Reply to Manuel and Colleagues Letter to the Editor

We thank and appreciate Dr. Manuel and his colleagues for the critical review of our manuscript.[1] Environmental health monitoring (EHM) is an exciting area of ongoing research in both the assessment of success and the measurement of weaknesses. Our manuscript serves the latter in its contributions to the growing body of research evaluating EHM and sentinel-free soiled bedding (SFSB).

The existing mouse health surveillance program in this study was a modified form of the typical soiled bedding sentinel (SBS) insofar as "typical" soiled bedding sentinel fecal samples were pooled with feces from colony animals. This already represented a variation of EHM that was further evaluated in our assessment of alternative methods to replace or otherwise modify the health surveillance program. "Sentinel fecal PCR" in Table 2 and Figures 2 and 3 should have been labeled as "soiled bedding sentinel method" or "SBS program" to remain consistent throughout the manuscript and to avoid the perception that it was a traditional SBS program.

In our hybrid system, SBS samples and flocked swabs from colony mice and the SBS cage were collected and pooled at the rack level, while SFSB samples were pooled at the room level; however, all results were considered at the room level. For our program and the intent of this study, if a rack in the room tested positive for a particular agent, then the entire room was considered positive. This same ideology was applied to all test methods so results could be considered at the room level as rooms across the vivaria varied in the number of racks housed in each particular space. Future studies could evaluate these same methodologies on a similarly large scale across multiple vivaria to evaluate performance under conditions with identical rack and cage numbers, although this scenario may differ from the real-world approach that was considered in this present study.

Our initial hypothesis was based on a "no worse than" model rather than statistical analysis, with the expectation that one or more EHM options could allow the reduction or elimination of live sentinel animals. We chose to use Cohen κ statistic because it was used to model previous similar studies[4] and allowed assessment at both the room and pathogen level based on available comparable data sets. The κ is a standardized value and can be interpreted the same across multiple studies.[3] The κ statistic was averaged per test method so that a cumulative agreement among both the test method and agent could be considered at a programmatic level in evaluating alternatives to our existing health surveillance program. Additional statistical analysis with ANOVA or logistic regression may show further differences in quantitative data. Figure 3 presents data on a percentage basis, with no statistical interpretation, and further supports the conclusions herein.

As acknowledged, it was unfortunate that complete data sets were not available. This highlighted the real-world practicalities of the alternatives we assessed and, on a programmatic level, was an important consideration when evaluating these methods for the modification of our health surveillance program. Facility C, with 18 rooms and 69 racks, was the only facility with a complete data set. Facility A, with 42 rooms and 115 racks, had a complete set except for swabs of the unoccupied soiled bedding cages. We could have evaluated these 2 facilities and omitted this swab technique to produce 2 full data sets for analysis; however, this again skirts compliance at a programmatic level, which was a core consideration of our study.

The use of EHM represents a significant shift in health surveillance monitoring techniques, and the active area of associated research shows both promising and proven results in carefully controlled settings.[2] In addition to evaluating the current literature, health surveillance programs should also assess methods for the replacement of traditional SBS within the context of their own vivaria as variations in engineering standards and facility practices may influence the success of various institutional murine animal health surveillance programs. In our assessment of our vivaria, we determined the existing SBS-EHB hybrid program provided the most reliable results. Our initial hypothesis was not met as results from total EHM options were less effective than our current hybrid system. Our data did show trends supporting a shift toward full EHM via colony animal sample collection for the specific IVC rack systems used throughout our vivaria, and this method could be evaluated further in future studies. We feel that sharing our experience is important because when institutions consider revisions to rodent health monitoring, training and consistency are important factors.

Sincerely,

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