Combined screening strategy to reduce the numbers of sentinel animals used, whilst maintaining confidence in results.

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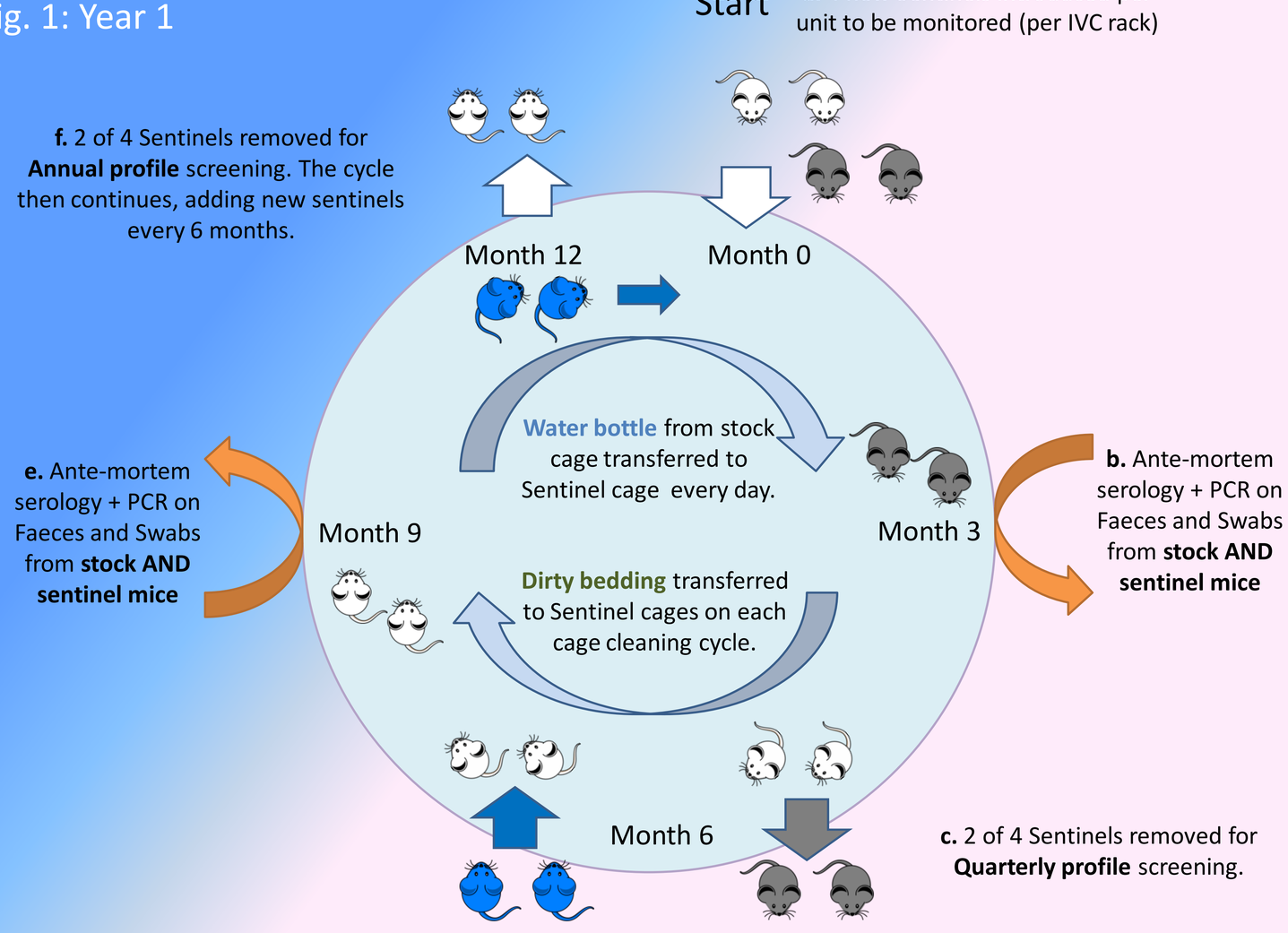
ABSTRACT

In recent times there has been a considerable focus on PCR/RT-PCR being sold as the "Gold Standard" in laboratory animal health monitoring. This however is sadly not the case. Whilst these molecular screening methods are very useful in the right situation, the use of PCR alone in the context of animal disease detection does not provide all the information required (as with any method, when used on its own, any limitations or "gaps" in data can potentially cause problems). What we propose here is a combined approach to LA Health monitoring where both the "traditional" methods (serology, microbiological culture and microscopy) are used alongside non lethal serology and molecular methods to give added confidence in results and a more rounded picture of a colony's health status, whilst at the same time allowing a significant reduction in the number of sentinel animals used.

Fig. 1: Year 1



ROLLING SENTINEL PROGRAMME

This is where 4 sentinels are housed in the sentinel cage on the rack. Then for each screening interval, 2 culled for sampling for screening via serology, microbiology and microscopy. If results are clear, then 2 new sentinels are introduced. On the next screen, the two older sentinels are used.

The advantages are...

1. You are you using 2 sentinels where originally the recommended 3 would have been used.
2. There is also a back-up. If you find unexpected results, there are 2 more sentinels to use in order to verify those/dismiss those results.

By using non-lethal sampling (Tail bleeds + PCR) for 2 of the quarterly screens at 3 and 9 months, between the live animals tests, the numbers of sentinel animals used would be reduced by up to 50%. In addition to this, you are doubling the sentinel exposure time.

