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Health & Welfare

Rapid test detects NHP in penaeid shrimp

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Low-cost test could monitor passage of infection between wild, cultured shrimp

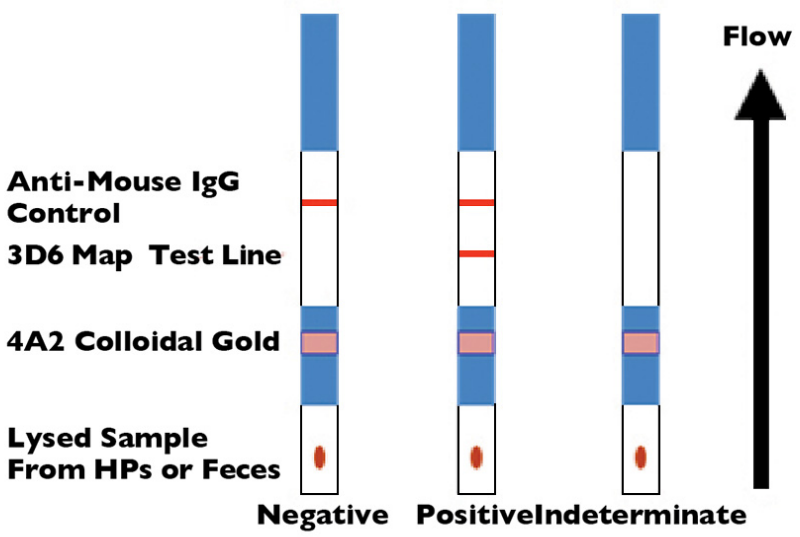

Fig. 1: Principle of the NHP-B rapid test.

Necrotizing hepatopancreatitis bacterium (NHP-B), a pleomorphic, gram-negative rickettsial-like organism, has been responsible for significant losses of cultured shrimp in the Americas and could be a threat to other worldwide sources. It was originally identified in the U.S. state of Texas, but has now been documented in aquaculture farms throughout North, Central and South America. NHP-B infection is potentially treatable if it is detected early and medicated food is applied before the shrimp cease feeding due to lethargy and anorexia – symptoms of the disease.

Current tests for NHP, such as polymerase chain reaction (PCR) or histology, require sending samples to centralized laboratories, which can take several days and be costly. Pondside analysis of wet mounts for melanized tubules can lead to misdiagnosis due to early similarities to septic hepatopancreatic necrosis from vibriosis.

NHP is currently being considered for listing by the World Organisation for Animal Health as a reportable disease, which points to the urgent need for faster and more cost-effective methods of detection. The development of a non-invasive test using fecal samples from broodstock or extracts from hepatopancreas tissue from cultured shrimp would enable more rapid detection and monitoring of potential infections, and earlier intervention.

Test development

With research funding through the United States Department of Agriculture Cooperative State Research, Education and Extension Service, the authors have developed a rapid lateral-flow immunoassay using monoclonal antibody combinations specific to NHP-B that can be administered on site at the pond and provide results in less than 15 minutes. The principle of the assay is shown in Fig. 1.

The monoclonal antibodies are directed to an antigen detectable in native and denatured NHP-B, and shown to be present on all geographical isolates tested. Such a low-cost test would also have applications in monitoring the passage of the infection between wild and cultured shrimp.

Two monoclonal antibodies shown by western blot to detect a 13kDa component in the bacterium were used in the assay development. The target antigen is as yet unknown, but believed to be a carbohydrate moiety present in Percoll gradient fractions of hepatopancreas enriched by flagellar components of the NHP-B.

During a typical test with the dipstick method, hepatopancreas tissue and fecal samples were collected from Pacific white shrimp (*Litopenaeus vannamei)* experimentally infected with the NHP bacterium and extracted with a detergent containing lysis buffer. Dipstick reactivity was observed in both sample types when infection was detected by PCR or immunohistochemistry. Examples of these reactions are summarized in Table 1.

Houghton, Reactivity of PCR positive and negative hepatopancreas samples, Table 1

		Dipstick Rapid NHP-B Assay +	Dipstick Rapid NHP-B Assay –
Polymerase	+	10	3
Chain Reaction	–	0	33

Table 1. Reactivity of PCR positive and negative hepatopancreas samples in dispstick assay.

Evaluations continue

The availability of this test can facilitate monitoring of NHP-B infection in the field and potentially reduce the significant production and revenue losses that are currently typical when infections of shrimp ponds with this organism occur. Ongoing field evaluations of the test will further determine its overall performance characteristics.

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