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Detection of Lactate Dehydrogenase Elevating Virus in a Mouse Vivarium Using an Exhaust Air Dust Health Monitoring Program

Kerith R Luchins,[1,2,*] Darya Mailhiot,[1] Betty R Theriault,[1,2] and George P Langan[1,2]

Lactate dehydrogenase elevating virus (LDV) continues to be one of the most common contaminants of cells and cell byproducts. As such, many institutions require that tumor cell lines, blood products, and products derived or passaged in rodent tissues are free of LDV as well as other pathogens that are on institutional exclusion lists prior to their use in rodents. LDV is difficult to detect by using a live-animal sentinel health monitoring program because the virus does not reliably pass to sentinel animals. After switching to an exhaust air dust health monitoring system, our animal resources center was able to detect a presumably long-standing LDV infection in a mouse colony. This health monitoring system uses IVC rack exhaust air dust collection media in conjunction with PCR analysis. Ultimately, the source of the contamination was identified as multiple LDV-positive patient-derived xenografts and multiple LDV-positive breeding animals. This case study is the first to demonstrate the use of environmental PCR testing as a method for detecting LDV infection in a mouse vivarium.

Abbreviations: LDV, lactate dehydrogenase-elevating virus; NSG, nonobese diabetic SCIDγ; PDX, patient-derived xenograft; PI, principal investigator; SBS, soiled-bedding sentinels

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Lactate dehydrogenase-elevating virus (LDV), a mouse-specific enveloped arterivirus, continues to be one of the most common contaminants of cells and cell byproducts.[23] Infected mice do not usually demonstrate clinical signs; however, the virus can cause multiple immunologic research effects as it propagates in mouse macrophages.[27] Natural transmission is rare, but infection induces lifelong viremia.[27] Therefore, many IACUC, including at our institution, require that tumor cell lines, blood products, and products derived or passaged in rodent tissues are free of pathogens that are on institutional exclusion lists prior to their use in rodents. LDV is difficult to detect by using a live-animal sentinel health monitoring program because the virus does not reliably pass to sentinel animals.[21] For reliable results, testing of the sample, infected mouse, or biologic is usually required.[23]

Exhaust air dust health monitoring has recently been evaluated for its efficacy in detecting pathogens, when used as a complete replacement or an adjunct to traditional health monitoring programs.[2,3,5,6,9-12,15,16,18-20,22,29] Exhaust air dust PCR analysis was found to be effective for detecting mouse hepatitis virus,[2,3,5] mouse norovirus,[16,29] Sendai virus,[5] astrovirus,[11] *Helicobacter* spp.,[2,15,16,20] *Rodentibacter pneumotropicus* and *R. heylii* (previously *Pasteurella pneumotropica*),[2,15,16,19] pinworms,[2,10,18] fur mites,[2,9,11,18] and enteric protozoa.[2,15] After switching to an exhaust air dust health monitoring system, our animal resources center was able to detect a presumably long-standing infection of LDV in a mouse colony. Because our institution uses compatible IVC racks, we implemented the associated commercial exhaust air dust health monitoring program. This program uses an adapter specifically designed for these IVC rack exhaust plenums; the adapter captures exhaust air dust on a collection media developed in partnership with a commercial testing laboratory; the exposed collection media is then submitted to the testing laboratory for PCR analysis. Compared with soiled-bedding sentinels (SBS), this health monitoring program provides increased sensitivity for the detection of multiple pathogens.[15,16,22] This report is the first to demonstrate the use of environmental PCR analysis as a method for detection of an LDV infection in a mouse vivarium.

Case Summary

During 2017 through 2018, the University of Chicago Animal Resources Center replaced the use of live-animal SBS with Sentinel EAD PCR testing (Allentown, Allentown, NJ) for the rodent health monitoring program in the majority of vivaria. Before the initial Sentinel EAD collection media was placed in each IVC rack, the racks were sanitized as described later. In July 2018, the first quarterly PCR results for one of the vivaria revealed that 4 IVC racks were positive for LDV in 2 adjacent rooms within a suite (Table 1). There was no previous history of LDV contamination in the health monitoring program at the university, and these racks had previously been monitored by using live-animal SBS. A typical procedure for positive results at the institution involves confirmatory horizontal exhaust plenum PCR testing, as described later. This procedure has been validated for agents such as fur mites[9] and *Corynebacterium bovis*.[17] The confirmatory testing was negative for LDV on all 4 racks. At that time, the presumption was that the initial results were false positives due to the low nucleic acid copy number (Table 1), given that the confirmatory testing was negative. However, for increased vigilance, the frequency of monitoring for LDV

