OIE Procedure for Registration of Diagnostic Kits

Abstract sheet

Name of the diagnostic kit: IQ 2000™ WSSV Detection and Prevention System
Manufacturer: GeneReach Biotechnology Corporation
OIE Approval number: 20080304
Date of Registration: May 2008

Disease: White Spot Disease

Pathogen Agent: White spot syndrome virus (WSSV) found in different penaeid shrimp species including *P. monodon*, *P. japonicus* and *L. vannamei* as well as other crustaceans, such as crab and crayfish.

Type of Assay: The IQ 2000™ WSSV Detection and Prevention System is a PCR test.

Purpose of Assay: Certified by the OIE in May 2008 as fit for the diagnosis of white spot syndrome in crustaceans and for the following purposes:

1. To certify freedom from infection (<10 virions/sample) in individual animals or products for trade/movement purposes;
2. To confirm diagnosis of suspect or clinical cases (confirmation of a diagnosis by histopathology or clinical signs);
3. To estimate prevalence of infection to facilitate risk analysis (surveys/herd health schemes/disease control).

Species and Specimen: Validated in shrimp but may be useful for detection of WSSV in other crustaceans.

1. Information on the kit

Please refer to the kit insert available on the OIE Registry web page or contact manufacturer at

GeneReach Biotechnology Corporation

TEL: 886-4-24639869

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2. Summary of validation studies

Analytical characteristics

Analytical sensitivity - The analytical sensitivity data were done by comparing the sensitivity of IQ2000™ WSSV Detection and Prevention System and the PCR diagnostic method listed by OIE (Lo, et al., 1996). DNA extracted from WSSV infected *L. vannamei* was 10 fold serial diluted, and subsequently taken as DNA template for both detection methods. The dilutants are ddH_2O or DNA extract of SPF *L. vannamei*. No apparent difference on sensitivity between the 2 detection methods was observed.

Analytical specificity - SPF *L. vannamei*, Monodon baculovirus or Baculovirus Penai infected *P. monodon*, as well as WSSV infected shrimp collected from different geographic areas were acquired from OIE reference laboratories. Results of all SPF samples and MBV or BP infected samples have shown no detectable non-specific band; while all WSSV infected samples can be detected by IQ2000™ WSSV Detection and Prevention System.

Repeatability data - The repeatability study was carried out using pleopods of *L. vannamei* with 3 production batches in triplicates. The data were consistent.

Diagnostic Characteristics

Two evaluation studies were carried out for the estimation of the diagnostic sensitivity and specificity:

- In one study, the OIE recommended nested PCR method (Lo, et al., 1996; Chapter 4.1.2. of the *Manual of Diagnostic Tests for Aquatic Animals*, 2007, available on the OIE website) was applied to classify certain populations of shrimp as either negative or positive. After screening, 300 white spot syndrome negative samples and 300 white spot syndrome positive samples were used for this study.

- In a second study, 100 un-defined samples collected from market were used to compare IQ2000™ WSSV Detection and Prevention System with the OIE recommended method.

Diagnostic sensitivity (DSn) and specificity (DSp) estimates with 95% confidence limits (CI)

Tests performed with virus negative and positive animals:

	WSS virus positive by reference method	**WSS virus negative by reference method**
Test Positive by IQ2000™	289	0
Test Negative by IQ2000™	11	300
Relative Diagnostic Sensitivity: 96.33%, CI [93.53 – 98.16%] Relative Diagnostic Specificity: 100.00%, CI [98.78 – 100.00%]		

Tests performed with undefined samples collected from market*:*

	WSS virus positive by reference method	**WSS virus negative by reference method**
Test Positive by IQ2000™	51	0
Test Negative by IQ2000™	0	49
Relative Diagnostic Sensitivity: 100.00%, CI [92.89 – 100.00%] Relative Diagnostic Specificity: 100.00%, CI [92.89 – 100.00%]		

Agreement between tests

The results presented above reveal that the consistency between IQ2000™ WSSV Detection and Prevention System and the OIE recommended method is very good.

Reproducibility

Different lots of IQ2000™ WSSV Detection and Prevention System were sent to three different laboratories in Chinese Taipei (including one OIE reference laboratory) to be tested. 35 shrimp samples (*L. vannamei*) were collected and sent to all 3 laboratories. Each sample was analysed with 3 different lots of IQ2000™ WSSV Detection and Prevention System. Results of the 3 independent laboratories were collected and the data were analysed statistically

The reproducibility data indicates that there is no significant difference in assay performance between laboratories. Furthermore, good reproducibility was demonstrated using three different lots of kits, suggesting that lot to lot variability is minimal.

Applications

The IQ2000™ WSSV Detection and Prevention System kit is currently used by several laboratories worldwide as an official test.

References

1. LO CF, LEU JH, HO CH, CHEN CH, PENG SE, CHEN YT, CHOU CM, YEH PY, HUANG CJ, CHOU HY, WANG CH, AND KOU GH (1996 a) Detection of baculovirus associated with white spot syndrome (WSSV) in penaeid shrimps using polymerase chain reaction. Dis Aquat Org 25: 133-141.
2. LO CF, HO CH, PENG SE, CHEN CH, HSU HC, CHIU YL, CHANG CF, LIU KF, SU MS, WANG CH, KOU GH (1996) White spot syndrome baculovirus (WSSV) detected in cultured and captured shrimps, crabs and other arthropods. Dis. Aquat. Org., 27,215-225.
3. NUNAN L.M., POULOS B.T. & LIGHTNER D.V. (1998) The detection of white spot syndrome virus (WSSV) and yellow head virus (YHV) in imported commodity shrimp. Aquaculture, 160, 19-30.
4. PENG SN, LO CF, HO CH, CHANG CF, KOU GH (1998) Detection of white spot syndrome baculovirus (WSSV) in giant freshwater prawn, *Macrobrachium rosenbergii* using polymerase chain reaction. Aquaculture 164:253-262.
5. THAKUR P. C., F. CORSIN, J. F. TURNBULL, K. M. SHANKAR, N. V. HAO4, P. A. PADIYAR, M. MADHUSUDHAN, K. L. MORGAN, AND C. V. MOHAN (2002). Estimation of prevalence of white spot syndrome virus (WSSV) by polymerase chain reaction in *Penaeus monodon* postlarvae at time of stocking in shrimp farms of Karnataka, India: a population-based study. Dis Aquat Org 49:235-243.
6. Chapter 2.2.6., White spot disease; *Manual of Diagnostic Tests for Aquatic Animals*, 2009, OIE, pp. 177-190.