Animal health monitoring: the value of a comprehensive programme at the Francis Crick Institute

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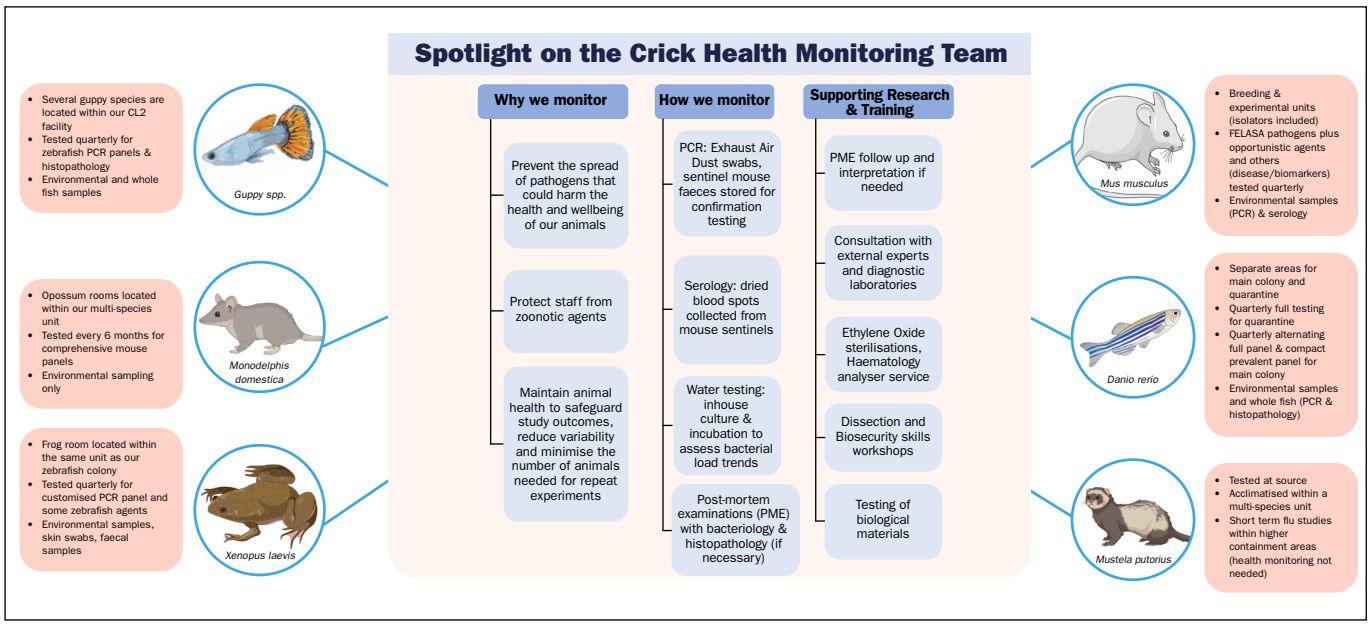
Effective animal health monitoring is essential to ensure both Animal Welfare and scientific validity in biomedical research facilities. As the health monitoring team, we play a crucial role in supporting the principles of the 3Rs by promoting proactive care, early detection of health issues and reliable research outcomes. This poster provides an overview of the health monitoring programme at the Francis Crick Institute which oversees routine health testing across a diverse range of species including mice, Zebrafish, guppies, frogs and opossums whilst also supporting ad hoc requests from research groups. It highlights the importance of facilitating rapid communication and the identification of emerging health trends through bacteriology, histopathology, serology and additional PCR testing. Finally, we will cover our work in reducing sentinel use, exploring the microbiome of our mouse colonies and preparing for the introduction of germ-free models.

Case study – *Xenopus laevis* mortalities

Import, quarantine and initial screening

- Frogs were imported from an external supplier and quarantined for ~2 months.
- PCR samples taken shortly after arrival and again around 6 weeks later showed no significant pathogens so quarantine restrictions were lifted.



Routine health surveillance findings

- During routine colony health checks (3 months post-arrival), *Mycobacterium ulcerans/liflandii* was detected – a pathogen not previously found in our facility.
- Around this time, the first mortality occurred. Over the next 2–3 months, six imported frogs died.

Diagnostic investigation

- A full diagnostic investigation was launched in collaboration with:
- Husbandry team – first line reporting of clinical signs.
- Veterinary team – clinical assessment, case management, diagnostic planning.
- External laboratories – specialist testing.

