IDEXX **BioAnalytics**

# REPLACE™ Your Sentinels

Sentinel-Free Soiled Bedding (SFSB) with IDEXX BioAnalytics REPLACE™


The IDEXX BioAnalytics **REPLACE™** matrix is a sentinel-free soiled bedding (SFSB) pathogen collection device that consistently detects higher copy numbers than other commercially-available environmental collection media. REPLACE™ matrices are validated to ensure absence of pathogenic nucleic acids and rigorously tested to verify pathogen capture and detection. Sentinel-free soiled bedding rodent health monitoring with REPLACE™ creates the most robust PCR-based surveillance method available while eliminating the use of sentinel animals. The following experiments summarize the ease and efficacy of SFSB with REPLACE™.

## Sentinel-Free Soiled Bedding Testing Detects More Pathogens Than Soiled Bedding Sentinels

- **Sentinel-free soiled bedding** detected all evaluated pathogens with nearly 100% sensitivity.
- **Sentinel animals** failed to detect 10 out of 22 pathogens.

Background | Environmental Health Monitoring methods are highly appealing alternatives to traditional soiled bedding sentinels (SBS). By eliminating unnecessary use of animals while simultaneously featuring vastly **improved pathogen detection**, these methods align with the 3R's precept. Sentinel-Free Soiled Bedding (SFSB) and Exhaust Dust Testing (EDT) are two primary PCR-based Environmental Health Monitoring (EHM) approaches to monitor 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). Moreover, it 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 (Bauer), SFSB provides a universal EHM approach for use with any rodent housing type.

Methods | 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 commercially available collection material 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.

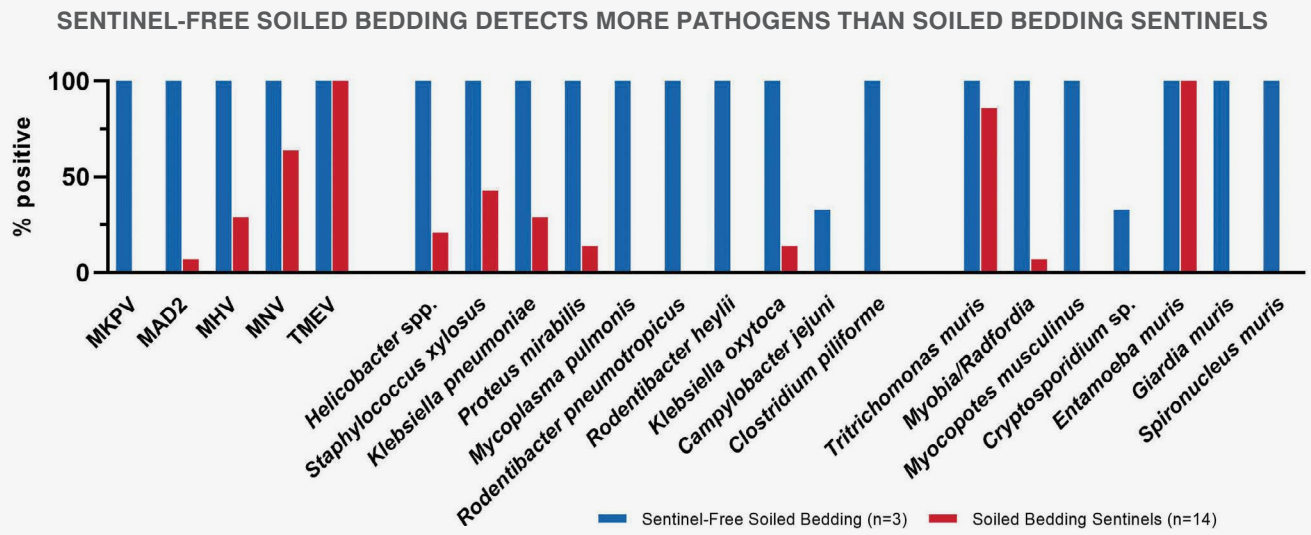



**Figure 1.** Comparison of sentinel-free soiled bedding to soiled bedding sentinels in detecting pathogens in a mouse colony infected with viruses, bacteria and parasites.

Results | **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.

# IDEXX REPLACE™ Matrix Outperforms Other SFSB Commercial Collection Media

- **REPLACE™** detected higher copy numbers for viruses, bacteria and parasites than other collection materials evaluated.

Background | Sentinel-Free Soiled Bedding (SFSB) testing relies on exposing a 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.

Methods | 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.

