Contaminating DNA can give false positives in “Sentinel Free” health monitoring by PCR on IVC exhaust air dust samples.

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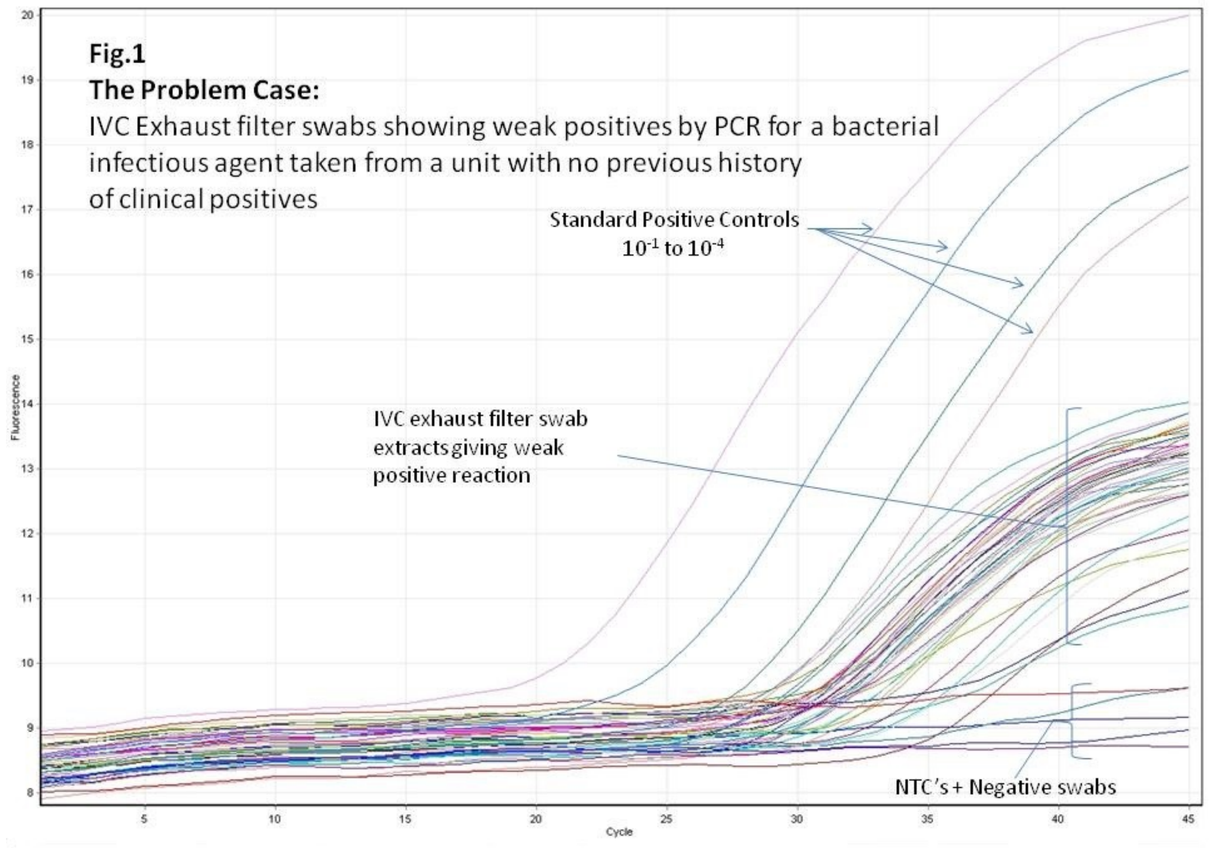
Abstract

