Free Soiled Bedding (SFSB) program?

Total annual cost was 7% lower when using SFSB program.

Cost	Sentinel ($)	SFSB ($)
Animal ordering	468	0
Animal shipping	292	0
Animal maintenance	2,956	0
Veterinary technician	154	47
Diagnostic testing	9,658	12,488
Total annual cost	**13,528**	**12,524**

Cost savings depend on multiple factors.

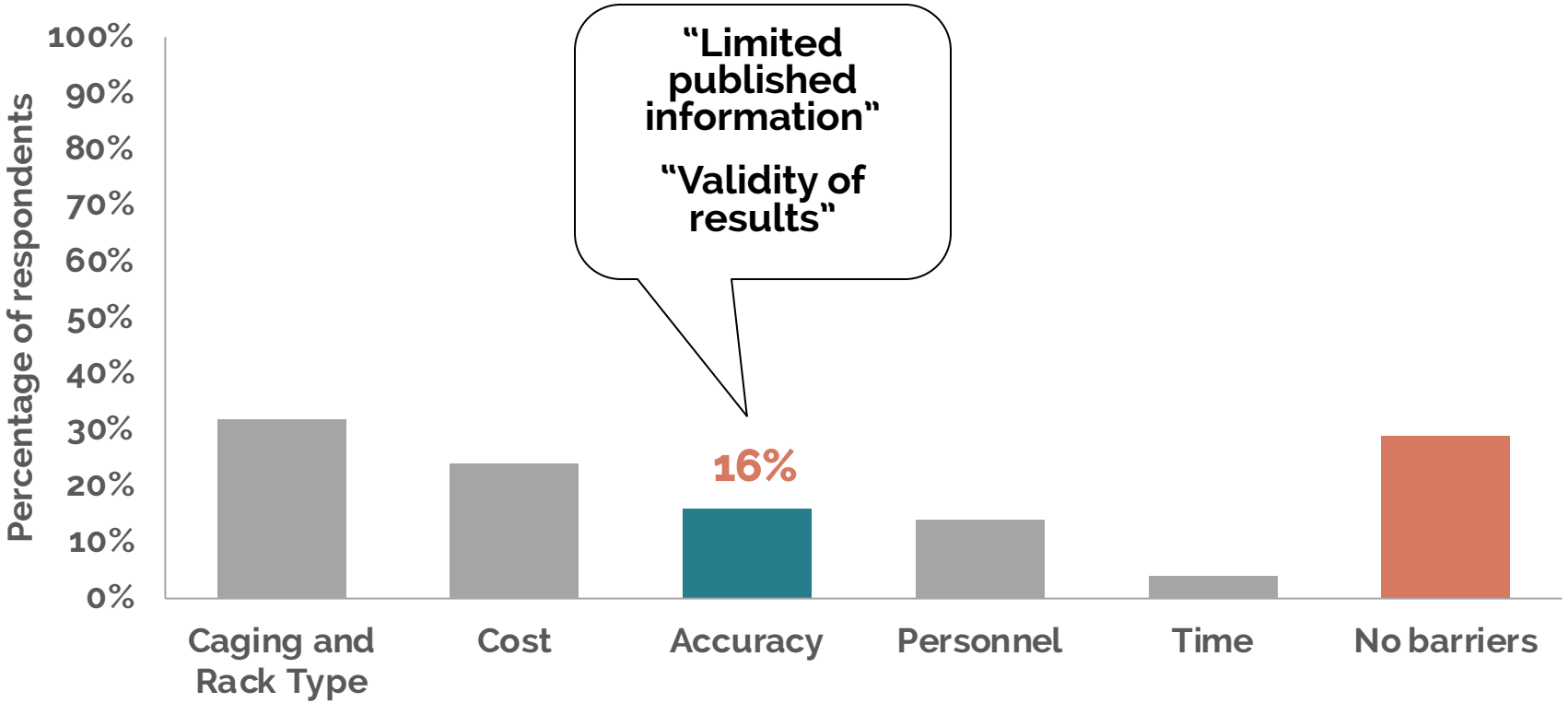


The takeaway

Both types of EHM were found to cost less than traditional soiled bedding sentinel programs.

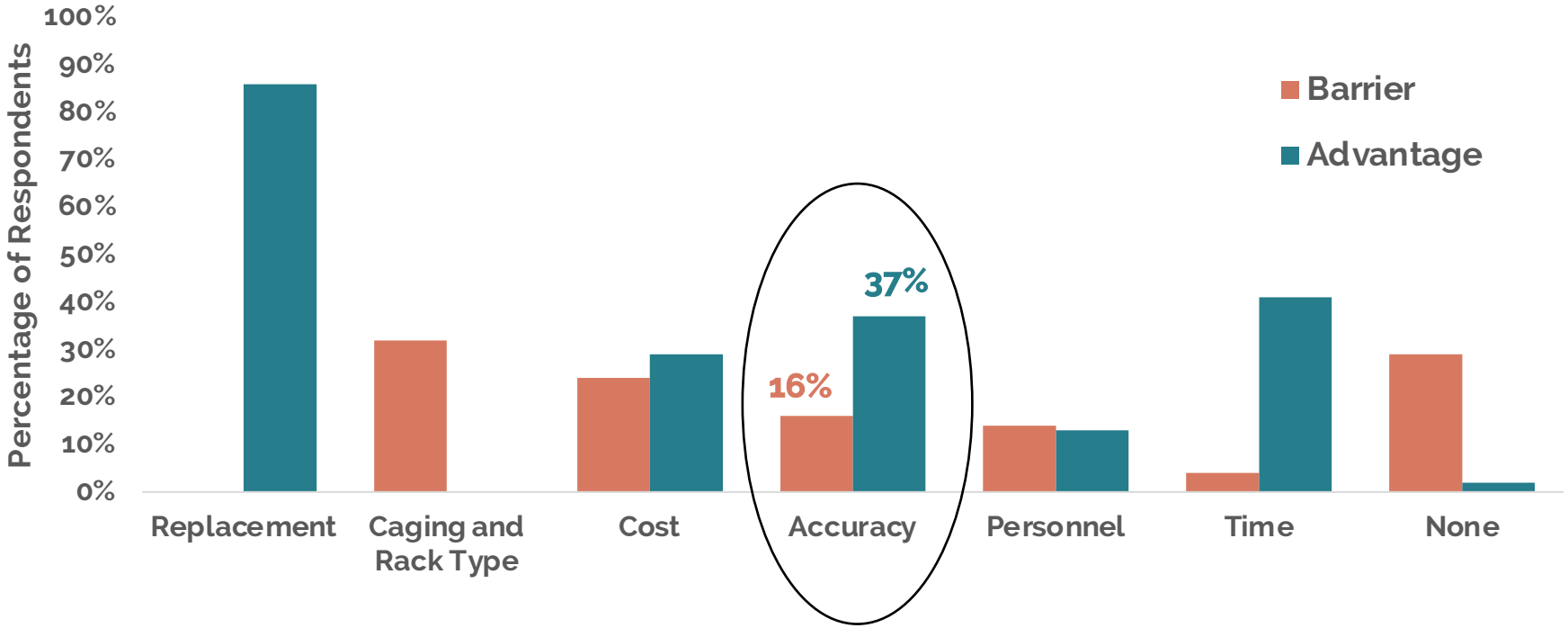
Ultimately, each program will need to complete their own cost analysis.

Accuracy was 3rd most common perceived barrier to EHM.



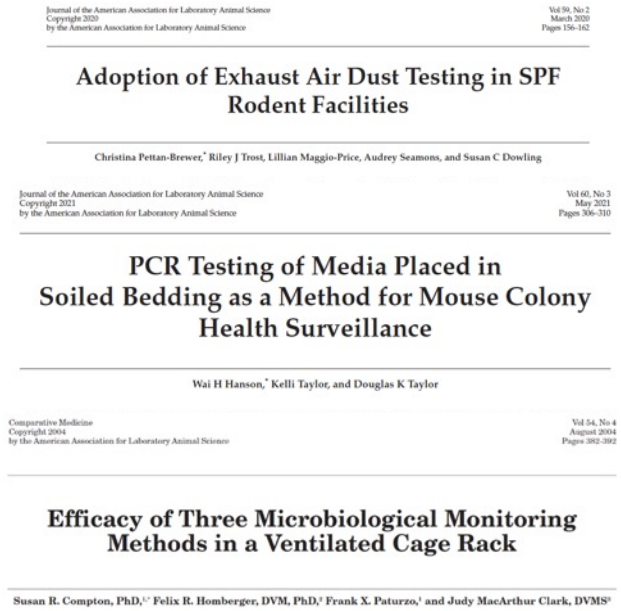
(n = 113 participants)

However, accuracy was considered an advantage to EHM more often than a barrier.



Research and publications on EHM are increasing.

Currently over 30 publications describing equal or improved detection with EHM.



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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

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

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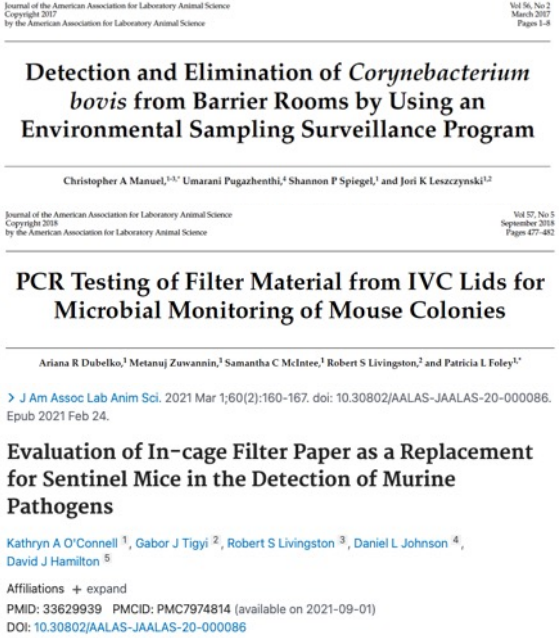
Vol 54, No 4
August 2004
Pages 382–392

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]

Journal of the American Association for Laboratory Animal Science
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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.

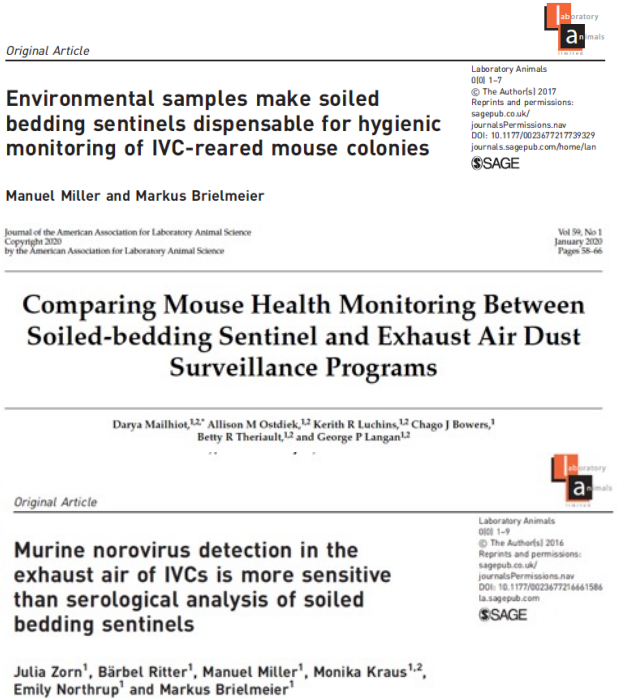
Evaluation of In-cage Filter Paper as a Replacement for Sentinel Mice in the Detection of Murine Pathogens

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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SAGE

