



COMPATH

HEALTH MONITORING RECOMMENDATIONS

SEPTEMBER 2021

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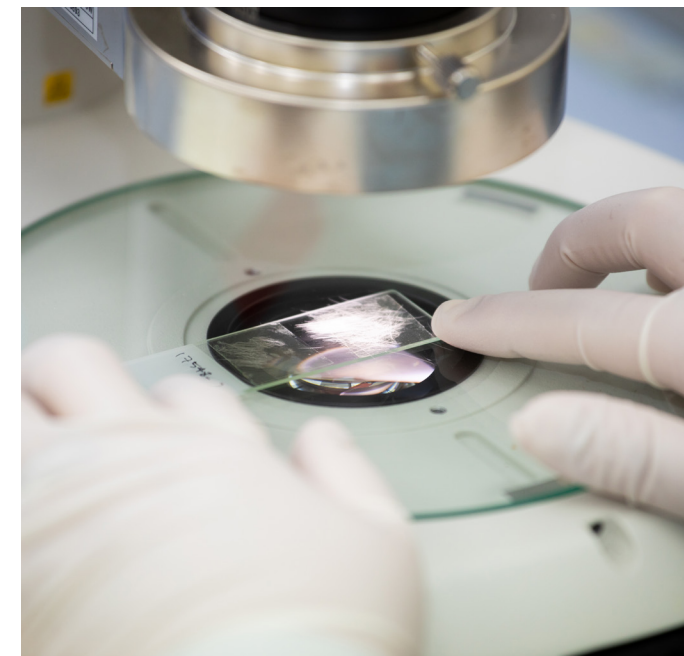
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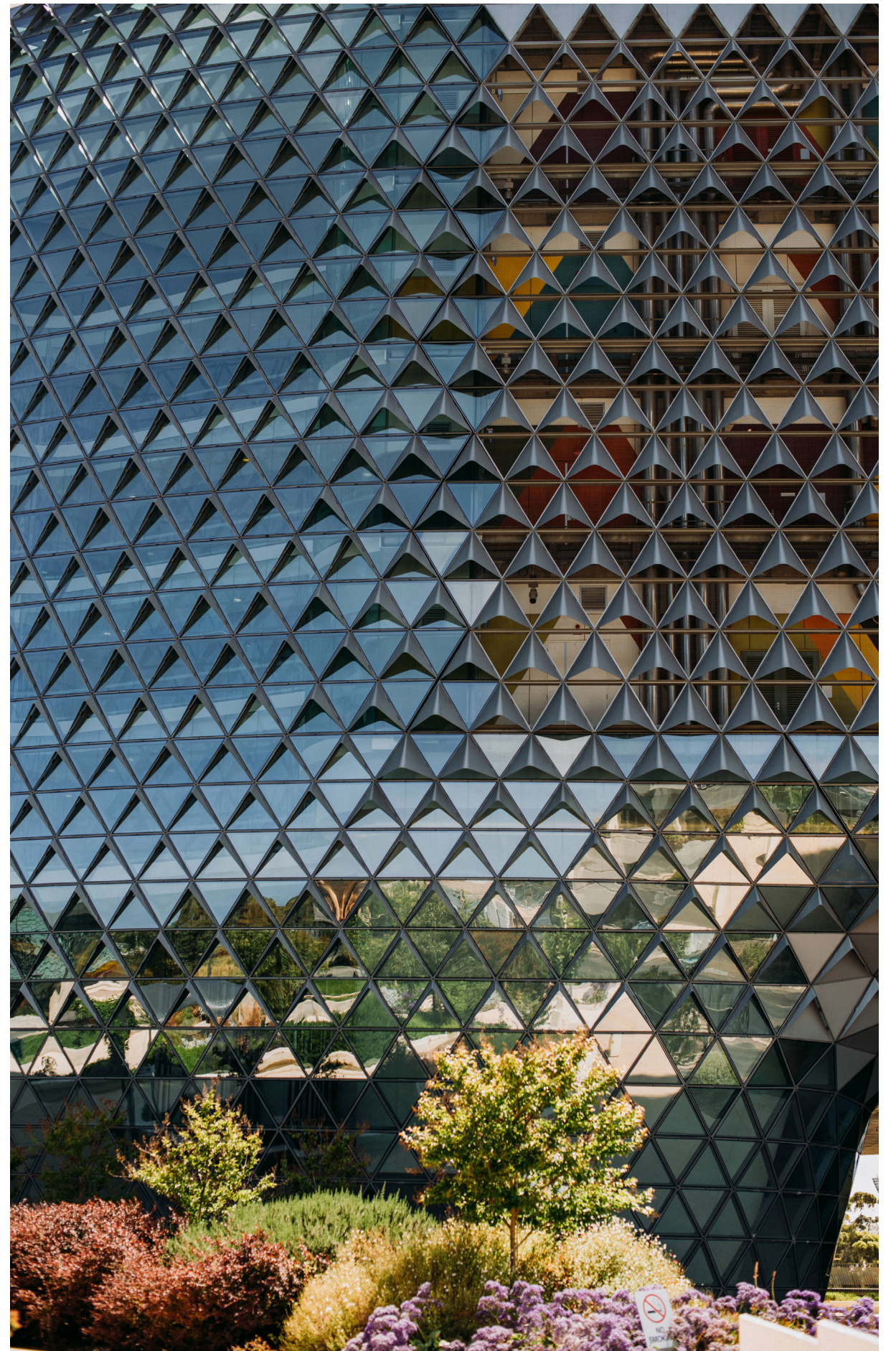
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Introduction

