



Evaluation of point-of-care (POC) tests for white spot syndrome virus (WSSV)

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Background

Project need identified following the 2016/2017 WSSV outbreak in the Logan River region, Queensland

Point-of-care (POC) pathogen testing = field-based, rapid, on-site pathogen testing

- potential to enhance biosecurity and outbreak response measures
- effectiveness dependent on reliability of test results

Project Objective

Determine the analytical and diagnostic performance characteristics (analytical sensitivity and specificity, diagnostic sensitivity and specificity, repeatability and applicability/diagnostic window) of 5 commercially available WSSV POC tests

Compare performance of POC tests

1. to each other
2. to laboratory reference tests (CSIRO and WOAHS WSSV qPCRs)

Purpose

To provide critical preliminary POC test performance information to government and industry so that regulatory authorities can make informed decisions regarding the fitness for purpose of the tests and management of their use in Australia



Proposed intended use of WSSV POC tests on Australian prawn farms

- preliminary on-site diagnosis of WSD where it is suspected in a clinically affected farmed population
- support existing laboratory-based systems for emergency diagnosis of WSD

Project Scope

- Evaluation designed to align with the World Organisation for Animal Health (WOAH) assay validation pathway
- Evaluation panels created from material relevant to Australian circumstances
- Diagnostic performance assessments primarily focused on the ability of the tests to detect WSSV in clinically affected *P. monodon*
- Laboratory-based side-by-side comparative evaluation (did not include field trials)



Evaluation Components

1. Analytical Specificity (ASp)

Cross-reactivity to other pathogens/antigens (exclusivity) and ability to detect different WSSV strains (inclusivity)

2. Analytical Sensitivity (ASe)

Limit of detection for WSSV

3. Relative Diagnostic Specificity (DSp)

Proportion of false positives

4. Relative Diagnostic Sensitivity (DSe) for Clinically Affected Prawns

Proportion of false negatives (for clinically affected prawns)

5. Repeatability

Ability to generate repeatable results for multiple preparations of the same sample

6. Applicability / Diagnostic window

Ability to detect WSSV throughout the course of infection

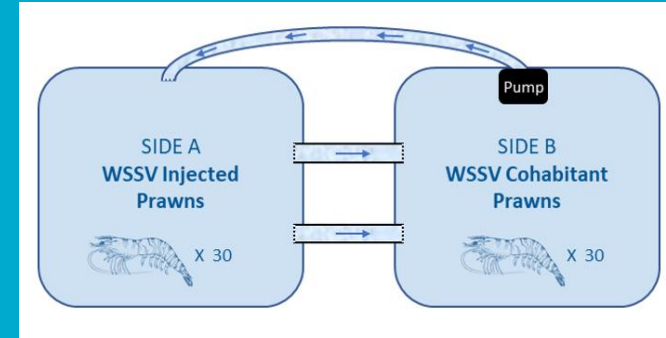
7. Operational Characteristics

E.g. clarity of kit instructions, ease of use, testing timeframe, cost, requirements for additional equipment, shelf-life etc.

- ❖ Evaluation panels prepared for each component and all panel samples tested in strict accordance with manufacturers' instructions

Generation of WSSV-infected prawns

- *P. monodon* experimentally infected with WSSV/Australia/2016-Logan River
- Natural route of infection simulated using cohabitation method



Summary of performance evaluation components completed for each test

Test Evaluation Panel	CSIRO and WOAH Reference Tests	Test A	Test B	Test C	Test D	Test E
1. Analytical Specificity (ASp)	✓	✓ Partial (56%)	✓	✗	✓	✓
2. Analytical Sensitivity (ASe)	✓	✗	✓	✗	✓	✓
3. Relative Diagnostic Specificity (DSp)	✓	✓ Partial (76%)	✗	✗	✓	✓
4. Relative Diagnostic Sensitivity (DSe) for Clinically Affected Prawns	✓	✓ Partial (91%)	✗	✗	✓	✓
5. Repeatability	✓	✗	✗	✗	✓	✓
6. Applicability / Diagnostic Window	✓	✗	✗	✗	✓	✓
7. Operational Characteristics	✓	✓	✓	✗	✓	✓

Test A: discontinued by the manufacturer part-way through the study therefore some components not assessed/partially completed

Test B: unable to detect WSSV/Australia/2016-Logan River therefore diagnostic performance characteristics and repeatability could not be assessed

Test C: unable to be procured therefore could not undergo performance evaluation

❖ *All attempts to obtain additional commercial rapid antigen style tests to include in the evaluation were unsuccessful*



