Achieving technical knowledge and establish a rapid diagnostic kit for Viral Nervous Necrosis for the purpose of rapid detection of (VNN) disease using Immune chromatography method

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Abstract

Rapid diagnosis of disease and fast removal of infected fish are needed to effective control strategies during disease outbreaks. Prompt action in the early stages of any disease problem can have a significant impact on the scale of the outbreaks. So, rapid methods which can be used outside the laboratory by unskilled persons can prevent the occurrence of the infections. Todays, the use of immunoassay tools and design of rapid methods for diagnosis are considered. Therefore, rapid, sensitive and precise tests have been designed and replaced with other sophisticated and expensive. The aim of this study was the development of a rapid and simple assay to detect infected-betanodavirus specimens. Gold possess optical properties and is easy to visualize, stable in liquid or dried form, easy to conjugate with biological material, due to which it is the most preferred label for the immune chromatographic test. For the preparation of antibody–gold particle conjugates, the concentration of antibodies and the pH of colloidal gold should be adjusted. In this research we added different volume of 1% sodium citrate to obtain desired gold nanoparticles size in 30 nm. Gold nanoparticles with size of 30 nm were conjugated with an appropriate concentration antibody and in specific pH, and then this conjugation was blocked with serum albumin bovine. Afterwards, both various volumes of antibody–gold particle conjugates were coated on the conjugate pad and different concentration of polyclonal antibody are used in a test line. To assure that the test strip will work correctly, the flow must also reach the control line, which has goat anti-mouse IgG (5 mg/ml) embedded in it. Both test and control lines were spotted on membrane by using Helena applicator. Finally, the components of strip were assembled and then the brain specimens of suspicious grey mullets and different dilution of virus were evaluated.

According to our result when we used 1 µg/µl of polyclonal antibody on the test line and 4 µl per strip of gold- antibody conjugates on the conjugate pad, the designed strip was able to detect $10^3 TCID_{50}$/ml concentration of VNN. In next step, 50 brain samples produced positive test results by PCR Supernatant solution from a brain homogenate of VNN-infected *Liza aurata* were confirmed negative for NVV by subsequent PCR an IHC testing, were evaluated using immune chromatography strip. It is generally recognized that the sensitivity and specificity of lateral-flow test strips are 64 and 100%, respectively. Considering the limitations of this study, the accuracy of the immune chromatography method is almost identical to the both real-time PCR and IHC.

Keywords: Antibody, Gold nanoparticles, Real-time PCR, IHC, Viral Nervous Necrosis, Rapid diagnostic kit