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Confirmation of Pathogen 'Burnout' in Mouse Colonies with Previous Evidence of Infection with Parvovirus and Rotavirus

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Pathogen monitoring and colony health management are critical components of any rodent research program. From an operational perspective, rodent facilities are protected from unwanted infectious agents by facility-specific bioexclusion criteria, sanitation of the physical environment, and personal protective equipment. Another important preventative measure is the use of room health levels to provide traffic patterns for animal care and research staff as they move between rooms of differing health status. For mice, our institution uses a tiered room level system with 6 defined categories, ranging from level 1 (strictest entry criteria) to 6 (least stringent entry criteria). Level 6 is defined as rooms with mice that have tested positive for mouse parvovirus (MPV) or mouse rotavirus (MRV) or both on sentinel serology at any point in time in the past and no decontamination. Because many of our mouse rooms had historically been positive for MPV and/or MRV and because of the high financial and logistic challenges of using repeated test-and-cull for elimination, we had tolerated the potential presence of MPV and MRV and had developed management practices that would promote 'burnout' (that is, elimination of infectious agents due to absence of susceptible hosts) of these pathogens. Analysis of sentinel data showed that we had 28 rooms in 4 facilities for which excluded pathogens had not been identified in 3 y or more. We therefore developed a hybrid testing strategy involving both PCR analysis and serology and implemented it in sentinels and in select colony mice to determine whether the rooms had undergone successful burnout and were free of MPV and MRV. All test results obtained during the assessment were negative for both viruses, and the rooms were subsequently upgraded to level 5 (free from excluded pathogens and allowing two-way movement in and out of housing room). All upgraded rooms have remained negative on subsequent quarterly routine sentinel serology for over 3 y. Our testing strategy for confirming pathogen burnout may be a useful and cost-efficient model for other academic rodent research programs that face a similar situation.

Abbreviations: MPV, mouse parvovirus; MRV, mouse rotavirus

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Introduction

The presence of pathogens in rodent research colonies is problematic for both researchers and animal care personnel. Infections with mouse parvovirus (MPV) and mouse rotavirus (MRV) can be particularly problematic for studies of the immune system, as well as other research using immunocompromised mouse strains or neonates.[4,6,8,29] In addition, the presence of pathogens in the facility often hinders sharing of mice between laboratories and collaborators at other institutions. Even when a room itself does not have a history of the presence of pathogens, many institutions will not accept mice that are housed in facilities where MPV or MRV has been documented and staff and resources are shared. This practice has been problematic for many investigators and was a driving force behind our effort to determine the pathogen status of rooms with a negative sentinel health history.

MPV and MRV are among the 4 most common viral infectious agents of research mice in North America, with reported incidences of 1.8% and 0.6%, respectively.[23] Although these viruses typically cause little mortality,[11,19] their presence is highly disruptive in research settings where they are considered excluded. Specifically, MPV can cause aberrant T-cell responses and function, including accelerated rejection of xenografts, allografts, and syngeneic grafts.[19] MRV can severely disrupt studies involving neonatal or infant mice, the gastrointestinal system, or the immune system.[11] These viruses may be associated with considerable monetary costs in terms of PPE, decontamination, and per diems, in addition to the lost efficiency and opportunities for publication and funding.[25] Both agents are shed through the feces,[7] are stable in the environment (permitting fomite transmission),[4,8] and are more likely to cause disease and productive infection in young and immunocompromised animals.[4] Of particular concern is the potential for persistent shedding by MPV-infected mice, in contrast to MRV, which shows some evidence for prolonged shedding[11] but not for a carrier status in which reactivation of viral shedding can occur.[28] In addition, intermittent and low-level viral shedding can cause the pathogens to go undetected in a dirty-bedding sentinel health-monitoring regimen due to inefficient or sporadic seroconversion.[5] Detection of either MPV or MRV typically triggers elimination strategies, including test-and-cull, decontamination, rederivation, and breeding moratoriums, concurrent with efforts to identify the source of the infection.[14,18,30]