Post-mortem findings

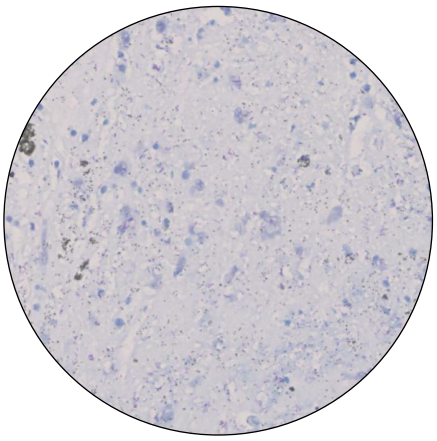
- Gross abnormalities in the heart, liver and spleen.
- Tissues collected for histopathology, bacterial and fungal culture, PCR and organ impression smears.
- Post-mortem analysis revealed multi-organ pathology including splenomegaly, hepatomegaly, cardiomegaly and coelomic effusion.
- No bacterial growth obtained from tissue cultures but Mycobacteria are notoriously difficult to culture requiring specialised media and extended incubation.
- PCR and histopathological testing of egg, liver and spleen tissue from one frog confirmed *Mycobacterium ulcerans/liflandii* infection.
- Notably, classic external skin lesions typical of mycobacterial disease were absent indicating an atypical presentation.

Outcome

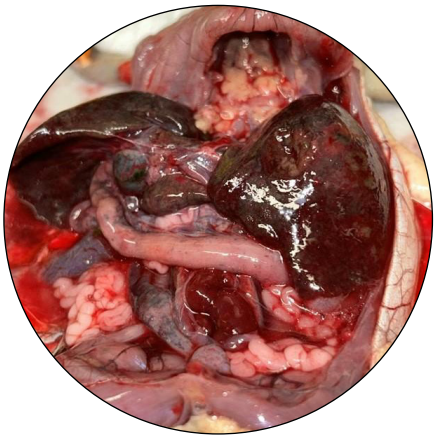
- Mortalities ceased after 2–3 months.
- Follow-up environmental screening did not detect *Mycobacterium ulcerans/liflandii*.

Key lessons and recommendations

- Importing live animals carries significant health risks to existing colonies.
- Reviewing supplier health history, implementing strict quarantine and conducting follow-up testing are essential safeguards.
- However as this case demonstrates, imports can still potentially impact the health of your colony despite precautionary measures.
- Transport stress may activate low-level or slow-growing infections, leading to disease and mortality even after initial clearance.
- Repeated routine testing is crucial for detecting infections that may be missed during the initial screening.
- A multi-modal diagnostic approach (PCR, culture, histopathology) is critical for identifying emerging health issues.
- Strong communication across husbandry, veterinary and laboratory teams is vital for early detection, coordinated response and protecting both animal welfare and research integrity.



Xenopus tissue section stained with Ziehl-Neelson Positive for Mycobacteria (dark blue spots)



Xenopus post-mortem with an enlarged spleen (splenomegaly)

Xenopus post-mortem with an enlarged heart (cardiomegaly)

Microbiome testing

Microbiome research has evolved from simply identifying which micro-organisms are present to understanding how microbial function and interact with the host to influence health. Establishing a Crick-specific microbiome baseline will support the interpretation of experimental outcomes and improve understanding of animal health. Our approach to microbiome sampling is centred on first characterising our core strains, while also considering animal role (breeder or stock), health status, age and sex.

How do we collect samples?

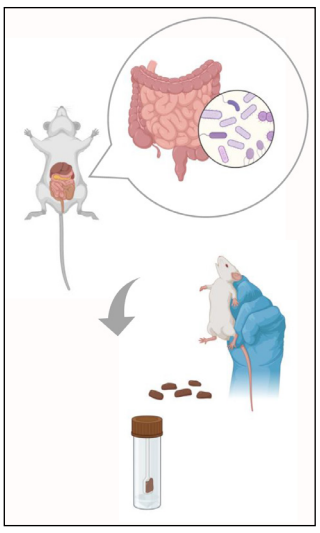
- Each mouse is placed in an empty container for up to 5 minutes to allow it to produce faecal pellets.
- Two pellets per animal are collected using sterile forceps and transferred into a sample vial.
- Alternatively the mouse may be gently scruffed and pellets placed directly into the vial.

What still needs to be established?

- We are refining the optimal sample size for each mouse strain and determining sampling frequency.
- Current work has focussed on core strains in breeding units but we still need to define the best approach for experimental animals, where breeder-to-stock ratios differ and sampling strategies may need to be adapted.

