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Necessary, appropriate, valid:

A mantra for facility managers in creating complete health strategies.

The challenge of maintaining colony health

The importance of health monitoring

Providing researchers with laboratory animals that are free from major pathogens, as well as being suitable and robust enough for scientific research, is a daily challenge for facility managers. It is, therefore, critically important to maintain and monitor the health status of research animals in a clean, stable and controlled environment.

To achieve this, a robust program of health monitoring is essential, and yet this is a complex area with a constantly changing landscape. It is well documented that the health of the animal can impact research results and that the consequences of infection are serious. Compromised experiments can result in significant delays and have serious financial implications. They can also result in the avoidable euthanasia of infected – and affected animals, as well as damage to the reputation of the facility in securing future research. It is, therefore, crucial that any contamination is detected as soon as possible, and rapid corrective action is taken to minimize impact.

Additionally, when phenotyping transgenic lines, it is critical to fully understand external parameters - including the health status of the animal – to be able to discriminate between mutation effect and environmental effects on the phenotype. It is important that the animals are free of pathogens that may impact health and, ultimately, research results.

Throughout Europe and the rest of the world, animal facilities are striving to maintain a fixed and clean health status that is specific pathogen-free (SPF) or specific and opportunistic pathogen-free (SOPF). Establishing and maintaining a clean SPF or SOPF facility with a reliable reputation requires investment of time, effort and money. To ensure a good reputation and working business, it is essential that any researchers and/or collaborators have confidence that the health status of the facility is correct.

It is the role of the facility manager to maintain and control the health status of animals within the research facility. The Federation of European Laboratory Animal Science Associations (FELASA) has published lists of pathogens that it recommends be eliminated from animal facilities, as well as recommended methods of testing for those pathogens.[1] A challenge for the facility manager is that FELASA guidelines on the health

screening of animals and monitoring methodologies are constantly evolving, and specialized research models and genetically modified models introduce additional specific health concerns.

In an ideal world, animals would be kept isolated and access to them would be carefully restricted to avoid the entrance or spread of unwanted pathogens in a research facility. However, this system is not practical when working with investigators and researchers who want to handle and check the animals in order to perform experiments. Space to house animals is also an issue with large colonies, with recent mandated changes to animal density requirements typical of the pressures facility managers face.

Individually vented cages (IVCs) are often used to keep animals apart and in good health, reducing the potential for the spread of infective agents, while managing economic and ergonomic considerations. This, however, creates different challenges for the facility manager when designing a health monitoring program. For example, how to determine which animals are representative of the colony and should be sampled for the presence of problematic pathogens? Or, if using sentinels, how can the facility manager be sure that the sentinel animal has been adequately exposed to the exact same conditions as the other animals in the colony, especially when looking for pathogens with low transmission rates?

If for any reason a pathogen does enter the facility, it is crucial to be able to identify the source as soon as possible and take action, in order to prevent the spread of the pathogen and the contamination of too many animals, as well as to maintain the integrity of the facility. If infected animals are unknowingly used for research, the results can be invalid. The costs of refreshing and cleaning a facility following widespread contamination can run into millions of dollars.

Case study 1:

A research group investigated liver inflammation and cellular repopulation in mice; however, the mice were found to be infected with the mouse hepatitis virus (MHV). This meant that the research team were unable to determine if the results of their experiments were linked to the transgene of interest or to the virus, and their data were, therefore, unusable. The research facility housed 250 transgenic lines and rederivation or revitalization of each transgenic line was needed to overcome this MHV outbreak. The cost was around $2,500 per line, which alone produced a bill for $625,000 and a delay of approximately four months (if enough material had not been available to carry out the rederivation, the delay would have been longer). On top of that, a new facility was needed that was specific pathogen-free (SPF), and this was built at a cost of $3 million and a further one-year delay. The reputation of the affected facility was damaged, and the time spent on the experiments had been wasted.

The pressures of developing an effective health monitoring program

1) Replacement, reduction, refinement (3Rs): which tests are necessary?

Until recently, traditional health monitoring has required a number of selected animals. It was standard practice to assess the health of the colony using live animals that were euthanized and then tested. The facility manager was responsible for selecting the right animal or sentinel and performing the appropriate tests to create the health monitoring report. However, according to *The Principles of Humane Experimental Technique*, when working with animals in experiments, every effort should be made to:[2]

- Replace them with non-sentient alternatives
- Reduce the number of animals used to a minimum
- Refine the experiments, so that they cause the animal the minimum pain and distress

The 3Rs principle has led to many institutions pushing facility managers to stop using live animals for health monitoring. Facility managers need to constantly look for alternative options and, where appropriate, switch from the use of live animal testing. To address the 3Rs, non-sacrificial panel (NSP) tests have been developed that only require samples (such as fecal, serum, fur etc.) to be collected from either animals and/or their environments. NSPs also typically deliver a cost benefit, as they reduce the need to ship animals.