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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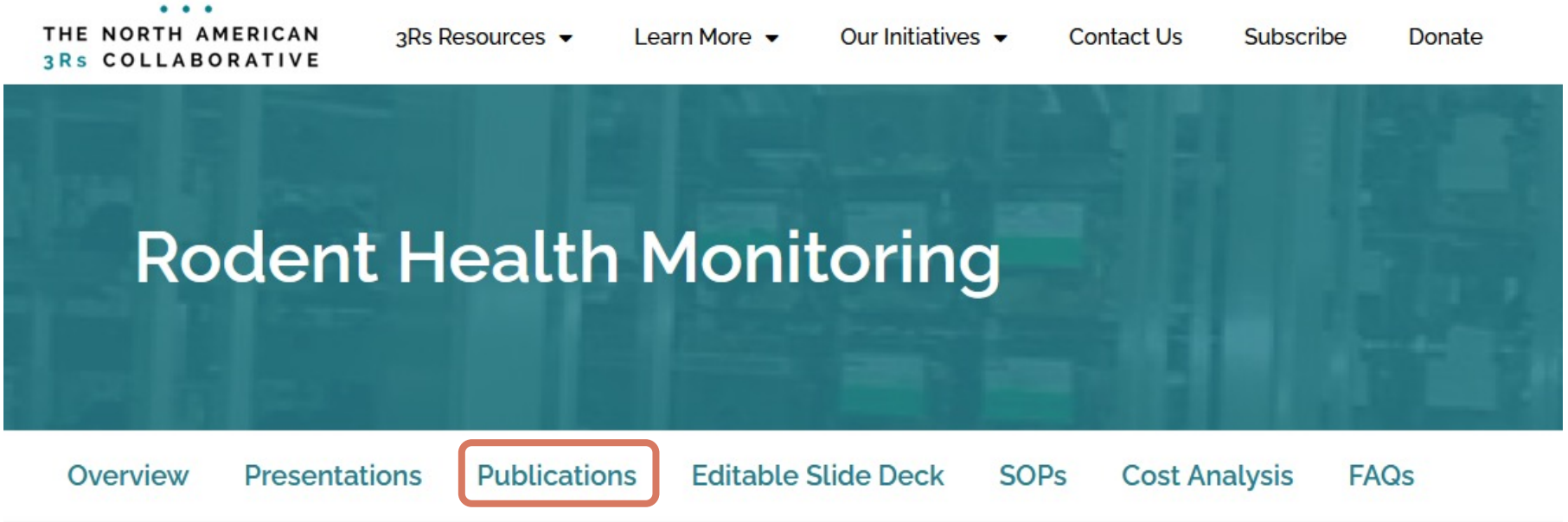
DOI: 10.1177/0023677216661586
la.sagepub.com

SAGE

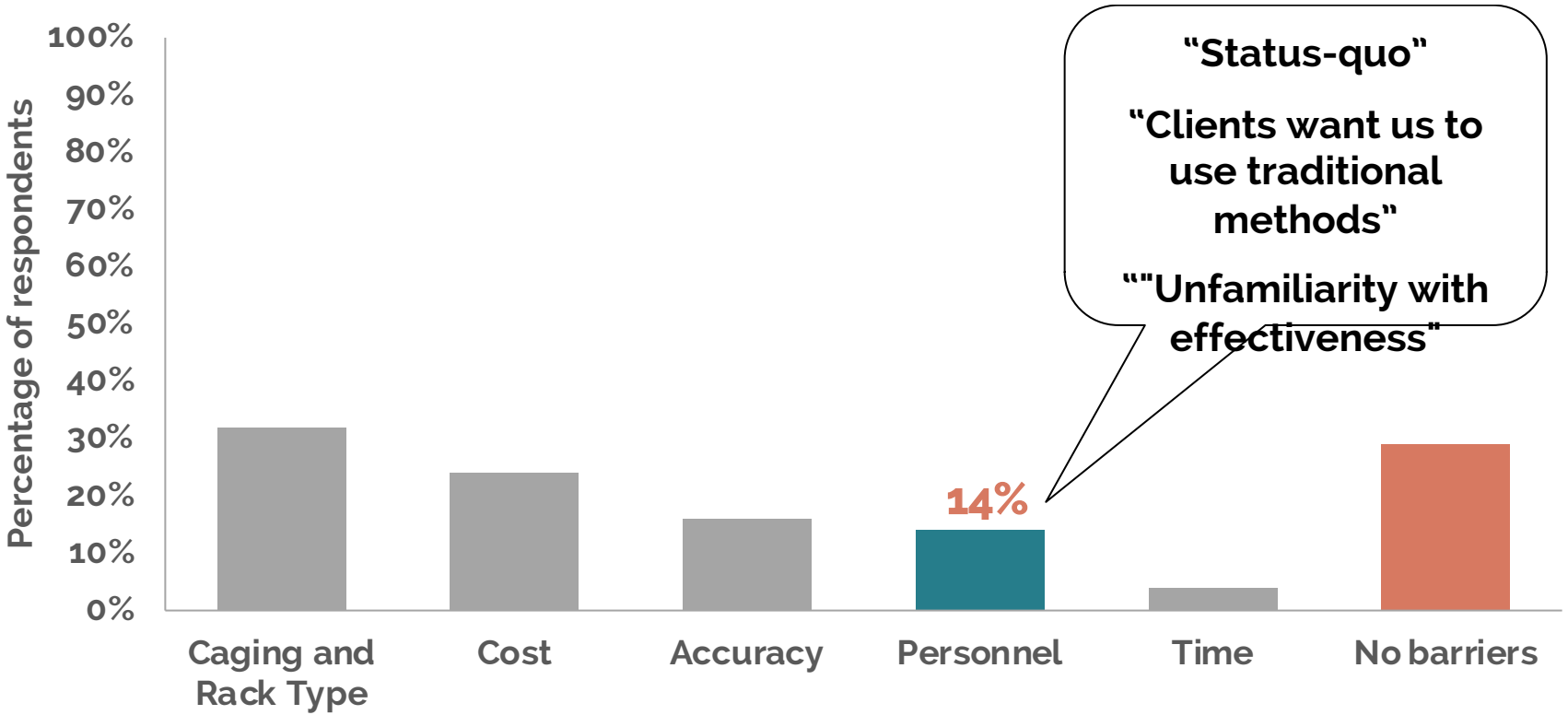
Murine norovirus detection in the exhaust air of IVCs is more sensitive than serological analysis of soiled bedding sentinels

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

These can all be found on NA3RsC website.

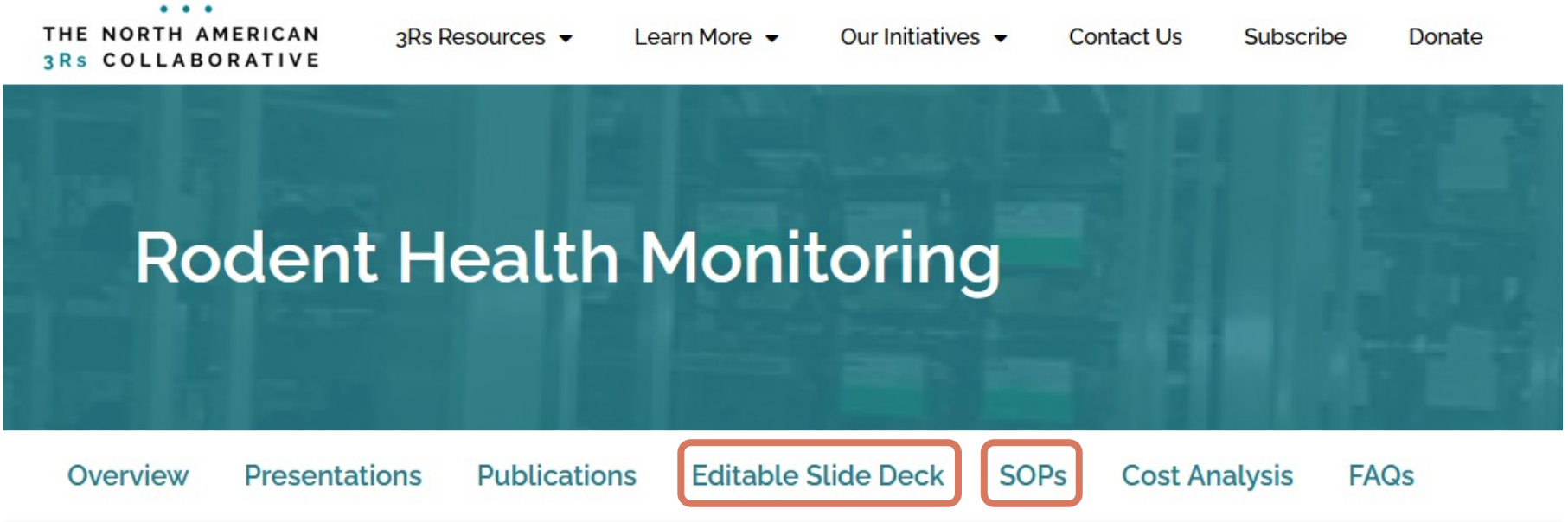


Personnel attitudes and expertise were the next perceived barriers to EHM.

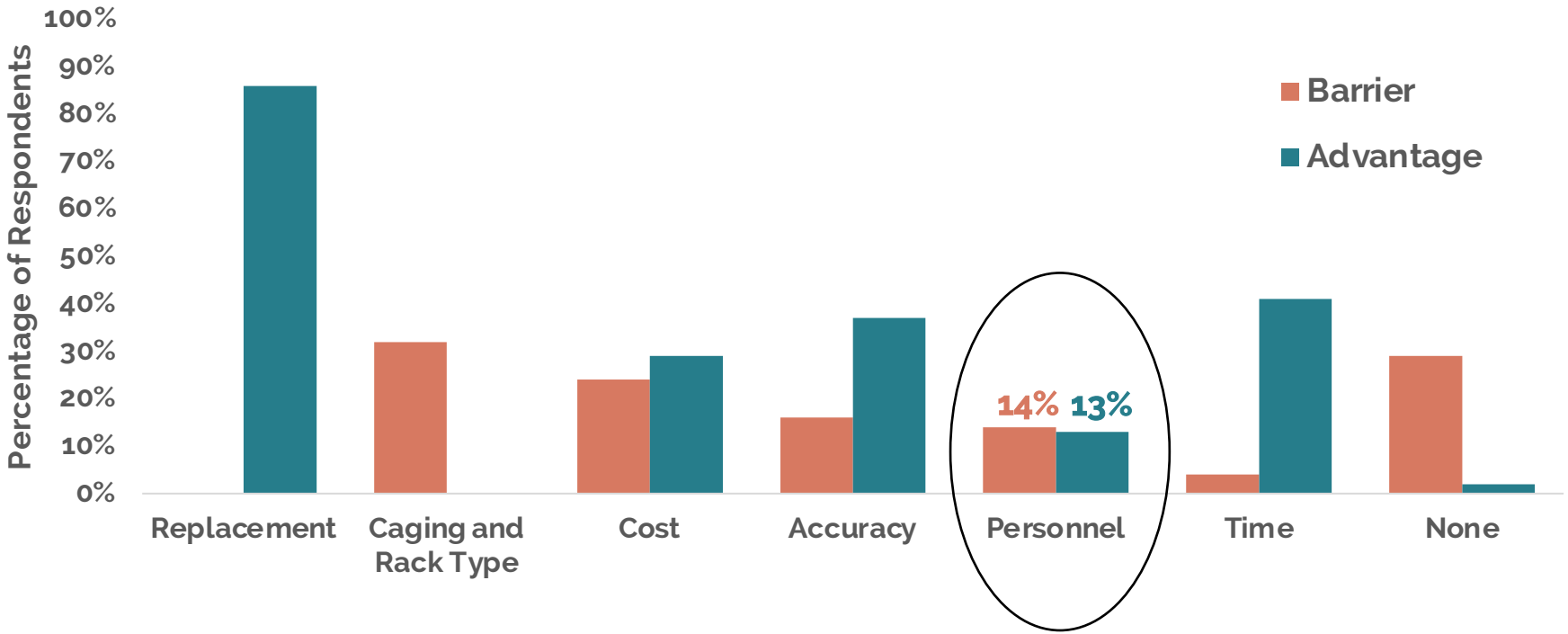


(n = 113 participants)

How do we change personnel attitudes and expertise?