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[1]*Animal Resources Center and* [2]*Department of Surgery, University of Chicago, Chicago, Illinois*
[*]*Corresponding author. Email: kluchins@bsd.uchicago.edu*

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Table 1. Timeline of quarterly Sentinel EAD PCR LDV results according to rack number and location

Rack	Room	July 2018 results/ copy no.	Sanitized before collection media placed?	October 2018 results/ copy no.	Sanitized before collection media placed?	January 2019 results/ copy no.	Sanitized before collection media placed?	April 2019 results/ copy no.	Sanitized before collection media placed?	July 2019 results
1	A	Positive / 12	No	Positive / 1	No	Positive / unknown	Yes	Positive / 12	Yes	Negative
2	A	Positive / 25	No	Negative	No	Negative	No	Negative	No	Negative
3	B	Positive / 3	No	Negative	No	Negative	No	Negative	No	Negative
4	B	Positive / 25	No	Negative	No	Negative	No	Negative	No	Negative

Nucleic acid copy number of positive samples is included when available. In addition, information regarding whether the rack was sanitized before collection media placement is provided.

was increased from yearly to quarterly. New collection media was then placed in these racks without their prior sanitization.

In October 2018, the next quarterly testing revealed that rack 1 was again positive for LDV (Table 1). Racks 2 through 4 were negative and remained negative throughout this study. Confirmatory horizontal plenum PCR swab testing was performed on rack 1 for LDV and was negative. After consultation with the diagnostic laboratory, we were informed that a sample is considered to be LDV-positive for any copy number detected, given that the virus is uncommon and the assay is very specific for it. Therefore, an outbreak was presumed on rack 1 based on this information and in light of the positive results from the previous quarter. In an abundance of caution, the rack handling order was changed so that rack 1 was handled last. No additional quarantine procedures were put in place due to the limited transmission properties of LDV and the fact that, as an enveloped virus, it is easily inactivated.[21,23,27]

Because LDV is a common contaminant of mouse-derived biologic materials,[23] the 2 principal investigators (PI1 and PI2), with animals housed on rack 1 were contacted to investigate whether these mice had been inoculated with tumor cell lines, blood products, or products derived or passaged in rodent tissues. PI1 had inoculated mice on rack 1 with 5 different patient-derived xenografts (PDX). Therefore, these 5 PDX were tested for LDV via PCR analysis, and all were found to be positive (Table 2). The animals inoculated with these tumors were culled, and the use of these LDV-positive tumors ceased for PI1. In addition, rack 1 housed breeding colony animals of PI1. Even though transplacental transmission of LDV is rare,[21,27] the dams from the breeding cages were tested to ensure complete examination of all animals housed on the rack and to ensure that LDV was not endemic in the colony. Dams were tested for LDV via PCR analysis of oral swabs as recommended by the diagnostic laboratory because it is a minimally invasive procedure. All dams tested were negative for LDV (Table 2). At this point, new collection media was placed to continue testing for the other excluded pathogens in the vivarium. Because testing of all tumor lines from PI2 was not complete, the rack was not sanitized. Sanitization was delayed until all diagnostic testing results were available. Therefore, the assumption was made that the subsequent quarterly LDV test results for rack 1 would be positive.

In December 2018, PI2 confirmed that they had inoculated mice with 3 of the same PDX as PI1, so these were assumed to be positive and not tested. PI2 confirmed that they would cease the use of these PDX tumors, and none of the current cages on the rack contained animals inoculated with these tumors. In addition, PI2 had inoculated mice with one mouse-origin and 4 human-origin tumor cell lines. These cell lines were tested to ensure thorough testing of all products used on the rack, and the cell lines were negative for LDV (Table 2). In January 2019, the next quarterly LDV results were positive for rack 1, as expected. Given that all diagnostic testing was completed, rack 1 was sanitized, and new collection media was placed. In addition, all animals with LDV-positive tumor lines had been culled, and the remaining cages of breeding animals were negative for LDV, according to the results from PCR testing of oral swabs. Therefore, rack 1 was expected to be negative for LDV at the next quarterly testing.

