Translating Best Practices From **Cleanroom Manufacturing**

Environmental Monitoring of IVC Production Rooms to Evaluate Risk of Bacterial Contamination

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ABSTRACT

Cleanrooms are facilities designed to maintain low particulate levels commonly implemented within the pharmaceutical or computer chip manufacturing industries. Cleanroom techniques may also have application to specific opportunist and pathogen free (SOPF) laboratory rodents. Many of our rodent production rooms use individually ventilated cages (IVCs) to provide biosecurity, though other measures including personal protective equipment (PPE) and high-efficiency particulate air (HEPA) filtration are implemented within the animal rooms to minimize contamination. Environmental monitoring programs survey microorganisms and particles within the environment, providing data to supplement animal health monitoring programs. We hypothesized that the environmental monitoring of our production rooms could serve the following purposes:

- Evaluate how well our PPE and processes restrict entry of microorganisms into the production room
- Assess the risk for contamination of IVC cages from excluded organisms
- Identify process breaks such as non-compliance with gowning or cleaning procedures.

We instituted an environmental monitoring program which consists of monthly room air sampling using a microbial air sampler known as the EMTEK P100. Air samples are cultured, and bacteria are identified to the species level. Results from 6-12 months of monitoring indicated that our IVC production rooms have low levels of bacterial contaminants, supporting the conclusion that our PPE and processes are effective. Environmental monitoring is useful in evaluating the risks of IVC cage contamination, as shown in correlations between our bacterial findings within the IVC cage and the room air samples. This data helps drive decisions for sample numbers in conventional animal health monitoring based on environmental risk and may be used in a variety of facilities. Within a conventional animal facility, it can provide feedback on process compliance or contamination of excluded agents, while in germ-free facilities monitoring aids in the understanding of isolator contamination and decisions towards cleaning procedures. Environmental monitoring is a relatively easy and inexpensive technique with benefits for rodent production.

MATERIALS & METHODS

Sample Collection

Air samples were collected for 5 minutes at 100 liters/minute using the EMTEK P100 (EMTEK, LLC.; Longmont, CO) as per room map (Figure 3). Samples were collected onto TSA-B agar plates (Northeast Laboratory; Waterville, ME). Surface samples were collected as per room map onto RODAC plates (Becton, Dickinson and Company; Franklin Lakes, NJ).

Culture and Identification

TSA-B and RODAC plates were incubated at 37°C for 48 hours. All colonies were counted and identified to the fullest extent possible. Identification was performed using MALDI-TOF.

Figure 1 Taconic Health Standards

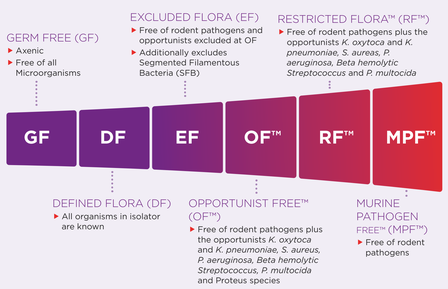


Figure 2 Standard PPE for Excluded Flora IVC locations

Figure 3 Sample location map

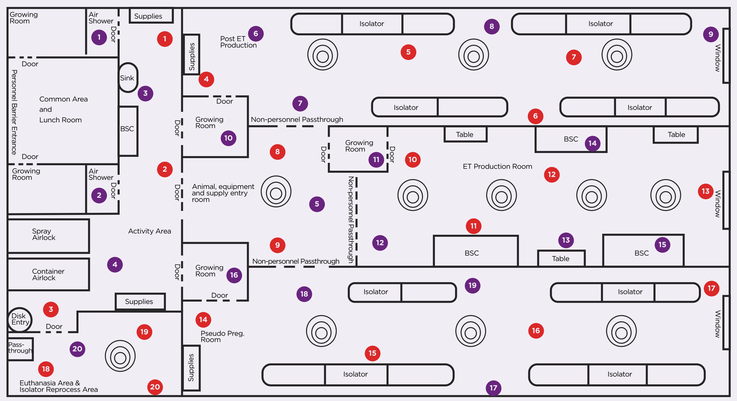
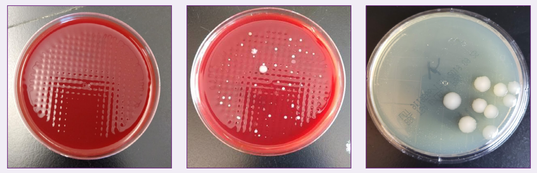