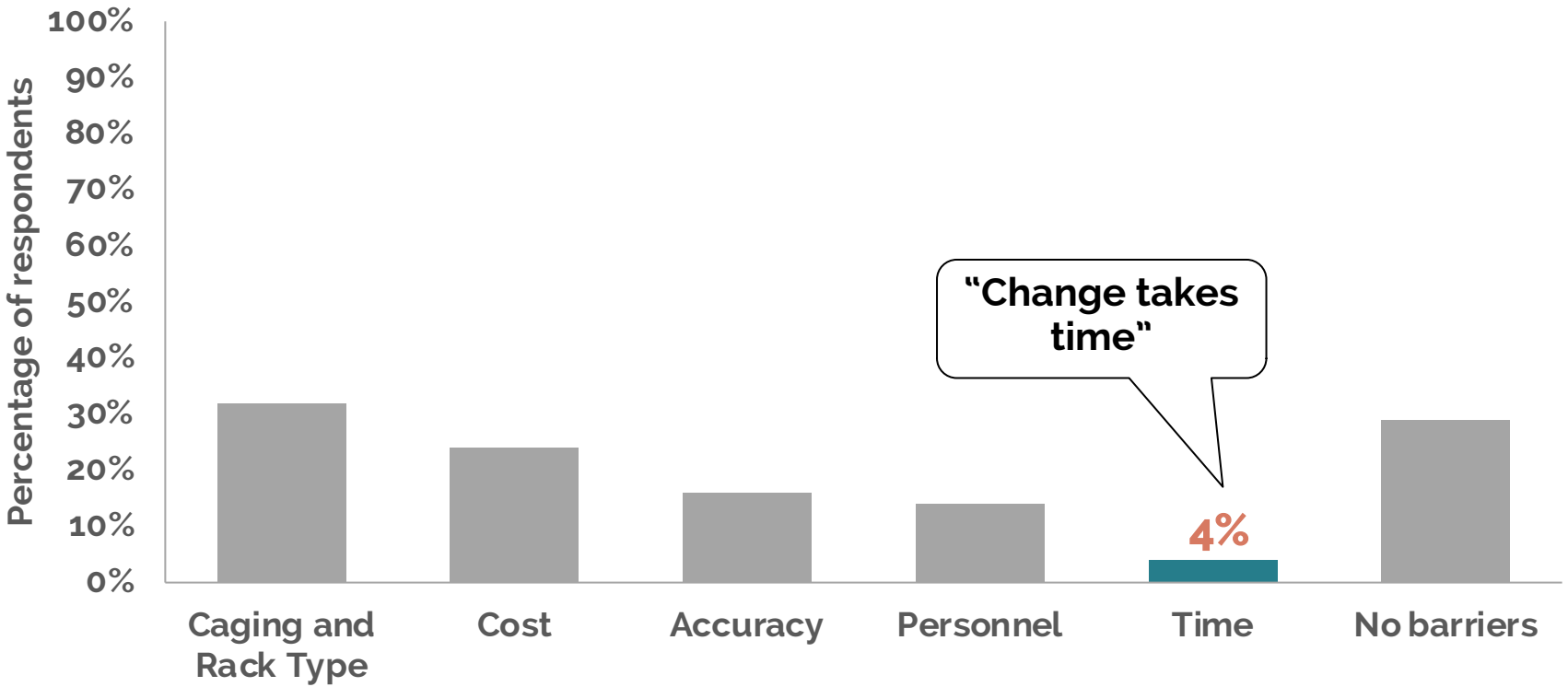
Personnel were recipients of the advantages of EHM almost as often as barriers.



Increased staff emotional burden from euthanasia of thousands of sentinel animals per year.

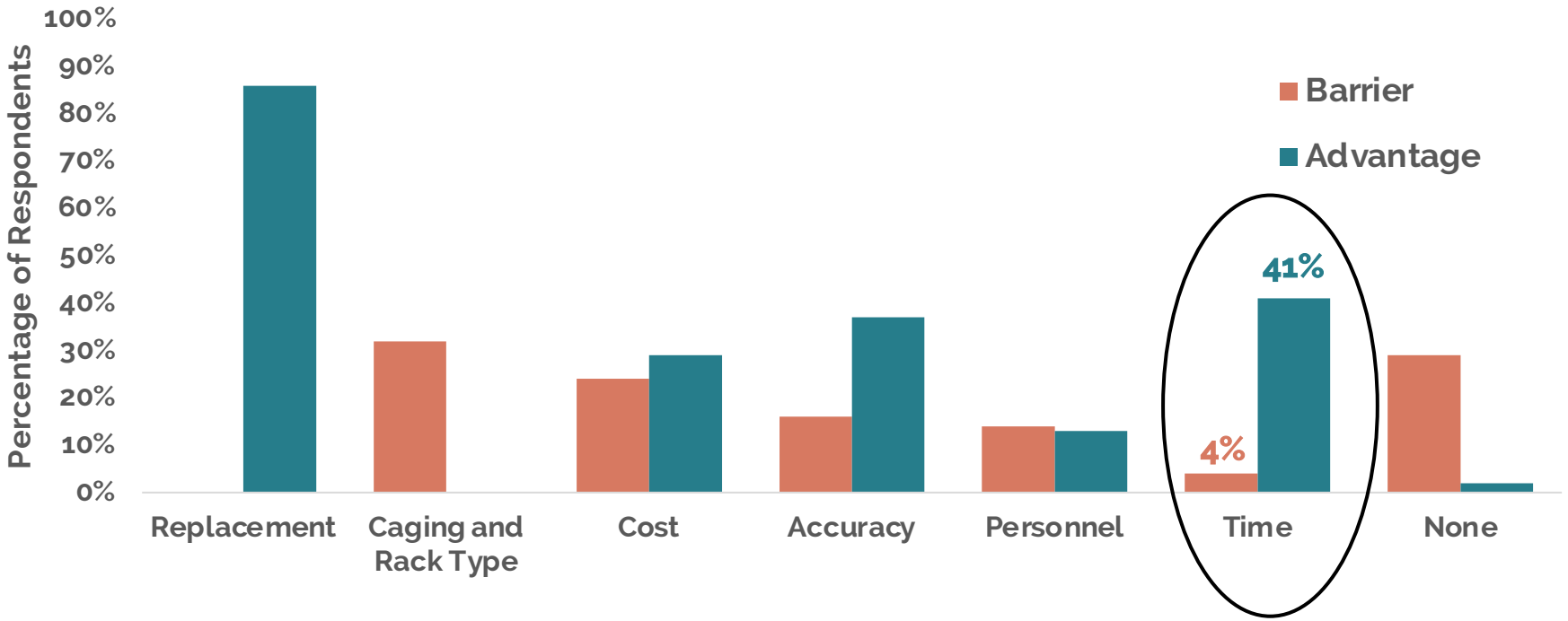
Pettan-Brewer et al., 2020

Time was the least common perceived barrier.



(n = 113 participants)

Time was considered an advantage to EHM more often than a barrier.

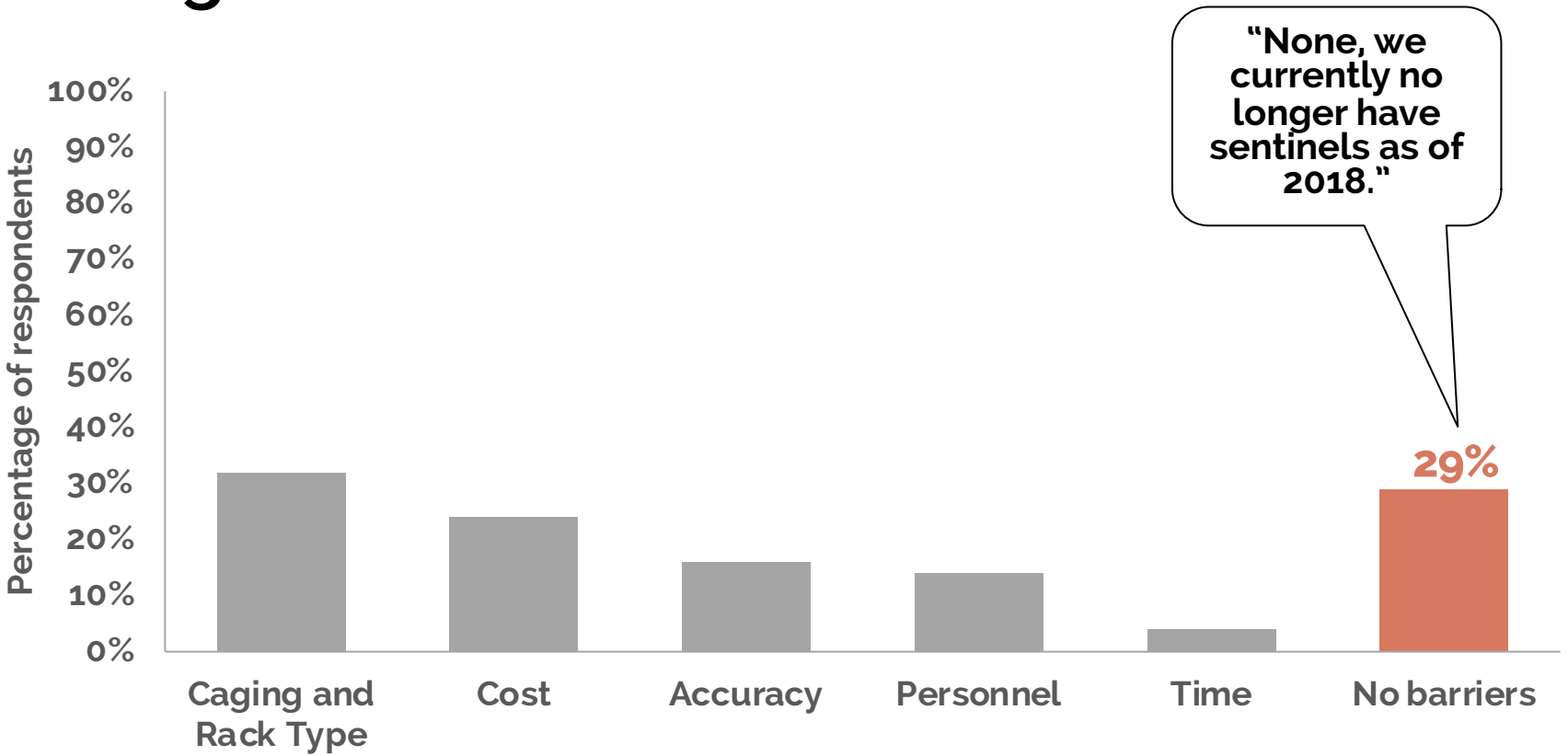


EHM reduced amount of staff time.

For veterinary technician, this amounted to ~1.5 hours per week per 10,000 cages.

Luchins et al., 2020

29% of respondents said there were No barriers to using EHM.



(n = 113 participants)

In 2022, when asked if their institution would accept rodents from other institutions that use EHM...

96%

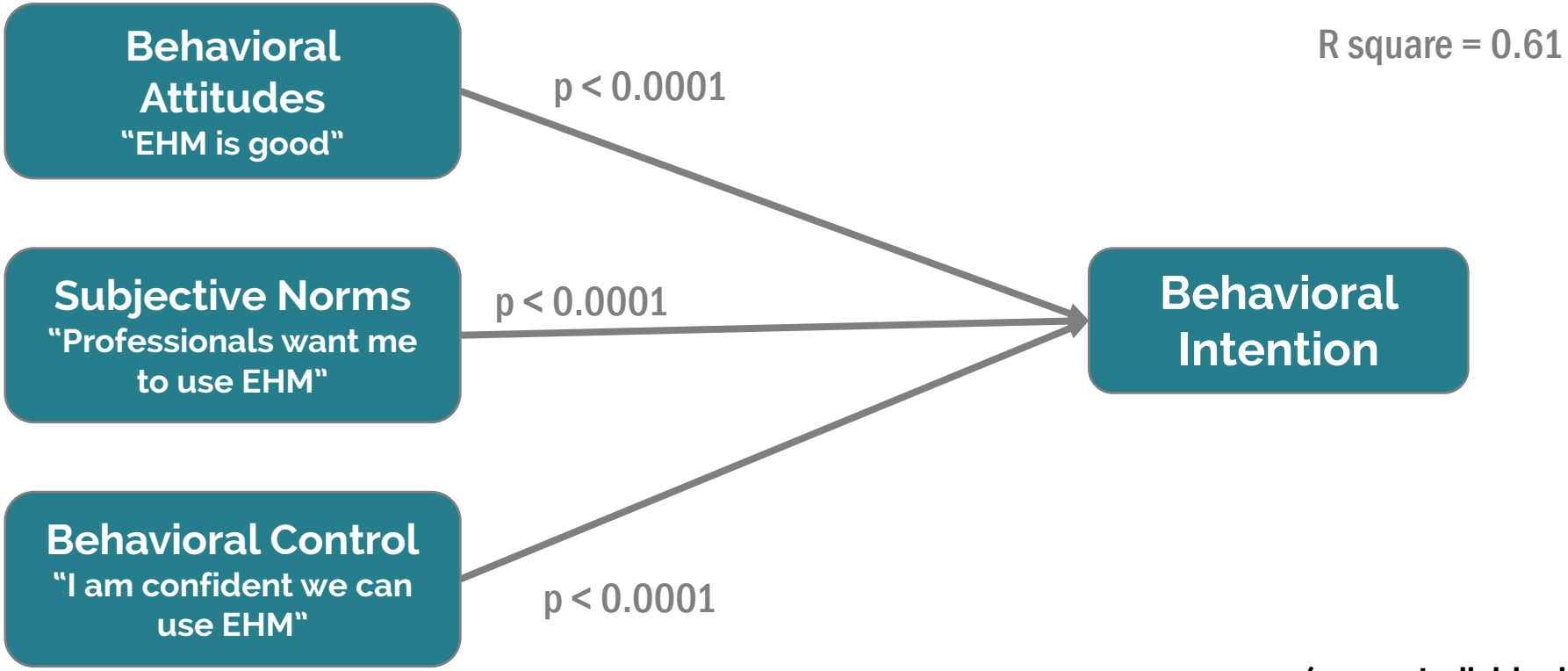
Said yes outright or with additional testing

(n = 73 institutions)

Quantitative data

Data taken from institutional representatives

Beliefs were associated with intention to use EHM.