However, in April 2019, quarterly testing again revealed a positive LDV result for rack 1. Both PI1 and PI2 confirmed they had not inoculated any mice with the LDV-positive tumor lines in the past 3 mo. The rack currently only contained PI1 breeding animals and PI2 animals inoculated with previously tested LDV-negative mouse tumor lines. At this point, the diagnostic laboratory recommended PCR testing of blood, given that both immunodeficient and immunocompetent mice are persistently viremic after infection and at a relatively high copy number.[27] Coincidentally, PI1 was planning on depopulating their breeding colony on this rack, and PI2's current studies involving mice on this rack were ending, so all animals were euthanized, and blood was collected terminally. One mouse from each cage that had been on the rack during the previous 3 mo was tested, which correlates to the timeframe that the collection media was present. The testing showed that 94% of PI1's breeding colony animals were positive for LDV (Table 2). None of PI2's experimental animals were positive (Table 2). The rack was sanitized again, and new collection media was placed. In July 2019, the quarterly LDV results for this rack were negative.

Materials and Methods

Animals and husbandry. The animals in this case were assigned to 2 individual principal investigator protocols whose research focuses on cancer. PI1 housed nonobese diabetic SCIDγ (NSG; NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ) mice on one side of the 140-cage double-sided rack (Jag 75 Micro-VENT Environmental System IVC racks, Allentown). PI2 housed athymic nude (Crl:NU(NCr)-*Foxn1*^{nu} *and Hsd:Athymic Nude- Foxn1*^{nu}) and C57Bl/6J mice on the other side of the rack. This rack was one of 155 in a vivarium that comprises 9 suites, each of which, on average, has 6 animal rooms. These racks were run at 60 air changes hourly. All mice were housed in Allentown Jag 75 Micro-Barrier (Allentown, Allentown, NJ) solid-bottom polycarbonate IVC (19.69 × 30.48 × 16.51 cm). Mice were housed on corncob bedding (1/4 in.; Teklad 7097, Envigo, Indianapolis, IN), provided ad lib. reverse-osmosis-treated water through an automatic watering system (Avidity Science [previously Edstrom Industries], Waterford, WI) and fed an irradiated diet

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Table 2. Timeline of LDV PCR testing performed on tumors and animals used on rack 1

PI	Sample type	Results received	Results before use at our institution	October–December 2018 results	April 2019 results	Final status
PI1	4 PDX tumors (3936, 4195, 4913, and 2147) from University A	4/4 negative in 2016	NA	4/4 positive	Not retested	In process of being cleared[14]
PI1	1 PDX Tumor: M1 from University B	1/1 positive in 2010 and then cleared[14]	1/1 negative in 2014	1/1 positive	Not retested	In process of being cleared[14]
PI1	NSG breeding colony mice	Initially from approved vendor	NA	9/9 negative (oral swabs)	15/16 positive (blood)	Euthanized
PI2	1 Human breast cancer cell line (MDA-MB-436) from nonprofit biologic reagent center	No testing required because not of rodent origin and not passaged in rodents	NA	1/1 negative	Not retested	NA
P12	2 Human breast cancer cell lines: BM1 derivatives from nonprofit biologic reagent center	No testing required because not of rodent origin and not passaged in rodents	NA	2/2 negative	Not retested	NA
P12	1 murine breast cancer cell line: LMB from Institute A	1/1 negative in 2016	NA	1/1 negative	Not retested	NA
PI2	Athymic nude and C57BL/6J experimental animals	From approved vendor	NA	Not tested	6/6 negative (blood)	Euthanized

(Teklad 2918, Envigo, Indianapolis, IN). Mice were provided cotton squares (NES 3600, Ancare, Bellmore, NY) or specialty paper (Enviro-dri, Shepherd Specialty Papers, Watertown, TN) for enrichment. All cages, bedding, and enrichment were autoclaved prior to use. Animal cages were changed every 14 d within a class II type A2 biosafety cabinet (NuAire, Plymouth, MN). Animal rooms were maintained on 12:12-h light:dark cycle with humidity ranging from 30% to 70% and temperatures ranging from 68 to 76 °F (20.0 to 24.4 °C), in compliance with the *Guide for the Care and Use of Laboratory Animals*.[8] The animal care staff checked the mice daily, to assure the animals were in good health and that appropriate food, water, and cage conditions were present. The Animal Resources Center is part of the AAALAC-accredited animal care and use program at the University of Chicago. All animal work was approved by the University of Chicago's IACUC.