Why Should I Test?

Knowing the health status of laboratory animals is critical for reproducible and accurate research outcomes. The health of an animal may affect their suitability for particular research and often these interfering pathogens will be asymptomatic, hence the need for a sensitive and robust surveillance program. In this document we will talk about routine health monitoring (HM) and how it is different to daily observation and assessment of animal health. Disease investigation also plays a vital role in an animal facility, but this differs from routine disease surveillance.



Test Samples

Which Samples Should I Use?

Sentinel and resident animals can be used for health monitoring and often a combination of the two will yield the best results. Theoretically, the number of animals to test is based on the prevalence of the pathogenic agent within the colony. In open-top cages, most introduced viruses can reach prevalence higher than 50% within a four-week monitoring period, making the testing of resident animals satisfactory but the spread of infection is drastically reduced in IVCs as the agent and animal is isolated. This means that more resident animals would need to be tested to detect the same agent. Prevalence as low as 1% is considered realistic for MPV infection and the sampling of sufficient numbers of resident animals is not feasible for this low-prevalence infection; therefore sentinels would be the obvious choice.

Resident Animals

- Useful alternative to sentinels for screening Quarantine animals if time is of a concern for introduction of animals into the research facility.
- Must be immunocompetent for serology screening.
- May give a better indication of parasite/bacterial infection than soiled-bedding sentinels especially if the resident animal is from an immunocompromised background.
- High numbers required to be screened to give certainty of detection for low-prevalence infections.
- Most test can be performed from survival samples.



Sentinel Animals

Soiled Bedding Sentinels (SBS)

- Primarily used for faecal-oral transmitting pathogens, ie. MHV, ROTA
- When cleaning cages add a handful of dirty bedding from each cage to a central bin. A few handfuls of pooled soiled bedding are then added to the sentinel cage. Sentinels should receive 100% soiled bedding for optimal results.
- For each 50-80 cage rack we recommend a minimum of one cage containing at least 2 sentinels.
- Introduced at 3-6 weeks of age.
- Testing performed quarterly, option to keep one sentinel going for up to six months. Sentinels should not be kept for longer than six months.
- Send one sentinel per cage for testing and set up new sentinel cage. Once results are received the “old” sentinels are euthanized or used for confirmation of any positive results or for difficult to detect pathogens eg. MPV.
- Ideally, you would use two cages of 2 different strain sentinels. One cage containing an outbred line (e.g. CD1 mice), the other containing an inbred line (e.g. Balb/c mice). This minimises the risk of genetic differences in disease susceptibility. Sentinels must be immunocompetent if being used for serology screening.

It is important to understand that sentinels are a compromise. If dirty bedding transfer is done well they become an acceptable compromise and enable more comprehensive surveillance to be achieved, particularly when IVCs are used. Incorporating testing of resident animals, particularly to gain a better picture of bacterial/ parasite status in GM or immunocompromised lines or in time-critical situations such as Quarantine release is advised.



Test Schedules

Environmental Samples (non-animal)

Environmental samples utilise the sensitivity of molecular based (PCR) testing to screen for presence of nucleic acids captured in dust particles.

Samples can include exhaust plenum swabs or exhaust plenum media from IVC racks or cage filter paper or cage swabs from static cages or cages with exhaust filtration. It is important to understand the type of caging system used to ensure that appropriate samples are taken for colony-wide surveillance.

Detection proves presence of nucleic acid only, not necessarily infection. Infection to be confirmed with direct animal samples.



How Often Should I Test?

