REDUCE. REFINE. REPLACE™

A Breakthrough Approach to Rodent Health Monitoring Using Sentinel-Free Soiled Bed-

INTRODUCTION

- Tens of thousands of soiled bedding sentinel animals are still used annually to monitor microbiological status of rodent colonies
- Environmental Health Monitoring (EHM) methods are highly appealing alternatives to traditional soiled bedding sentinels (SBS)
- EHM includes direct colony sampling, exhaust dust testing (EDT) and sentinel-free soiled bedding (SFSB) methods
- PCR-based EHM monitors health status of rodent colonies without the need for sentinel animal import, husbandry, sample collection and euthanasia
- EHM has increased sensitivity in pathogen detection versus SBS (Hanson 2021, Miller 2016, Pettan-Brewer 2020, Zorn 2016)
- EHM has the additional benefit of an overall decrease in health monitoring program cost (Luchins 2020)
- While EDT is applicable only with racks having an open airflow design, SFSB provides a universal EHM approach for use with any rodent housing type.

MAIN OBJECTIVES

1. Evaluate soiled bedding sentinels against sentinel-free soiled bedding testing.
2. Compare three commercially-available media for binding capacity.
3. Identify lowest labor method for SFSB.
   a. Compare agitated vs. dredged matrices.
   b. Compare frequency of agitation.

EXPERIMENTS AND DATA

Objective 1: Evaluate SBS detection against SFSB PCR-based testing.

We directly compared the effectiveness of collection materials placed into agitated SFSB cages to traditional SBS for pathogen detection in a colony of naturally infected mice. Mice were confirmed upon intake to be positive by fecal PCR for a variety of viruses, bacteria and parasites. Each SFSB cage (n=3) contained a matrix and each SBS cage (n=7) contained two 6-8 week old CD-1 mice. At two-week intervals, for a total of 12 weeks, soiled corn cob bedding from colony mice was pooled and mixed thoroughly. Two ounces of composite soiled bedding was added to each SFSB and SBS cage. Twice a week SFSB cages were agitated for 15 seconds using an elliptical “stir-fry” motion to expose SFSB material to soiled bedding. SFSB collection material was moved to the new SFSB cage at regularly scheduled 2-week cage change intervals. At the end of the 12-week study, SFSB material was collected and nucleic acids were extracted and tested for pathogens by real-time PCR. SBS mice were tested for bacteria and endoparasites by fecal PCR, ectoparasites by fur swab PCR and viruses by MFI serology.

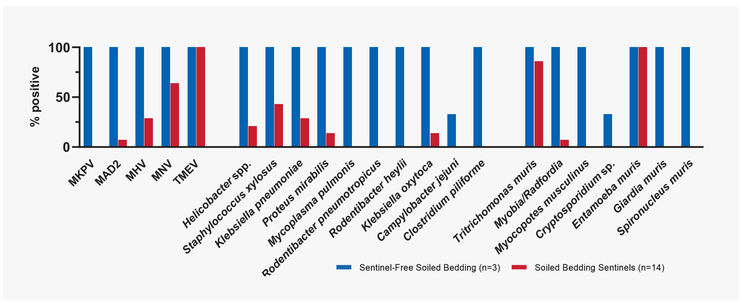

Figure 1. Comparison of sentinel-free soiled bedding to soiled bedding sentinels in detecting pathogens in a mouse colony infected with viruses, bacteria and parasites.

Objective 2: Compare three commercially available matrices for binding capacity.

Sentinel-Free Soiled Bedding (SFSB) testing relies on exposing sample collection material to soiled bedding at regularly scheduled cage change intervals over the course of the health monitoring period. Collection material binds pathogens, or their components, allowing detection by real-time PCR analysis. SFSB collection materials with **higher binding capacity** can provide **higher diagnostic test sensitivity**, especially when pathogen burden is low. Binding efficiency of REPLACE™ matrix was directly compared to two other commercially available EHM collection media. Five replicates of all three materials were placed in soiled corn cob bedding collected from mice naturally infected with viruses, bacteria, and parasites. Bedding was agitated using an elliptical “stir-fry” motion for 60 seconds. Following agitation, the SFSB materials were collected, nucleic acids extracted, and real-time PCR testing for pathogens was performed.

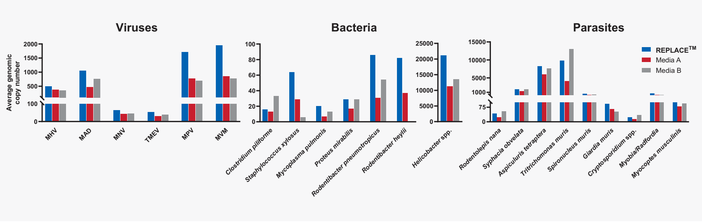

Figure 2. Average genomic copy number from technical triplicate evaluation of three different collection matrices/media after 1 minute of agitation in soiled bedding from mice known positive for viruses, bacteria and parasites.

Objective 3: Identify lowest labor method for performing SFSB with REPLACE™.