Racks were sanitized in a cage and rack washer (Basil 9500, STERIS, Mentor, OH) as noted. This method has been validated to provide adequate sanitization of IVC racks at this institution.[16] Racks in the facilities are not sanitized before every new quarterly Sentinel EAD collection media placement, because sanitization is performed on an as-needed basis. To ensure that an appropriate sanitation temperature (180 °F [82.2 °C]) is achieved, a temperature-indicating strip (TempTape 180, Pharmacal Research Laboratories, Naugatuck, CT) is placed in the cage washer at the start of each day.

Health monitoring. Sentinel EAD collection media (Allentown) was placed into the exhaust plenum of the racks and collected and replaced quarterly for PCR testing, according to the manufacturer's instructions and as previously described.[15,16,22] Gloves were changed before inserting or removing the collection media. Horizontal exhaust plenum swabs of the IVC rack were performed for confirmatory testing after positive Sentinel EAD results. To perform this procedure, the exhaust plenum was opened, and an adhesive swab provided by the diagnostic laboratory (3-in. Double Cotton-tipped Swab, Puritan Medical Products, Guilford, ME) was used to swab each row manifold. The 10 swabs were pooled. Prior to the use of exhaust air dust testing, health monitoring at our institution was performed by using live-animal SBS, as described previously.[16] In short, every 14 d, all cages on the same rack or rack side were changed before the sentinel cage, and approximately 5 g of soiled bedding from the dirtiest area of each cage was collected by using a 5-finger pinch method and added to the new sentinel cage. Yearly, after 12 wk of exposure to dirty bedding, LDV infection status was tested through PCR analysis of feces from the sentinel animals. The diagnostic laboratory recommended against testing SBS for LDV due to its limited transmission properties[21,27] and low prevalence of the virus. However, fecal samples were already being submitted for PCR analysis of various pathogens. LDV was added to this testing panel because this virus may be present in feces.[24,26,27] Serology is not recommended for LDV due to the presence of antigen–antibody complexes.[27] Sentinel EAD collection media, horizontal plenum swabs, and feces were submitted to Charles River Research Animal Diagnostic Services (Wilmington, MA) for PCR testing.

Tumor cells. PI1 used 5 PDX tumors on rack 1. PDX tumor M1 was contaminated with LDV before use in the University of Chicago's vivarium and cleared using a previously described process.[14] Once this PDX was confirmed to be LDV-negative, it

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was inoculated in mice described in this report (Table 2). Before use, PDX tumors 3936, 4195, 4913, and 2147 were tested for pathogens on the University of Chicago's exclusion list and were negative (Table 2). These 5 PDX tumors were then maintained by subcutaneous passage in NSG mice in the same vivarium as described herein. Before 2018, the basement membrane matrix Matrigel (BD Biosciences, San Jose, CA) was used with these PDX. In 2018, this reagent was changed to Cultrex Basement Membrane Extract, PathClear (R and D Systems, Minneapolis, MN), because each batch of this compound is certified to be free of common rodent pathogens (including LDV); consequently, the University of Chicago IACUC does not require rodent pathogen testing before use. PI2 used 3 human breast cell cancer lines and one murine breast cancer line in mice on rack 1 (Table 2).

PCR testing for LDV. For PDX, the earliest passage of each tumor in the lab's tumor bank was tested. Approximately 100 mm^3 of each PDX tumor and cell line (n = 10) were placed in microcentrifuge tubes. When oral swabs were performed to test the NSG breeder dams of PI1, 9 breeding cages were present; these 9 dams were tested. When blood was collected to test one mouse from each cage that had been on the rack during the previous 3 mo, 16 NSG breeder animals of PI1 and the 6 experimental animals of PI2 were bled via terminal cardiac collection (total n = 22). Additional information regarding the number of animals in each cage and the age of the animals is unavailable. The tumor samples, oral swabs, and serum were submitted to Charles River Research Animal Diagnostic Services (Wilmington, MA) for LDV reverse-transcription real-time PCR testing. Specimen preprocessing and total nucleic acid isolation by magnetic purification were performed as previous described.[7] Briefly, tumor cells were homogenized with steel beads in lysis buffer as previously described for lung tissue, oral swabs were washed with lysis buffer as previously described for fur-perineal swabs, and serum was added to an equal volume of lysis buffer and homogenized.[7] Prior to magnetic isolation of total nucleic acid and reverse transcription, a proprietary exogenous control RNA was added to each sample for lysis and homogenized as previously described to monitor for nucleic acid recovery, functional reverse transcription and PCR inhibition.[7]