Results – Analytical Specificity

Prawn Pathogen	CSIRO WSSV qPCR	WOAH WSSV qPCR	Test E	Test D	Test A	Test B
Yellow Head Virus 1 (YHV1)	ND	ND	ND	ND	nt	ND
Yellow Head Virus 7 (YHV7)	ND	ND	ND	ND	nt	ND
Gill Associated Virus (YHV2)	ND	ND	ND	ND	nt	ND
<i>Vibrio parahaemolyticus</i> (Vp _{AHPND})	ND	ND	ND	ND	nt	ND
Taura Syndrome Virus (TSV)	ND	ND	ND	ND	nt	ND
Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV)	ND	ND	ND	ND	nt	ND
Hepatopancreatic Parvovirus (HPV)	ND	ND	ND	ND	nt	ND
WSSV Strain	Mean C _t			Qualitative result		
WSSV/Australia/2016-Logan River	12.94	14.66	16.98	+ve	+ve	ND
WSSV/Vietnam/2017	18.93	20.38	23.01	+ve	+ve	+ve
WSSV/China/2017	14.90	16.45	19.88	+ve	+ve	+ve
WSSV/Australia/2022-NSW Broodstock	15.79	17.46	17.80	+ve	+ve	+ve
Prawn Species						
<i>P. monodon</i> (Giant Tiger Prawn)	ND	ND	ND	ND	ND	ND
<i>P. esculentus</i> (Brown Tiger Prawn)	ND	ND	ND	ND	ND	ND
<i>P. merguensis</i> (Banana Prawn)	ND	ND	ND	ND	ND	ND
<i>M. plebejus</i> (Eastern King Prawn)	ND	ND	ND	ND	ND	ND
<i>M. latisulcatus</i> (Western King Prawn)	ND	ND	ND	ND	ND	ND

No cross-reaction to heterologous prawn pathogens

Test B unable to detect WSSV/Australia/2016-Logan River

No cross-reaction to prawn host genome

ND: no positives detected nt: not tested

Results – Analytical Sensitivity

- Limit of detection determined for 3 WSSV strains
- Laboratory reference qPCRs the most sensitive tests for all WSSV strains
- Test E (PCR-based) 10-fold less sensitive than reference qPCRs
- Test D (PCR-based) 1000-fold less sensitive than reference qPCRs
- Test B (rapid antigen style) 10,000-fold less sensitive than reference qPCRs for China and Vietnam strains and unable to detect WSSV/Australia/2016-Logan River

WSSV/Australia/2016-Logan River					
WSSV genome copies/ μ L in prawn tissue homogenate	CSIRO WSSV qPCR	WOAH WSSV qPCR	Test E	Test D	Test B
	C_T value (mean \pm SD), No. +ve/6	C_T value (mean \pm SD), No. +ve/6	C_q value (mean \pm SD), No. +ve/6	No. +ve/6	No. +ve/6
1×10^5	16.83 \pm 0.13, 6/6	18.17 \pm 0.10, 6/6	20.11 \pm 0.03, 6/6	6/6	test unable to detect this WSSV strain
1×10^4	20.31 \pm 0.06, 6/6	21.77 \pm 0.11, 6/6	23.50 \pm 0.10, 6/6	6/6	
1×10^3	23.52 \pm 0.15, 6/6	24.90 \pm 0.21, 6/6	27.20 \pm 0.12, 6/6	6/6	
1×10^2	26.57 \pm 0.17, 6/6	27.99 \pm 0.25, 6/6	30.47 \pm 0.22, 6/6	4/6	
1×10^1	30.76 \pm 0.19, 6/6	31.92 \pm 0.10, 6/6	34.21 \pm 0.40, 6/6	ND	
1×10^0	33.77 \pm 0.51, 6/6	35.11 \pm 0.83, 6/6	36.15 \pm 0.07, 2/6	ND	
1×10^{-1}	35.81 \pm 0.70, 3/6	37.21 \pm 0.77, 2/6	ND	ND	
1×10^{-2}	ND	ND	ND	ND	
Rank	1	1	2	3	4

