Practicalities of incorporating Exhaust Air Duct (EAD) testing into a mouse health monitoring programme

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1. Introduction

1.1 Changes to mouse housing and the impact on health surveillance

Health surveillance in large laboratory mouse colonies has always been challenging. Collecting samples from individual mice in numbers sufficient to detect disease of low prevalence is cost and time prohibitive, prompting the development of the 'dirty bedding' sentinel system. When wire topped cages were used without filters, dust from other cages complemented the exposure from dirty bedding transfer to the sentinel. But when individually ventilated cage systems (IVCs) were introduced, the sentinel's exposure to pathogens decreased significantly. While the use of in-cage sentinels or random testing of colony mice provides alternatives, each presents challenges, especially in detecting diseases of low prevalence. The advent of polymerase chain reaction (PCR) screening of environmental samples such as Exhaust Air Duct (EAD) samples provides a break-through in the screening of large laboratory mouse colonies housed in IVCs.

1.2 Sensitivity in health screening

Published comparisons of EAD versus dirty bedding health surveillance methods highlight improved sensitivity across a range of pathogens following EAD testing. [1,2,3] Studies involving the detection of Helicobacter spp, Murine norovirus, Rodentibacter spp, Radfordia affinis and Myobia musculi found an increased sensitivity in the detection of all pathogens when EAD testing was used.

Despite the enhanced sensitivity seen in the published studies, a basic understanding of PCR technology and the factors impacting sample collection is needed to optimise results and minimise the risk of false negative or false positive results.

2. PCR testing on EAD samples

2.1 How does a PCR test work?

In a PCR test, DNA is subjected to a high temperature melt, then primers bind to each DNA strand. The strands are amplified by TAQ polymerase cycling between melting and amplification temperatures. The amplification cycles are repeated multiple times to increase the PCR product making PCRs very sensitive. There are several different ways of visualising PCR results. Gel electrophoresis can be used for end point analysis, or using fluorescent labelled probes, real-time observation of the increased PCR product is possible.

2.2 Advantages/ disadvantages of Exhaust Air Duct testing

Advantages

PCR testing is extremely sensitive. It does not rely on the mouse to elicit an immune response to a pathogen. PCR can be used on samples collected directly from mice or from the environment such as an EAD filter. Because an EAD filter collects dust from every cage on a rack over a defined period, typically 1-3 months, it overcomes the challenge of intermittent pathogen shedding.

Disadvantages

PCRs are designed to detect specific pathogens and will not detect other pathogens. This prevents any adventitious discoveries.

PCR will detect the presence of genetic material in live and dead pathogens, so environmental contamination from non-viable pathogens is a risk, especially given PCR's sensitivity.

In Australia, serology and culture remain cheaper than PCR, making it difficult to transition to EAD testing.

Table 1- Comparison of different health screening methodologies

Characteristics	Serology	PCR	Culture
Does the method confirm an active infection?	Not necessarily- serology indicates historic infection	No – both active and inactive pathogens are detected	Yes
Does the method detect pathogens outside the panel?	No	No	Yes
Can the method screen multiple cages with a single sample?	Only by using a sentinel	Yes	Only by using a sentinel
Can the method be used with immunodeficient mice?	No- relies on the host mounting an immune response	Yes	Yes
Can the method be used for all types of pathogens	Primarily (but not exclusively) used for viral pathogens	Yes	Mainly bacterial culture used

3. Practical consideration in setting up EAD screening

3.1 Rack and cage type

Several IVC suppliers have developed sample collection devices to facilitate EAD testing. However, any dust collection system located in a centralised rack exhaust duct can provide sample material. Because dust emanating from mouse cages is needed in some quantity, rack design, housing density and cage/ rack hygiene may impact sample quantity and quality.

3.2 Rack design, housing density and cage/ rack hygiene

Rack design can have a significant impact on dust collection in the exhaust air ducts. A 2016 study by Bauer et al. [4] found that IVC systems with cage exhaust level filtration can decrease the efficiency of EAD dust collection. In addition, the study found that dust collection from horizontal exhaust plenums may provide some advantages over collection from vertical exhaust systems.

Cage population densities on racks impact the dust collection on EAD filters. If few cages are populated and empty cages occupy most slots there is a high dilution factor. Depending on the rack design & maintenance, empty cage slots may be a source of air leakage into the system also diluting the sample.

The frequency of cage sanitation will impact on the amount of contaminated dust reaching the sample collection device. Weekly cage and lid change will decrease the amount of contaminated dust compared to fortnightly cage base change with a lower frequency of cage lid change.

The timing of IVC rack sanitation compared to sample collection may impact the success of EAD testing, especially if the sample collection device is changed. The sample collection device must be exposed to dust from the rack for ideally a month or more to ensure that the sample collected is relevant.