Quarterly testing is the default frequency used within most animal facilities. Animals generally seroconvert to most agents within 14 days except for MNV, which may require up to 8 weeks. Highly infectious and prevalent agents should be tested frequently. Rare or exotic agents may only require annual testing.

Quarterly testing is recommended for environmental or non-animal samples. As the results will be a historic record of infection, the greater the elapsed time between screens, the greater the difficulty in pinpointing the infection source. Facilities wishing to adopt environmental sample type screens should consider doing so initially in parallel with their traditional sample types. Evaluation of these results over a period of time will help dictate the best approach moving forward and often a combination of methods will form the most economical and efficient HM program.



Test Methods

How Should I Test?

Serology

Serology screens are the most commonly used method for testing viral agents as they are cost-effective, able to screen large populations and samples are easy to collect. Serology screens are a historic record of infection. Animals must be immunocompetent for serology testing to be useful.

Molecular Diagnostics

PCR screening is more sensitive and indicates an active (current) infection if the sample has been obtained from the animal itself (faeces, liver etc.). PCR screening on environmental or non-animal samples indicates that the DNA/RNA from the organism is present but not whether or not it is viable. It is generally more expensive than serology for screening viral agents but plays a useful role in outbreak management as animals do not need to have seroconverted to yield a positive result. It is also an important method for screening pathogens that do not have a serology test available (*Helicobacter* sp.) or are difficult to isolate by microbiological methods (*Streptobacillus moniliformis*) or for those animals unable to mount a serologic response. Cost-efficiencies can be made by pooling samples, for example, with *Helicobacter* sp. we have performed validation studies to demonstrate that up to 10 faecal pellets can be pooled without compromising sensitivity.

Bacteriology/Parasitology

Microbiological examination of nasopharyngeal washings and colonic swabs continues to be an important aspect of any health monitoring program. Although many bacteria and protozoa can be considered non-pathogenic and even commensal inhabitants of rats and mice, they can act as an indicator of hygiene practices within the colony. Each facility should have an exclusion list of unacceptable microorganisms and clearly indicate these to ComPath. What may be considered an acceptable commensal in one facility might comprise part of the defined, excluded flora in another. It is recommended that facilities screen for bacterial and protozoal microorganisms that comprise their exclusion list by PCR methods annually, as this test is 10-fold more sensitive than traditional methods.

Histopathology

Routine histopathology on grossly normal tissues is not considered an important aspect of typical health monitoring programs. However, *Pneumocystis carinii* is one of the most prevalent infections of rats so histopathologic examination of immunocompromised rat lungs should be considered. Histopathology remains an important tool in disease investigation.



What to Test For

Which Agents Should I Test For?

Animal facilities should test for those agents which could interfere with research conducted within that facility and any agent whose detection would invoke an immediate course of action. All viruses can alter the function of infected cells and trigger host defence mechanisms and should therefore, ideally, be excluded. Bacteria and fungi are of concern only when they invade tissues or elaborate toxins. Metazoan parasites (Pinworms, Tapeworms etc.) are of primary concern while protozoan parasites (*Entamoeba* sp., *Sprionucleus* etc) are of differing levels of concern.

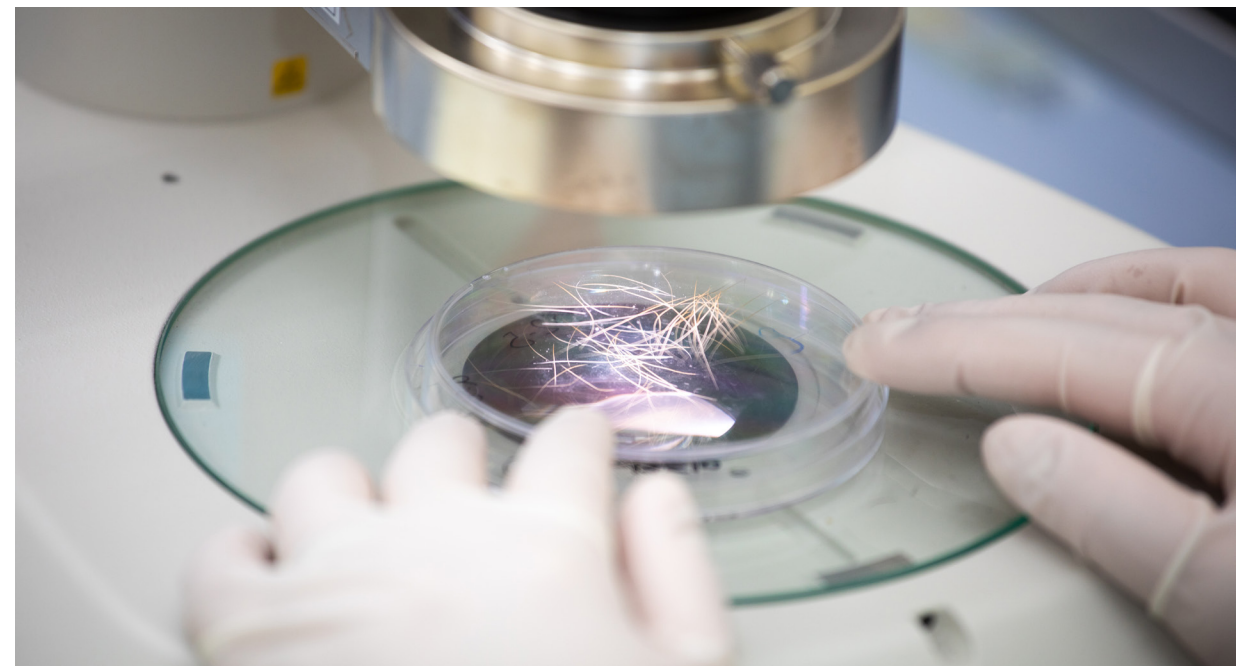
Our health monitoring profiles are based on the Federation of European Laboratory Animal Science Association (FELASA) recommendations and those outlined by leading global Health Monitoring Providers. As every facility has differing needs these recommendations should be used as a guide only. We are happy to discuss specific requirements further and adapt the Health Monitoring Program accordingly for individual facilities.