(n = 151 Individuals)
2022 Data Only

Other factors were NOT associated with intention to use EHM.

Knowledge

Country

Rack Design

Familiarity

Institution

Caging Type

Takeaway

Future directions

Increase education about Sentinel-Free Soiled Bedding (SFSB) for static & cage-level filtration caging

Addressing attitudes, norms, & control beliefs

Institutions have changed.

Your institution can change.

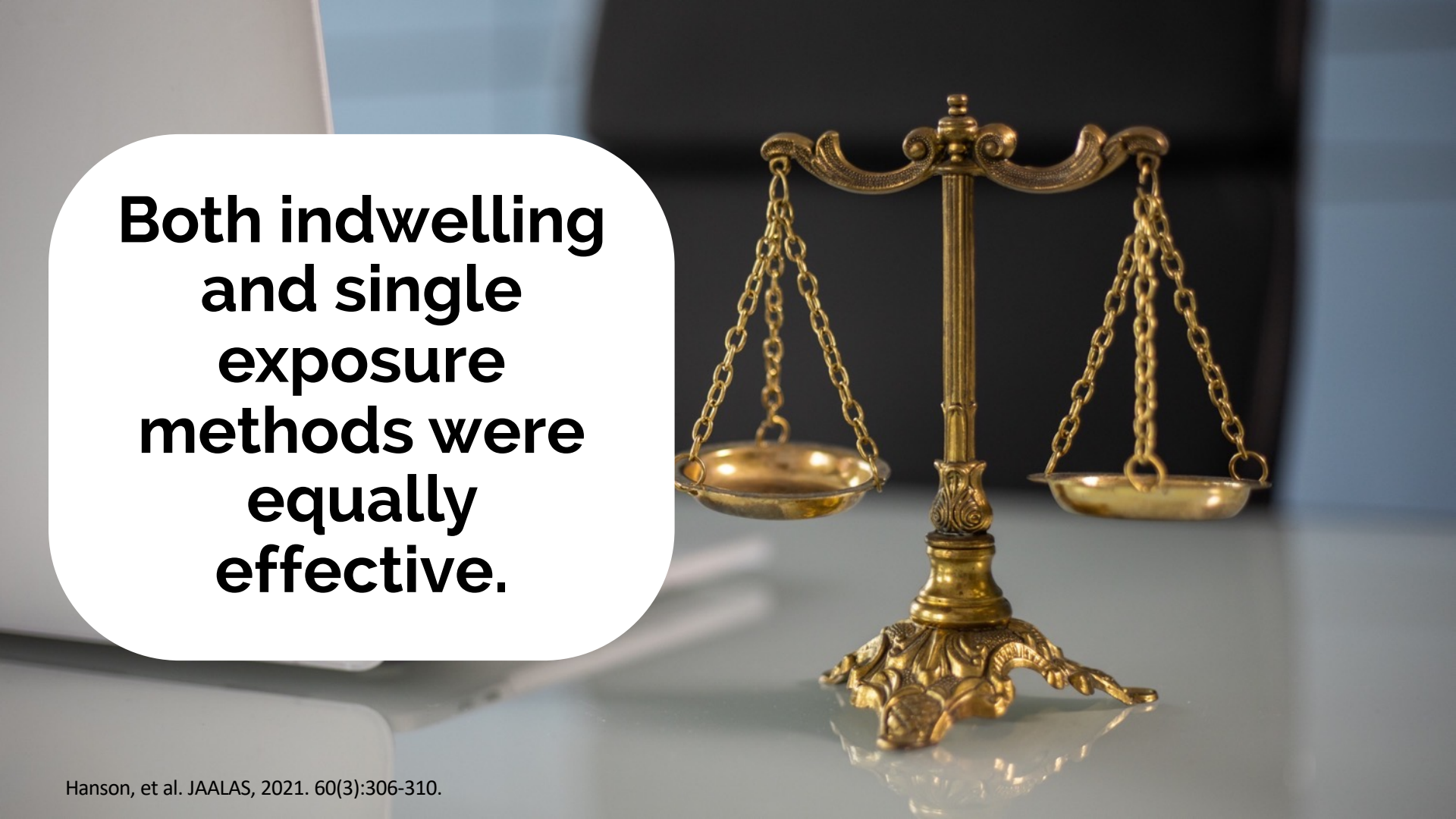
We can help with implementation barriers.

NA3RsC website: www.na3rsc.org/health-monitoring/

Contact me: kluchins@bsd.uchicago.edu

Environmental samples were equally or more effective at detecting all 3 pathogens of interest.

Hanson, et al. JAALAS, 2021. 60(3):306-310.



Hanson, et al. JAALAS, 2021. 60(3):306-310.

Other institutions have accepted our exports without concern.

Goals and Considerations

Effective Pathogen Detection

Replace Sentinel Animals

Reduce Emotional Fatigue

Reduce Labor?

Save Time & Money?

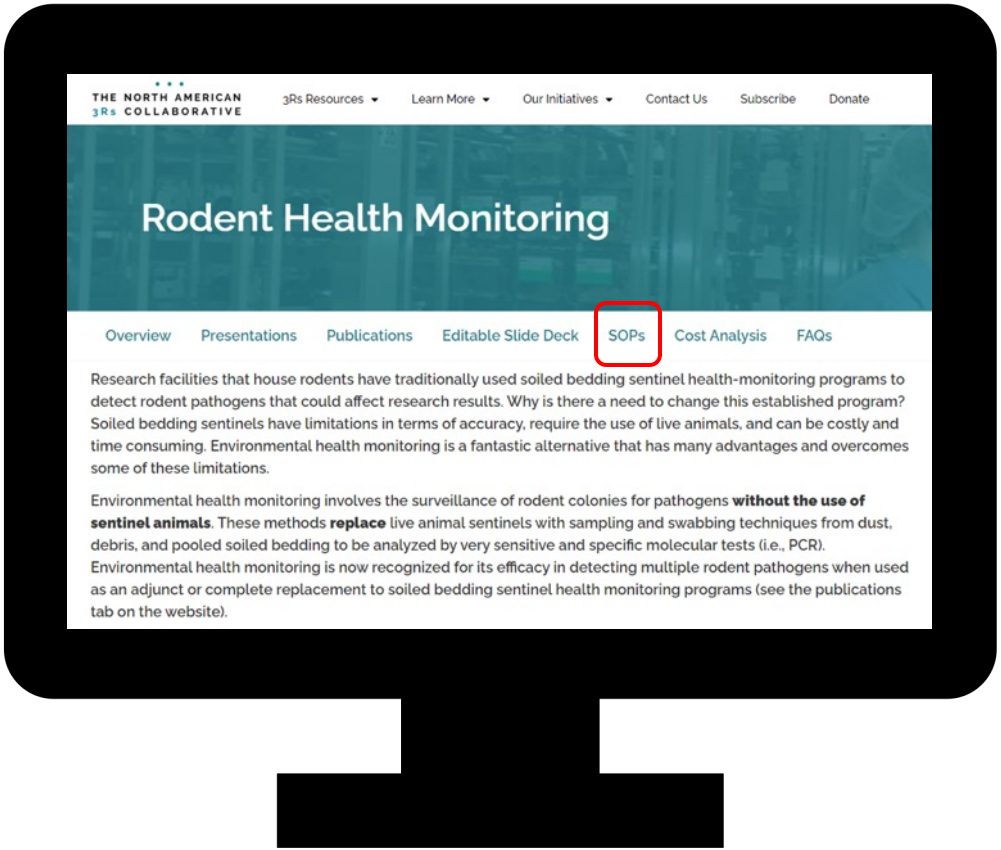
Variations in SOP

Indwelling vs. Single Exposure

Swabs vs. Media

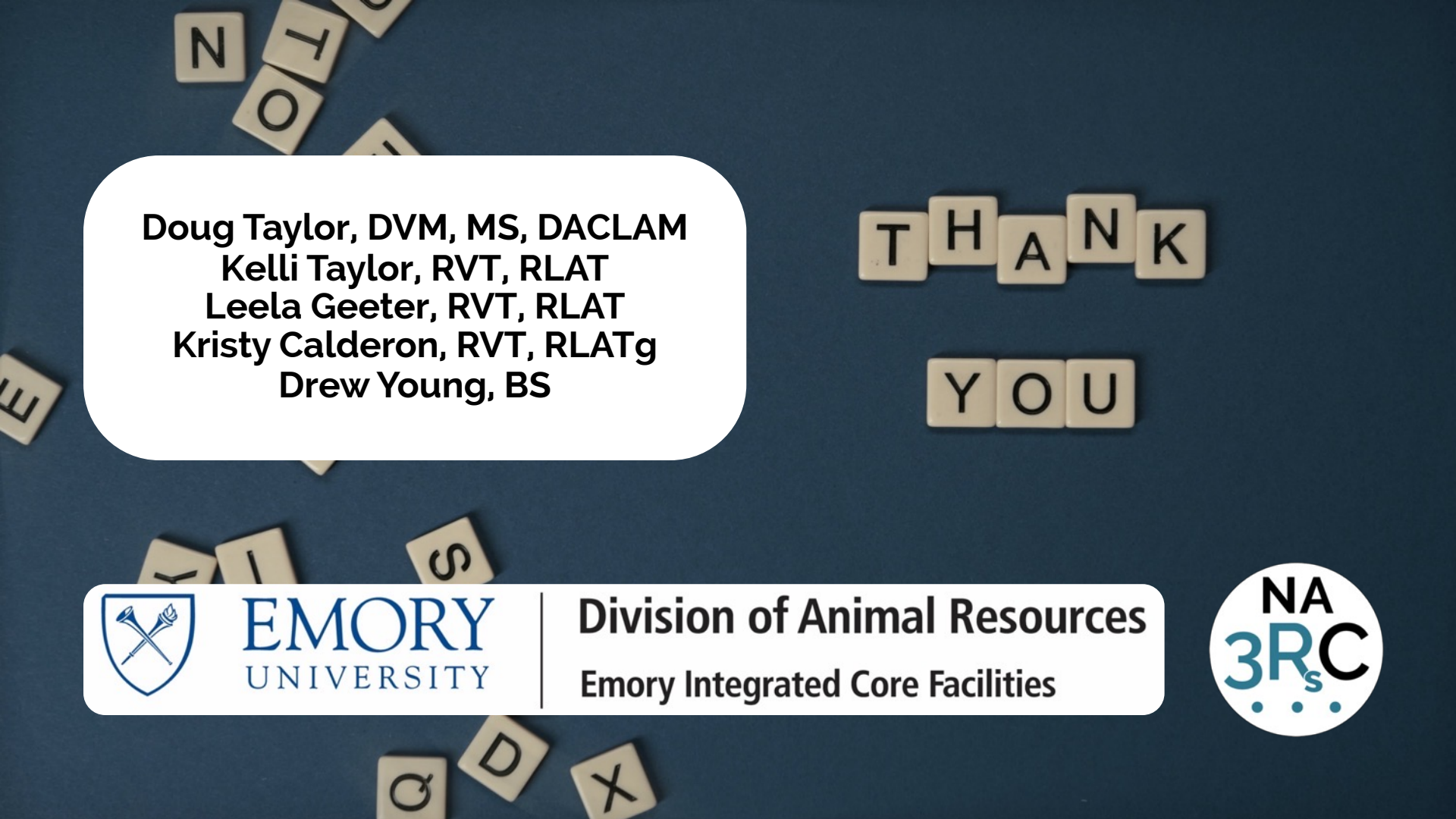
+/- Fecal Pellets

NA3RsC.org/health-monitoring



Contact your diagnostic laboratory to make sure that the materials and pooling procedures meet the lab's acceptance criteria for submission.

It can be done, and people are doing it!



