Testing of penaeid broodstock for multiple pathogens - should it be mandatory?

*By: Stephen G. Newman, Ph.D.**

Shrimp farming has expanded rapidly over the last three decades. Current estimates are that somewhere between 3.5 and 5 million metric tons of farmed shrimp are produced annually.

Much of this is the white shrimp, *Penaeus vannamei*. Most production is from a handful of countries with the crown for the world's largest producer shifting as quickly as can the score in a football game. The reasons for this are complex but tend to center around costs of production. The lower the cost of production the lower the potential selling price. Some producers cannot produce shrimp profitably unless the prices are high. More often than not this is because of the impact of disease on the crops. This reduces survival rates and can dramatically increase costs.

It was recognized some years ago that Specific Pathogen Free (SPF) broodstock, known to be an effective tool in terrestrial agriculture, could be of benefit. It is important to appreciate that SPF does not mean free of all pathogens. It also has no bearing on the susceptibility of these animals to the specific pathogens that they are free from, although tolerance and resistance can be present in SPF stocks. These are not population traits that result from being SPF. Creating SPF animals is a process, not a claim based on periodic testing. Constant screening and history are both needed to establish that a population is truly SPF.

Selling SPF shrimp broodstock has become big business. With well over one million adults being sold annually as the source of post larval

shrimp (PLs) for farms all over the world and a myriad of companies focusing on developing strains of shrimp that are uniquely theirs, the risks are significant that exotic diseases can and will be spread. This is not necessarily a result of inherent dishonesty, although as with any business "caveat emptor-let the buyer be aware" is always the wisest approach to take.

Most shrimp broodstock companies use conventional approaches towards screening for the presence of pathogens. Long term histories of the performance of PLs from a given source of broodstock is a critical element in ensuring that one is not moving pathogens between shrimp and is essential in the process of developing SPF animals. This data is very hard to get at and in most cases of limited usefulness as farmers do not routinely engage in proactive animal health practices and are unaware of what is killing their shrimp. Very few companies can claim to have this data.

The conventional approach to testing entails the use of the polymerase chain reaction (PCR). This is a powerful technique that allows one to detect very small amounts of specific DNA/RNA via amplification with homologous nucleic acid sequences. The overall test utility is based on sampling of a population and blue book fishery statistics that are applicable to fish populations. These were derived for using PCR to test for the presence of specific pathogens in fish and were not developed for screening broodstock on a population basis. PCR is not a viable tool for this and its widespread use in shrimp farming has done little to stem the movement of pathogens across borders. Given that this was the only tool available for many years the perception has been that it is better than doing nothing. That may have been the case but conventional PCR testing is ready to be replaced by a better tool. Some of the reasons for this are explained in Table 1:

Table 1

The myth	*The reality*
When you test a population for the presence of a given pathogen and the sample is negative it means that the pathogen is not present in the population	All one can say is that a given PCR result is positive or negative. It cannot be used to state that a given pathogen is not present in the population if the test result is negative. The sample is negative- not necessarily the population.
Testing at a 95% to 98% level of detection is good enough.	To achieve this level of detection requires at least three things. One is that the sample is random. This is not as simple as it seems. The second is that the technology is 100% accurate. Most PCR tests are highly sensitive and accurate (specific). However that does not mean invariably that they are used properly. As an example, detection of the WSSV requires that the animals being tested are held at water temperatures that are consistent with development of a viremia. Most PLs and broodstock held at 31 C or above will test negative. The third consideration is that pooling samples reduces test sensitivity. Pooling animals, while it saves money, reduces the value of the results.
The presence of a pathogen means that the population is diseased.	For broodstock, where even a single animal can result in the presence of undesirable pathogens in PLs and end up on the farm affecting animals, 95% to 98% detection is not good enough. If one tests post larval shrimp using 150 PLs and pools them, and the individual tests that are performed come back negative, for each 1 million PLs being tested, even if the 98% detection were accurate, there could still be 20,000 PLs carrying a given pathogen. Pathogens are often present in the absence of pathological processes that are the result of active disease. While it is important to know if a given pathogen is growing in a population of animals and how this impacts production, the mere presence of the pathogen does not mean that disease will be the inevitable outcome.

The next step in PCR testing

In the last few years a new technology has appeared. This allows one to screen for a large number of potential pathogens from single samples at levels of specificity and sensitivity that are equal to or better than single tests. This multiplex technology uses a different approach for quantifying amounts of DNA or RNA present in the sample and has resulted in a dramatic drop in the costs to perform real time PCRs. Currently testing for 13 pathogens in a single sample, the price per individual PCR (based on testing for all 13 at once) is low enough to test EVERY individual broodstock. This is a powerful tool that ensures, when done properly, that a given pathogen is not present at any level in a population.