WSSV/China/2017					
WSSV genome copies/ μ L in prawn tissue homogenate	CSIRO WSSV qPCR	WOAH WSSV qPCR	Test E	Test D	Test B
	C_T value (mean \pm SD), No. +ve/6	C_T value (mean \pm SD), No. +ve/6	C_q value (mean \pm SD), No. +ve/6	No. +ve/6	No. +ve/6
1×10^4	20.02 \pm 0.10, 6/6	21.34 \pm 0.11, 6/6	22.87 \pm 0.11, 6/6	6/6	6/6
1×10^3	23.26 \pm 0.08, 6/6	24.57 \pm 0.05, 6/6	26.73 \pm 0.16, 6/6	6/6	2/6
1×10^2	26.84 \pm 0.05, 6/6	28.12 \pm 0.22, 6/6	30.25 \pm 0.22, 6/6	4/6	ND
1×10^1	29.99 \pm 0.33, 6/6	31.15 \pm 0.38, 6/6	34.04 \pm 0.72, 6/6	ND	ND
1×10^0	33.40 \pm 0.57, 6/6	34.22 \pm 0.52, 6/6	36.18 \pm 1.05, 2/6	ND	ND
1×10^{-1}	36.48 \pm 0.14, 3/6	37.27 \pm 1.02, 4/6	ND	ND	ND
1×10^{-2}	ND	ND	ND	ND	ND
Rank	1	1	2	3	4

WSSV/Vietnam/2017					
WSSV genome copies/ μ L of prawn tissue homogenate	CSIRO WSSV qPCR	WOAH WSSV qPCR	Test E	Test D	Test B
	C_T value (mean \pm SD), No. +ve/6	C_T value (mean \pm SD), No. +ve/6	C_q value (mean \pm SD), No. +ve/6	No. +ve/6	No. +ve/6
1×10^4	20.20 \pm 0.12, 6/6	21.58 \pm 0.11, 6/6	23.20 \pm 0.37, 6/6	6/6	6/6
1×10^3	22.45 \pm 0.19, 6/6	23.90 \pm 0.21, 6/6	27.12 \pm 0.63, 6/6	6/6	ND
1×10^2	26.67 \pm 0.21, 6/6	28.00 \pm 0.11, 6/6	30.75 \pm 0.69, 6/6	4/6	ND
1×10^1	30.18 \pm 0.21, 6/6	31.43 \pm 0.19, 6/6	34.94 \pm 0.56, 6/6	1/6	ND
1×10^0	33.96 \pm 1.00, 6/6	35.85 \pm 0.80, 6/6	36.95 n/a, 1/6	ND	ND
1×10^{-1}	36.34 \pm 0.08, 2/6	37.79 \pm 0.16, 4/6	ND	ND	ND
1×10^{-2}	ND	ND	ND	ND	ND
Rank	1	1	2	3	4

ND: no positives detected

Blue highlight denotes LOD. LOD defined as the final dilution where all 6 test replicates are positive.



Results – Relative Diagnostic Sensitivity (experimentally infected clinically affected prawns) and Relative Diagnostic Specificity

- Determined using 240 *P. monodon* of confirmed WSSV infection status
 - DSp panel = 100 WSSV-negative *P. monodon* (wild-caught and farmed)
 - DSe panel = 140 WSSV-positive experimentally infected clinically affected *P. monodon* (reference qPCR C_T values between 10 - 20)

- All POC tests: 100% relative DSp (no false positives)
- Test D (PCR-based): 100% relative DSe (no false negatives)
- Test A (rapid antigen style): 98% relative DSe (2% false negative)
- Test E (PCR-based): 82% relative DSe (18% false negative)

Test	Relative DSe (95% CI)	Relative DSp (95% CI)	Rank
CSIRO WSSV qPCR	100.00% (97.40% - 100.00%)	100.00% (96.38% - 100.00%)	1
WOAH WSSV qPCR	100.00% (97.40% - 100.00%)	100.00% (96.38% - 100.00%)	1
Test D	100.00% (97.40% - 100.00%)	100.00% (96.38% - 100.00%)	1
Test A	98.44% (94.47% - 99.81%)	100.00% (95.26% - 100.00%)	2
Test E	81.68% (73.98% - 87.89%)	100.00% (96.38% - 100.00%)	3