A proprietary fluorogenic 5′ endonuclease LDV TaqMan PCR assay, which targets a conserved region among all publicly available and proprietary Charles River Research Animal Diagnostic Services LDV genomic sequences, was used to analyze the reverse transcribed nucleic acid. The LDV PCR assay has been validated to detect 1 to 10 copies of target nucleic acid within a PCR reaction. Samples submitted for assessment regarding panels of multiple infectious agents were initially tested on the OpenArray PCR platform.[7] Initial positive findings were confirmed by processing retained sample from lysis through the entire TNA isolation process and tested by using LDV PCR analysis on a 96-well or 384-well PCR platform. Samples submitted to Charles River Research Animal Diagnostic Services specifically for LDV PCR assay were analyzed via the 96- or 384-well platform for initial and confirmation testing. Samples with a cycle threshold values translating to approximately 1 copy or higher for both the initial and confirmation test were interpreted as positive.

Results

Health monitoring. Quarterly Sentinel EAD PCR analyses showed that the animals in this vivarium were free from the following viral, bacterial, and parasitic agents: mouse hepatitis virus, Sendai virus, pneumonia virus of mice, mouse parvovirus, minute virus of mice, Theiler murine encephalomyelitis virus, reovirus type 3, mouse rotavirus, ectromelia virus, lymphocytic choriomeningitis virus, mouse cytomegalovirus, mouse adenovirus 1 and 2, hantavirus, *Mycoplasma pulmonis*, *Salmonella* spp., *Citrobacter rodentium*, *Clostridium piliforme*, *Streptobacillus moniliformis*, *Filobacterium rodentium*, *Corynebacterium kutscheri*, pinworms (*Syphacia obvelata* and *Aspicularis tetraptera*), fur mites (*Myobia musculi*, *Myocoptes musculinus*, and *Radfordia affinis*), and *Giardia* spp. However, mouse norovirus, *Rodentibacter pneumotropicus* and *R. heylii* (previously *Pasteurella pneumotropica*), and *Helicobacter* spp. are endemic in the vivaria, except for a few designated rooms. The first quarterly testing revealed that the collection media was positive for LDV on racks 1 through 4; for subsequent quarters, testing for LDV was positive on rack 1 only (Table 1). There was no previous history of LDV contamination in the institutional health monitoring sentinel program. All confirmatory horizontal plenum swabs were negative for LDV via PCR testing.

PCR testing for LDV. The 5 PDX tumors were positive for LDV, but the 4 human- and one murine-origin cell lines were LDV-negative (Table 2). Oral swabs from the NSG dams were negative for LDV (Table 2). A total of 15 of the 22 (68%) blood samples submitted were positive for LDV; all 15 samples were from the NSG breeder cages belonging to PI1 (Table 2). None of PI2's experimental mice were positive for LDV (Table 2).

Discussion

This report details the use of environmental PCR testing as a way to detect a long-standing LDV infection in a mouse vivarium; this infection was not previously detected by using live-animal SBS. Directly after implementation of an exhaust dust health monitoring program, this virus was detected in the colony. An outbreak investigation identified one source as multiple LDV-positive PDX tumors. In addition, multiple LDV-positive breeding colony animals were identified; however, the source of their infection was never confirmed.

Initially, 4 racks were positive for LDV. However, because 3 of these racks, racks 2 through 4, were negative for LDV after subsequent testing without sanitization of the racks, the assumption is that the initial results for these 3 racks were most likely due to false-positive results. One cause of false-positive results could have been due to human error during the handling of the collection media. However, staff change gloves before insertion or retrieval of the collection media. If the IVC racks are not sanitized before placing a new collection media, residual nucleic acid in the exhaust plenum can lead to continued positive results in subsequent quarterly results.[18] Therefore, given that these racks continued to be negative, we assumed that these initial results were not true positives. Other possibilities were considered for these positive results. However, because the investigators with mice on these racks had not used any products derived or passaged in rodents and because the racks continued to be LDV-negative, these alternatives were not investigated further.

After the positive LDV results, confirmatory horizontal plenum swab testing was performed, as is the typical follow-up procedure for positive exhaust air dust testing results at our institution. Given that the confirmatory horizontal plenum testing results were negative, the initial quarterly results for all racks were considered to be false positives. From their inhouse studies, the diagnostic laboratory later confirmed that the nucleic acid copy number is generally higher on the Sentinel EAD collection media than the horizontal plenum swab for the standard adhesive swab technique.[6] Therefore, the number of viral particles on the plenum swab may have been inadequate for detection by PCR analysis. This finding demonstrates that

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horizontal plenum swab testing is not the best method for confirmatory testing of LDV, given that the virus is usually present in low copy numbers due to its limited transmission properties.[21,27] Therefore, other confirmatory testing methods should be considered for this agent.