Doug Taylor, DVM, MS, DACLAM
Kelli Taylor, RVT, RLAT
Leela Geeter, RVT, RLAT
Kristy Calderon, RVT, RLATg
Drew Young, BS

EMORY UNIVERSITY

Division of Animal Resources

Emory Integrated Core Facilities

NA 3Rs C

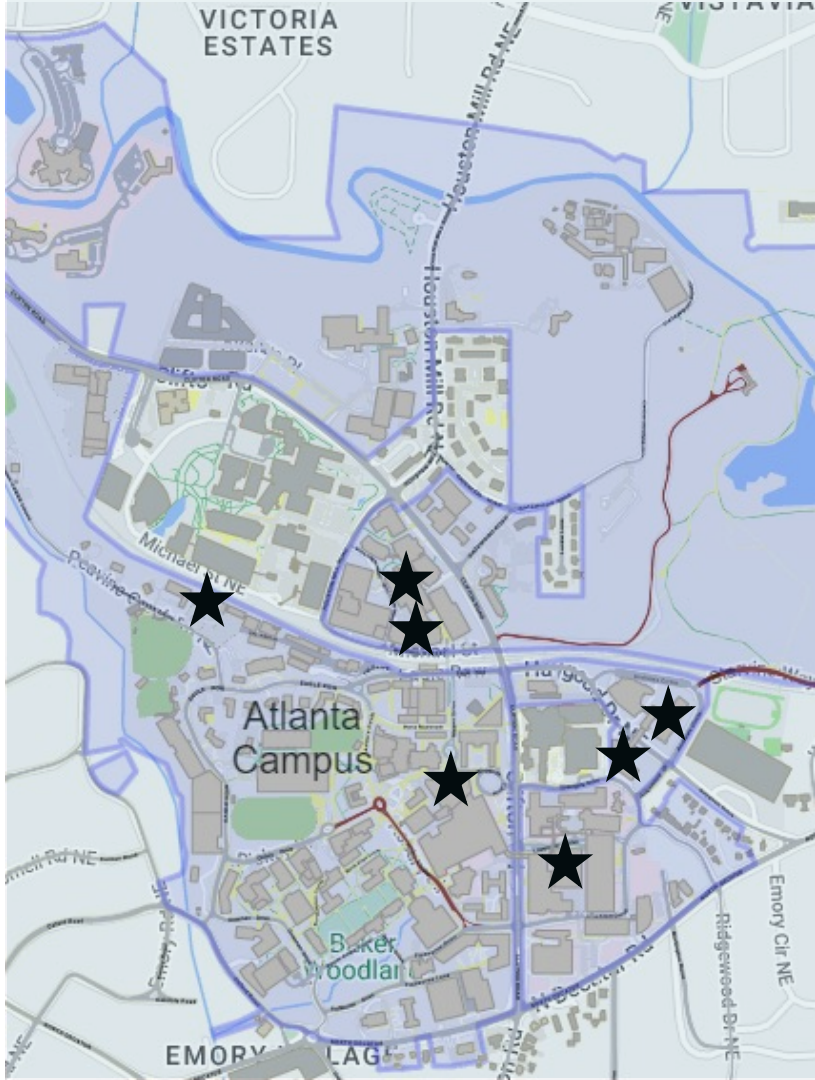
Cage Level Filtration and EHM: Sentinel-Free Soiled Bedding

Wai Hanson, DVM, PhD, DACLAM
Emory University

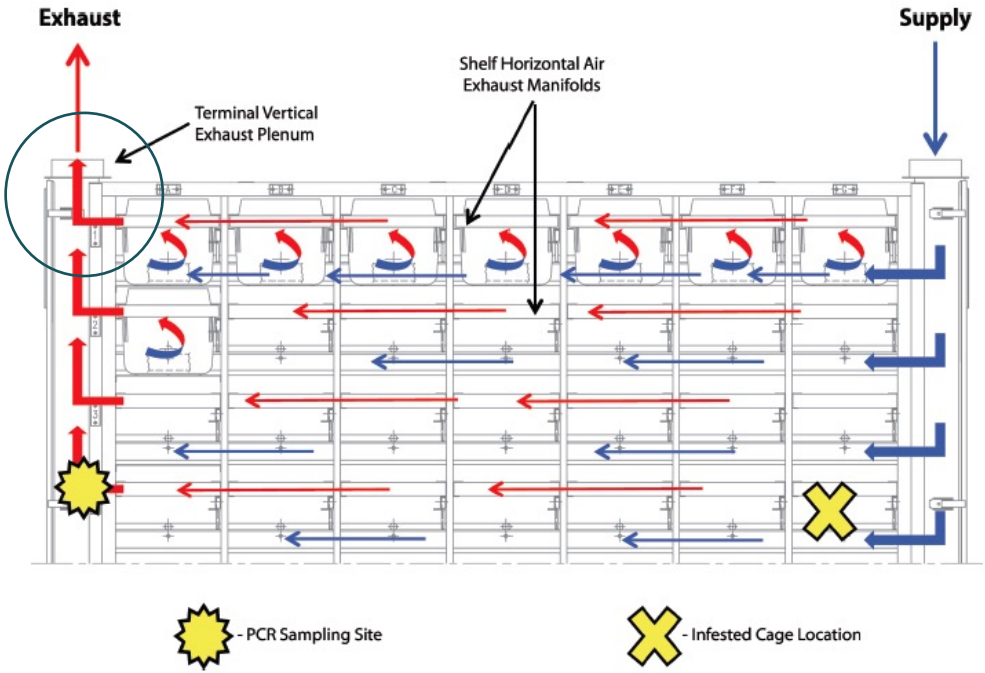
The North American 3Rs Collaborative

Emory University

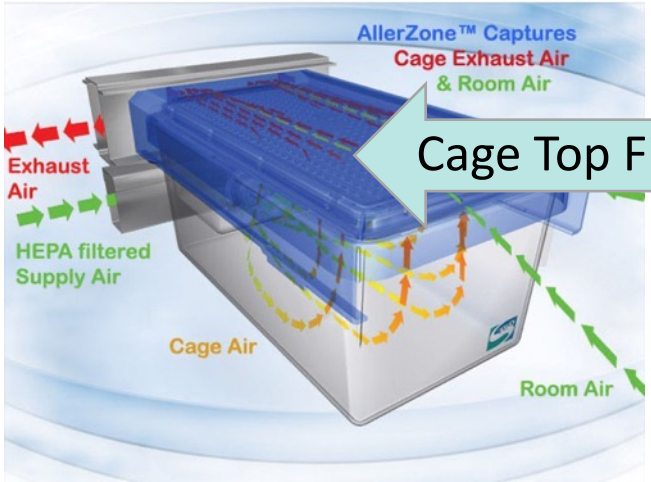
- 7 facilities on 1 campus
- 20,000 cages of mice
  - 80% IVC + 20% static
  - All LabProducts, LLC. caging
- 3,200 sentinel mice each year



Why perform EHM at the cage level?



Jensen, et al. JAALAS, 2013. 52(1): 28-33.



LabProducts, LLC.
Innovive
Animal Care Systems
Thoren

LabProducts, LLC. AllerZone™

Goals and Considerations

Effective Pathogen Detection

Replace Sentinel Animals

Reduce Emotional Fatigue

Reduce Labor?

Save Time & Money?

Investigation of Sentinel-Free Soiled Bedding (SFSB)

- **Method:**
  1. **Collect soiled bedding as before**
     - **Soiled bedding cage: Empty with no animals**
  2. **Collect samples at the end of the quarter**

Hanson, et al. JAALAS, 2021. 60(3):306-310.

Samples

- Swabs
- Filter media
- Fecal pellets

Hanson, et al. JAALAS, 2021. 60(3):306-310.

Sample Collection

Indwelling

Single Exposure

Figure 1: Each side of the media is passed through the SBC 10 times.

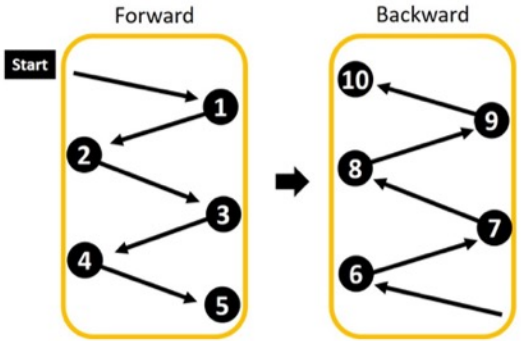


Figure 2: All swabs complete 10 rotations in the SBC at the same time.

Hanson, et al. JAALAS, 2021. 60(3):306-310.

Investigation of Sentinel-Free Soiled Bedding (SFSB)

MOUSE NOROVIRUS (MNV)

HELICOBACTER SPP.

FUR MITES

Hanson, et al. JAALAS, 2021. 60(3):306-310.

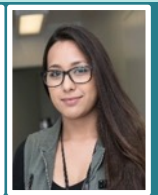
Academic Perspective:
Making the switch across caging types

Chris Manuel, DVM, PhD, DACLAM
Senior Associate Director, Office of Laboratory Animal Resources
Associate Professor, Department of Pathology

Environmental Health Monitoring (EHM)

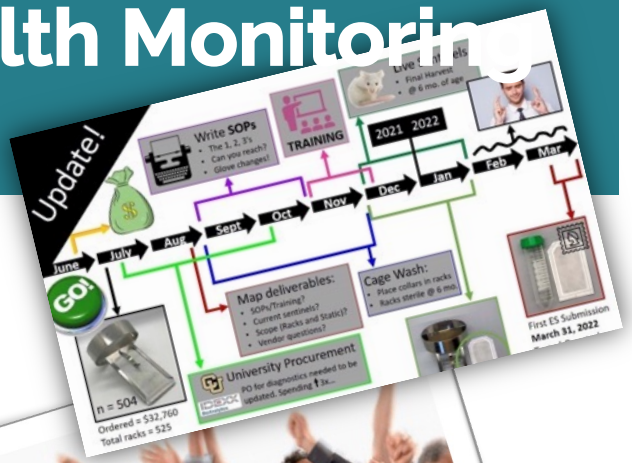
CU Anschutz Stats

- ✓ Started March 2022
- ✓ 11 months to implement
- ✓ Est. $41,500 savings/yr.
- ✓ 2,200 rodents saved/yr.
- ✓ Widely Accepted
- ✓ Nominated and Won
  CU Efficiency Award 2022



Lauren Habenicht, DVM, MS, DACLAM (left)
Sentinel Program Veterinarian

Christina Avena-Roman, CVT, ALAT (right)
Sentinel Program Coordinator

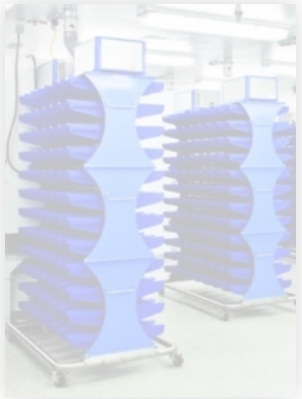


EDT
Exhaust Dust Testing

Allentown

98%
~23,000

SFSB
Sentinel-Free Soiled Bedding



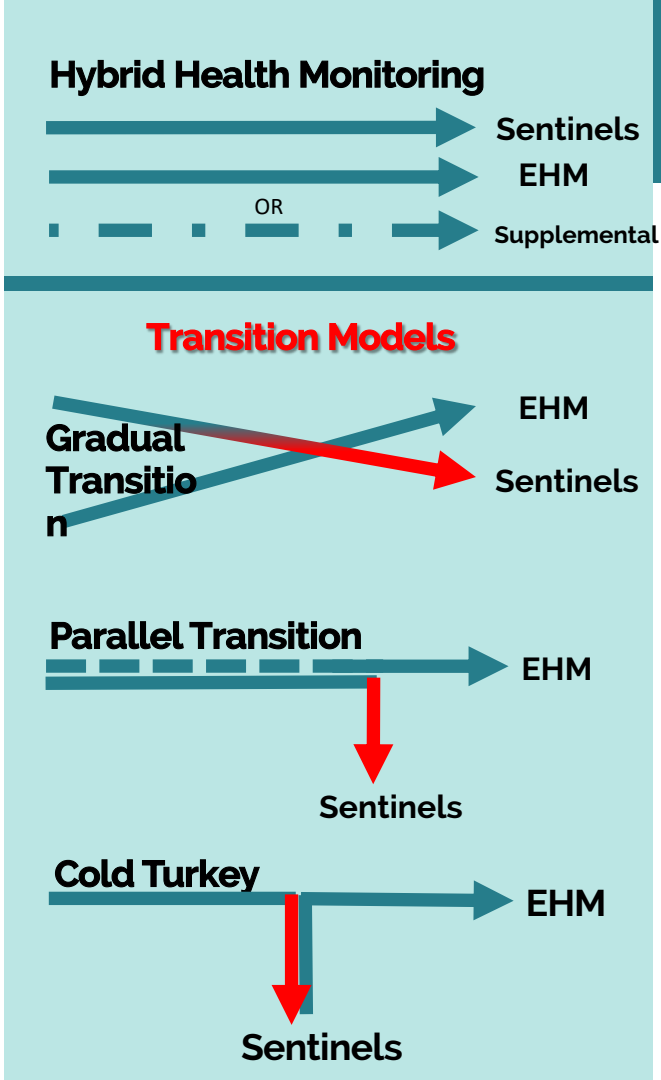
Animal Care | Systems

Allentown

inno vive

Static Caging

2%
~400



EDT Decisions

- 

- Sentinel™ media holders @ $37,510
  - Long run; maximize staff efficiency
  - Decrease sampling variability
- Stagger facility starts by 1 month
- January – March 2022
  - Nation wide short staffing begins…..
  - Vet Techs = harvest SBS program
  - Vets = startup EDT program
- All IVC racks except ABSL2+ & 3
- COVID restriction still in effect

EDT Training:
Dismantling a 50 Year Old Program

How to insert media
How to move media
How to collect media
Starting a new rack?
Removing a rack?
Moving a rack?
Glove changes
Rack + media in cage wash?