Results – Repeatability

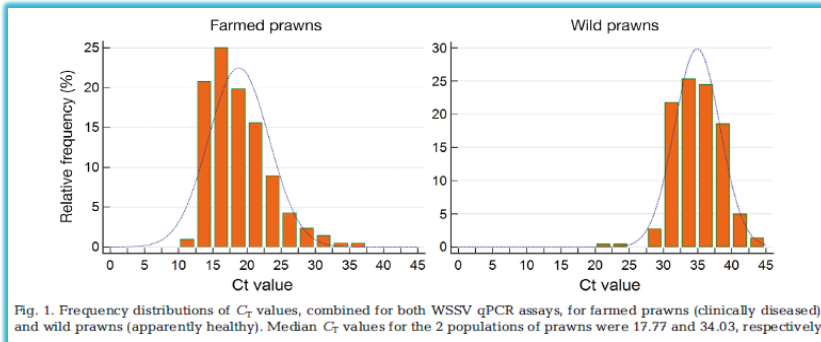
- Determined using negative, strong positive and weak positive prawn tissue homogenate replicates
 - 216 test replicates per test, conducted by 2 operators over 12 different days
- Strong positive or negative homogenates = 100% concordance between test replicates within runs, between runs and between operators.
- Weak positive homogenates = reduced repeatability for POC tests (55% to 78% of weak positive replicates detected)

Results – Applicability / Diagnostic Window

- *P. monodon* experimentally exposed to WSSV via cohabitation and periodically sampled through the course of infection
- First WSSV-positive detections occurred via reference qPCR at 24 hours post exposure
- First positive detections by POC tests (D and E) occurred at 48 hrs post exposure
- Overall, POC tests detected a lower proportion of positives compared to reference tests in the early stages of infection, when clinical manifestation was mild or when reference test C_T values were weak

Aligning POC test performance with reference qPCR C_T value ranges provides useful insight into how POC tests may perform in different populations

- E.g. clinically diseased vs apparently healthy WSSV-infected prawns



Moody et al, 2022

Vol. 150: 169–182, 2022 https://doi.org/10.3354/dao103687	DISEASES OF AQUATIC ORGANISMS Dis Aquat Org	Published online August 18
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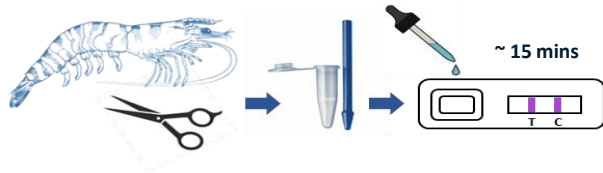
Performance characteristics of two real-time TaqMan polymerase chain reaction assays for the detection of WSSV in clinically diseased and apparently healthy prawns

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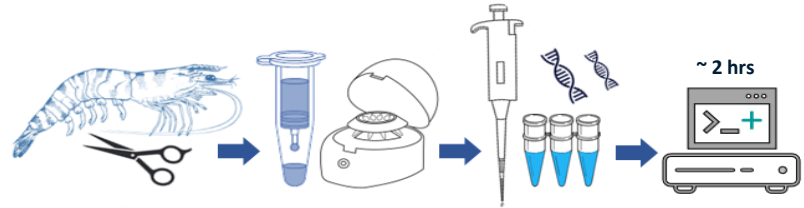


Results – Assessment of Operational Characteristics



Rapid Antigen Style Tests (Tests A & B)

- Easy to conduct and simple to interpret – no special skills or training required
- Minimal processing steps – quick turnaround (~15 mins)
- Cheap (\$10 - \$15 per test)
- No specialised equipment required
- Kit instructions clear
- Room temperature storage (refrigeration not required)
- Shelf-life – 2 years from date of manufacture
- Customer service satisfactory



PCR-Based Tests (Tests D & E)

- More technically complex to conduct – some basic laboratory skills required (e.g. micropipetting)
- Result interpretation easy for Test D; moderately complex for Test E
- On-site training required for Test E
- More processing steps – longer turnaround (~2hrs)
- Specialised equipment and consumables required
- More expensive (\$15 to \$20 per test plus cost of specialised equipment and consumables)
- Kit instructions clear for Test D; printed instructions for Test E lacked critical information.
- Refrigerated storage required for some test components
- Shelf-life – 1 to 3 years from date of manufacture
- Customer service satisfactory

Summary

- Evaluation essential to understand the unique pros/cons and benefits/risks of each test
- Main advantage of rapid antigen style WSSV POC tests was simplicity, however...
 - less sensitive than PCR-based POC tests
 - Test A = discontinued, Test B = specificity issues, other RATs could not be procured
- Main advantage of PCR-based WSSV POC tests was increased analytical sensitivity, however...
 - complexity could pose challenges for operator training and maintaining competency
- Overall, WSSV POC test performance was reduced compared to laboratory reference tests (expected outcome given the simplified design that make POC tests appropriate for field use)

More Information

- Research Project Report has been provided to the APFA, Biosecurity Queensland and SCAAH and will soon be available online at <https://www.frdc.com.au>



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