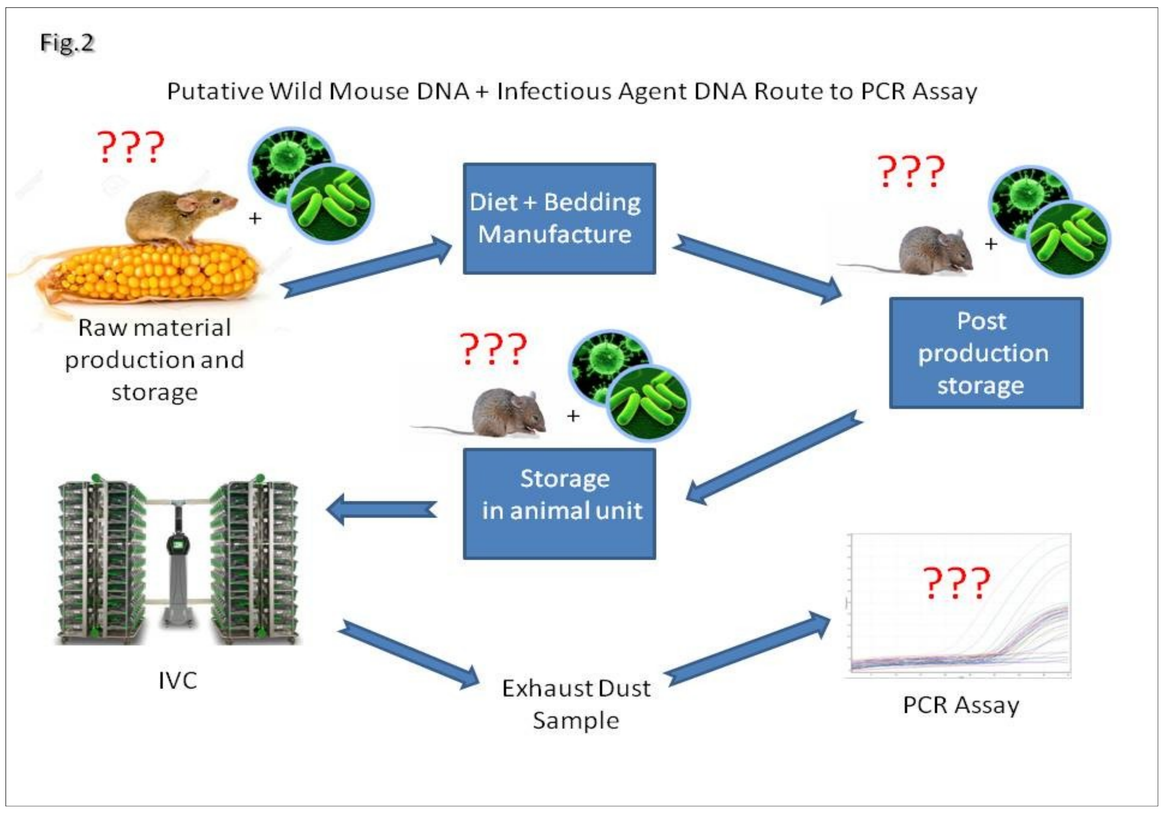
Following the introduction of “sentinel free” animal health monitoring by PCR on the dust collected on filters in the exhaust plenums of IVC (Individually Ventilated Cage) racks, we present a case where exhaust filters gave positives by PCR for an infectious agent which had not previously been found by microbiological culture in that colony on multiple live animal samples across the lifespan of the IVC exhaust filter.

Our own experience, along with other accounts of false positives from other users, possibly due to the presence of contaminating DNA of an environmental source such as diet and bedding, led us to the hypothesis that the sentinel free PCR method could be prone to false positives due to the presence of contaminant DNA from infectious agents of mice. This could be due to the ingress of wild rodents during the storage of raw materials prior to the manufacturing process and in subsequent storage. The diet and bedding used in IVCs both contribute to the dust collected on the plenum filters which will then be sampled and tested, subsequently, any agents detected will be assumed to be present in the animals. There may also be a concentrating effect in play if filters are left in situ for prolonged periods.

Using Real-Time PCR for detection of the mouse Cox1 (Cytochrome Oxidase 1) gene, we found that mouse DNA was indeed present in “clean” diet and bedding at a low level. This therefore could be a mitigating factor in the reliability of health monitoring by PCR on plenum filters alone.