**REPLACE™ HAS HIGHER PATHOGEN BINDING CAPACITY COMPARED TO OTHER EHM COLLECTION MATERIALS**

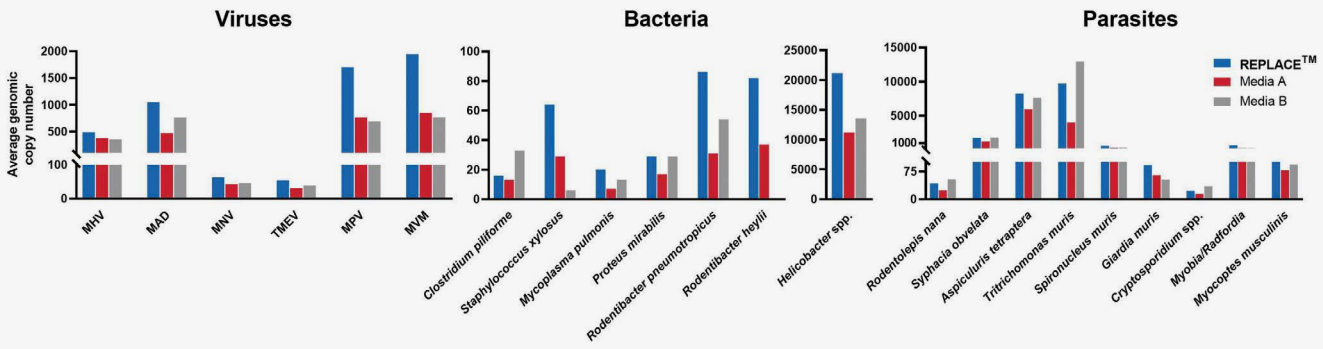
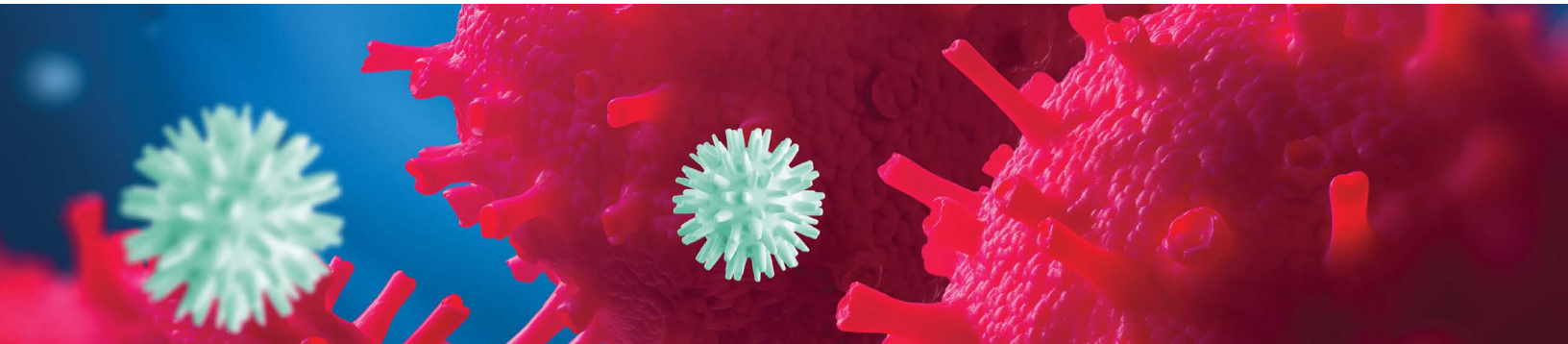



**Figure 2.** Average genomic copy number from technical triplicate evaluation of three different collection matrices/media after 1 minute of agitation in soiled bedding from mice known positive for viruses, bacteria and parasites.

Results | **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.



# Agitation of SFSB Cages with REPLACE™ is the Most Simple, Effective Method for Pathogen Detection

- **REPLACE™** in an agitated SFSB cage detected higher copy numbers for viruses, bacteria and parasites than dredging.

Background | Successful Sentinel-Free Soiled Bedding (SFSB) testing relies on SFSB collection material capturing pathogens when exposed to soiled bedding. Varying methods of exposure have been described and include agitation or stirring with soiled bedding or swiping/dredging soiled cages or bedding at various time periods and frequencies. To assess the most sensitive exposure method, we evaluated **agitation by shaking or by swiping** environmental matrices and media through dirty bedding (dredging).

Methods | 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.

**AGITATED VS. DREDGED SFSB COPY NUMBER DETECTION**

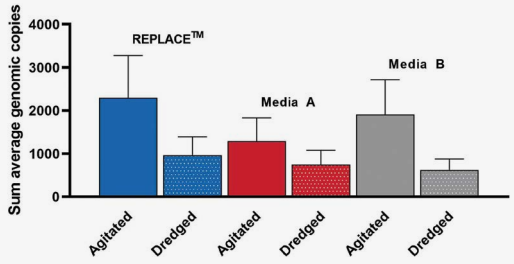



**Figure 3.** Sum of average detected copy numbers for all detected pathogens compared amongst three matrices/media tested in agitated and dredged SFSB cages with bedding from colony known positive for viruses, bacteria and parasites. REPLACE™ matrix in an agitated SFSB cage outperformed all other testing methods.