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MPV in particular has been the subject of study regarding differential susceptibility among mouse strains, best testing modality for determining true infection in a mouse colony, and means of colony-wide eradication. In comparison with MRV, more variables appear to affect the epidemiologic triad (that is, the pathologic agent, the susceptible host, and their shared environment that supports transmission from source to host) of MPV. In terms of the host, C57BL/6 strains tend to be least susceptible to MPV, with BALB/c and C3H being intermediately susceptible, and ICR and Swiss Webster being the most susceptible immunocompetent strains.[4,6,18,19] This variability creates a particular challenge in transgenic colonies, which are largely B6-based, because the relative disease resistance of the B6 background must be weighed against the functional immune status created through genetic modification; as such, transgenic mice are considered to have a higher potential for prolonged viral shedding.[11] Age also plays a role in susceptibility to MPV: younger mice are considered more highly susceptible to infection,[4] and seroconversion after MPV challenge is diminished if exposure occurs after 8 wk of age.[6] Individual differences in seroconversion have also been documented.[5] Although fecal viral shedding at a magnitude that facilitates efficient transmission is believed to occur for only 2 to 4 wk after infection,[28] infectious virus has been recovered from mesenteric lymph nodes at 6 wk after infection, and viral DNA has been detected in lymphoid tissues for as long as 9 wk after infection in immunocompetent mice, with the potential for shedding extending to 24 wk in immunocompetent mice[19] and beyond 25 wk in SCID mice.[4] Considerations regarding the agent and environment come into play in the dirty-bedding sentinel context. Mice exposed to multiple doses of soiled bedding from a colony with 5% MPV incidence did not seroconvert.[7] When mice are chronically exposed to low doses of MPV, they tend not to seroconvert consistently.[5,18] Dilution and the age of the soiled bedding[5,7] as well the interval after MPV infection of the mice providing soiled bedding[19] all play a role. In addition, various husbandry measures, including the provision of irradiated feed, have been associated with reducing MPV infection.[25]

Infection elimination response plans that do not involve large-scale depopulation or rederivation have been laid out previously,[15] with both modified breeding moratoriums[11] and a targeted sentinel approach[18] reportedly used successfully for MRV and MPV, respectively. In each of these circumstances, at least 3 consecutive rounds of negative serology were required to deem the infection cleared. The challenge lies in the fact that to determine a testing strategy, the prevalence of the agent must be known or approximated. When the estimated prevalence is 10%, representing the lowest threshold for the industry, 20 to 30 mice must be tested to achieve 95% confidence for detecting infection in a colony of at least 100 mice.[19] The reality is that, in modern mouse colonies where biocontainment practices are rigorous, an enzootically infected colony may have a prevalence of 1% or less,[19] driving the sample size—and manpower and cost of testing—much higher.

Due to the large number of rooms that were historically positive for MPV and MRV at our institution and the potential financial and logistical problems with using a repeated test-and-cull method of elimination, we had tolerated the potential presence of MPV and MRV, and had implemented practices that promote 'burnout' of these pathogens (that is, pathogen elimination due to absence of susceptible hosts). For the current project, we assumed a low disease prevalence and hypothesized that a hybrid testing strategy involving both PCR and serology in targeted colony mouse sampling and enhanced sentinel testing would successfully detect or confirm burnout of MPV and MRV infection with lower cost and veterinary technician effort as compared with traditional elimination testing strategies. Throughout the second quarter of 2018, all targeted colony and enhanced sentinel testing results were negative, and the health levels of all 28 affected rooms were subsequently upgraded to MPV- and MRV-free health status. Routine sentinel serology from these areas has remained negative for more than 3 y after the cleanup initiative.

Materials and Methods

Vanderbilt University Medical Center. The Vanderbilt University Medical Center Animal Care and Use Program supports the biomedical research and teaching programs at the Vanderbilt University Medical Center, Vanderbilt University, and the Veterans Affairs–Tennessee Valley Healthcare System. Vanderbilt has been accredited by AAALAC International since 1967. All animal use and accompanying procedures were in accordance with the *Guide for the Care and Use of Laboratory Animals*[13] and approved by the Vanderbilt IACUC.

Animal housing and husbandry. Mice are housed in IVC (Lab Products LPI Super Mouse 750) with automatic watering or water bottles and either corncob (Enrich-o'Cobs, The Andersons, Maumee, OH) or cellulose paper (AlphaDri Plus, Shepherd Specialty Papers, Watertown, TN) bedding. Cages are changed every 2 wk, and ventilated racks are sanitized every 6 mo. Cage changes and the manipulation of mice in housing rooms are performed in laminar flow cage change stations or biosafety cabinets by using microisolation technique and the disinfectant MB10 (100 ppm; Quip Laboratories, Wilmington, DE). Mice receive unrestricted access to irradiated PicoLab Laboratory Rodent Diet 5L0D or Advanced Protocol PicoLab High Energy Mouse Diet 5LJ5 (LabDiet, St Louis, MO) and reverse-osmosis–purified or filtered and acidified water. Mice are maintained on a 12:12-h light:dark cycle via recessed fluorescent lights (150 to 400 lux), and temperature (68 to 79 °F [20 to 26.1 °C]) and humidity (30 to 70%) are controlled in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals*.[13]