Positive Results

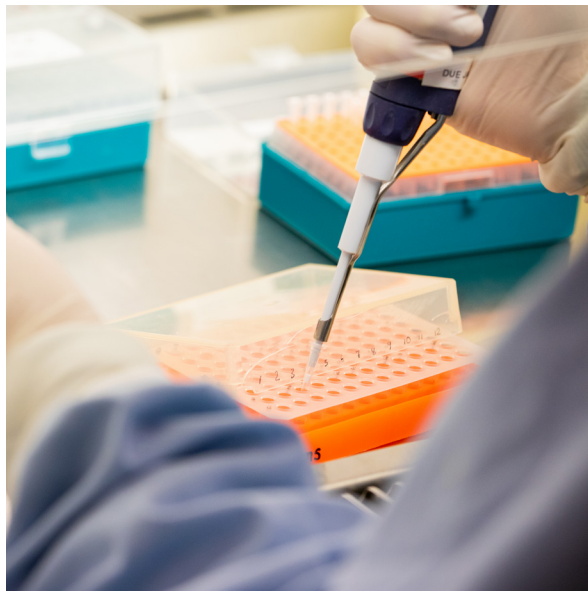
What Should I Do Next?

Firstly, do not panic. A single positive result does not constitute an outbreak. It is important to recognise that even the best assays can give false positive results so careful interpretation and repeat testing is necessary. Positive results should always be confirmed by a secondary method or sample and drastic action is discouraged on the basis of a single positive result. ComPath can advise on the selection of further samples and methodologies for follow-up testing.



Other Considerations

- PCR Panels – routine testing of pooled faeces (up to 10) for most prevalent or facility-critical pathogens
- Additional PCRs- e.g. PPN (*P.pneumotropica*) for mice, PCAR (*P.carinii*) for rats every 6 months
- Quarantine testing for imports/exports
- Biologics screening- e.g. Tumour cell lines, PDX tissue
– contact ComPath to design your tailored screen



Disease Investigation

Disease Investigation is a critical component of every animal facility and needs to be considered in the design of the Health Monitoring budget. Any animals displaying clinical signs of illness must be investigated thoroughly. ComPath provides free expert advice and consultation on any matter and can help recommend the best choice of samples to collect and tests to perform in order to get the answers you are looking for.



Expertise

We're Here to Help

ComPath has a strong reputation as a leader in Laboratory Animal Science and Health Monitoring testing. We pride ourselves on keeping up with the latest trends in testing and our team of resident veterinary specialists and scientists are dedicated to supporting you and your facility by providing free expert advice and consultation on any matter. Our Veterinarian consultants have extensive experience in the management of animal facilities; the advice and insights that can be offered are directly relevant and unmatched by other providers. Whether it is regarding animal health, symptoms, outbreak management, treatment, or minimisation strategies and how to integrate this into your animal facility Standard Operating Procedures, we are your partners in laboratory animal science.

Through our contact network at SAHMRI and SA Pathology we have access to internationally recognised specialists in all fields of testing which we can utilise to confirm results or perform additional services such as bacterial sequencing and comprehensive microbial sensitivity testing.



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