



WOAH Procedure for Registration of Diagnostic Kits Validation Studies Abstract

Name of the diagnostic kit: Innocreate Bioscience WSSV RP Rapid Test Kit

Manufacturer: Innocreate Bioscience Co., Ltd.

Procedure /Approval number: 082132

Date of Registration: May 2023

Disease: Infection with white spot syndrome virus in shrimp

Pathogen Agent: Whispovirus, White Spot Syndrome Virus (WSSV)

Type of Assay: Lateral flow immunochromatographic assay (LFIA)

Purpose of Assay

The Innocreate Bioscience WSSV RP Rapid Test Kit is a qualitative detection kit for WSSV infection in shrimp. The lateral flow immunoassay device is designed for the following purposes:

1. Field based confirmatory diagnosis of clinical cases (includes confirmation of suspect cases and a positive screening test)
2. Estimate the prevalence of infection to facilitate risk analysis in production system shrimp farms to aid in management practices. (The test kit should not be used to estimate prevalence in broodstock or post larvae shrimp for risk analysis prior to translocation to other farms or across borders).
3. For use in conjunction with other tests or diagnostic procedures as an aid in the diagnosis or other clinical or epidemiological assessments.

Species and Specimens : 2-3 small pieces of shrimp gill.

1. Information on the kit

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

Website: <https://www.innocreatebio.com/>

Email: info@innocreatebio.com

2. Summary of validation studies

Analytical specificity

Conclusion:

AHPND-caused *Vibrio parahaemolyticus*, Infectious hypodermal and hematopoietic necrosis virus (IHHNV), EHP (*Enterocytozoon hepatopenaei*), Monodon Baculovirus (MBV), Yellow head virus (YHV) and Taura syndrome virus (TSV) infected shrimp were tested. All infected samples showed negative results.

Analytical sensitivity

Conclusion:

Serial dilution of recombinant WSSV target protein with homogenized shrimp tissues was used to estimate the limit of detection (LOD). The LOD was estimated to be 0.4 ng/ test.

Repeatability

Conclusion:

Within run repeatability was assessed using quadruplicates of 6 samples with various levels of infection, which were tested by the same operator on 5 separate days. Between run repeatability was performed by testing of the 6 samples by 3 different operators using 3 different lots of kits on 5 days. The results for both the within- and the between-runs were reproducible with kappa values of 1.0.

Diagnostic characteristics

Threshold determination and Diagnostic sensitivity (DSe) and specificity (DSp) estimates:

Threshold determination:

Innocreate Bioscience WSSV RP Rapid Test Kit is an immunochromatographic assay designed for the qualitative detection of WSSV infection in shrimp. The pink purple band needs to be observed at both the test line (T) and control line (C), to indicate that the shrimp was infected with WSSV. If the pink purple band appears only on the control line (C), this indicates that there is no infection of WSSV or light infection beyond the sensitivity of the kit. The threshold is determined by the analytical sensitivity as 0.4 ng of the target protein.

Diagnostic sensitivity (DSe) and specificity (DSp) estimates:

1. Diagnostic sensitivity and specificity estimates – with defined reference animals

Two hundred and fifty two specific pathogen free shrimp were either used as negative controls (n=105) or challenged (n= 147) with an injection of 100 ul of WSSV infected hemolymph to determine the diagnostic sensitivity and specificity in samples from defined reference animals. Of the 147 challenged and WOH TaqMan real time PCR (Durand & Lightner, 2002) positive samples, 125 tested positive by the WSSV RP Rapid Test Kit, while 22 samples were considered false-negative. The infected level of these 22 samples was considered very light (Ct>32.5). From the 105 WOH TaqMan real time PCR Negative non-challenged samples, no false-positive results were observed.

Reference Animal:

Innocreate Bioscience WSSV RP Rapid Test Kit		Target Species/Specimen: Gill	
		WOAH TaqMan real time PCR method	
		Ct<32.5 considered positive	Ct<40 considered positive
Diagnostic sensitivity	N	(126)	(147)
	DSe	(99.21%)	(85.03%)
	CI	(99.66% - 99.98%)	(78.22% - 90.38%)
Diagnostic specificity	N	(105)	(105)
	DSp	(100%)	(100%)
	CI	(96.55-100%)	(96.55-100%)

2. Diagnostic sensitivity and specificity estimates – Production shrimps

A total of 465 shrimp from 4 batches of production systems were tested, of which 45 of 465 shrimp were classified as symptomatic, and 64 of 465 were classified as positive (Ct<40) on qPCR testing by the WOH TaqMan real time PCR method (Durand & Lightner, 2002).