Approximately 25,000 cages of mice are housed in 100 different rooms in 10 facilities on campus. The population is composed of both immunocompetent and immunocompromised mouse strains. Husbandry technicians, veterinary technicians, and veterinarians are assigned to multiple facilities, and researchers may have mice housed in multiple rooms and facilities. Anyone entering rodent housing areas is required to respect room entry order, working from 'cleanest' (most restrictive, level 1) to 'dirtiest' (least restrictive, level 6), using the designated PPE. For levels 1 through 4, required PPE includes disposable caps, gowns, masks, gloves, and shoe covers; PPE for levels 5 and 6 comprises disposable gowns and gloves. Mice may not return to rooms at levels 1 through 4 once they leave the room (that is, one-way traffic) but may leave the facility for procedures in laboratories and other shared resources and return to rooms at level 5 or 6 (that is, 2-way traffic). Housing room levels are assigned according to sentinel testing history and traffic flow (Figure 1).

Sentinel program. The Division of Animal Care monitors all mouse housing rooms for excluded pathogens by using a dirty-bedding sentinel program. The targeted agents are: mouse hepatitis virus, MPV, minute virus of mice, mouse adenovirus, lymphocytic choriomeningitis virus, Sendai virus, pneumonia virus of mice, epizootic diarrhea of infant mice (MRV), Theiler mouse encephalomyelitis virus, ectromelia virus, reovirus, *Mycoplasma pulmonis*, pinworms, and fur mites. A sentinel cage

Room health level	Movement of mice from room	Room health status
1	One-way	SPF; free from excluded pathogens; negative for MNV and *Helicobacter* spp.
2	One-way	Free from excluded pathogens; negative for MNV
3	One-way	Free from excluded pathogens; negative for *Helicobacter* spp.
4	One-way	Free from excluded pathogens
5	Two-way	Free from excluded pathogens
6	Two-way	MPV or MRV or both pathogens historically identified on sentinel testing

Figure 1. Definitions of health levels for mouse rooms. Room entry order is from most restrictive ('cleanest,' level 1) to least restrictive ('dirtiest,' level 6). Health levels are assigned according to colony health monitoring and traffic flow. Rooms with one-way traffic (mice cannot return when they are removed from the room) are entered prior to rooms that allow 2-way traffic (mice may leave the room and return).

is placed on each rack side and houses 2 or 3 ICR (CD1) female mice. Sentinel mice are ordered from Charles River Laboratories (Wilmington, MA) and placed in sentinel cages at 4 to 6 wk of age with 1 or 2 older females. Mice are identified by using ear punches, which are linked to a unique barcode identifier on the cage cards that is tracked in an electronic database. At cage change every 2 wk, approximately 1 teaspoonful of dirty bedding from each colony cage (maximum of 70 cages per rack side) is collected and placed in the sentinel cage. Sentinel mice are euthanized and tested quarterly for excluded pathogens. Replacement mice are placed on a rolling basis to ensure approximately 3 mo contact time between the newly placed and older sentinel mice, for a total of approximately 6 mo spent in contact with soiled bedding before testing.

Quarterly, the oldest sentinel is removed from the cage and submitted for comprehensive testing. Each mouse undergoes gross necropsy, blood collection for serology, cecal wash, fecal floatation, anal tape testing, and fur plucks. Blood is sent to an outside diagnostic lab (Charles River Research Animal Diagnostic Services, Wilmington, MA) for serologic assays (multiplexed fluorometric immunoassays). Parasitology testing (fecal float, cecal wash, pelt exam, and tape test) is conducted inhouse. A positive test for an excluded agent on multiplexed fluorometric immunoassay automatically triggers single-agent retesting of the same sample via immunofluorescence analysis to confirm the result. All results are recorded in an electronic database; 2 veterinarians are assigned to monitor health summarize and deliver results to facility managers, veterinarians, and research staff through quarterly meetings and letters.

