Caveats of PCR

An Overview of the Caveats of PCR as a Primary Method for Laboratory Animal Health Monitoring.

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The **Polymerase Chain Reaction (PCR)** is a process used in molecular biology to amplify a single copy or a few copies of a piece of DNA by several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence so that the presence of the DNA sequence can be demonstrated, for example by gel electrophoresis (end-point PCR) or by a specific fluorescent labelled probe (real time or quantitative PCR). If the sequence of DNA is unique to a particular organism then the presence of the DNA is taken to indicate the presence of the organism and with quantitative PCR, the number of organisms present in the sample.

In the field of laboratory animal (LA) health monitoring there has recently been a trend to rely increasingly upon PCR as the primary method for detecting infectious agents. PCR is indeed a very valuable tool in LA health monitoring and has been used successfully for many years as confirmatory testing of positive samples found by serology and bacteriology, but it would seem that the caveats of using PCR as a primary method for animal health screening have been ignored or minimised when PCR screening is offered as an alternative to the "traditional methods".

The movement towards wholly PCR based screening raises some concerns which we aim to illustrate here.

PCR will only detect the agent that it is looking for. Citrobacter rodentium (a biotype of C. freundii), is included in PCR screening programmes, but the specificity of the test may be problematic. We have seen a case where a hydrogen sulphide producing Citrobacter freundii (not rodentium) was detected by culture in rats but was not detected by PCR as this is very specific for this particular rodentium biotype. Although C.freundii is not a problem for the rats themselves, it does pose a potential health risk to staff handling these animals.

Another example is one of Acinetobacter baumanii, which can be used as a research model for pneumonia infections. This was found by culture in an animal facility and would be a serious problem for respiratory research. Acinetobacter baumanii is not in the FELASA guidelines and would have been missed if using a PCR only screen. Culturing of live bacteria therefore has the advantage of detecting unexpected infections, which although not immediately part of the FELASA recommendations, may have serious consequences.

PCR will amplify DNA/RNA from both live AND non-viable organisms. There is the possibility PCR will detect nucleic acid from non-viable agents in the environment. This is especially relevant as PCR is now being used to test the exhaust plenums and filters in IVC (Individually Ventilated Cage) racks which will trap dust produced from sources such as diet and bedding in addition to the wanted particulate material from the animals. In our own experience we have detected Helicobacter spp DNA in diet but we are not aware if any studies have been done to check if these residual levels of DNA in diet, or those potentially in bedding, can affect results of PCR testing of exhaust air, or indeed whether the exhaust plenums and/or filters can, as we suspect, concentrate environmental DNA contamination. Diet and bedding suppliers are presumably exposed to the risk of ingress from wild rodents in raw material storage facilities and therefore is a possible source of contaminating DNA. Finally, because of its sensitivity, this method of testing is prone to false positives in subsequent testing if routine cleaning is not sufficiently thorough to remove residual nucleic acid 1.

Many viruses are not continuously shed, so an infection could possibly be missed.

MPV is only shed in mouse faeces for approximately 6 weeks.n Testing by PCR alone has a relatively small

window of opportunity to detect its presence. This is where serology of sentinels has a real advantage, detecting the "footprint" of an infection for a much longer time after the infection has occurred.

PCR Sensitivity. PCR is able to detect a small number of copies of target DNA/RNA, in some instances as low as n=1, representing 1 infectious agent in the sample tested. While this level of detection is impressive, the significance of this high sensitivity depends of course on how many infectious organisms constitute an infectious dose and therefore such results must be interpreted with caution and with full knowledge of the agent in question.

PCR specificity. The specificity of the primers used is extremely important and should be carefully considered when developing a PCR system and also when interpreting results. For example, some PCRs will be designed to show the presence of a particular genus, e.g. Helicobacter spp, but others will distinguish between biotypes of a particular species e.g the Jawetz and Heyl biotypes of Pasteurella pneumotropica. Careful design of primers is vital, ensuring that they do really correspond to unique sequences in the target organism, for example there have been cases where a unit was thought to have a pinworm infection. On closer examination the test was found to also detect non-pathogenic Rhabditid worms since initially, it hadn't been determined that the target sequence was not unique to the pinworms 2.

There has also been a case in a facility where Clostridium piliforme had been consistently detected by PCR. This was later found to be false positives due to inadequate verification of the primers being used, so detecting other Clostridia species.

PCR requires a certain amount of expertise both in designing primers (see above) and also in interpretation before giving results to researchers. PCR can at times behave in an unpredictable manner and a certain amount of scrutiny is needed, which can only be done by people with a good experience of performing PCR.

We believe that PCR is a very useful method to have at your disposal, it's one tool in the box, but mostly as an adjunct to "traditional" methods of screening not as a replacement for them, additionally it is important to use an appropriate sample and number of samples in relation to the agents being screened for, the size of the colony and the likely incidence and persistence of organisms in infected individuals. PCR, ideally should used as a confirmatory test for positive results from bacteriology, serology and parasitology. One situation where wholly PCR based screening could be acceptable is in the case of quarantine isolators where numbers of animals are limited and traditional invasive sampling is not possible. There are also some instances where there is no alternative, for example with immuno-incompetent animals when serology cannot be used, or to detect organisms which are extremely difficult to culture such as Helicobacter.

The use of PCR, properly applied, can lead to a reduction in the number of animals needed for health monitoring which is a very desirable outcome, but it is a real concern that without properly considering the limitations of PCR testing as outlined above, there may be more animals used in the longer term in research confounded by missed infections which may subsequently come to light.

With these points in mind it would seem that a multi-disciplinary approach to LA health monitoring, using the most appropriate methods would be the way forward, giving the most informative results possible.

References.

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