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Pond Site Diagnosis: Monoclonal Antibody-Based Farmer Level Tests to Detect the Acute Hepatopancreatic Necrosis Disease in Shrimp

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Abstract : Early mortality syndrome (EMS)/Acute Hepatopancreatic Necrosis Disease (AHPND) has emerged as a major obstacle for the shrimp farming around the world. It is caused by a strain of Vibrio parahaemolyticus. The possible preventive and control measure is, early and rapid detection of the pathogen in the broodstock, post-larvae and monitoring the shrimp during the culture period. Polymerase chain reaction (PCR) based early detection methods are good, but they are costly, time taking and requires a sophisticated laboratory. The present study was conducted to develop a simple, sensitive and rapid diagnostic farmer level kit for the reliable detection of AHPND in shrimp. A panel of monoclonal antibodies (MAbs) were raised against the recombinant Pir B protein (rPirB). First, an immunodot was developed by using MAbs G3B8 and Mab G3H2 which showed specific reactivity to purified r-PirB protein with no cross-reactivity to other shrimp bacterial pathogens (AHPND free Vibrio parahaemolyticus (Indian strains), V. anguillarum, WSSV, Aeromonas hydrophila, and Aphanomyces invadans). Immunodot developed using Mab G3B8 is more sensitive than that with the Mab G3H2. However, immunodot takes almost 2.5 hours to complete with several hands-on steps. Therefore, the flow-through assay (FTA) was developed by using a plastic cassette containing the nitrocellulose membrane with absorbing pads below. The sample was dotted in the test zone on the nitrocellulose membrane followed by continuos addition of five solutions in the order of i) blocking buffer (BSA) ii) primary antibody (MAb) iii) washing Solution iv) secondary antibody and v) chromogen substrate (TMB) clear purple dots against a white background were considered as positive reactions. The FTA developed using MAbG3B8 is more sensitive than that with MAb G3H2. In FTA the two MAbs showed specific reactivity to purified r-PirB protein and not to other shrimp bacterial pathogens. The FTA is simple to farmer/field level, sensitive and rapid requiring only 8-10 min for completion. Tests can be developed to kits, which will be ideal for use in biosecurity, for the first line of screening (at the port or pond site) and during monitoring and surveillance programmes overall for the good management practices to reduce the risk of the disease.

Keywords : acute hepatopancreatic necrosis disease, AHPND, flow-through assay, FTA, farmer level, immunodot, pond site, shrimp
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