Maximising Efficacy of your Health Monitoring Programme

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ABSTRACT

There are a multitude of health monitoring techniques/methods available. It can be difficult to know which of these to use and when to use them. This is an attempt to simplify, maximise the efficacy of and ultimately **reduce** the number of animals used in Health Monitoring programmes. Whilst this is not exhaustive, and may not suit all situations it may provide some information that allows you to optimise your current methods and give greater confidence in HM results.

ROLLING SENTINEL PROGRAMME

This is where 2 to 4 sentinels are housed in a sentinel cage on each rack. For each screen interval (usually every Quarter) 1 or 2 are sampled by necropsy. If results of the screen are clear, then 1 or 2 new sentinels are introduced to the sentinel cage. On the next screen, the older sentinels are used.

Advantages…

Using only 1 or 2 sentinels where there would have originally been 3 used in each screen.

The sentinels left in the cage can be re-sampled and tested if there are any unexpected results, confirming or discounting the original result.

By using non-destructive sampling (tail bleeds + PCR) for 2 of the quarterly screens at 3 and 9 months, alternating with the destructive screens, the numbers of sentinels used can be reduced by 50%. This method also increases the sentinel exposure time therefore increasing the likelihood of any infections being detected.

Identifying "new" and "old" sentinels is essential for this to work.

APPROPRIATE SAMPLING

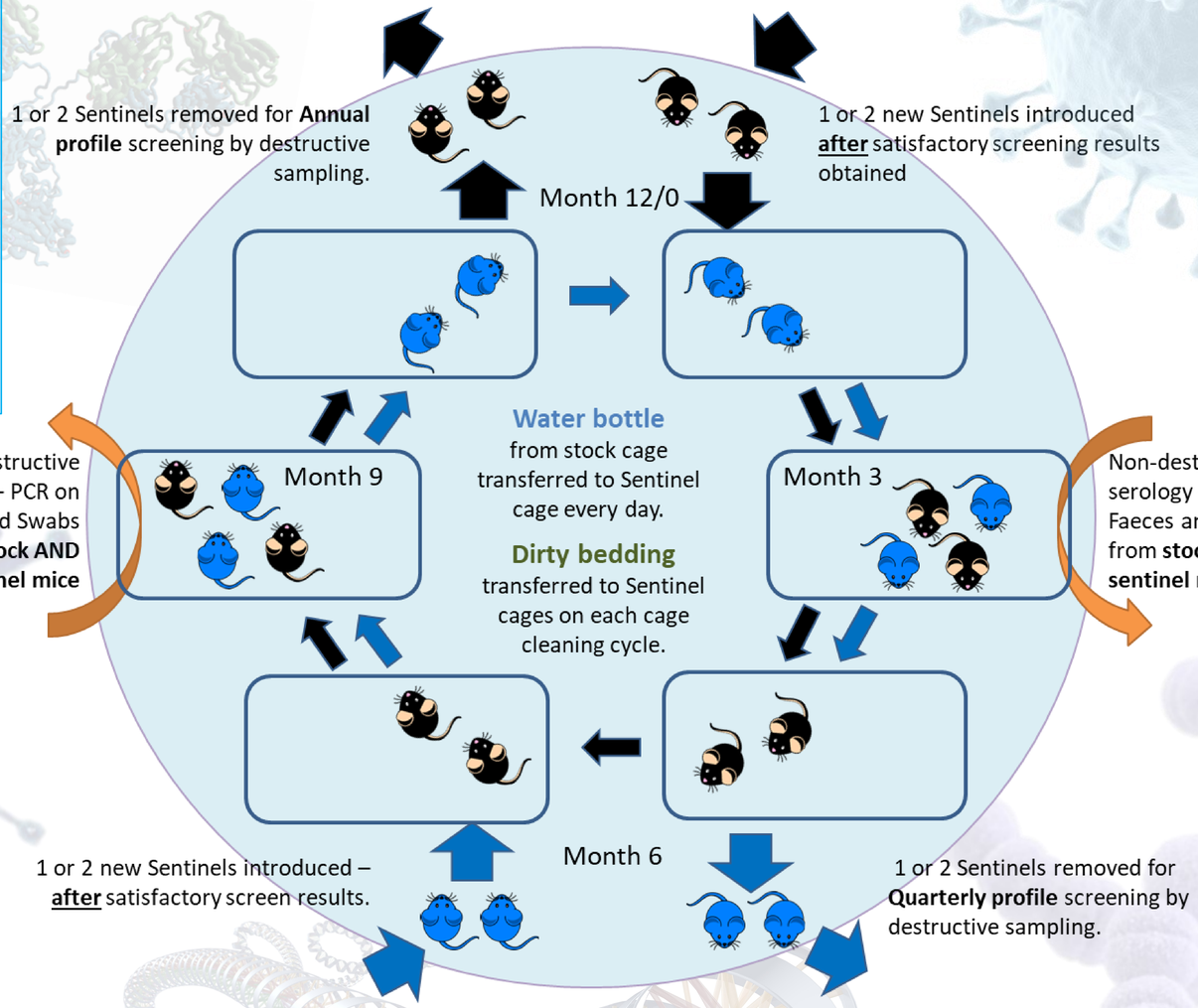
Wherever possible the samples taken for screening should be appropriate to the agents being screened for; faeces for GI infections, throat swabs for respiratory infections and so on. PCR allows samples to be pooled (up to 10 individuals) so decreasing the cost of screening.