Since the term SPF is not widely appreciated as being the result of a process that includes consistent and constant screening, historical information and pond performance of the offspring, some companies selling SPF animals are potentially selling broodstock that still contain pathogens, and in some cases they are using inadequate PCR detection technologies to test for the presence of these pathogens.

This multiplex technology was developed in Australia by CSIRO and has been validated repeatedly (Genics Pty Ltd.). Recently, shrimp farmers in Australia have been able to use it to largely eliminate the negative impact of a shrimp virus that, prior to development of this technology, historically has been widely described as being endemic: IHHNV. This has resulted in consistent increases in profit.

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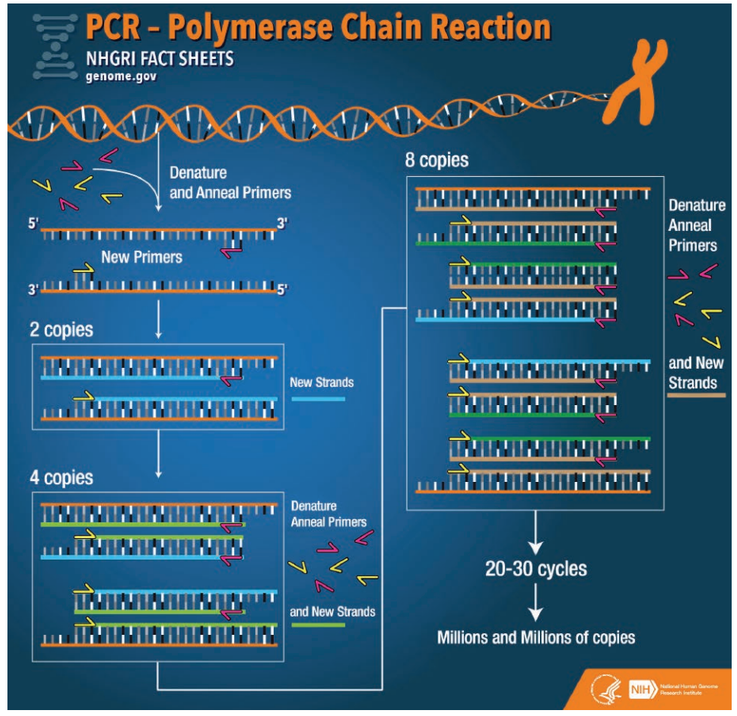

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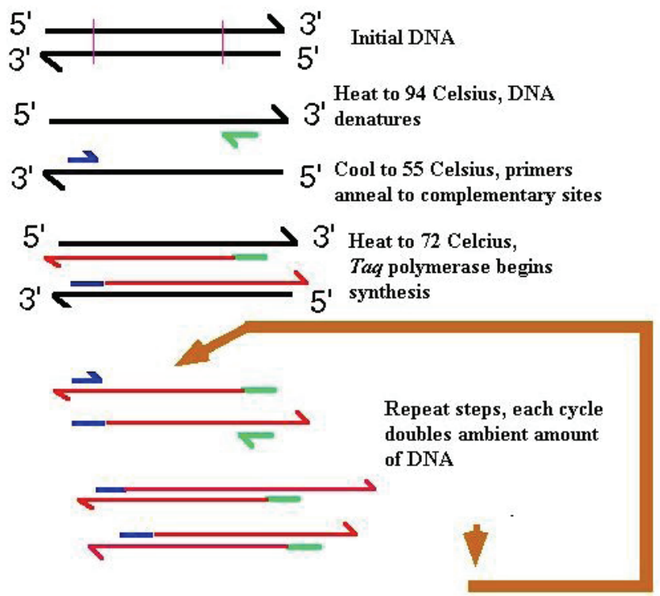

Figure 1. There are three basic steps involved in PCR (polymerase chain reaction).
• Denaturation at 94oC. By heating the mixture up to 94 degrees C, the DNA to be copied is forced to "denature" (unwind and become single stranded). Also present are other ingredients such as primers (to cut the DNA at pre-determined points and allow subsequent replication), the Taq polymerase that provides the synthesis of replicated DNA, and raw materials for synthesis (known as nucleotide triphosphates or NTP's).
• Annealing at 55oC. Cooling the mixture down to 55 degrees allows the primers to anneal to the DNA, meaning they stick to complementary sites on the DNA that we wish to replicate.
• Extension at 72oC: Raising the temperature to 72 degrees (which is the temperature optimum for Taq polymerase) starts the "extension" process, where Taq polymerase will work off the primers provided and generate new DNA strands, making each strand of the original DNA double stranded again.

The entire process is repeated several-to-many times to create a potentially enormous amount of DNA, all copied from the section of interest in the initial double strand.

The impact of shrimp diseases on the global industry has been and continues to be significant. Over the last decade many billions of dollars in lost revenues have been directly attributable to disease. While efforts have been made to limit this, these efforts are weakened by the use of random sampling and testing regimes of broodstock using conventional approaches. The ability to cost effectively screen each brood animal and populations of PLs and shrimp in production ponds for a very low cost should open the door to widespread testing of, at the very least, entire broodstock populations.