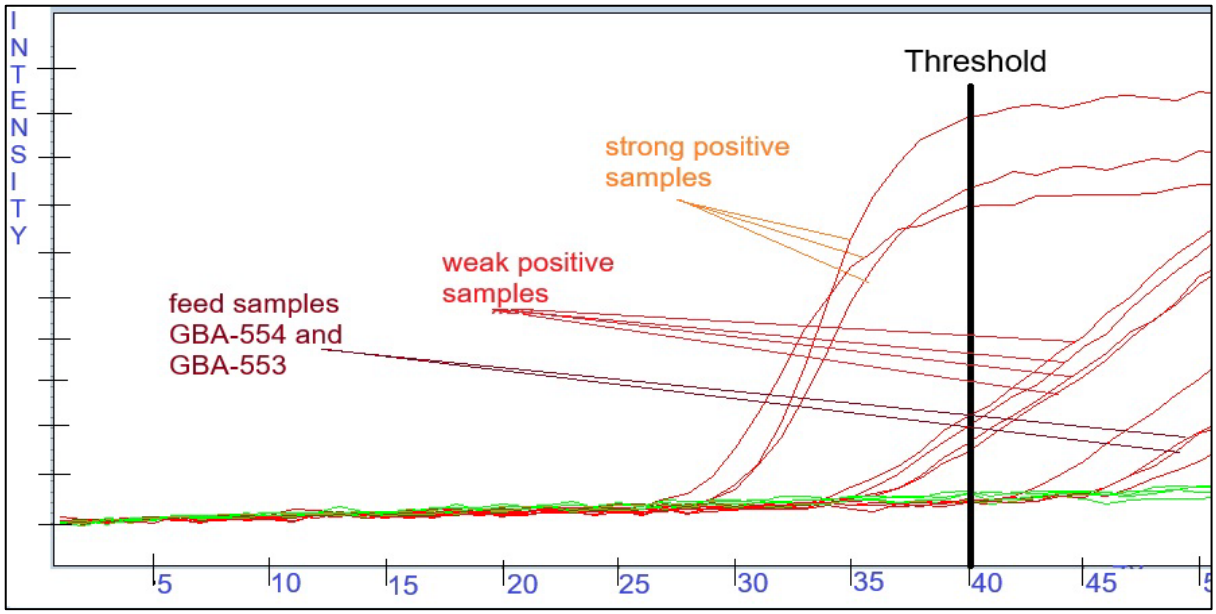
Once a pathogen has been detected on a rack, effective rack sanitisation to denature any remnant DNA is essential. Sterilisation via the autoclave is optimal to denature DNA, however physical cleaning combined with sanitisation using chemicals such as chlorine dioxide can also be effective. If the rack is not effectively sanitized following pathogen eradication, false positive results may occur in subsequent testing.

Follow up testing of the EAD sample collection system is advisable post sanitation to ensure any remnant DNA has been removed or denatured.

3.3 Working with the testing laboratory on a positive threshold

Working closely with the laboratory testing EAD samples is important when a new EAD surveillance programme is introduced. Real-time PCRs are extremely sensitive and may detect background contamination that does not reflect the presence of living pathogens. An appropriate threshold for positive results is required to distinguish true positive from false positive results.

In the example provided below the real time melt-curve results shows positive samples of varying strengths and compares these to environmental contamination from gamma irradiated feed (GBA-554, GBA-553). Strong positive results appear as a curve between 25-30 cycles, while weak positive results (may be reported as equivocal) appear as a curve at 35 cycles. When the threshold for a true positive result is placed at 40 cycles the false positive results from environmental contamination (in this case remnant DNA from gamma irradiated feed) are eliminated.



3.4 Selection of pathogens & cost

For many animal facilities the cost of PCRs is the biggest deterrent to changing to EAD health surveillance. However, establishing a short panel and using this in combination with other testing methods can provide a cost-effective option. Selection of pathogens to include in the PCR panel will depend on-

- *Facility use- breeding vs experimental*. Short term housing of experimental mice, especially if most are purchased from suppliers, can be treated with reduced health surveillance compared to long term breeding colonies.
- *Pathogens commonly found in Australia*. Laboratories provide PCR panels specifically targeting common pathogens.
- *History of previous outbreaks.* If the facility has had cases of pathogens not on established panels, on-going testing post eradication will be needed to ensure the facility remains disease free.
- *Immune status of colony*. Immunocompromised mice are much more likely to become infected with opportunistic pathogens like S.aureus or C.bovis, so regular screening of these pathogens may be added to panels.
- *Housing of other species housed within the facility*. Other species, especially if they are conventional, may increase the risk of pathogen outbreaks eg. E cuniculi from rabbits.

3.5 Can PCRs be used with other methods?

A PCR panel will only test for the nominated list of pathogens. As cost may limit the number of pathogens included on a PCR panel, complimentary testing using other methods will be needed. Samples collected from random colony mice or in-cage sentinels are alternatives. The use of culture from fresh samples, special stains on histopathology or follow up testing from postmortems can provide additional information to the core PCR panel.

3.6 Validating the EAD testing system

It is important to validate a new EAD system to ensure that the system provides enough inherent sensitivity to detect pathogens. One way of testing the system is to trial EAD sample collection on a rack housing mice that are known to be positive for one or more pathogens. A side-by-side comparison with other testing methods will provide assurance about the sensitivity. A trial needs to replicate the proposed screening schedule, including exposure time.

3.7 Follow up screening after positive EAD results

When a positive result is received from EAD testing, follow up screening is needed to ensure the result is a true positive, reflects an active infection and to determine which cages are infected. The follow up screening will depend on the immune status of the mice, pathogen found, and type of holding eg breeding versus experimental.

Follow up screening may include-

- Direct screening of mice - swabs for culture to confirm an active infection with or PCR confirmation.
- Screening of cages to determine which have positive mice– eg PCR on exhaust cage filter or swab from cage exhaust.
- Serology from immunocompetent mice to check for a history of infection.
- Direct screening and clinical investigation of sick mice.
- Environmental screening of gamma irradiated feed, bedding or environmental enrichment items.

It is important in follow up screening that as many cages as possible on the rack are tested to validate the EAD result, especially for pathogens of low incidence.

Conclusion

The integration of EAD testing into colony health screening provides enhanced sensitivity in pathogen detection. This method circumvents the need to use dirty bedding sentinels and does not involve procedures on mice, providing animal welfare advantages.

However, the method is expensive, rack/ cage design and hygiene can impact effectiveness, and alternate complementary methods should be considered to ensure health surveillance covers all relevant pathogens.

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