Facility controls to reach burnout. To reach the goal of eventual burnout of unwanted pathogens in our facilities, several controls were put in place over a 10-y period to minimize pathogen spread and decrease the prevalence of MPV and MRV in our facilities. In an effort to maintain valuable genetically manipulated and immunocompromised mice, a SPF mouse facility was created. This provision allowed many researchers to establish and maintain a clean breeding colony via rederivation or approved-vendor purchases and to house experimental mice in rooms outside of that barrier. Around the same time that the barrier facility opened, the health-level–associated room entry order was established to ensure that animal care staff and researchers work in mouse rooms in a manner that prevents cross-contamination between health levels. In addition, we transitioned our PPE from individual-owned cloth lab coats to disposable gowns, thus ensuring that only clean PPE that had not been exposed to other rodent areas was used in the animal facility.[16] The mouse chow provided in the animal facilities was changed to an irradiated version of the existing diet, which has been associated with a reduction in MPV in mouse colonies.[1] Finally, training of animal care and research staff has focused on handling mice only in biosafety cabinets and laminar flow hoods while using careful microisolation technique to minimize pathogen spread within animal rooms. The combination of these practices helped reduce the prevalence of pathogens and isolated them to specific, clearly identified housing areas. This decreasing prevalence and eventual burnout of MPV and MRV was closely tracked via sentinel testing over the past 10 y to a point where upgrading the health status of the level 6 rooms appeared possible. Given the large number of level 6 rooms (28) that had no positive sentinel results for MPV and MRV, we sought to determine a testing methodology that could feasibly be used to confirm the burnout of these pathogens in a cost-effective manner.

Health level upgrade plan. Veterinarians responsible for health-monitoring reviewed our electronic sentinel health record database. They collated a list of level 6 rooms in which no excluded pathogens had been identified on sentinel testing in 5 or more consecutive years (Table 1) or in which no excluded pathogens had been identified on sentinel testing in 3 or more consecutive years (Table 2). These rooms were considered eligible for upgrade to health level 5.

As previously described for other health-monitoring programs, we performed a cost analysis to compare the potential financial and work burdens of the proposed hybrid testing strategy to those of the traditional testing strategy used to eliminate MPV and MRV from rooms at our institution.[17] This traditional testing methodology includes serologically testing a single mouse from each colony cage. This testing is performed 3 times at monthly intervals until 3 consecutive rounds of negative testing are obtained.[18] The hybrid testing strategy requires fecal PCR testing of 20% of the colony mice, with concurrent PCR testing of the associated sentinel mice. Routine quarterly sentinel serology testing continues during both the traditional and hybrid testing processes. Costs for testing were based on current facility discounted costs for single-agent serology and PCR testing (Charles River Research Diagnostic Services, Wilmington, MA). The average technician time needed to collect each fecal sample for the hybrid testing method and each blood sample via the submandibular vein was estimated at 2 min per cage. The cost of technician time was calculated by using the average veterinary technician annual salary at our institution. Routine quarterly sentinel serology was not included in the cost analysis, because this cost is the same for both strategies. Collection of the spleen from sentinels was not included in the calculation of technician effort because this activity took place during the routine necropsy of sentinels during quarterly testing in the diagnostic lab. However, the cost of the PCR testing of sentinel mice was included because it is an addition to routine quarterly sentinel testing.

We began with testing in rooms with a 5-year history (or more) of being negative for excluded pathogens and then proceeded to the rooms with at least a 3-year history of no excluded pathogens (Figure 2). Testing took place during the second and third quarters of 2018 (January through June), in light of the reported possible seasonality of MPV infection.[25] Investigators with mice in these rooms were notified of upcoming testing so that any unneeded cages could be removed or consolidated. Based on an expected low disease prevalence, the threshold of colony

Table 1. Rooms with at least 5 consecutive years of no excluded pathogens identified on sentinel testing

Facility and room[a]	Cage census at time of testing	Days elapsed between sentinel testing and colony testing	Cumulative health history prior to 5-y mark	Proportion of positive sentinels at last positive test
A1	381	8	MPV	Not available
A2	326	3	MPV	2 of 12
A3	373	3	MPV	13 of 43
A4	663	11	MPV and MRV	1 of 5; 1of 10
A5	300	13	MPV	5 of 8
B1	677	40	MPV	23 of 60
B2	491	20	MPV and MRV	18 of 60; 3 of 8
B3	615	10	MPV	12 of 57
B4	304	1	MPV	4 of 16
B5	276	10	MPV	19 of 31
C1	102	8	MPV	3 of 4
C2	253	1	MPV	1 of 3
C3	660	11	MPV	1 of 6