Key lessons learned and considerations

- Keep sample numbers consistent across strains and ensure they are large enough to capture natural microbiome variation (our pilot used 20 mice per strain).
- Recommended not to collect samples from more than two mice per cage to capture wider variability between cages.
- Clarify the purpose of sampling (testing vs. long-term storage/reconstitution), as this determines the vial type and storage method.
- Samples in DNA buffer can be stored at room temperature for up to two years.
- Samples intended for longer term storage at -80°C should be kept on ice immediately to prevent bacterial overgrowth that could affect results.
- Sampling may not always follow the exact protocol – record all deviations and maintain complete metadata to support accurate interpretation.



Towards sentinel-free monitoring

Sentinel use and health monitoring approach:

- All sentinel mice are CD1 females that were not successfully plugged. These are surplus animals rather than mice bred specifically for sentinel purposes.
- Blood samples are collected post-mortem and tested by serology to identify antibodies to infectious agents. Serology provides historical health information, indicating whether a mouse has been exposed to an infectious agent within the previous ~ lifetime exposure to dirty bedding
- PCR testing is performed on exhaust air dust (EAD) from AMUs, giving a real-time snapshot of infectious agents present in a particular sample.

Current challenges

- Some microbes do not reliably infect sentinels due to insufficient dirty bedding transfer/dilution/ poor environmental survival, meaning they may go undetected.
- PCR testing is highly sensitive and can detect environmental DNA from outside IVC racks or after autoclaving which may lead to false-positive colony results.

Ongoing improvements

We are conducting trials across our mouse units to evaluate sentinel-free IVC systems, using specialised filter materials designed to:

- reduce use of sentinel animals
- minimise false positives and improve the accuracy of true colony-level health monitoring

Aims

- Increase the overall sensitivity of our health-screening programme.
- Speed up confirmatory testing of unexpected results with a lower risk of environmental contamination.
- Enable more frequent monitoring for opportunistic pathogens particularly in immunocompromised lines.

Key considerations

- Environmental filter pooling is still under evalution and not yet validated and could lead to increased costs.
- Removing serology from the programme would require a review of our environmental PCR testing panels to ensure all relevant agents continue to be captured while maintaining the confidence of long term detection.

Supporting germ-free colonies

Germ-free (GF) mice are completely devoid of detectable micro-organisms and are maintained in sterile isolators with continuous positive pressure. These animals offer a powerful model for studying how the absence of a microbiota influences host physiology. GF mice can also be deliberately colonised with a single microbial species or with defined microbial communities to create gnotobiotic mice – animals with a known, controlled microbiome.

This enables researchers to investigate how specific microbes affect biological processes, disease development and drug metabolism.

Our team is collaborating with one of our experimental units to establish purpose-built isolators suitable for housing germ-free mice, expanding research opportunities in this area.

Sterilisation and validation

All materials and husbandry consumables are sterilised using steam (autoclave) or gas (ethylene oxide) depending on the material type. Delicate items require lower temperature sterilisation methods.

The effectiveness is confirmed by using chemical indicators and biological indicators which require an incubation period.

Fumigation

- Sampling materials are placed inside the isolator before fumigation with a sporicidal disinfectant.
- Minimum contact and ventilation times must be followed typically carried out over a weekend.

Collect samples for microbiological testing

- Multiple swabs are taken from the fumigated isolator for microbiological assessment.
- In-house testing: swabs are incubated in lysogeny broth where turbidity indicates bacterial growth.
- External laboratory testing: swabs placed in PBS and cultured for bacterial and fungal detection.

Import consumables and repeat testing

- No microbial growth should be detected in any samples.
- If a positive result occurs, repeat testing is required for confirmation.
- Once negative results are obtained, sterilised consumables can be imported into the isolator.
- The entire testing process is repeated one week later.

Import germ-free mice

After mice are introduced into the isolator, minimise and log port openings to reduce contamination risk. Establish a schedule for ongoing microbiological monitoring including:

- environmental samples: surface swabs, water, diet
- mouse samples: faeces, fur swabs

Challenges

- Risk of false positives due to benchtop contamination.
- Risk of false negatives if the sterilant inadvertently contacts the sample.
- Some bacterial species are slow-growing or require highly specific media or environmental conditions making culture difficult.
- Procedures require significant planning, preparation and scheduling.
- Contamination risk to the isolator when importing consumables.
- Physiological differences in germ-free mice compared to conventional mice (e.g. enlarged caecum), which must be considered during research, licensing needs and animal care.

Research values

Working with germ-free colonies enables researchers to investigate:

- How microbes influence host physiology.
- Interactions between specific microbes and the host.
- How individual microbial species affect drug metabolism.
- Enhances experimental clarity, reduces variability, and improves the consistency of research outcomes.

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