Even though a single nucleic acid copy was present on the collection media for rack 1 during the second quarterly testing, the presence of 2 consecutive positive results initiated an outbreak investigation. The lab considers samples to be LDV-positive regardless of copy number detected because it is an uncommon virus and the assay is very specific for it. Therefore, the initial positive results should have been investigated more thoroughly at the time. However, because the frequency of monitoring for LDV was increased from yearly to quarterly, the outbreak was detected 3 mo later and investigated at that time. Due to the limited transmission properties of the virus,[21,27] the virus did not spread and jeopardize the animal program.

The preliminary investigation involved LDV-testing of the tumor lines inoculated in mice during the previous 3 mo, which correlates with when the collection media was present. Even though transplacental transmission is rare,[21,27] the dams from the breeding cages were tested via PCR analysis of oral swabs to ensure thorough examination of all animals housed on the rack. All PDX tumors used on this rack were positive for LDV, and all other samples were negative. Because 1) the mice inoculated with the PDX were culled, 2) the use of these LDV-positive tumors ceased, and 3) the rack was sanitized, the assumption was that the next quarterly testing results would be negative. However, when the rack was positive again for LDV the next quarter, the 2 PI were contacted again to ensure that no new tumor lines, blood products, or products derived or passaged in rodent tissues were used during the previous 3 mo. Because they confirmed that this was the case, the decision was made to test all of the animals housed on rack 1 for LDV via PCR analysis of blood, as mice with LDV infection are persistently viremic.[27] The 2 PI allowed this testing on their experimental mice, because all of the studies were coming to completion. One animal per cage was tested, and the animals that were positive were the NSG breeder animals.

We were unable to identify the cause of the LDV-positive results in the breeder cages: those mice were never inoculated with PDX or any other compound, nor were they progeny of those that had been. In light of the transmission properties of LDV,[21,27] the positive animals on this rack are very unlikely to have transferred the virus to the breeders. In addition, we presumed that some of the initial breeder animals that were tested by oral swabs were actually LDV-positive at the time but that oral swab testing failed to detect this virus. This presumption is further strengthened by the fact that records showed that animals from 2 of the cages that tested negative by oral swabs in October 2018 were positive for LDV by blood samples in April 2019. Using oral swabs could have missed the detection of LDV, given that to viral excretion into saliva decreases significantly after the first week of infection.[21] This pattern suggests that testing saliva by oral swabs may be a poor method for diagnostic detection of long-standing LDV infections. Historically, plasma lactate dehydrogenase levels were measured as a diagnostic test for this virus because they rise after infection and remain significantly elevated for life.[21,27] However, this testing method can lead to false-positive results because a rise in enzyme concentration is not specific for LDV infection.[1] For example, lactate dehydrogenase is found in RBC and can be falsely elevated due to hemolysis.[25] Therefore, plasma lactate dehydrogenase levels were not measured for any animals in this report because PCR testing is now the recommended diagnostic tool.[1] Overall, as with the majority of pathogen outbreaks in mouse vivaria, the original cause for the positive results in these breeder animals was never fully determined. Most important, however, is that the outbreak was detected and resolved without additional consequences to the animal program.

Because this rack was previously monitored by using SBS, LDV was not detected, even though these PDX were used to inoculate mice on this rack for as long as 4 y before the start of this case study. This result is due to the fact that LDV does not reliably pass to sentinel animals; rather, mechanical transfer is required.[21,27] This case is the first that demonstrates the use of environmental PCR testing as a method for the detection of an LDV infection in a mouse vivarium. The findings show the potential for increased detection of LDV by using an exhaust air dust health monitoring program compared with SBS, similar to what has been shown for multiple other pathogens.[2,3,5,6,9-11,15,16,18-20,29] Future prospective studies are needed to compare the efficacy of exhaust air dust relative to SBS for the detection of this virus.