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

Results | When overall average pathogen copy number for each collection device was compared, REPLACE™ in an agitated cage **outperformed dredged samples, as well as agitated media** from other manufacturers (Figure 3). Figure 4 shows the same data broken down into individual agents. The average genomic copy number for **viral, bacterial and parasite detection was approximately double for agitated** vs. dredged samples for REPLACE™.

**REPLACE™ IN AN AGITATED SFSB CAGE OUTPERFORMS MORE LABOR-INTENSIVE DREDGING METHOD**

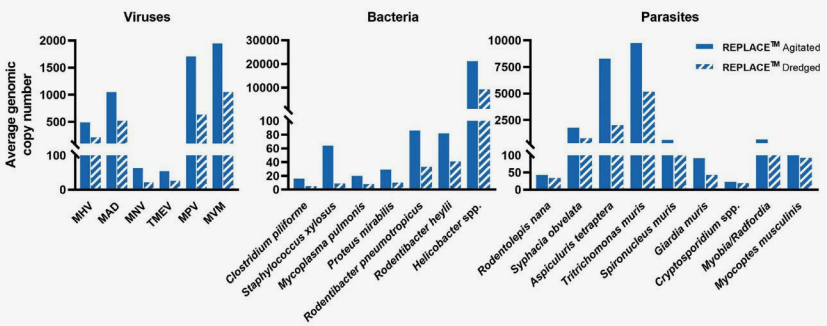



**Figure 4.** Copy number comparison of agitated vs. dredged exposure of REPLACE™ matrix. REPLACE™ was evaluated with two methods against pooled dirty bedding from a colony known to be positive for a broad list of pathogens. REPLACE™ agitated in an SFSB cage outperformed REPLACE™ dredged through the same pooled dirty bedding.

# Agitation of REPLACE™ Matrices Every Two Weeks is Sufficient for Pathogen Detection

- **REPLACE™** matrices exposed to agitation for 30 seconds at two timepoints resulted in equivalent copy number detection as more frequent agitation methods.

Background | Ideal matrix agitation frequency and duration in SFSB cages remains unproven. Balancing caretaker workload and maximum pathogen detection is necessary to facilitate successful SFSB practices. To determine an agitation method that will provide **sensitive detection of pathogens while decreasing labor time**, we evaluated agitation with varying time length and frequency by comparing copy number detection in REPLACE™ matrices exposed to the same dirty bedding.

Methods | Two experimental groups of five replicates of REPLACE™ matrices were placed in soiled corn cob bedding collected from mice naturally infected with viruses, bacteria, and parasites. Matrices were exposed over a two-week period. **Group 1** Agitation twice per week: Bedding was agitated using an elliptical "stir-fry" motion for 15 seconds twice weekly. **Group 2** Agitation at setup and collection: Bedding was agitated using an elliptical "stir-fry" motion for 30 seconds at initial cage set up and again for 30 seconds just prior to matrix collection.

Results | This study revealed **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 (Figure 5). This is beneficial to facilities and husbandry staff as it **decreases technician SFSB cage handling in half** while **maintaining accurate pathogen detection**.

**REPLACE™ AGITATION CAN BE PERFORMED TWICE PER TWO WEEK PERIOD**

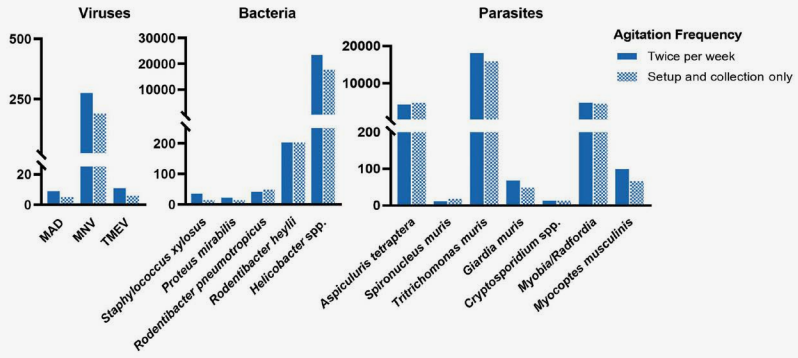



**Figure 5.** Results from a two-week study evaluating pathogen detection with different agitation frequencies. Group 1 (solid blue bar) represents REPLACE™ agitated in SFSB cage twice per week throughout the two-week exposure period. Group 2 (hashed blue bar) represents REPLACE™ agitated in SFSB cage 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.

In summary these experiments support SFSB as providing superior pathogen detection compared to SBS. The use of REPLACE™ in SFSB health monitoring maximizes pathogen detection, allowing confident elimination of the use of sentinel animals. Adding REPLACE™ to an SFSB cage is straightforward and can be applied to any caging configuration and bedding type. REPLACE™ has been thoroughly tested against a wide range of viruses, bacteria, and parasites ensuring the detectability of pathogens.

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