Figure 4

A. EMTEK user interface

B. EMTEK on tripod during sampling session

Figure 5 Culture Plates



A. Impingement zone on TSA-B plate

B. Bacterial growth on impingement zone of TSA-B plate

C. Surface sample with growth on RODAC plate

RESULTS

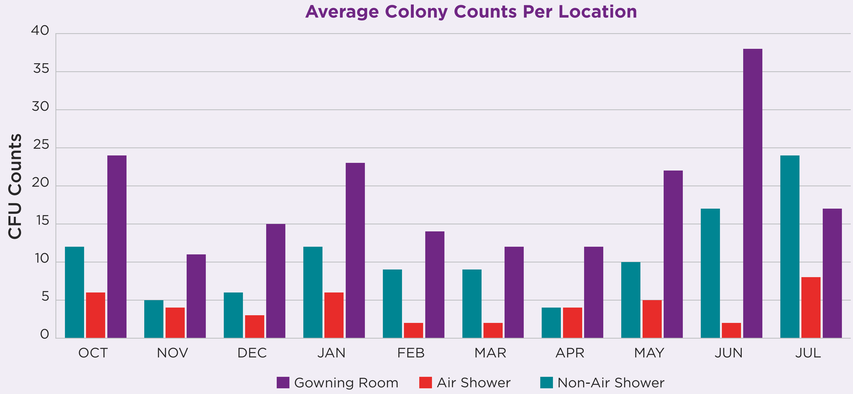
Taconic Biosciences provides animals to researchers at various health standards to meet scientific needs (Figure 1). To meet these health standards, animals are raised in various types of housing ranging from open-top caging to IVCs and isolators. For this study, we focused on our Excluded Flora (EF) health standard in which animals are housed in IVCs inside of HEPA-filtered barriers. This particular health standard excludes many bacterial opportunists which are commonly found on human skin, therefore biosecurity measures are extremely important. In addition to exclusive use of autoclaved supplies, all caretakers wear extensive PPE including a type of Powered Air Purifying Respirator (PAPR)(Figure 2) to minimize the risk of contaminating the animals.

While all husbandry procedures follow detailed processes, it is still difficult to ascertain how effective the air filtration, cleaning procedures, biosecurity measures, and PPE really are at reducing the bioburden in the barrier. To examine this, we borrowed techniques from cleanroom facilities and instituted an environmental monitoring program for our IVC barriers.

We created sampling maps that covered active and passive areas of the barriers (Figure 3) and began monitoring EF locations using both air sampling (Figures 4 and 5) and surface sampling to assess bioburden. For all locations, all colonies are counted and identified using MALDI-TOF. Samples with less than 30 colony forming units (cfu) are considered “passing” while anything over 30 cfu is considered a “fail”. Samples that fail require recleaning of the area and resampling. In addition, if any excluded bacteria are identified, the area must be recleaned and resampled.

Generally, we see very low cfu counts (<10 cfu) in our EF barriers (Figure 6) and have only found an excluded organism in two samples during the time we have been monitoring. Notably, we observe a difference in bioburden between locations with and without air showers. We use these results to make data-driven decisions about sample numbers for routine barrier monitoring and to risk assess procedural changes.

Figure 6 Air sampling results over time for a barrier with an air shower, a barrier without an air shower, and a typical non-HEPA filtered gowning location.



SUMMARY

- Environmental monitoring can be used as a tool to assess barrier processes and procedures
- Barriers with air showers consistently have lower bioburden than barriers without air showers
- Environmental monitoring can provide information about the effectiveness of facility HEPA/HVAC systems

Reference: EMTEK.P100.001v01 User Manual (First Edition). October 2013.