Pooling large numbers of samples is tempting but can also be a problem. Faecal samples can vary in the amount of positive material present, so a weak signal can be diluted out in pools of large numbers, leading to false negative results .

Conversely, a positive result from a large pool will require further tests to pinpoint the originator(s) of the positive material.

EXHAUST AIR DUST SCREENING

A recent development in the application of PCR as a screening method is the use of exhaust air dust from the exhaust plenum filter of each rack/AHU. The main problem being that you cannot control for any background levels of nucleic acid from infectious agents originating from diet (from wild mice during manufacturing process or during storage) so its use as a primary method of screening is potentially problematic.



SOILED BEDDING TRANSFER PROTOCOL

There are some basic changes that can be made here in order to maximise the transfer of any infectious agents.

During each change, only 1 row on the IVC should be transferred to the sentinel cage.

Eg. Week 1—Row 1 and so on until all rows have been transferred at least once.

Only occupied cages should have their bedding transferred and the volume of bedding transferred should be adjusted to ensure maximum levels of exposure occurs.

Once all rows on a rack have been transferred, there should be a period of 4 weeks where the sentinels have clean bedding to allow any sero-conversion to take place prior to screening.

The transfer of dirty cage lids and dirty water bottles from stock cages to the sentinel cage on each soiled bedding transfer can help to increase the chance of transmission of any infectious agents to the sentinels, particularly those agents which don't transmit easily by soiled bedding alone. Any enrichment can also be transferred to the sentinel cage.

NON-DESTRUCTIVE SAMPLING

It is now possible to sample and screen animals effectively by using non-destructive methods…

-Tail bleed for viral screening by serological testing.

-Faecal, throat and fur-swab PCR for bacteria and parasites.

By combining these methods with a rolling sentinel programme, we can reduce the numbers of animals used by up to 50% without compromising the robustness of your screening. There may even be an increase in the effectiveness of sentinels by increasing their overall exposure time to any infections.

LIMITS OF TECHNIQUES USED

It is always worth bearing in mind that each technique used in health monitoring has its own limits. Such as, serology being indirect detection of viruses and PCR indicates only the presence/absence of nucleic acid of infectious agents with no information on infectivity. By combining these techniques with direct observation methods such as microbiological culture, parasitology by microscopy and gross morphology we increase the likelihood of zeroing in on genuine infections.

ASK YOUR SCREENING PROVIDER FOR ADVICE.

Health Monitoring is a complex process and it can be hard to know which techniques, or which tests to use. Also the interpretation of results and the significance of any findings can be confusing. The laboratory that carries out your screening for you, should be able to provide any support and advice needed, free of charge.

DISCUSSION

It is worth highlighting that all health monitoring assays currently available to us are not 100% "perfect". That is to say that each particular method on its own will have certain limitations on the knowledge that can be gained from its results. Eg Serology will only tell you if an infection has occurred at some point in the past, rather than an active infection. PCR can only tell you if nucleic acid from a specific agent is present or absent, with no information on whether the agent is dead or alive or indeed a product of an active infection. The more direct methods available in health monitoring of microbiological culture and parasitology by microscopy can only be carried out effectively by necropsy, but these methods do demonstrate that an active/viable infection is happening at the time of sampling, along with providing opportunity to gain an overall picture of the animals general health.

With these points in mind, the logical way forward, if we want to reduce the numbers of animals used in health monitoring programmes, without compromising the confidence in results, it is recommended that a programme which uses a combination of the methods available to us is used.

Relying on one or two methods for health monitoring potentially raises the likelihood of missing infections or the occurrence of false positives, which may be acted upon without the possibility of checking results by another method. It could be said that the "gold standard" in health monitoring should be to detect an infectious agent in the animals themselves by more than one method. The combined screening approach allows this to happen, as well as reduce the overall numbers of animals used. This approach also goes some way to fulfilling the recently proposed 5R's (Reduce, Refine, Reuse, Robustness and Repeatability).

REFERENCES

1. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig and rabbit colonies in breeding and experimental units. Laboratory Animals 2014. Vol 48(3). 178-192
2. Caveats of PCR. An overview of the caveats of PCR as a Primary Method of Laboratory Animal Health Monitoring. Dickinson. A, et al. LASA Forum, Summer 2016
3. False Positive Results after Environmental Pinworm PCR Testing due to Rhabditid Nematodes in Corncob Bedding. Leblanc. M et al. J Am Assoc Lab Anim Sci. 2014 Nov; 53(6): 717-724
4. If PCR is always the answer, then perhaps you are asking the wrong questions. Thompson A. Guest Editorial, Laboratory Animals Vol 12(8) Europe Aug 2012
5. Comparison of faecal PCR with traditional methods in the detection of Syphacia obvelata and Pasteurella pneumotropica. (Poster) *Dickinson et al,* IAT Animal Technology and Welfare 2015 Dec: 14(3): 221-222
6. Contaminating DNA can give false positives in "Sentinel Free" health monitoring by PCR on IVC exhaust air dust samples. (Poster) *Dickinson et al.* Laboratory Animal Science Association Annual Conference. November 2016
7. Efficacy of Direct Detection of Pathogens in Naturally Infected Mice by Using a High-Density PCR Array. Henderson. K.S. et al. JAALAS Nov 2013 Vol 52(6) p763-772.
8. Combined screening strategy to reduce the numbers of sentinel animals used, whilst maintaining confidence in results (Poster) Dickinson A, IAT Congress March 2017

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