MPV, mouse parvovirus; MRV, mouse rotavirus
[a]Uppercase letters indicate facilities; numbers indicate rooms
[b]Data are given as no. of sentinels that tested positive/total no. of sentinels tested

Table 2. Rooms with at least 3 consecutive years of no excluded pathogens identified on sentinel testing

Facility and room	Cage census at time of testing	Days elapsed between sentinel testing and colony testing	Health history at 3-y mark	Proportion of positive sentinels at last positive test
A6	112	2	MPV	1 of 4
A7	356	3	MRV	4 of 10
A8	71	5	MPV and MRV	2 of 6; 3 of 6
A9	389	9	MRV	1 of 8
A10	148	4	MPV	1 of 4
A11	585	6	MRV	2 of 14
A12	414	4	MRV	2 of 12
A13	488	7	MRV	1 of 11
A14	768	10	MPV	1 of 12
A15	163	9	MRV	1 of 7
C4	306	13	MPV	1 of 6
C5	339	5	MPV and MRV	1 of 7; 1 of 7
C6	477	3	MRV	1 of 7
C7	288	3	MRV	1 of 6
C8	228	10	MPV	1 of 5

MPV, mouse parvovirus; MRV, mouse rotavirus
[a]Uppercase letters indicate facilities; numbers indicate rooms
[b]Data are given as no. of sentinels that tested positive/total no. of sentinels tested

animal testing was set at 20% of the cage census. Room cage census numbers were determined and, from these, the number of pooled fecal PCR samples to be collected from each room ($n = 0.2 \times$ cage number/10) was calculated. A map of each room was created, and every cage was uniquely labeled as fecal PCR samples were collected so that cages could be linked to pooled sample numbers. Cages containing young mice, including breeding cages, were targeted preferentially due to their increased susceptibility to MPV infection.[4,6] Fresh fecal samples from colony mice were collected into nuclease-free microfuge tubes and shipped to an outside diagnostic lab that limits pooling of fecal pellets for PCR testing to 10 pellets per tube. A single fecal pellet was obtained from each selected cage and pooled by rack (up to 10 fecal pellets per PCR sample). Cages were marked and their identification and location documented for tracking purposes. No mouse or cage movement was permitted between the collection of fecal PCR samples and release of the results. Concurrently, routine sentinel testing was augmented by splenic PCR analysis in which both MPV and MRV tests were run at the same diagnostic laboratory and half of each spleen was retained at −80 °C in until the final testing results were obtained. Spleen was selected because it previously had been used for PCR detection of MPV,[12,24] is easily identified by technicians collecting samples, and is large enough to freeze a portion of the tissue for confirmation testing, if needed. The colony and sentinel testing were conducted within the same quarter at an average of 11 d apart (range, 1 to 40 d) for the 5-y group (Table 1) and 6 d apart (range, 2 to 13 d) for the 3-y group (Table 2). Immediately after completion of colony and sentinel testing, brightly colored health level upgrade signage was placed on the room's entry door, and an email was sent to appropriate research staff communicating the change from level 6 to level 5. This plan was reviewed and approved by Vanderbilt's Small Animal Advisory Committee prior to deployment.

Results

Costs of the traditional elimination strategy. The total cost for 3 rounds of MPV and MRV serology for one mouse from each of the 10,877 colony cages in the 28 level 6 housing rooms was USD $247,560.52 (Table 3).

Collection of blood from a single mouse via the submandibular vein from each colony cage was estimated at 2 min per cage. Based on this estimate, 1088 h of veterinary technician time would be required to collect samples from all 10,877 colony cages eligible for health level upgrade, totaling USD $30,140.17 (Table 3).

The total costs of diagnostic testing and technician time were added to calculate the total cost of performing the traditional elimination testing strategy for all 28 eligible rooms: US $277,700.69 (Table 3).

Costs of the PCR-based hybrid strategy. The total cost for PCR for MPV and MRV for 221 pooled colony mouse fecal samples and 229 splenic samples from sentinel mice in the rooms eligible for upgrade was USD $39,312.00 (Table 3).

Collection of a fecal sample from a single mouse in each cage of 20% of the colony cages was estimated at 2 min per cage. Based on this estimate, 70 h of technician time would be required to collect samples from 2107 colony cages in the rooms eligible for health level upgrade. The cost for that veterinary technician effort was USD $1946.17 (Table 3).

The total costs of diagnostic testing and veterinary technician time were added to calculate the total cost of performing the PCR-based hybrid testing strategy for all 28 eligible rooms: USD $41,258.17 (Table 3).

