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ACKNOWLEDGEMENTS. We thank ICAR and National Agricultural Technology Project, New Delhi, for infrastructure and financial assistance.

Received 27 August 2002; revised accepted 22 October 2002

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Early diagnosis of white spot viral syndrome using rapid gill staining technique

White spot viral syndrome caused by the white spot syndrome virus (WSSV) is the major cause of mortality and morbidity in shrimps, resulting in huge losses to shrimp farmers in coastal areas. It has been reported that during 1994–95, this disease struck the booming aquaculture industry and caused a loss of around 20,000 tons of shrimp worth about Rs 250–350 billion[1]. This is a dreadful disease and is difficult to identify in the early stages. The diagnosis of viral infection is made possible with PCR and monoclonal antibody-based techniques[2,3], but these methods are expensive and the facility is not available everywhere. Early diagnosis will provide an opportunity to manage the disease in a comparatively better manner and will also help in preventing the rapid spreading of the disease. In general, the prevalence of WSSV can be observed by visual symptoms like appearance of white spots on the carapace and other external parts of the body. However, diagnosis at this stage of infection is of no use, as the shrimps start dying due to the disease. Thus, diagnosis of WSSV before it shows external symptoms is of practical significance. This will be useful at least to regulate the disease in the preliminary stage. In the present correspondence, a tool for rapid diagnosis of WSSV has been described as a reproducible protocol for the aqua industry. Diagnosis of the disease can be made by adopting wet squash preparation of the tissue and observing it through a microscope after fixing and staining the tissue for observation of hypertrophied nuclei (symptom for WSSV), as the WSSV is known to replicate in the nucleus of the host by forming inclusion bodies[4,5]. This is a novel method of identification of infection in the asymptomatic phase using a nuclear stain, developed in our laboratory based on the principles of existing stains. For developing the stain, the components were mixed in the proportions shown in Table 1.

For employing the rapid gill staining technique, live shrimps were collected from the culture ponds for experimental analysis. Staining experiments were conducted on the gill filaments obtained from shrimps showing external symptoms as well as those not showing any symptoms. However, the shrimps were collected from the same ponds. After plucking the gill filaments from the shrimps, they were placed on a glass slide for squash preparation and the smear was prepared using another glass slide. The gill filament was chosen because WSSV is a systemic ectodermal mesodermal baculovirus. The smear was fixed on the glass slide with gentle heating. After fixing the smear, the slide was flooded with the stain for 5 min. The glycerol component in the stain is useful to prevent the rapid drying of the stain and phenol will act as cytotoxicant for integration of stain. The slides were washed and observed under different objectives of the compound microscope for the occurrence of hypertrophied nuclei. Under the 40× objective, the early stages of infection with WSSV were observed; which revealed the occurrence of basophilic, blue-coloured, hypertrophied nuclei in the gill filament. This might be because of the onset of basophilic, central inclusions surrounded by marginated chromatin. In the later stages of infection, some of the eosinophilic nuclei have shown Cowdry type-A inclusions which are characterized by a central dark region followed by a clear zone of chromatin in circular shape (Figure 1). In the advanced stage of infection, large hypertrophied, basophilic and blue-coloured nuclei were distinctly seen (Figure 1). It was quite interesting to

Table 1. Composition of the developed stain

Component	Quantity
Trypan blue	0.60 g
Eosin Y	0.20 g
Phenol crystals	0.50 g
Sodium chloride	0.30 g
Glycerol	20.00 ml
Distilled water	80.00 ml
pH	7.0–8.0

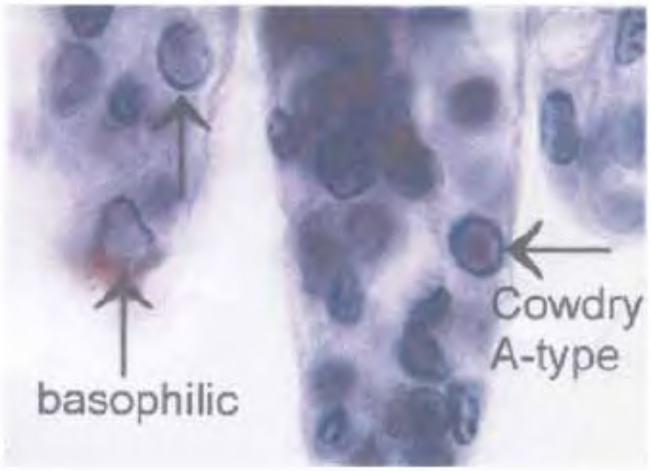

Figure 1. Gill filaments showing hypertrophied nuclei of different stages (basophilic and Cowdry type-A) with Trypan blue and Eosin stain.

note that all the 56 samples tested with this stain showed the hypertrophied nuclei with various stages of inclusions. Thus, the occurrence of hypertrophied nuclei in the gill filament in the early stages of infection even before the onset of physical symptoms, can be considered as symptoms of WSSV prevalence.

This method of staining and diagnosis can be adopted for checking of cultured shrimps at regular intervals for the possible occurrence of white spot disease. Detection of the virus under asymptomatic condition will provide an opportunity for culturists to delay the onset of WSSV by controlling the various stress-inducing factors like bacterial infections and abnormalities in water quality parameters like increase in temperature (temperature stress leads to the mortality in 5 to 7 days after WSSV infection)[6], concentration of ammonia and decreased oxygen levels in the culture ponds. Thus the present observation is useful to the small farmers in the aqua industry to detect the dreaded WSSV syndrome in its early stages for necessary remedial steps, as this method of diagnosis is relatively cheap (it costs only Rs 2.50 each analysis).

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Received 30 November 2002; revised accepted 25 June 2003

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