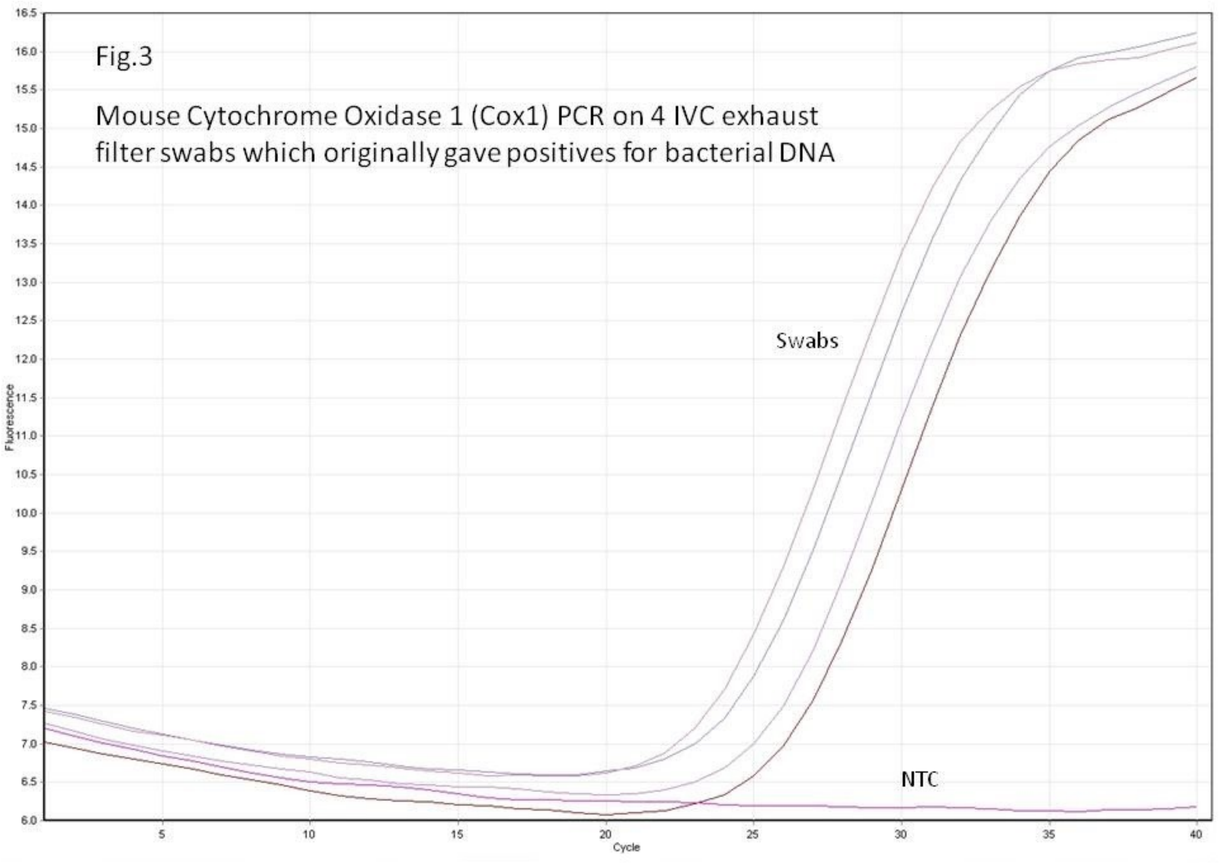
These false positives on plenum filters cost the research facility a considerable amount of time and money through subsequent extra testing to track down the source of the problem.