English
Español
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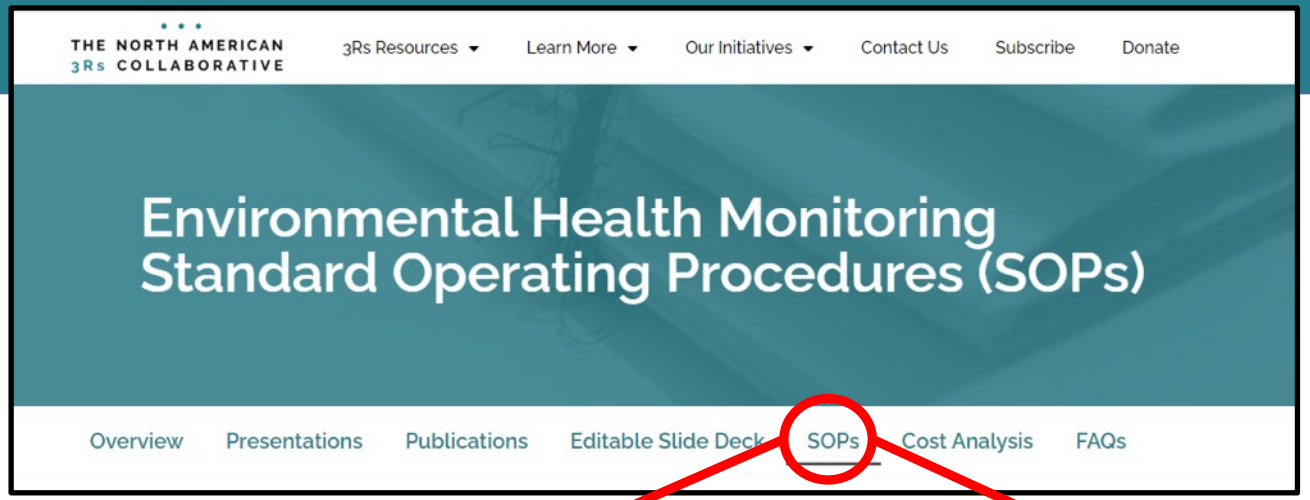
I hate writing SOPs…



Google: "NA3RsC health-monitoring"
www.na3rsc.org/health-monitoring/sops/

NA3RsC Standard Operating Procedures for Environmental Health Monitoring

1. Exhaust Dust Testing with Allentown Racks
2. Exhaust Dust Testing with Tecniplast Racks
3. Sentinel Free Soiled Bedding Sampling (for static, open top, or racks filtering at the cage level)
4. Direct Colony Sampling
5. Room and Equipment Monitoring

Physically…

Making the Switch

How many racks?

Existing: ~27 holders

3/2021: 409 holders

Uh…Chris, we don't have enough

2/2022: ~~40~~ 95 holders

Chris…, we are short on holders

\+ 7/2022: 20 holders

551 racks

How do you hide 115....
2022 Chevy Spark's ?

Physically...

Making the Switch

How many racks?

Existing: ~27 inserts
3/2021: 409 inserts
2/2022: 95 inserts
\+ 7/2022: 20 inserts

551 racks

Lots of... media and holders

Is it in there?

Reminders !!!!

Rack Sanitation Interval

- Every 6 months
- 2-3 racks are changed-out/day
- Racks move!
- Media in rack at cagewash?

EDT
Exhaust Dust Testing

Allentown

98%
~23,000

SFSB
Sentinel-Free Soiled Bedding

Animal Care | Systems

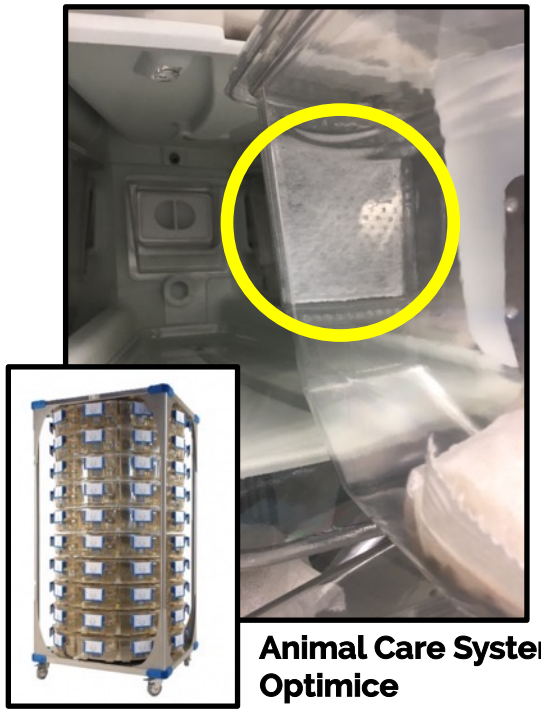
Allentown

innovive

Static Caging

2%
~400

Cage Level Air Exhaust Filters



Animal Care Systems
Optimice

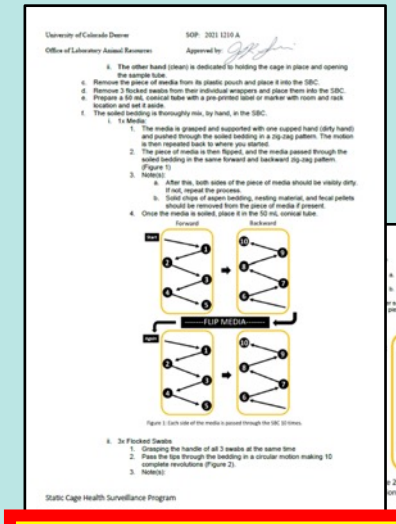
Innovive

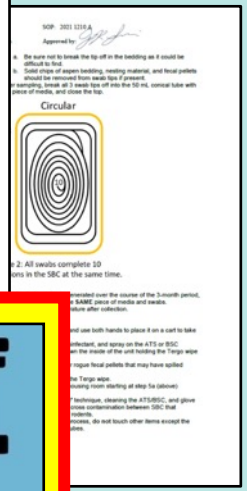
Bauer *et al.* 2016 Influence of Rack Design and Disease Prevalence on Detection of Rodent Pathogens in Exhaust Debris Samples from Individually Ventilated Caging Systems. *J Am Assoc Lab Anim Sci.* 55(6):782-88.

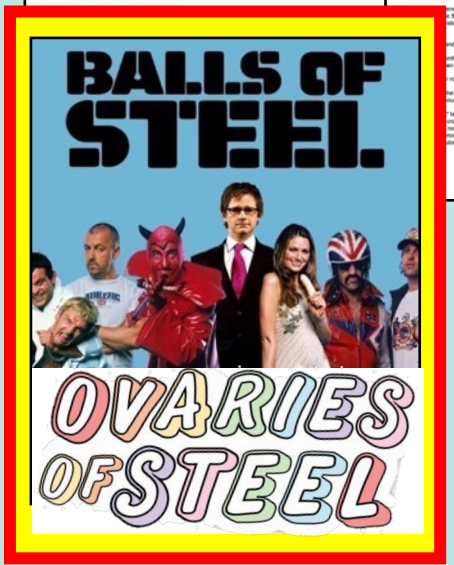
SFSB Decisions

- No parallel period
- SFSB (Sentinel-free Soiled Bedding)
  - Qualifiers:
    - Dredge Allentown filter media
    - Swirl 3-flocked swabs
    - Single media exposure @ 3 months
      - i.e. non-indwelling
    - No agitation
    - No fecal collection

Contact your diagnostic lab to help make these decisions!







Balls of Steel (2005)
3 Seasons
British Comedy Series

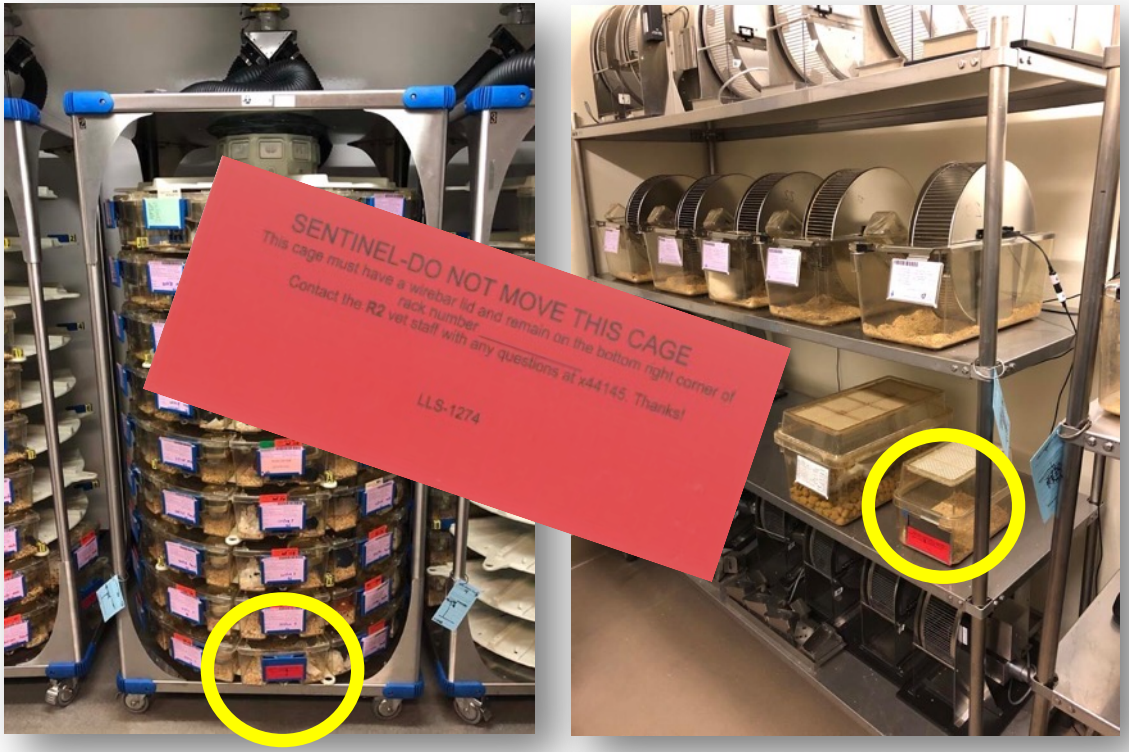
SFSB Training:
Dismantling a 50 Year Old Program

English
Español
አማርኛ

- Scope
  - Significantly less leg work
  - Hands on training with:
    - 8 animal care technicians
    - 2 veterinary technicians

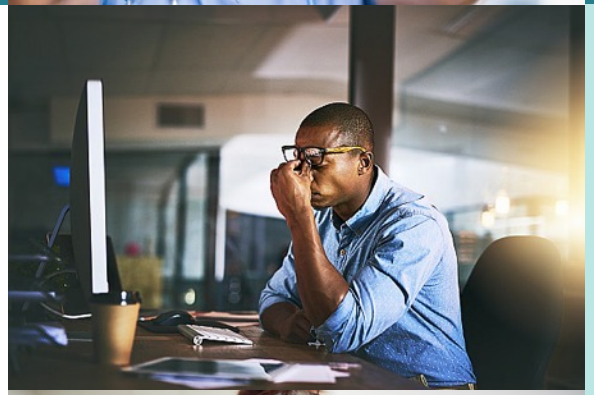
Physically...

