OIE Procedure for Registration of Diagnostic Kits

Abstract sheet

Name of the diagnostic kit: IQ Plus™ WSSV Kit with POCKIT System
Manufacturer: Genereach Biotechnology Corporation
OIE Approval number: 20130108
Date of Registration: May 2013

Disease: White Spot Disease

Pathogen Agent: White Spot Syndrome Virus (WSSV)

Type of Assay: Insulated isothermal Polymerase Chain Reaction

Purpose of Assay: Certified by the OIE in May 2013 as fit for the detection of white spot disease in target tissues (Shrimp tissue of ectodermal and mesodermal origin) of *Litopenaeus vannamei* and for the following purposes:

1. To certify freedom from infection (<10 virions/reaction) 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: *Litopenaeus vannamei*; Shrimp tissue of ectodermal and mesodermal origin.

1. Information on the kit

Information can be found by emailing: sales@genereach.com, or by visiting http://www.iq2000kit.com/products_2.php?bgid=3&gid=6&sgid=34.

In summary, IQ Plus™ WSSV Kit with POCKIT System was designed for qualitative detection of WSSV DNA based on multiplex insulated isothermal PCR technology (iiPCR; Chang *et. al.,* 2012; Tsai *et. al.,* 2012a; Tsai *et. al.,* 2012b). IQ Plus™ WSSV Kit with POCKIT System is designed to be used with a compact and portable iiPCR compatible instrument, POCKIT™ Nucleic Acid Analyzer (POCKIT™). IQ Plus™ WSSV Kit with POCKIT System is highly sensitive and specific for WSSV DNA detection from aquaculture specimen and suitable for onsite viral DNA detection. Specific primers and probe (520-nm fluorescent signal) are designed to detect WSSV DNA in samples. In addition, internal control (IC) primers and probe (550-nm fluorescent signal) are used to target a house-keeping gene of *Penaeid* shrimps. The assay has been simplified for easy and fast operation in POCKIT™ for pond-site WSSV DNA detection.

2. Summary of validation studies

Analytical characteristics

Repeatability:

Various types including negative and positive WSSV-infected shrimps (*L. vannamei*) were selected and three production batches were tested. Each sample was tested in quadruplicates per run. The data showed 100% agreement among the test results.

Analytical specificity:

WSSV-, Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV)-, or Hepatopancreatic Parvovirus (HPV)-infected *L. vannamei* were used to test the specificity of IQ Plus™ WSSV Kit with POCKIT System. The IHHNV- and HPV-infected samples were confirmed to be WSSV-negative by IQ 2000™ WSSV Detection and Prevention System (DPS). Signals were generated from only WSSV-infected, not from IHHNV- and HPV-infected samples in IQ Plus™ WSSV Kit with POCKIT System.

Analytical sensitivity:

Analysis using standard plasmid (pWSSV1) and purified WSSV genomic DNA of known copy numbers shows that the ⩾95% detection rate of IQ Plus™ WSSV Kit with POCKIT System was 23.7 and 16.9 copies pWSSV1 and WSSV DNA, respectively, per reaction. Furthermore, analysis of DNA extracted from WSSV-infected *L. vannamei* serially diluted with ddH2O or DNA extracts of SPF *L. vannamei* shows that the detection endpoint (10^4 dilution) of IQ Plus™ WSSV Kit with POCKIT System was similar to that of IQ2000™ WSSV DPS.

Diagnostic Characteristics

Test Cut-off Determination

IQ Plus™ WSSV Kit with POCKIT System, based on iiPCR and fluorescent probe detection principles, is designed to work in an iiPCR-compatible instrument, POCKIT™. The cut-off for POCKIT™ device were determined on the basis of fluorescent signal of numerous NTC and positive reactions of iiPCR assays developed for various targets at GeneReach (confidential data).

Readouts of the results are determined as follows:

1. When "+" is displayed on POCKIT™, the sample is classified as WSSV positive.
2. When "-" is displayed on POCKIT™, the sample is classified as WSSV negative.
3. When "?" is displayed on POCKIT™, the test result is indeterminate and should be repeated.

Diagnostic sensitivity (DSn) and specificity (DSp) estimates

Diagnostic sensitivity was evaluated by comparing the test results of IQ Plus™ WSSV reaction of positive reference animals which were identified by IQ2000™ WSSV DPS. Pleopods of 400 WSSV-positive samples were sampled and tested in this study. Negative reference animals selected by IQ2000™ WSSV DPS were also subjected to analysis by IQ Plus™ WSSV Kit with POCKIT System.

		IQ2000™ WSSV DPS	
		Positive	Negative
IQ Plus™ WSSV Kit with POCKIT System	Positive	374	9
	Negative	26	291

In summary, this validation testing was conducted on 700 samples. The results are: sensitivity: 93.5% [95% confidence interval (CI): 90.61–95.56%], specificity: 97.0% [95% CI: 94.31–98.50%]).

Comparative performance

See "*Diagnostic sensitivity (DSn) and specificity (DSp) estimates".*

Agreement and discrepancies

The results revealed that when compared to IQ2000™ WSSV DPS, with defined reference animals, the diagnostic sensitivity of IQ Plus™ WSSV Kit with POCKIT System was 93.5% with a 95% CI of 90.61% - 95.56%, and the diagnostic specificity was 97.0% with a 95% CI of 94.31% - 98.50%. In addition, with un-defined reference animal, IQ Plus™ WSSV Kit with POCKIT System showed 100% agreement (100/100) for both sensitivity and specificity. For this experiment, pleopods of 100 un-defined shrimps obtained randomly from a local farm were sampled and examined by both IQ2000™ WSSV DPS and IQ Plus™ WSSV Kit with POCKIT System. Statistical analysis using one-tailed binomial test suggested that results from these two experiments (defined reference animals and un-defined animals) agreed with each other.

Reproducibility

Different lots of IQ Plus™ WSSV Kit with POCKIT System were sent to three different labs located in Chinese Taipei and USA (including two OIE Reference Laboratories) to be tested. Trunk muscles of total of 64 *L. vannamei* samples were aliquoted, preserved in 95% ethanol, and sent to all three laboratories. Each sample was analysed with 3 batches of IQ Plus™ WSSV Kit with POCKIT System.

Chi-square test for homogeneity was conducted to analyse the experimental results generated from the three labs.

The results showed that there was no difference among different laboratories with three batches of IQ Plus™ WSSV Kit with POCKIT System.

Applications

The kit is being used worldwide by different laboratories (Private and Public)

References

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