As well as NSP techniques, animal welfare can be enhanced by taking a common-sense approach to the testing of certain pathogens. For example, it would be reasonable to reduce the frequency of testing pathogens such as cryptosporidium or Hantaan virus, considering their low prevalence in laboratory rodents. This helps to reduce stress on animals, as well as cost to the facility. Similarly, it may be deemed unnecessary to subject an animal to a retest to confirm positivity for pathogens such as Streptobacillus Moniliformis or Clostridium Piliformi that present in an obvious and symptomatic manner in an animal.

The number of NSP health monitoring sampling techniques and testing methods has increased markedly in recent years, accompanied by an ever-increasing portfolio of pathogen targets available for testing. The latter has added to the pressure of health monitoring, as it is not always clear which pathogens are important and which are not. The risk of over-identifying unknown pathogens is that, upon detecting an outbreak, a laboratory immediately shuts down the facility and euthanizes animals at great cost and delay, when the pathogen identified may actually be harmless and its presence would have had no known adverse effect on research outcomes or be simply part of normal commensal flora. Monitoring strategies need to capture all necessary pathogens, and not waste time and money screening for unnecessary ones.

When running different panels of tests with different suppliers at different times of the year, it is also important that potentially problematic pathogens are not missed and leave gaps in the health monitoring that risk contamination and shutdown of the facility.

2) Is the test appropriate?

The challenge is not only to make sure that necessary tests are being carried out, but that they are the appropriate tests to clearly demonstrate effective health monitoring.

NSP testing is preferred to animal testing, but only where it can accurately detect the pathogens that it has been designed to identify. Each pathogen has its own infectious pattern of detection in an environment depending on the type of sample collected and the location of collection (oral cavity, fur, environment, fecal samples). Facility managers, therefore, need to understand that the agents screened for, and the detection techniques, will vary and produce a range of results with different reliabilities.

A mix of techniques should be used to ensure that the most appropriate method is being used for each target pathogen, and that the most appropriate sample is taken from the animal to best detect any contaminant based on its specific pattern of spreading. The scientific researchers and investigators must be confident that the health status is as accurate as possible and represents the current health of the whole colony.

Case study 2:

To validate pathogen screening results, Laboratory A was asked by its researchers to send 10% of the health screening samples to a third-party laboratory (Laboratory B). Laboratory A was confident in its testing procedure and the results; however, Laboratory B identified a number of contaminants. Analysis confirmed that Laboratory A's method was not sensitive enough to detect a number of agents. When sending different samples to different service providers, it is important to be sure that the observed results are representative of what is happening in the research facility, and not a reflection of the testing laboratory. The laboratory performing the screening must be able to provide formal robust scientific validation of its methods.

3) Has the test been validated?

When replacing live animal testing with NSP alternatives, it is important to have a full understanding of method test validity. Not all of the NSP tests available on the market can show a clear like-for-like comparison with live animal health monitoring. When selecting a technique, it is important to understand the strength of results, comparing published scientific validation (if available) with marketing claims made. When a facility manager selects test methods for required pathogen panels, it is important to understand the validity of the test to reduce the risk of false positive and false negative results, and if necessary to run additional, alternative tests to verify a result.

For example, a positive result from an environmental sample may require an additional test with a different methodology in order to verify the result. However, this does question the validity of a negative result from an environmental sample, which would not normally prompt a facility manager to seek a secondary verification test to be conducted. Utilizing scientifically validated techniques for target pathogens is, therefore, good practice, reducing the risk of missing an outbreak, and minimizing its impact on the wider colony, should it occur.

Interpreting results

Using multiple service providers can complicate health monitoring, and facility managers need to have confidence in their testing partners to determine an overall picture of animal health. Facilities that employ multiple laboratories to test for specific pathogens may receive little more than a "binary" positive or negative result, leaving the facility manager to collate reports into an overall health statement.

One might think that so long as all the results come back negative that a complete picture can be formed. One might also reasonably turn to verification testing of specific pathogens should one of the tests return a positive result. However, such binary reports make it difficult to form a conclusive overall picture with results from different samples and different labs conducting tests on different animals at different times.