DIRTY WATER BOTTLE TRANSFER

It is known that respiratory pathogens don't transmit to sentinels efficiently via transfer of dirty bedding alone, so it is recommended that the use of water bottles may be a good way to compensate for this . Every day, one of the bottles from a stock cage on the rack is moved to the sentinel cage so increasing the chances of any respiratory pathogens being transferred to the sentinels.

EXHAUST AIR DUST SCREENING

A recent development in the use 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 DNA from infectious agents which may originate from wild mice (during the diet manufacturing process) so its use as a primary method of screening is potentially misleading. If this method is to be used, it might be as a confirmatory test to back up the combined method described here but this would need to be verified.

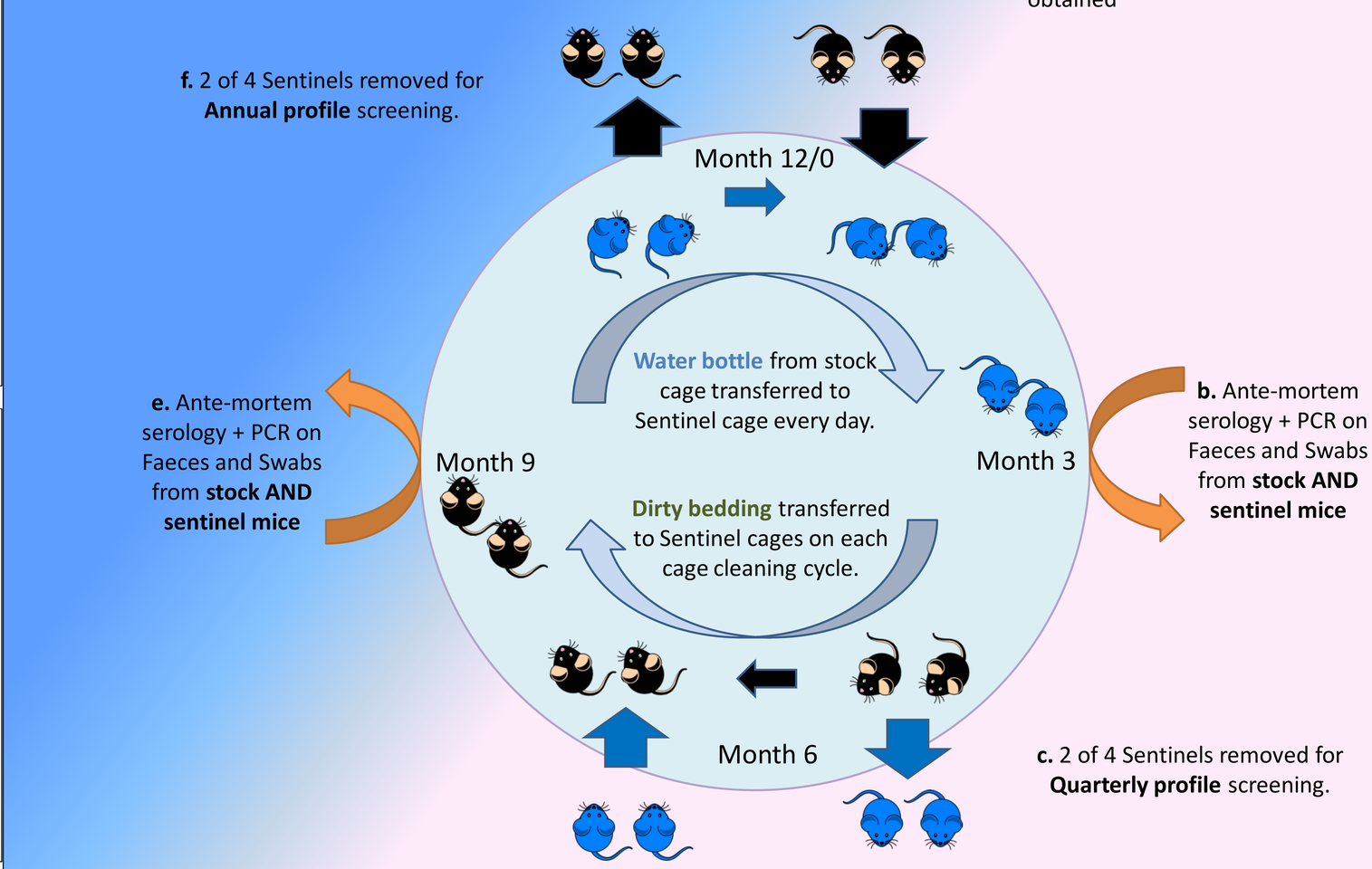
DISCUSSION

Unfortunately there are no "perfect" techniques available for laboratory animal health monitoring, we can however seek to minimise the risk of false positive and false negatives in the technologies we have currently. The combining of ante-mortem serology and molecular methods with the more traditional screening techniques is a step towards this and should give a clear and robust overall picture of the health status of the colony being tested and ultimately reduce the number of animals sacrificed for screening by as much as 50%.

Relying on one method for health monitoring potentially raises the likelihood of missing infections. It could be said that the "Gold standard" in health monitoring should be to detect an infectious agent in the animals themselves, preferably by more than one method.

There could be potential to reduce the numbers of animals used further by omitting the Quarterly live screen , but this may compromise the confidence in results.

Fig. 2: Subsequent years



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)
   Hazelby 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.

ACKNOWLEDGEMENTS

Thank you to A. Thompson, H. Donnelly, O. Hazelby, F. Ward and D. Pimbley for their support.

Hello to Jason Isaacs