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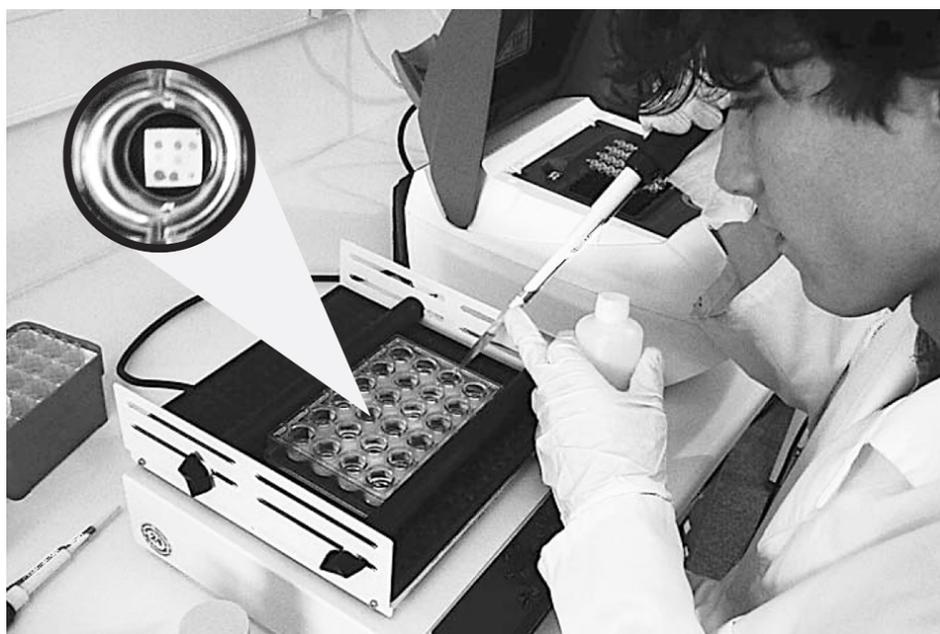
Health & Welfare

Dual detection in shrimp disease prevention

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By Jean Robert Bonami, Ph.D.

Mini-array diagnostic method offers efficiency, sensitivity



The mini-array test method requires little more than basic lab equipment. Here a technician “dots” the test membranes in the individual wells of the kit.

Diagnostic tools that are highly sensitive and specific are needed to manage and prevent shrimp diseases, particularly viral diseases, that have caused losses estimated at billions of dollars. After the development of genomic probe diagnostic systems in the 1990s, followed by polymerase chain reaction- (PCR) based systems, a new mini-array method now allows one-step detection of multiple infections that are common in crustaceans.

The first mini-array colorimetric kit, developed in a collaboration between Skuld Tech and ADIMAQUA of France, provides simultaneous diagnostics for two different viruses: White Spot Syndrome Virus and Infectious Hypodermal and Hematopoietic Necrosis Virus. Both viruses are important because of their wide range and significant impact on shrimp production.

Mini-array method

The mini-array method is based on detection that combines target nucleic acid amplification by PCR and specific hybridization using enzyme-linked antibodies that produce a dark-blue precipitate on a nylon membrane.

This method has numerous advantages over other molecular diagnostic tools, including higher sensitivity, high specificity, greater efficiency and reliability, ease of use, and straightforward interpretation. It eliminates the need for tedious electrophoresis and the use of ethidium bromide, a carcinogenic dye.

Multiple detection possible

Multiple detection is one of the major advantages of mini-arrays. Multiple sets of specific primers are used, and the different amplicons issued from the PCR hybridize with the homologous probes plotted in pre-defined positions on the nylon membrane. Interpretation of results viewed on the membrane can easily be done by the naked eye (Fig. 1).

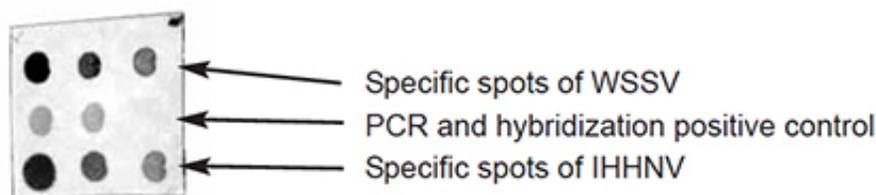


Fig. 1: Spots on the test membrane indicate the presence of pathogens.

An internal control is added to each reaction. It is a PCR and hybridization- positive control DNA fragment used to prevent false negatives due to experimental errors. This test is considered positive if the corresponding spots dotted on the membrane show the dark-blue positive staining.

High sensitivity

The mini-array method significantly increases the power detection limit of pathogens and is a highly sensitive diagnostic method that contributes to virus prophylaxis. Its sensitivity is 100 times higher than the regular detection in electrophoresis of PCR-amplified products, and 10,000 times higher than the dot-blot method.

Mini-array is an ideal method for the detection of low-level or early infections. Such early warnings can help operators limit the risks of horizontal transmission by systematically eliminating virus carriers, and the risks of vertical transmission by selecting healthy broodstock individuals.

Conclusion

The mini-array test is fast; between one and 24 reactions can be done in parallel in only six hours. Interpretation of results is easy, as the presence or absence of blue dots can be detected by the naked eye. Sample processing is also easy, involving simple heating in boiling water.

The membranes provided with the mini-array kits are ready to use, dotted with the probes, and pre-hybridized. Only a few items of equipment are required. Except for the thermocycler, only basic equipment such as a water bath, small bench centrifuge and shaker are necessary. Thus, the mini-array method can be easily implemented at shrimp farms or hatcheries that already use PCR as a diagnostic tool.

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