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COLLOIDAL NANOGOLD IMMUNE ASSAY FOR RAPID DETECTION OF *EDWARDSIELLA TARDA* IN *LABEO ROHITA*

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ABSTRACT

Edwardsiella tarda infections resulting in important economic losses have been reported from a variety of cultured fish in Asia, especially India and Japan. Disease is enzootic in different parts of India also. To date, the disease has been reported from various fish species, including channel catfish, Japanese eel, mullet, tilapia, Chinook Salmon, Flounder, common carp, stripped bass, turbot, koi, European eel and Senegalese sole. The symptoms are lethargy, "hang" at the surface, and swim in a spiraling or erratic pattern. The fish often develop small, cutaneous ulcerations; in advanced cases, however, larger depigmented areas mark the sites of deep muscle abscesses. Detection of a disease causing organism is the pre-requisite for the diagnosis of any disease. The diagnosis of *E. tarda* is based on isolation and identification of etiological agent. Conventional plating and culturing methods commonly used to identify causative bacterial pathogens have a low degree of accuracy and conclusive results typically require periods of 72 h or more. Although histopathology, haematology, molecular diagnosis, Dot ELISA, indirect or competitive ELISA and latex agglutination test have been used for the detection of *E. tarda*, these processes require time and sophisticated equipment for development of the tests. Sero-diagnosis is a routine methodology in diagnosis. There is a constant need to improve the performance of current diagnostic assays as well as develop innovative testing strategies to meet new testing challenges. The nanoparticles promise to help promote *in vitro* diagnostics to the next level of performance. Quantum dots (QDs), gold nanoparticles (GNPs) and superparamagnetic nanoparticles are the most promising nanostructures for *in vitro* diagnostic applications. Colloidal gold conjugates have found widespread use due to their high stability and unique optical properties of GNP (Jain *et al*., 2006). Nano size is within the size range of biomolecules and cellular organelles. This would allow one-on-one interaction between the nanoparticles and the biomolecules of interest. Furthermore, nanoscale surfaces provided by colloidal gold particles could accelerate antibody-antigen reaction sufficiently, which provide an amplified signal for immunoassay. Especially, the results can be read directly without the use of instruments, which ensures the convenience of assay on-site. It has been reported that although, the use of polyclonal antibody generated by immunization with whole bacteria as antigen is associated with the problem of low specificity, cross reactivity hamper the application of polyclonal antibodies but surprisingly, coupling of polyclonal antibodies with colloidal gold particles results in considerably high specificity of detection. By considering these, an attempt was made to develop anti *E. tarda* rabbit IgG gold conjugate to detect *E. tarda* in fishes. Herein we report the development of a specific immunoassay using gold-conjugated polyclonal antibodies for the rapid detection of E. tarda. Positive reactions of development of purple red agglutination indicated presence of E. tarda.

The other bacteria gave a negative reaction indicating the specificity of the test developed. The antibody coated particles were stable upto 21 weeks at 4^0C and detected 10^5 CFU/ml.

KEYWORDS: *Edwardsiella tarda,* Cutaneous, Nanoscale, Nanoparticles