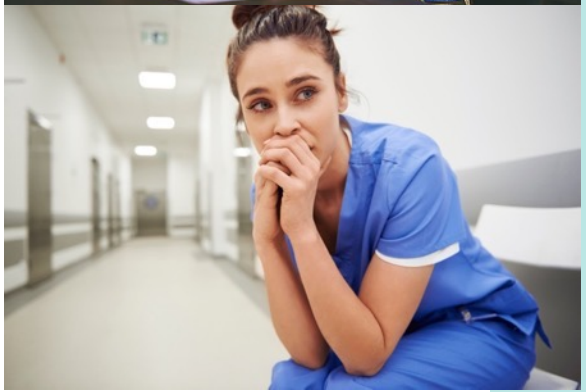
Making the Switch



Lesions Learned

- **Detail Oriented People**
  - Sentinel Program Coordinator
  - Tough not knowing the entire program
- **Managing Expectations**
  - No hole-in-one on the first swing
  - Build in room for mistakes
- **Communication**
  - It can always be better!
  - Hands on training!
  - Don't expect people to read emails!



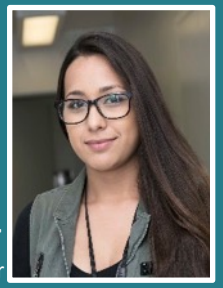


Summary

- **Anticipating a 15% media loss in 1st quarter**
  - Actual loss < 2% (n = 5-10)
- **Program fully implemented?**
  - Primary Facility: March 1st, 2022
  - Second & Satellites Facilities: April 1st, 2022
- **Easiest to switch EDT vs. SFSB?**
  - SFSB was the easiest
  - Potential bias with only ~400 cages?
- **Detection comparison evaluation?**
  - MNV detected at same rate
  - No new agents detected
- **Financial and time comparison?**
  - Pending

Thank you

Lauren Habenicht, DVM, MS, DACLAM
Sentinel Program Veterinarian



Christina Avena-Roman, CVT, ALAT
Sentinel Program Coordinator

Chris.Manuel@cuanschutz.edu

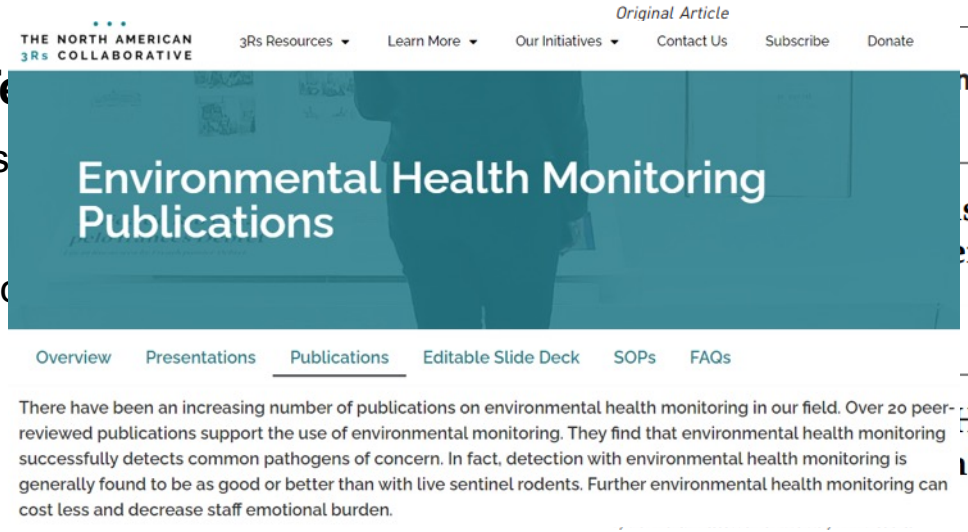
Rodent-Free Health Monitoring for SPF mice

A Multi-Site Initiative to Utilize Rodent-Free Health Monitoring Methods with Mixed Caging Systems in a Pharmaceutical Setting

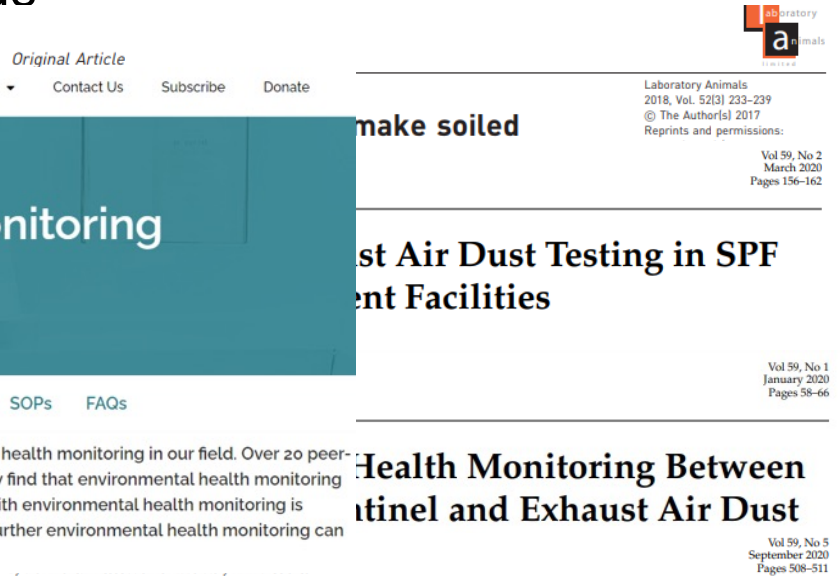
Beth Bennett, DVM (Pearl River, NY)
Caroline Winn, DVM, MS, DACLAM (Cambridge, MA)

There is an abundance of data to support replacement of live animal sentinel programs with animal-free methods

- **Exhaust Dust Te**
  - Caging systems
    - Plenums
    - In-line colle



https://www.na3rsc.org/health-mor


Original Article

make soiled

Laboratory Animals
2018, Vol. 52(3) 233–239
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Vol 59, No 2
March 2020
Pages 156–162

st Air Dust Testing in SPF
nt Facilities

Vol 59, No 1
January 2020
Pages 58–66

Health Monitoring Between
ntinel and Exhaust Air Dust

Vol 59, No 5
September 2020
Pages 508–511

Cost Comparison of Rodent Soiled Bedding Sentinel and Exhaust Air Dust Health-Monitoring Programs

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

The 4 Pfizer Vivaria

La Jolla, CA
90% EDT Compatible
35,000 rodents

Cambridge, MA
0% EDT Compatible
35,000 rodents

Groton, CT
70% EDT Compatible
6,500 rodents

Pearl River, NY
80% EDT Compatible
29,500 rodents

Unified Pfizer Research Facilities Rodent Health Standards (Exclusion List)

Can we reduce Pfizer’s sentinel animal use even further?

Other Agents Detected by Media Exposed to Soiled Bedding

Media Type(s) in soiled bedding	Cage Systems*	Agents Detected	References
Filter, flocked swabs	Vendor 1	**MNV, *Helicobacter* spp., fur mites**	Hanson et al., 2021
Filter	Vendor 2	**MHV, MNV, MPV, MVM, TMEV, *S. obvelata, A. tetraptera***	O'Connell et al., 2021
Rear filter, flocked swabs, sticky swabs	Vendor 3	***Entamoeba, Helicobacter* spp., *Rodentibacter heylii, R. pneumotropicus, MuCPV, Chilomastix, Tritrichomonas, K. oxytoca, K. pneumoniae, MNV, Astrovirus, Ps. aeruginosa*** ***Note: Sentinel mice were better at detecting *P. mirabilis* and *S. aureus***	Presentation - 2021 National AALAS Meeting, U. of Tennessee
Filter vs. EDT	Vendor 2	***Helicobacter spp., K. oxytoca, K. pneumotropicus, MNV, Ps. aeruginosa, Tritrichomonas, R. pneumotropicus, R. heylii***	Poster - 2021 National AALAS Meeting, West Coast Institution
Filter, flocked swabs, sticky swabs	Vendor 3	***Helicobacter* spp., *R. pneumotropicus, R. heylii, Tritrichomonas,* Astrovirus, MNV, *Entamoeba***	Presentation - 2021 National AALAS Meeting, U. of Alabama

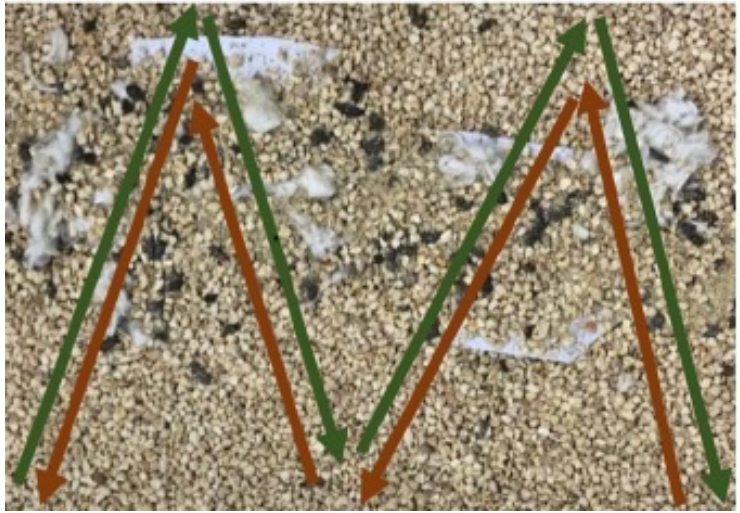
*Cage Systems that exhaust at the cage level / Non-EAD compatible

Growing support for sentinel-free monitoring methods in non-EDT compatible caging

In-cage filter paper detected TMEV, MNV, MHV, MNV, MPV, MVM, *S. obvelata* and *A. tetraptera* as well as sentinel mice, and outperformed sentinel mice in detection of *Helicobacter* spp. for a 1- and 2-month period[1]

Shaken, mouse-free cage filters outperformed sentinel cage filter PCR in detecting *Helicobacter* spp., MNV, *R. pneumotropicus, E. muris* and *S. muris* over a 3-month period[2]

Flocked swabs and filter media in soiled bedding in both IVCs and static caging detected MNV, *Helicobacter* spp., and fur mites[3]



1. O'Connell KA et al. 2021 In-Cage Filter Paper as a Replacement for Sentinel Mice in the Detection of Murine Pathogens. 60(2): 160-7.
2. Dubelko AR et al. 2018. PCR Testing of Filter Material from IVC Lids for Microbial Monitoring of Mouse Colonies. 57(5): 477-82.
3. Hanson WH et al. 2021. PCR Testing of Media Placed in Soiled Bedding as a Method for Mouse Colony Health Surveillance. 60(3): 306-10.

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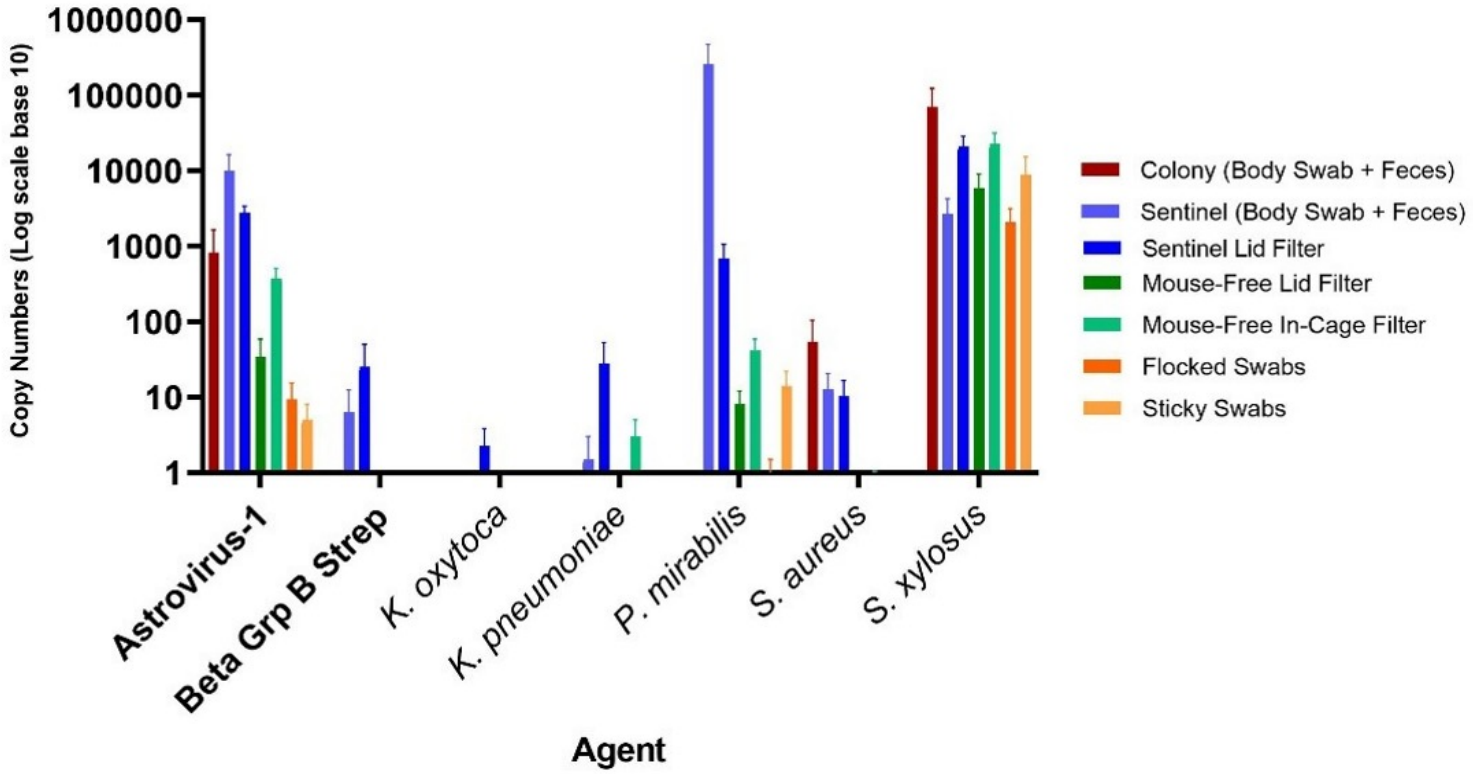
Vol 99, No 9
Month 2022
Pages 1–9