Cost and effort comparison of the traditional elimination strategy with the PCR-based hybrid strategy. The cost of using the PCR-based hybrid of targeted colony and sentinel mice was $236,442.52 less than the traditional strategy, equivalent to a cost savings of 85%. The technician effort required to collect the samples for the PCR-based hybrid strategy was 1017 h less than the traditional elimination strategy. These savings in cost and effort, combined with the low risk of MPV and MRV positivity associated with the long history of negative sentinel results for these rooms, drove the decision to proceed with the hybrid testing strategy.

Implementation and results of the PCR-based hybrid testing strategy. Samples from sentinel mice and 20% of colony mice were collected and submitted for PCR analysis during the same quarter of 2018 (Figure 2). Rooms with a 5-y history of negative test results for MPV and MRV were tested first (Table 1), and all results were negative for MPV and MRV. Upon completion of the rooms with at least a 5-y negative testing history, the same process was followed for rooms with a 3-y history of negative sentinel results for MPV and MRV (Table 2). Again, all PCR testing results were negative for MPV and MRV. Consequently, all 28 rooms tested were upgraded to level 5 health status, increasing the total available level 5 capacity by nearly 11,000 cages and eliminating all level 6 rooms on campus. This status has been maintained for more than 3 y, with a total of 15 rounds of negative serology for MPV and MRV in every sentinel cage of all 28 rooms that were upgraded as well as for all other mouse housing rooms on campus.

Discussion

The traditional testing strategy used to eliminate MPV and MRV from the colony is labor-intensive, and in our case, cost-prohibitive. This strategy was used initially at our facility in an attempt to exclude MPV and MRV from our mouse colonies. However, due to the number of rooms that had historically positive results for MPV or MRV, the decision was made to accept the presence of those pathogens in a subset of housing rooms. Those MPV- and MRV-positive rooms were incorporated into our traffic flow pattern as level 6 rooms, but practices were implemented to avoid introduction and prevent spread. The traditional method of testing all cages with the goal of reaching 3 consecutive rounds of negative testing before initiating a health level upgrade was reserved for rooms that allowed only one-way traffic and were intended to have a slightly higher level of biosecurity (levels 1 through 4). Burnout of MPV and MRV seemed to have been achieved 10 years after the implementation of the room entry order, opening of the barrier facility, modified PPE usage, and strict use of microisolation technique.

The hybrid testing paradigm used to confirm the negative MPV and MRV status of the colony was developed for an expected low prevalence (less than 1%) of MPV and MRV. In addition to routine testing of sentinels via serology, these mice were tested by splenic PCR analysis. This combined testing captures both high-sensitivity virus detection and antibody responses due to prior exposure.[26] To further ensure that the viruses were no longer present in these rooms, a proportion of colony mice was also tested. The colony mice were selected to ensure that every rack in the room was tested, with preference given to recently weaned and young mice, because they are more likely to be recently infected and actively shedding virus that could be detected by fecal PCR assay.[6,10] By testing 20% of the cages in each room, we exceeded the minimal number of mice necessary to detect viruses present at 10% prevalence while maintaining a sample size that was feasible from budgetary and labor standpoints.

In our mouse colony, the presumed MPV- or MRV-positive health status significantly reduced the efficiency of animal husbandry and the limited available space for housing of mice that are free of our excluded pathogens. Husbandry technicians and veterinary technicians work through rooms, from most to least restrictive heath level. This situation means that once a room considered potentially positive for excluded pathogens (level 6) is entered, cleaner rooms cannot be serviced unless the technician or veterinarian showers and changes uniforms. This requirement limits the time of day that these rooms can be entered by the staff or wastes valuable technician time before animal care duties can be resumed. Our new testing process allowed us to eliminate level 6 rooms from our mouse facilities, which has resulted in more flexibility in staffing. The detection and treatment of animal health concerns can now occur earlier in the day, which has been beneficial for veterinary and research staff and for mice.

Over the past decade, researcher awareness and demand for animal housing rooms that are free of excluded pathogens, including MPV and MRV, has increased markedly. In addition, many of these researchers use our robust shared core resources, resulting in the need to remove mice from the facility for procedures and return them to housing for longitudinal studies. Together, these factors created a bottleneck in the housing space and left the level 6 rooms underutilized while the level 5 rooms reached maximum capacity. By creating 28 additional level 5 housing rooms due to elimination of the level 6 designated rooms, we have been able to relocate colonies to allow for expansion and have more consistent utilization of all housing rooms. In addition to creating more space, the elimination of MPV and MRV from our colonies has permitted greater collaboration and sharing of valuable mouse strains both within and outside of our institution.

