ALLIANCE™
(https://debug.globalseafood.org)

Responsible Seafood ADVOCATE

Health & Welfare

Polymerase chain reaction: How useful is it?

1 November 2014

By Stephen G. Newman, Ph.D.

PCR is a powerful tool, but not a total solution

The mere presence of a pathogen in a culture system at the low levels that can be identified by PCR does not, in itself, result in disease.

Reflecting on how disease diagnosis has evolved over the last 100 years or so, one sees a gradual progression in specificity and sensitivity. Today, nucleic acid-based detection technologies are currently at the forefront.

Pathologists can look at how tissues change as a result of disease processes and describe what they believe to be the cause of a given problem based on these changes. However, this does not always give exacting information about the causative agent. Sometimes the etiologic agents are not characterized, while at other times, the changes could be due to any number of potential pathogens.

The discovery of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), and the subsequent invention of the technology that underlies polymerase chain reaction (PCR) testing yielded a very powerful tool that can, when properly used, detect very low levels of a given pathogen. However, as with all technologies, there are practical limitations.

Defined procedures?

The American Fisheries Society's Fish Health Section publishes the *Blue Book, Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens*. This outlines the procedures one should follow for diagnosing disease in fish and, by extension, shellfish such as shrimp. The manual also details a statistical basis for population sampling based on assumed prevalence levels to ensure high levels of confidence that specific pathogens are or are not present in populations.

Screening for the presence of pathogens is an essential step in limiting their potential impacts. The mere presence of a pathogen in a culture system at the low levels that can be identified by PCR does not, in itself, result in disease.

The sampling protocols as outlined in the *Blue Book* are based on a number of assumptions. The first is that the technology used to look for pathogens is 100 percent accurate and will always detect them, if present. The second is that random samples can be taken. Finally, the technology is accepted as specific for a given pathogen and will not react with similar pathogens.

These requirements are theoretical and rarely, if ever, achievable in the real world. Thus, the ability of DNA detection technologies to screen for the presence of a pathogen with a high degree of confidence and that the results represent the population is in fact oversimplified. The current challenges with early mortality syndrome (EMS) in shrimp serve to highlight this.

Meaningful accuracy

The etiologic agent(s) of EMS, more accurately described as acute hepatopancreatic necrosis, are strains of *Vibrio parahaemolyticus* that carry toxin-producing genes on plasmids that allow these strains to produce the characteristic pathology. PCR probes that are specific for these genes have been devised, but the problem of sensitivity is an issue.

Ultimately, the focus is on the minimum assumed prevalence level. When one is concerned that even a very low level of prevalence can potentially be problematic, then one must screen the population in a manner that is consistent with finding the very few animals that are carrying the pathogen.

The *Blue Book* states that to have a 98 percent level of confidence that a given pathogen is not present, 150 animals must be tested for populations that are greater than 100,000 animals. This 98 percent figure is based on random sampling and 100 percent test sensitivity.

Random sampling is not straightforward, and the ability of any given test to provide definitive and utile results should never actually be based on a single series of test results. The presence of disease symptoms is important, as is the history of the population. All of these factors must be considered in concert to ensure that the conclusions reached from PCR-based screening are as close to valid as the tools can give.

Although it remains to be proven, the bacterial strains that cause EMS are likely ubiquitous once they become established in marine environments. The genes are readily spread among bacteria. As part of a responsible screening program, where the goal is to avoid introduction of the pathogen to clean environments, screening of broodstock, postlarvae and even potential vectors in incoming water supplies and pond environments is suggested.

If broodstock sampled in a maturation facility are found negative, one can only be confident they are, in fact, not carriers if the history of the facility is consistent with ensuring that infection cannot take place. An example of this would be a nuclear breeding facility that has been closed to external factors for generations. This facility is much less likely to carry the bacteria than one where the animals have not been held indoors in highly controlled production systems for years. Screening of the wrong tissues, too few animals, etc. can lead to conclusions that a population is free of the bacteria when it is not.

In theory, for a meaningful level of biosecurity, each adult should be screened in those facilities where there are real risks of contamination. This is costly, stressful and therefore usually not done. Screening postlarvae in hatcheries is also potentially problematic.

First of all, getting a random sample from a tank is challenging if not impossible. Secondly, when bacteria are present at very low levels, there is a risk of false negatives. To maximize the ability of the PCR to detect very low levels of prevalence, it is smart to enrich. Considered the global standard for the

detection of *Salmonella* and *Escherichia coli*, enrichment entails using selective media to encourage the growth of the organism of interest so that it can be detected. Yet there are many circumstances in which PCR results are negative when, in fact, bacteria are present.

Perspectives

Many questions remain unanswered about EMS, although it appears stressors are important in impacting susceptibility, a common component of many shrimp diseases. Also, the mere presence of the pathogen itself does not necessarily result in disease. Similarly, the fact that PCR is negative should not be taken as a universal affirmation that pathogens are not present.

Nonetheless, it is in producers' best interests to ensure that stocking infected animals does not occur. PCR is a powerful tool, but not a total solution. Many other factors must be considered to ensure that results are real and not a result of limitations in the technology.

(Editor's Note: This article was originally published in the November/December 2014 print edition of the Global Aquaculture Advocate*.)*

Author

STEPHEN G. NEWMAN, PH.D.

President and CEO
AquaInTech Inc.
6722 162nd Place Southwest
Lynnwood, Washington 98037-2716 USA

sgnewm@aqua-in-tech.com (mailto:sgnewm@aqua-in-tech.com)