Using Filter Media and Soiled Bedding in Disposable Individually Ventilated Cages as a Refinement to Specific Pathogen-free Mouse Health Monitoring Programs

Caroline B Winn,[1,*] Renee N Rogers,[1] Rose A Keenan,[1] Philip M Gerwin,[2] Kristin A Matthews,[3]
Julita A Ramirez,[4] Terese E Bennett,[4] Cheryl L Perkins,[5] Kenneth S Henderson[5]

- **Mouse-free in-cage sampling**
- **3-month period (1 quarter)**
- **Multiple vivaria with same caging type**
- **Same health monitoring criteria and protocol for husbandry, media selection, agitation, and sampling**

In-house pilot data supported use of filter media based on highest copy numbers



Winn CB et al. Using Filter Media and Soiled Bedding in Disposable Individually Ventilated Cages as a Refinement to Specific Pathogen-free Mouse Health Monitoring Programs. J Am Assoc Lab Anim Sci. 2022 Jun 24. doi: 10.30802/AALAS-JAALAS-22-000013. Epub ahead of print. PMID: 35750479.

In-cage mouse-free filter outperformed direct testing from direct colony sampling & sentinel mice

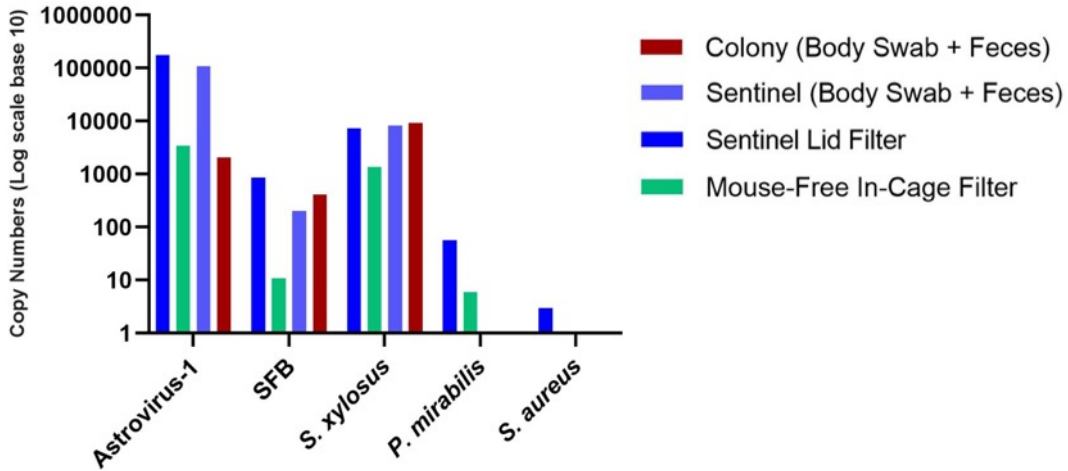
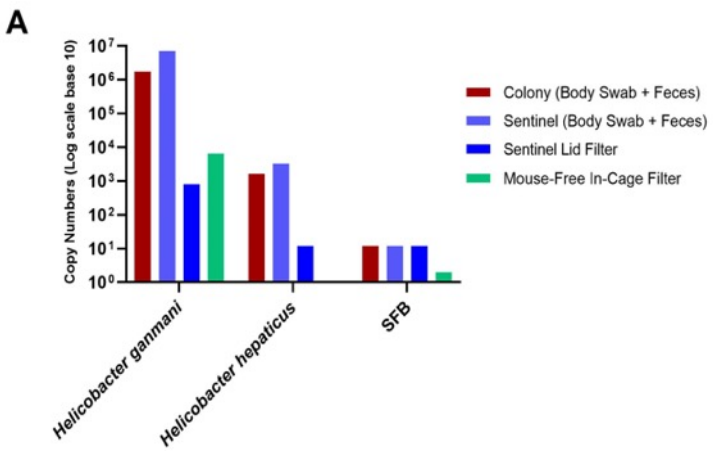


Table 1. Pathogen detection by Rack ($n = 4$) for site A with SPF colony mice after 3 mo

	Sentinel Mice		Colony Mice Mouse-Free Cages	
Agent	Fecal and Body Swab PCR	Lid Exhaust Filter PCR	Fecal and Body Swab PCR	In-Cage Filter PCR
Astrovirus-1	1/4	4/4	2/4	4/4
SFB	1/4	4/4	2/4	4/4
S. xylosus	2/4	4/4	4/4	4/4
S. aureus	0/1	1/1	0/1	0/1
P. mirabilis	0/3	3/3	0/3	1/3

Winn CB et al. Using Filter Media and Soiled Bedding in Disposable Individually Ventilated Cages as a Refinement to Specific Pathogen-free Mouse Health Monitoring Programs. J Am Assoc Lab Anim Sci. 2022 Jun 24. doi: 10.30802/AALAS-JAALAS-22-000013. Epub ahead of print. PMID: 35750479.

Similar results found at collaborative sites in mice inoculated with wild microbiome



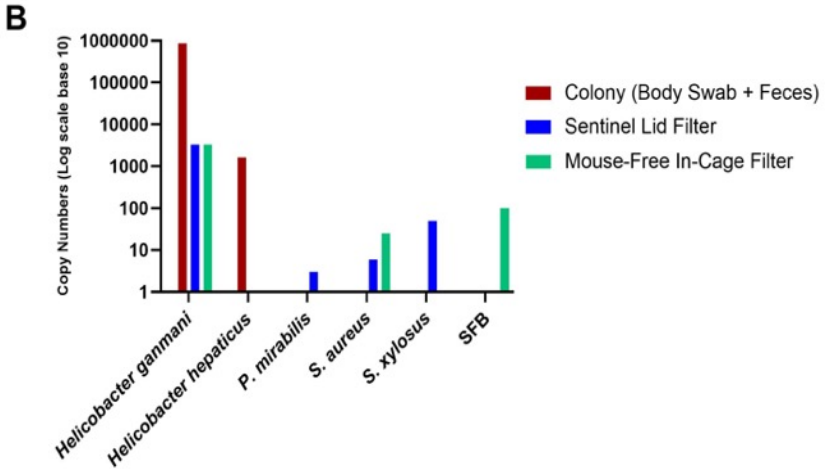


Table 2. Pathogen detection (±) denoted from the Rack ($n = 1$) at sites B and C after 3 mo with wild microbiome-inoculated study mice

	Sentinel Mice				Study Mice		Mouse-Free Cage	
	Fecal and Body Swab PCR		Lid Exhaust Filter PCR		Fecal and Body Swab PCR		In-Cage Filter PCR	
Agent	Site B	Site C	Site B	Site C	Site B	Site C	Site B	Site C
Helicobacter genus	+	–	+	+	+	+	+	+
H. ganmani	+	–	+	+	+	+	+	+
H. hepaticus	+	–	+	–	+	+	–	–
SFB	+	–	+	+	+	–	+	–
*S. xylosus**		–		–		–		+
*S. aureus**		–		–		+		+
*P. mirabilis**		–		–		+		–

*Agent(s) not detected at site B

Winn CB et al. Using Filter Media and Soiled Bedding in Disposable Individually Ventilated Cages as a Refinement to Specific Pathogen-free Mouse Health Monitoring Programs. J Am Assoc Lab Anim Sci. 2022 Jun 24. doi: 10.30802/AALAS-JAALAS-22-000013. Epub ahead of print. PMID: 35750479.

Current Pfizer Environmental Health Monitoring Status

La Jolla, CA
100% EHM

Cambridge, MA
95% EHM

Groton, CT
90% EHM

Pearl River, NY
100% EHM

US Comparative Medicine
96% Sentinel Animal-Free

OVERCOMING STANDARDIZATION CHALLENGES

1. Variety of stakeholders

- GLP vs non-GLP
- Research units (vaccines, oncology, etc.)
- Rodent health status (outbred immunocompetent vs GEMMs, etc.)

2. Different caging systems

- Mainly use two manufacturers at Pfizer

3. Closed colonies

- Source all rodents from SPF vendors
- Minimal cross-site animal movement

4. Staff training, competency, confidence

- SOP Compliance (especially for facilities with multiple caging systems)

Benefits of incorporating in-cage filter media testing: Pfizer Cambridge Vivarium

	2020 Q1/Q3: Serology Q2/Q4: PCR of Filter	2022 Q1-Q4: Pooled PCR of In-cage Filter + Colony Fecal Pellets
Est. HM Program Costs	$25,000	**$20,000**
Sentinel Rodents	N=360	**N=16**
Time, Supplies (Husbandry)	Same	**Reduced**
Time (Veterinary Care)	Approx. 80hr	**0hr**
Staff Feedback	Neutral	**Positive**
Health Monitoring Results	Negative	**Negative**
Events of Receipt of “Contaminated” Sentinel rodents from vendor	2	**0**

Future Directions

Segmented filamentous bacteria (SFB) may serve as a good positive control

- Use to optimize health monitoring strategy for SOPF and/or immunodeficient rodents?

Continue Pfizer Global collaboration

Pfizer
Worldwide Research, Development and Medical
Comparative Medicine

Incorporate 3Rs+ health monitoring strategies

THE NORTH AMERICAN 3Rs COLLABORATIVE

Danzeisen, Jessica & Calvert, Alamanda & Noll, Sally & McComb, Brian & Sherwood, Julie & Logue, Catherine & Johnson, Timothy. (2013). Succession of the turkey gastrointestinal bacterial microbiome related to weight gain. PeerJ. 1. e237. 10.7717/peerj.237.

Takeaways

This work would not be possible without:

Rose Keenan

Renee Rogers

CM Technical Staff

CM Husbandry Staff

CM Biosafety Team:

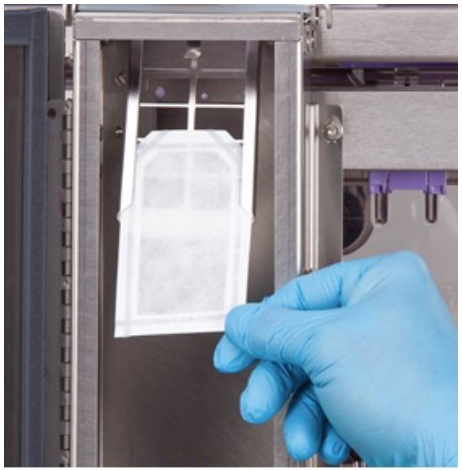
- Philip Gerwin (Groton, CT)
- Kristin Matthews (La Jolla, CA)
- Julita Ramirez (Pearl River, NY)
- Michael Wadanoli (Cambridge, MA)
- Chandra Williams (Cambridge, MA)

Charles River Laboratories

Ken Henderson

Cheryl Perkins

NA3RsC



https://www.allentowninc.com/sentinel/

Thank you!

Terese.Bennett@pfizer.com
Caroline.Winn@pfizer.com

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1. **Compton SR, Homberger FR, Paturzo FX, Clark JM.** 2004. Efficacy of 3 microbiological monitoring methods in a ventilated cage rack. Comp Med **54**:382–392.
2. **de Bruin WCC, van de Ven EME, Hooijmans CR.** 2016. Efficacy of soiled bedding transfer for transmission of mouse and rat infections to sentinels: A systematic review. PloS one **11**(8):e0158410.
3. **Dubelko AR, Zuwannin M, McIntee SC, Livingston RS, Foley PL.** 2018. PCR testing of filter material from IVC lids for microbial monitoring of mouse colonies. J Am Assoc Lab Anim Sci **57**(5):477–482.
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