Successful Sentinel-Free Soiled Bedding (SFSB) testing relies on exposure of environmental matrices to dirty bedding. Methods of exposure vary and include agitation or stirring with soiled bedding or swiping/dredging soiled cages or bedding for various time periods and frequencies. To assess the most sensitive exposure method, we evaluated agitation by shaking or by swiping environmental matrices through dirty bedding (dredging).

Two experimental groups of five replicates of REPLACE™ matrices, commercial media A and commercial media B were placed in soiled corn cob bedding collected from mice naturally infected with viruses, bacteria, and parasites (verified by real-time PCR testing prior to study initiation).

Group 1: Bedding was agitated using an elliptical “stir-fry” motion for 30 seconds.

Group 2: REPLACE™ matrices or commercial media were held in a gloved hand parallel to bedding surface and wiped through dirty bedding using a zig zag pattern. The inner cage periphery was wiped at the bedding – cage interface using a circular motion. Once completed, the matrices or media were flipped over, and the process repeated.

Nucleic acids were extracted from REPLACE™, commercial media A and commercial media B and real-time PCR testing for pathogens was performed, maintaining procedures and volumes identical for all samples tested.

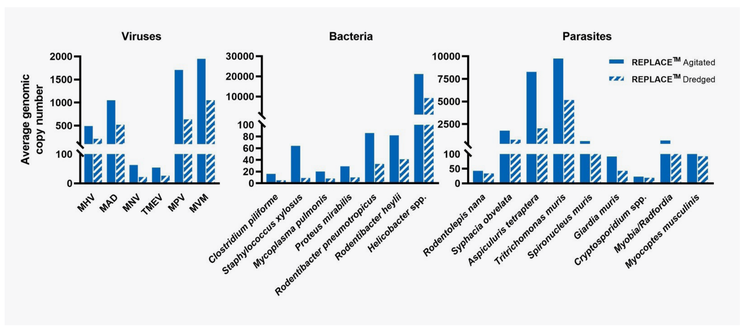

Figure 3. REPLACE™ matrices used in an agitated cage outperformed matrices dredged through dirty bedding when exposed to the same bedding from a mouse colony known to be positive for viruses, bacteria and parasites. Agitation results in higher copy number detection with lower labor efforts, further reducing technician workload and repetitive motion.

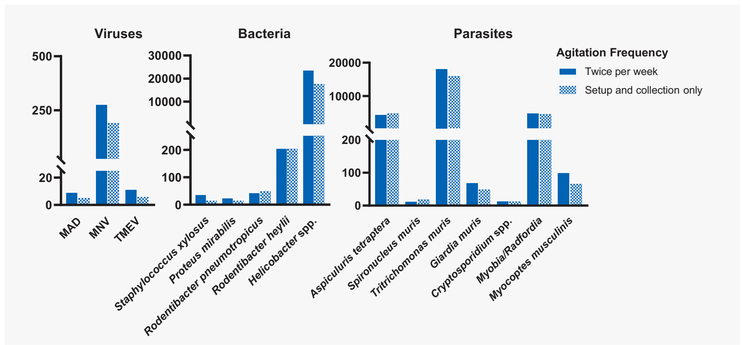

Figure 4. A two-week study evaluating low-prevalence pathogens shown above compared agitation frequency. Group 1 (solid blue bar) represents REPLACE™ agitated in SFSB cage twice per week throughout the two-week exposure period. Group 2 (hashed blue bar) represents REPLACE™ agitated in SFSB cage once at cage setup, and once at the two-week collection point, with no cage manipulation in between. Even with low prevalence agents, minimal manipulation resulted in copy numbers similar to more frequent agitation, allowing confidence in reducing labor and time related to SFSB.

CONCLUSIONS

Objective 1: SFSB outperformed SBS for viral, bacterial and parasite detection (Figure 1). Of the 22 pathogens detected by SFSB, only 12 were detected by SBS. SFSB detected positives in 3/3 replicates for all agents tested, with the exception of *Cryptosporidium* spp. and *Campylobacter jejuni* which were at low prevalence in colony mice based on fecal PCR testing.

Objective 2: REPLACE™ matrix repeatedly **outperformed other media** by detecting higher genomic copies per pathogen (Figure 2). The high binding capacity of **REPLACE™ resulted in detection of higher pathogen genomic copy numbers.** In modern rodent colonies where disease prevalence is often low, the enhanced binding capacity of REPLACE™ can result in improved pathogen detection.

Objective 3: When overall average pathogen copy number for each collection device was compared, REPLACE™ in an agitated cage **outperformed dredged matrices**, as well as agitated media from other manufacturers (data not shown). Individual agents are shown in Figure 3, comparing agitated vs. dredged REPLACE™ matrices. The average genomic copy number for **viral, bacterial and parasite detection was approximately double** for agitated vs. dredged samples. Figure 4 reveals that there was no difference in pathogen detection or copy number for viruses, bacteria and parasites when REPLACE™ matrices were agitated twice weekly or only at cage setup and REPLACE™ collection. This is beneficial to facilities and husbandry staff as **it decreases technician SFSB cage handling in half while maintaining accurate pathogen detection.**

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