If PCR is always the answer, then perhaps you are asking the wrong questions

There has been an increasing move by some companies to offer complete screening services based solely on a panel of polymerase chain reaction (PCR) tests, mostly carried out on faeces. Although this seems to offer a fantastic opportunity to screen an entire unit by just submitting a few pooled faecal samples and possibly some fur swabs to look for mites, I am concerned that this methodology is giving some facilities a false sense of security that they are clean when, in fact, pathogens are present but were overlooked.

Using PCR to screen for multiple viruses and bacteria was first done on a regular basis to screen cell lines and transplantable tissues for contamination. This was seen as the perfect replacement for mouse antibody production (MAP) and rat antibody production (RAP) testing, in which cells were injected into a mouse or rat to infect the animal with any murine pathogens present in the cells. The animal was then left for a few weeks to seroconvert to any infections that had been transmitted and then tested serologically to look for antibodies. PCR has two major advantages over the traditional MAP and RAP methods. First, no animals are used, and second, the turnaround time is reduced from weeks to days. This still is a perfect use for PCR technology: testing an aliquot of what should be a homogeneous sample, so any sample is representative of the entire vial of cells.

Faecal pellets: single, pooled, perhaps not at all

The major difference between this and the testing of faecal pellets is that a single faecal pellet from a rodent may or may not be representative of the mouse or rat that produced it. It may or may not contain genomic material from any pathogens with which the animal is infected. The chances of finding viruses or bacteria in faeces depend on which particular organ or tissue is targeted by the organism. An agent that affects the gut is much more likely to be shed in the faeces than an agent that resides in the lungs. Some agents are shed in the faeces for only a short time post-infection, and some shed intermittently. Others may not be reliably excreted in the faeces at all.

Many PCR-based screening protocols will suggest that one pellet from each of ten cages can be pooled into one sample that will cover all the animals in all ten cages. These protocols also suggest that oral swabs and fur swabs be submitted to detect the entire range of pathogens included in the panel. This one sample per cage does not serve as a reliably representative sample from those ten cages of animals, especially if only faeces is tested, as is often the case.

There are other reasons to be concerned that PCR-based screens may not detect all the infectious agents present. When an animal is examined for ectoparasites, all arthropods seen will be collected and identified. The fur mite PCR tests currently available will detect many common mites, including Myobia and Mycopte,s but not Ornithonyssus. A negative PCR result could give a false sense of security that the animals were mite-free, when they were not. Similarly, by using a mixture of specific and non-specific agars, routine bacteriology will detect any possibly pathogenic bacteria, not just a strictly defined list. An example of this is that many PCR-based tests look just for Pasteurella pneumotropica, whereas the Federation for Laboratory Animal Science Associations (FELASA) suggests reporting any bacteria from the family Pasteurellaceae.

A positive is not always positive and vice versa

At the other end of the spectrum, there is always the chance with PCR that inconsequential results may be raised to the level of positive results. Some companies now claim that their PCR tests can detect as little as a single organism. What is the clinical significance of a single organism, especially when that organism could be dead? A few years ago, a client was comparing Surrey Diagnostics with another laboratory for Helicobacter PCR. We were finding positives where the other laboratory was not. This perplexed the client, since we were both consistent in our findings. The client ran a blind trial where both laboratories were sent the same samples. We found half strongly positive and half weakly positive; the other laboratory only detected the strong positives. The samples consisted of duplicates of positive samples, where one had been autoclaved and one had not. Does this result mean that we had the superior methodology, as we could still detect Helicobacter DNA at the much lower levels found in the autoclaved samples, or that our test was inferior as it was too sensitive? Do we really want to detect what could be clinically insignificant levels of genomic material from non-viable organisms, particularly when this could lead to expensive searches for potential nonexistent infections?

Many people think that PCR will give a 'black and white' answer, but, like with many other techniques, the results come in shades of grey. The experience and expertise of the person interpreting the results can make the difference.

PCR-based screening is an important tool that was developed for very good reasons. But no one method should be relied upon completely to give the most accurate results. Traditional screening also has limitations: the sacrificing of animals for screening may not always be possible, and not all agents are easily transferred to sentinel animals in individually ventilated caging dirty bedding programmes. PCR can give a snapshot of the current status of an animal, for example, whether it is shedding virus and is therefore a major threat to a facility. It is immediate and can detect new infections but may miss older infections where shedding has decreased or stopped. Serological techniques, on the other hand, give a historical perspective of what the animal has been exposed to during the entire screening period but are not appropriate for detecting infections that are only days old. Using PCR-based screening methods as an adjunct to conventional screening, when necessary and appropriate, will give screening results an added level of confidence and should be considered the gold standard.

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