Unfortunately this is slow to catch on. Having worked with the industry for almost 30 years I think that I understand why. First of all, for many it is better not to look. That way you do not have to deal with the consequences of finding pathogens where they are not supposed to be. It is easy to test for certain pathogens and not others and claim animals are SPF. The second reason has to do with being short sighted. Why test each animal when you do not have to? Why screen for the whole range of potential pathogens when you do not have to? No one is forcing it and regulators seem unable to appreciate the importance of ensuring individual testing, at least until it can be established that a population is indeed SPF for all of the OIE notifiable pathogens. It is certain some of the pathogens that impact farmed shrimp come into the farm in PLs that are carrying them, typically as a result of broodstock carriers and inadequate efforts taken to exclude a given pathogen.

Long term histories of the performance of PLs from a given source of broodstock is a critical element in ensuring that one is not moving pathogens between shrimp and is essential in the process of developing SPF animals.

Given that PCR testing was the only tool available for many years the perception has been that it is better than doing nothing. That may have been the case but conventional PCR testing is ready to be replaced by a better tool.

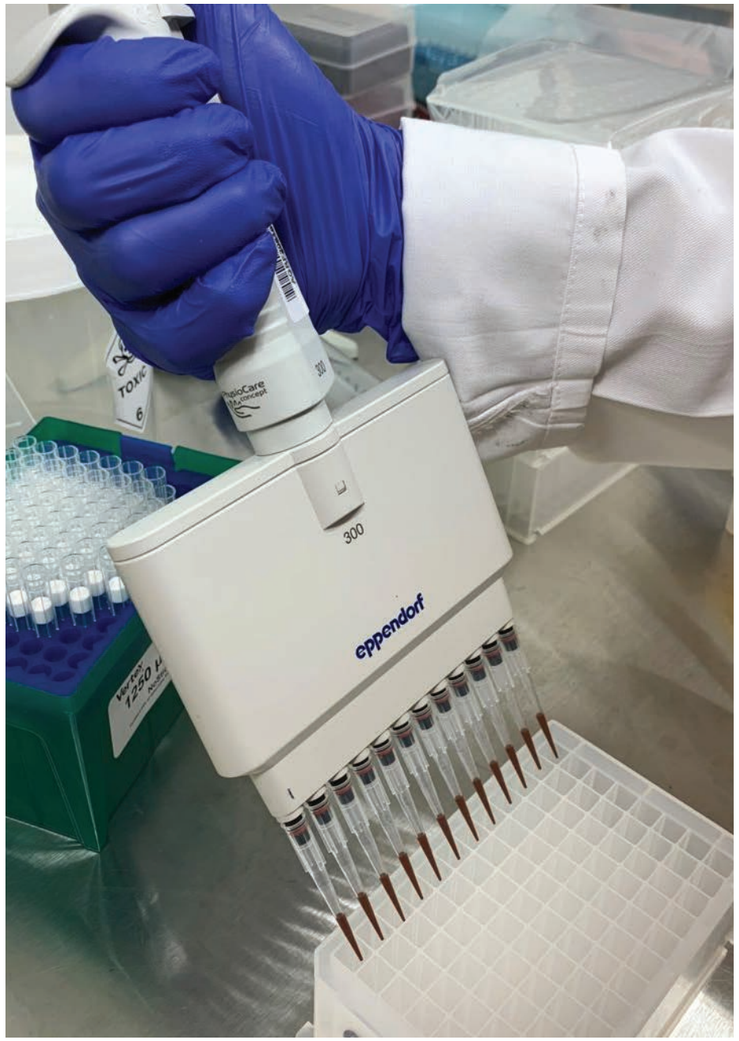

Figure 2. Photograph courtesy Genics Pty. Ltd.

If the global industry is truly serious about preventing the movement of these pathogens between shrimp populations then multiplex screening is absolutely essential. At the very least, broodstock companies that do not track the performance of their strains on their customers' farms should test each animal until an adequate history is established to ensure that the PLs from these broodstock are not carrying any known pathogens into the production system with them. All companies that buy broodstock to sell PLs should be required to test each individual brood animal for the complement of known possible pathogens. Responsible providers of broodstock, without adequate histories, should elect to do this voluntarily and build the costs into the price of the broodstock.

While this is not the only avenue that needs to be addressed, it is the only way to ensure that shrimp farming will ever becoming truly sustainable. Conventional screening based on testing small, often pooled, samples should not be acceptable to regulators. The risks are real and until the industry stops the movement of potential pathogens between stocks there is no chance that shrimp farming will ever move away from this endless cycle of massive global disease outbreaks. No one is invulnerable to this.

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