PDX tumors are commonly contaminated with LDV, and, historically, the basement membrane complex Matrigel has been identified as a cause of LDV contamination.[4,21,23,28] Matrigel produced before mid2008 was not guaranteed to be LDV-negative.[14] This biologic could have been the source of contamination in this situation; however, the Matrigel used in the mice reported here was purchased more recently than 2008. The tumors underwent PCR testing and were LDV-negative before use in this vivarium. Therefore, the testing performed resulted in potentially false-negative results, as previously described.[13] Alternatively, because LDV persists in transplantable tumors,[14] it may have infected the tumors at some point and then later transferred to subsequent tumor generations. Because LDV is on the institutional exclusion list, PI1 was required to eradicate the PDX tumors of this virus. The PI chose to eradicate the virus from the PDX tumors by using FACS.[14] Alternatively, the literature describes passaging the tumors in nude rats.[1]

This report highlights the need for vigilant pathogen testing of all products derived or passaged in rodents that are used in mouse vivaria. Because a complete history is not always available for PDX tumors, institutions should consider requiring rodent pathogen testing before their use in mouse vivaria, to ensure that they are LDV-negative. With increasing use of exhaust air dust health monitoring programs, LDV may be detected more readily, given that this methodology may provide improved detection of this virus.

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References

1. **Barthold SW, Griffey SM, Percy DH.** 2016. Mouse, pp 1–118. In: Pathology of laboratory rodents and rabbits. Ames (IA): Wiley–Blackwell.
2. **Bauer BA, Besch-Williford C, Livingston RS, Crim MJ, Riley LK, Myles MH.** 2016. Influence of rack design and disease prevalence on detection of rodent pathogens in exhaust debris samples from individually ventilated caging systems. J Am Assoc Lab Anim Sci **55:**782–788.
3. **Brielmeier M, Mahabir E, Needham JR, Lengger C, Wilhelm P, Schmidt J.** 2006. Microbiological monitoring of laboratory mice and

http://prime-pdf-watermark.prime-prod.pubfactory.com/ | 2025-02-05

biocontainment in individually ventilated cages: a field study. Lab Anim **40:**247–260. https://doi.org/10.1258/002367706777611497.