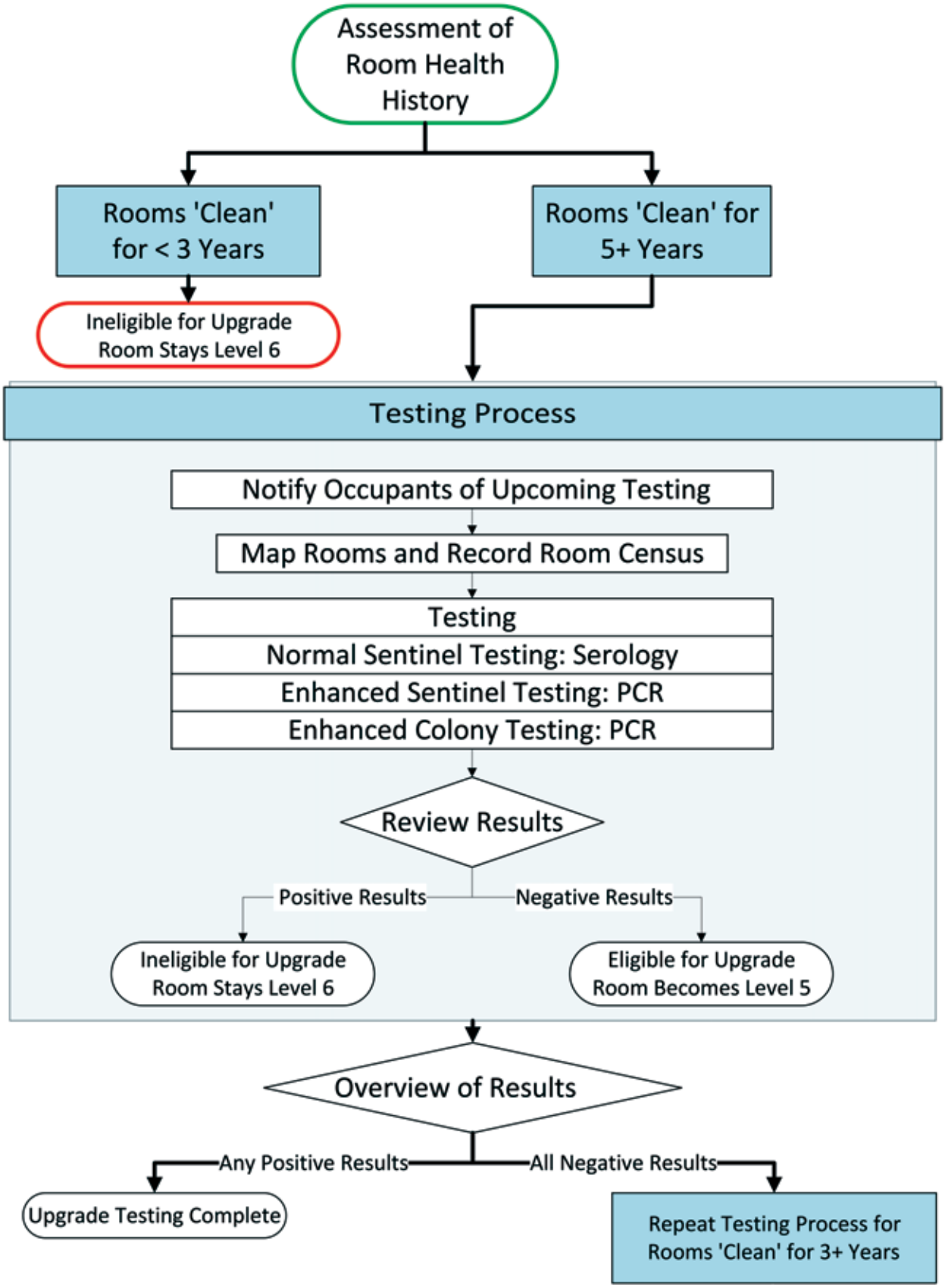

Figure 2. Plan for upgrading room health levels. This flow chart outlines the process for assessing room eligibility for upgrading, the steps in conducting the hybrid PCR testing plan, and the actions that will be taken according to test results. This plan includes contingencies for positive test results for MPV or MRV.