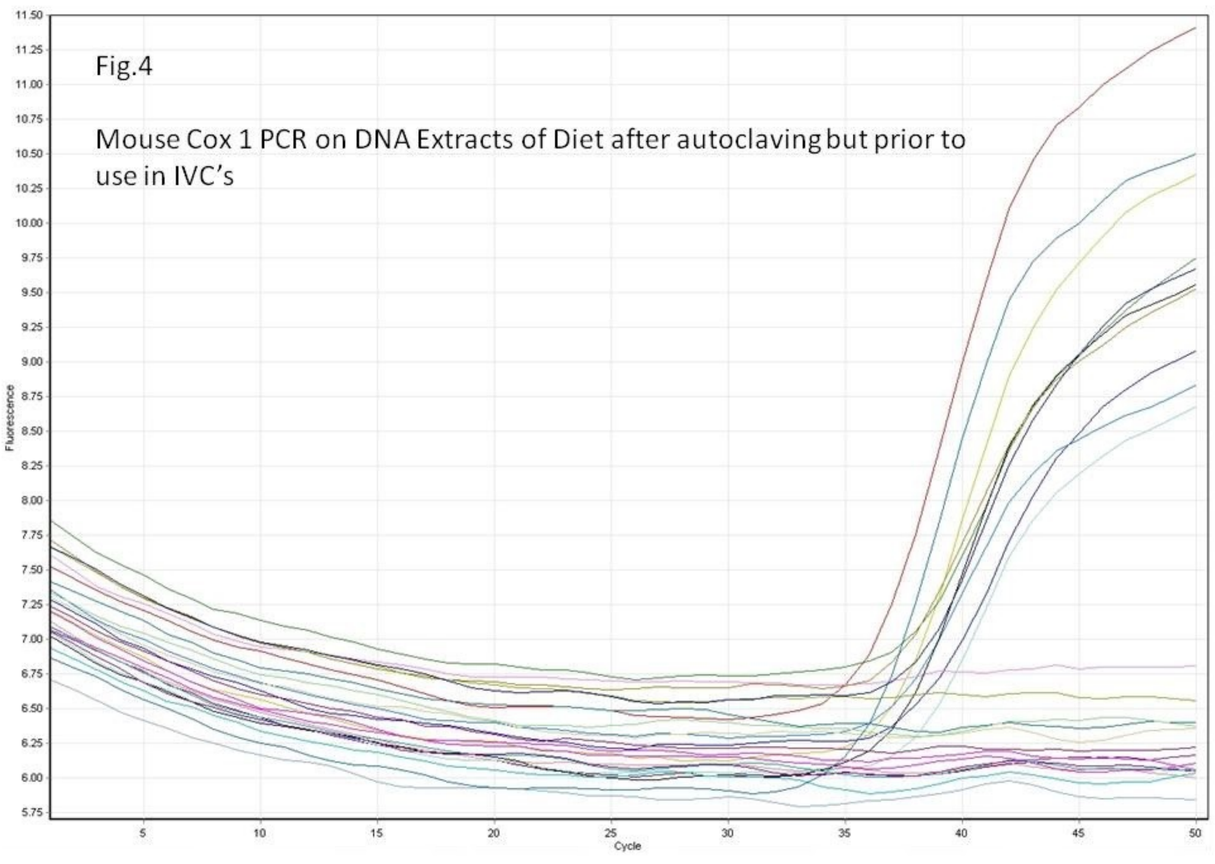




Materials and Methods

All filter swabs were supplied by the client along with 13 diet samples. The diet samples were collected before being used in IVC racks and after autoclaving at 115°C for 20 minutes with 15 minutes cooling. 6 additional diet and 4 bedding samples were collected from various suppliers. All extractions were performed according to the relevant standard operating procedures. Extracted DNA was analysed with a NanoDrop One and normalised samples, along with standard controls (Mouse Lung DNA extract) and Non-Template Controls (NTC’s) were amplified using Real-Time PCR on a Qiagen Rotor-Gene Q using Mouse Cox 1 specific primers and a fluorescent labelled probe.





Discussion

The results clearly show the presence of Mouse DNA on both the IVC exhaust filter swabs (Fig.3) and in the diet samples (Fig.4). A sample of bedding was also found to be positive for Mouse DNA (data not shown). It is not surprising that there is a strong reaction in the swabs as a large amount of mouse DNA would be coming from the mice housed In the IVC rack and collecting on the exhaust filter. However, significant numbers of the “clean” diet samples show a weaker reaction than the swab samples, but at a level which cannot be ignored. From this data we can surmise that the Mouse DNA detected in the diet must be from the ingress of wild mice at one or more of the points highlighted in Fig 2. From this we can deduce that the low level response from samples in Fig.1 is detecting DNA from the infectious agent carried by the wild mice. The DNA from such infectious agents, whilst not causing any clinical disease has the real potential to give false positive results in IVC exhaust dust screening by PCR. This adds to the mounting evidence that DNA from infectious agents is present in feed and bedding. Parvovirus (1) and Pinworms (3) have been detected by PCR which have later been found to be from an environmental source.

It is a real concern that false positives from this method of screening could lead to the unnecessary loss of healthy animals along with valid research data.

PCR remains to be a very useful method in laboratory animal health monitoring provided that it is **used in the right context**, **on the most appropriate sample types**. The limitations of the use of PCR in different situations need to be understood and advice should be given to the end user.

It is hoped that future development and refinement of health monitoring techniques will enable the screening of IVC racks by exhaust dust without false positives. Until this happens, it is recommended that if this method is to be used, it should be in conjunction with other proven techniques (Serology, Bacteriology, Microscopy and PCR on appropriate samples from live animals).

References

1. “Risk of introducing parvovirus through food”. van de Ven E. et al, QM Diagnostics, Netherlands. Presentation given at FELASA 2016.
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. Advances in PCR Improves Health of Animal Research Colonies. Henderson K. R&D Magazine. Jan 2015

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