4. **Carlson Scholz JA, Garg R, Compton SR, Allore HG, Zeiss CJ, Uchio EM.** 2011. Poliomyelitis in MuLV-Infected ICR-SCID mice after injection of basement membrane matrix contaminated with lactate dehydrogenase-elevating virus. Comp Med **61:**404–411.
5. **Compton SR, Homberger FR, Paturzo FX, Clark JM.** 2004. Efficacy of 3 microbiological monitoring methods in a ventilated cage rack. Comp Med **54:**382–392.
6. **Henderson KSPC, Benigni S, Bilecki S, Momtsios P.** 2015. Investigation of an In-Line Filter Manifold to Simplify and Improve PCR Detection of Rodent Pathogens on an IVC Rack. Abstract PS82 presented at the American Association for Laboratory Animal Science National Meeting, Phoenix, Arizona, 1–5 November 2015. J Am Assoc Lab Anim Sci **54:**665.
7. **Henderson KS, Perkins CL, Havens RB, Kelly MJ, Francis BC, Dole VS, Shek WR.** 2013. Efficacy of direct detection of pathogens in naturally infected mice by using a high-density PCR array. J Am Assoc Lab Anim Sci **52:**763–772.
8. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
9. **Jensen ES, Allen KP, Henderson KS, Szabo A, Thulin JD.** 2013. PCR testing of a ventilated caging system to detect murine fur mites. J Am Assoc Lab Anim Sci **52:**28–33.
10. **Kapoor P, Hayes YO, Jarrell LT, Bellinger DA, Thomas RD, Lawson GW, Arkema JD, Fletcher CA, Nielsen JN.** 2017. Evaluation of anthelmintic resistance and exhaust air dust PCR as a diagnostic tool in mice enzootically infected with *Aspiculuris tetraptera*. J Am Assoc Lab Anim Sci **56:**273–289.
11. **Körner C, Miller M, Brielmeier M.** 2019. Detection of Murine Astrovirus and *Myocoptes musculinus* in individually ventilated caging systems: Investigations to expose suitable detection methods for routine hygienic monitoring. PLoS One **14:**1–12. https://doi.org/10.1371/journal.pone.0221118.
12. **Leblanc M, Berry K, Graciano S, Becker B, Reuter JD.** 2014. False-positive results after environmental pinworm PCR testing due to rhabditid nematodes in corncob bedding. J Am Assoc Lab Anim Sci **53:**717–724.
13. **Lipman NS, Henderson K, Shek W.** 2000. False negative results using RT-PCR for detection of lactate dehydrogenase-elevating virus in a tumor cell line. Comp Med **50:**255–256.
14. **Liu H, Bockhorn J, Dalton R, Chang YF, Qian D, Zitzow LA, Clarke MF, Greene GL.** 2011. Removal of lactate dehydrogenase-elevating virus from human-in-mouse breast tumor xenografts by cell-sorting. J Virol Methods **173:**266–270. https://doi.org/10.1016/j.jviromet.2011.02.015.
15. **Mahabir E, Durand S, Henderson KS, Hardy P.** 2018. Comparison of two prevalent individually ventilated caging systems for detection of murine infectious agents via exhaust air particles. Lab Anim **53:**84–88. https://doi.org/10.1177/0023677218785929.
16. **Mailhiot D, Ostdiek AM, Luchins KR, Bowers CJ, Theriault BR, Langan GP.** 2020. Comparing mouse health monitoring between soiled-bedding sentinel and exhaust air dust surveillance programs. J Am Assoc Lab Anim Sci **59:**58–66. https://doi.org/10.30802/AALAS-JAALAS-19-000061.
17. **Manuel CA, Pugazhenthi U, Leszczynski JK.** 2016. Surveillance of a ventilated rack system for *Corynebacterium bovis* by sampling exhaust-air manifolds. J Am Assoc Lab Anim Sci **55:**58–65.
18. **Miller M, Brielmeier M.** 2018. Environmental samples make soiled bedding sentinels dispensable for hygienic monitoring of IVC-reared mouse colonies. Lab Anim **52:**233–239. https://doi.org/10.1177/0023677217739329.
19. **Miller M, Ritter B, Zorn J, Brielmeier M.** 2016. Exhaust air dust monitoring is superior to soiled bedding sentinels for the detection of *Pasteurella Pneumotropica* in individually ventilated cage systems. J Am Assoc Lab Anim Sci **55:**775–781.
20. **Miller M, Ritter B, Zorn J, Brielmeier M.** 2016. Exhaust air particle PCR detects *Helicobacter hepaticus* infections at low prevalence. J Vet Sci Technol **7:**343. https://doi.org/10.4172/2157-7579.1000343.
21. **National Research Council.** 1991. Infectious diseases of mice and rats. Washington (DC): The National Academies Press.
22. **Niimi K, Maruyama S, Sako N, Miyata K, Yoshimoto T, Bilecki B, Henderson K, Takahashi E.** 2018. The Sentinel™ EAD program can detect more microorganisms than bedding sentinel animals. Jpn J Vet Res **66:**125–129. http://doi.org/10.14943/jjvr.66.2.125
23. **Peterson NC.** 2008. From bench to cageside: Risk assessment for rodent pathogen contamination of cells and biologics. ILAR J **49:**310–315. https://doi.org/10.1093/ilar.49.3.310.
24. **Plagemann PGW, Gregory KF, Swim HE, Chan KKW.** 1963. Plasma lactic dehydrogenase elevating agent of mice: distribution in tissues and effect on lactic dehydrogenase isozyme patterns. Can J Microbiol **9:**75–86. https://doi.org/10.1139/m63-008.
25. **Pritchett-Corning KR, Cosentino J, Clifford CB.** 2009. Contemporary prevalence of infectious agents in laboratory mice and rats. Lab Anim **43:**165–173. https://doi.org/10.1258/la.2008.008009.
26. **Wagner AM, Loganbill JK, Besselsen DG.** 2004. Detection of lactate dehydrogenase-elevating virus by use of a fluorogenic nuclease reverse transcriptase-polymerase chain reaction assay. Comp Med **54:**288–292.
27. **Whary MT, Baumgarth N, Fox JG, Barthold SW.** 2015. Biology and diseases of mice, p 43–149. In: Fox JG, Anderson LC, Otto GM, Pritchett-Corning KR, Whary MT, editors. Laboratory animal medicine. Boston (MA): Academic Press.
28. **Whittle JR, Lewis MT, Lindeman GJ, Visvader JE.** 2015. Patient-derived xenograft models of breast cancer and their predictive power. Breast Cancer Res **17:**1–13. https://doi.org/10.1186/s13058-015-0523-1.
29. **Zorn J, Ritter B, Miller M, Kraus M, Northrup E, Brielmeier M.** 2017. Murine norovirus detection in the exhaust air of IVCs is more sensitive than serological analysis of soiled bedding sentinels. Lab Anim **51:**301–310. https://doi.org/10.1177/0023677216661586.

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