Replacement of rodents with PCR for infectious agent screening

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Abstract
In addition to rodents used for research, additional rodents are commonly used to monitor biological materials or study rodents for infectious agents. Virus PCR assay panels have replaced the majority of rodent use for antibody production tests. To accommodate large numbers of virus PCR panel submissions and assays, we integrated a high-density qPCR array and thereafter used this same platform to develop a PCR rodent infectious agent (PRIA) array to screen production barrier rooms and isolators for reportable infectious agents. Subsequently, we investigated PRIA as a quarantine alternative to sentinels by using pet shop mice to simulate a quarantine scenario. CD-1 contact and bedding sentinels were screened by traditional methods and PRIA at 4, 8, and 12 wks (n=3 mice/time point) post exposure. Fecal pellets, fur/perianal hair swabs, and oral swabs were pooled at day 3, 4, 5, 6, 7 and 10 for each of four pet shop mice in these cages and analyzed by PRIA. Of the agents present in the pet shop mice, *M. pulmonis*, *P. pneumotropica*, and *Giardia* were not detected in contact sentinels. Additionally in bedding sentinels, *Spironucleus*, *Cryptosporidium*, and adenovirus were not detected and fur mites and *Helicobacter* were poorly detected. PRIA detected all agents at early time points. These results demonstrate that the direct testing of quarantined rodents by PCR can replace sentinel rodents, improve detection, and reduce the quarantine time. Preliminary data also demonstrated that infectious agents can be detected in exhaust air samples from ventilated racks suggesting that PCR could potentially be used to reduce bedding sentinel use for routine health monitoring.

Introduction
Mice and rats are used for biological research when in vitro research models can not be used to investigate a hypothesis. In addition to the mice and rats used for research, a second set of mice and rats, denoted as sentinels, are used only for the purpose of monitoring for the presence of infectious agents. A good example of the successful elimination of sentinel rodent use is the replacement of an adenovirus production testing by PCR for the detection of rodent viruses in research biologics (Figure 1). The antibody production test commonly uses 10-12 rodents that are inoculated or sham-inoculated with test material. Serological testing of rodents for virus antibodies is performed 8-10 weeks later to determine the presence of viruses in the test specimen. PCR became the method of choice because it detects less than an infectious dose, takes only a week to complete, and replaces the need for rodent use (Figure 1).

To accommodate a large number of TaqMan Real-time PCR assays for testing research biologics we integrated the use of a high-density real-time PCR array (Figure 1), which is the equivalent of using 28 96-well PCR plates. Using this same platform, we then developed the PRIA panel of commonly reported or excluded rodent pathogens and investigated alternatives to contact and bedding sentinel use as described in the abstract. Additionally, we initiated limited feasibility testing to determine the potential of using air exhaust samples in place of sentinels.

Conclusion
PCR methods have already and continue to eliminate rodent use for health monitoring by improving detection and reducing time and labor. Air Exhaust testing has the potential to replace some or all routine health monitoring that has traditionally relied on sentinel use.