Compared with the WOH TaqMan real time PCR method, the overall DSe of the WSSV RP Rapid Test was 92.50% when Ct<32.50 is considered as positive, 84.00% when Ct<36 is considered as positive, or 65.62 % if Ct<40 is considered as positive; DSp was 100%.

As for diagnostic performance in symptomatic production system shrimp, DSe was 93.33%, and DSp was 100% when using Ct<40 as the cut-off. Positive predictive value (PPV) and Negative predictive value (NPV) were 100% and 99.3%. For samples with Ct<32.5 (Moderate to High infection, >=100 copies), the DSe was 92.50%. Overall the Innocreate Bioscience WSSV Rapid test kit shows high agreement on diagnosis.

Production shrimps:

Inncreate Bioscience WSSV RP Rapid Test Kit		Target Species/Specimen: Gill			
		WOAH TaqMan real time PCR method			
		Symptomatic	Ct<32.5 considered positive	Ct<36 considered positive	Ct<40 considered positive
Diagnostic sensitivity	N DSe CI	(45) (93.33%) (95.66%-99.98%)	(40) (92.50%) (79.61%-98.43%)	(50) (84.00%) (95.66%-99.98%)	(64) (65.62%) (52.70%-77.05%)
Diagnostic specificity	N DSp CI	(420) (100%) (99.13%-100%)	(425) (100%) (99.14%-100%)	(415) (100%) (70.89%-92.83%)	(401) (100%) (99.08%-100%)

Conclusion:

The WSSV Rapid test kit is fit-for-its defined purposes and demonstrates high overall sensitivity in identifying moderate and heavy levels of WSSV infection or when used on samples from symptomatic shrimp, and the test has very high specificity. The high PPV and NPV of the assay and fast turn-around time (15-30 min onsite vs. >4hrs plus shipping time) make it a powerful tool for identifying potential outbreaks.

We recommend users apply the test to shrimp, that present with behavioural changes (lethargy, decreased or absence of feed consumption, and abnormal swimming behaviours such as slow swimming, swimming on side, swimming near the water surface, or gathering around edges of rearing units) either on a regular basis or when environmental stress, such as rapid changes in salinity, or when suspecting a WSSV outbreak.

Reproducibility

Analytical reproducibility

Conclusion:

Analytical reproducibility evaluation was carried out by 2 laboratories. Six samples with various levels of infection (2 Light, 2 Moderate, 1 Heavy and 1 Negative infection) as determined by the WOH TaqMan real time PCR method (Durand & Lightner, 2002) were selected and provided 'blinded' to the 2 laboratories. Testing was repeated on 4 occasions and a Kappa value was calculated on the results of the 24 repeated assays. There was no misclassification in all assays (20 positive and 4 negative). The agreement of the two methods was 100%, and Kappa =1.0.

Diagnostic reproducibility

Diagnostic reproducibility was conducted by the five laboratories in Taiwan and Thailand, including one WOH reference laboratory. The test panel consisted of 25 samples (with various virus-infected levels, comprising 5 'known' samples (3 positive with target protein concentrations of 1.6, 0.8 or 0.4 ng and 2 negative) and 20 unknown 'blind' samples. The participating laboratories followed the procedures described in the instruction manual of the Inncreate Bioscience WSSV RP Rapid Test Kit.

Conclusion:

Samples were analysed by each of the 5 laboratories using Inncreate Bioscience WSSV RP Rapid Test Kit. Results show high reproducibility. The 5 laboratories showed 100% agreement on the 5 known samples. Four of the 5 laboratories showed 100% agreement on all of the 'blinded' samples, whilst results from one laboratory showed a slight discrepancy for one sample. Chi-square test for homogeneity was conducted to analyse the experimental results from the five labs. Independent Chi-squared test p-value = 0.998 (Hsu et al., 2022).

Reference

- 1) Durand, S., & Lightner, D. V. (2002). Quantitative real time PCR for the measurement of white spot syndrome virus in shrimp. *Journal of Fish Diseases*, 25(7), 381-389.
- 2) Hsu, J. C.-K., Hsu, T.-K., Kannan, J., Wang, H.-C., Tassanakajon, A., & Chen, L.-L. (2022). Diagnostic performance of a Rapid Test Kit for white spot syndrome virus (WSSV). *Aquaculture*, 558, 738379. <https://doi.org/https://doi.org/10.1016/j.aquaculture.2022.738379>.