Case study 3:

During routine screening of animal samples from a client's facility, Laboratory A directly observed intestinal parasites. The client submitted the equivalent of 100 fecal samples to a different commercial laboratory (Laboratory B) for fecal PCR and the results were negative. Upon retesting, using another method (fecal flotation), the parasite was detected. This example highlights that it is really important to understand the capabilities of the assays being carried out, and to use a range of tests if needed.

About the author

Emmanuel Gomas is the European Services Manager at Envigo. Emmanuel has 20 years' experience of working with laboratory animals, mainly in managing transgenic models colonies by either breeding or assisted reproductive technologies. He has collaborated with academic laboratories in France, as well as with industrial companies such as Charles River Laboratories and Envigo. Emmanuel is currently focused on new techniques in health monitoring and the potential benefits these can provide in colony management and animal welfare. Here he talks about the future direction of facility management and how Envigo is staying one step ahead in maintaining the health status of laboratory animals.

References

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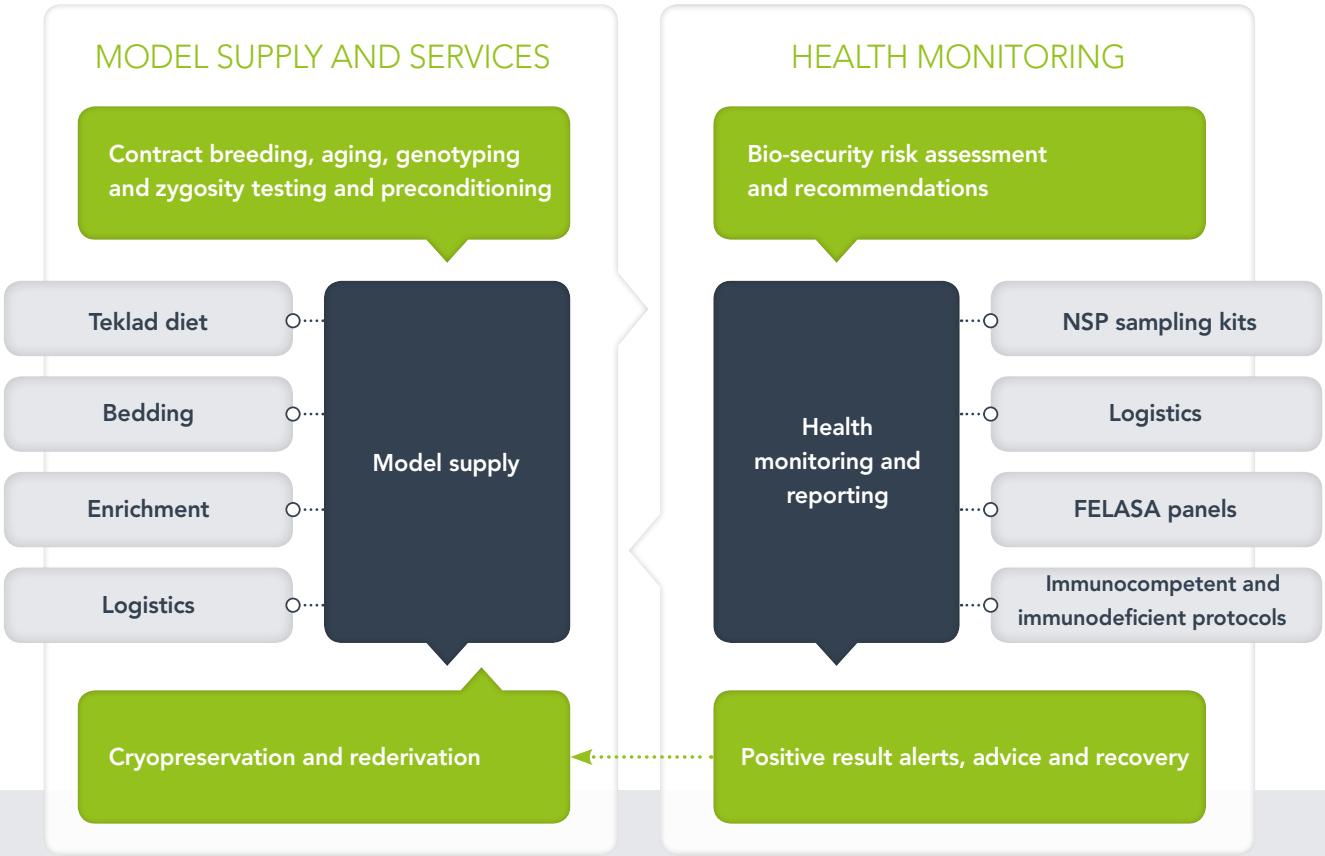

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