Table 3. Comparison of the total costs of the traditional testing and hybrid PCR testing approaches

	Traditional testing (3 serologic tests of all colony animals)	Hybrid PCR approach (Analysis of 20% of colony animals and all sentinels)
Number of samples submitted	10,877	450
Cost of diagnostic testing	$247,560.52	$39,312.00
Cost of veterinary technician time	$30,140.17	$1946.17
Total cost	$277,700.69	$41,258.17

A limitation of our strategy is the length of time required for the pathogens to burn out before confirmation testing can be performed. Although our approach clearly offers cost and labor savings, it also demands considerable resource investment in implementing controls that promote containment and eventual burnout of pathogens. This investment was feasible because ample housing space was available to segregate MPV- and MRV-positive colonies and establish new breeding colonies in cleaner rooms, allowing investigators to continue with research that might be hindered by these pathogens. Without the ability for controls and space to segregate mice, our hybrid strategy likely would not be a practical approach to eliminating pathogens. In addition, this strategy acknowledged that seroconversion of dirty-bedding sentinels exposed to low doses dirty bedding is inconsistent, perhaps due to dilution associated with sampling from a large number of colony cages in the presence of low prevalence of the pathogen in the colony.[5] However, the 3- to 5-y history of negative serology in all rooms provided some level of confidence that burnout had occurred. Another limitation of our strategy is the sample size. We assumed that the prevalence of MPV and MRV was extremely low (less than 1%), which increases the number of mice that have to be tested.[19,27] Although testing 20% of the cages would not necessarily detect the virus with 95% confidence at a very low prevalence (less than 1%), this approach was suitable for confirmation testing after 3 to 5 y of negative quarterly sentinel testing when incorporating both targeted colony cages and multiple testing modalities, and thus was more likely to accurately capture the status of the colony. We also considered that fecal PCR only detects MPV and MRV during shedding and therefore could miss chronically infected mice. Serology of the same colony mice could be used in conjunction with fecal PCR analysis of colony mice for more comprehensive testing. The additional cost and labor associated with the blood sample collection and serologic testing likely would have made this project unfeasible at our institution. To address the concern regarding fecal shedding, we targeted young colony mice to increase the likelihood of capturing active shedding.[2] Finally, we acknowledge that our study was not validated by statistical analysis of our results. However, the 85% savings in testing and labor cost of using the hybrid testing paradigm compared with the traditional approach to confirm burnout of MPV and MRV and eliminate the level 6 rooms from our campus demonstrates that the hybrid approach can be an efficient way to improve colony health status.

We considered the use of environmental testing by performing PCR analysis of ventilated rack plenum exhaust air test filters as either an alternative or adjunct to our testing paradigm. This strategy has been demonstrated to effectively detect many common mouse pathogens at the rack level without the need to test individual animals or cages.[20,22] However, this method of sampling is only effective in ventilated racks with unfiltered exhaust air flow, and the ventilated racks in all of the level 6 housing rooms included an inline filter between the cage and the exhaust.[3] Although a sample of the filter-top paper from the sentinel cages could be used as an alternative, we determined that noninvasive sample collection from colony mice would be more time efficient and provide more direct data from young mice that were most likely to be infected.[9,21] All new ventilated racks that are purchased at our institution are designed with unfiltered exhaust air flow with the intent that we will eventually transition to a completely PCR-based exhaust air dust colony health monitoring program. If excluded mouse pathogens are detected in housing rooms that use this ventilated rack style, PCR analysis of exhaust air dust is used in the elimination strategy.

The presence of mouse pathogens in a research colony can significantly diminish the health of the mice and the validity of their data. Negative sentinel serology results for viruses that were previously present at an extremely low incidence without the initiation of decontamination can be problematic. Although rooms might be presumed to be truly disease-free after a protracted period of subsequent negative testing, confirming this status prior to changing practices that could compromise the health status of other mice in the colony is important. However, the presumed-positive level 6 categorization at our institution resulted in a considerable burden to our research and care staff, as well as increased pressure on our level 5 housing rooms, which are in high demand due to their being free from excluded pathogens and still allowing 2-way traffic. Our hybrid testing strategy for confirming burnout of MPV and MRV allowed us to upgrade the status of nearly 11,000 mouse cages relatively rapidly and at considerable savings in cost and labor as compared with our traditional pathogen elimination strategy. Furthermore, all upgraded rooms have remained negative for MPV and MRV on subsequent quarterly routine sentinel serology for over 3 y. Our hybrid testing strategy may be a useful model for other large academic rodent research programs to confirm burnout of adventitious infectious agents and upgrade the health status of their rodent colonies.

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