Final Project Report: FRDC Training Program

Growing disease testing capability in Northern Australia: Molecular diagnostics for aquatic animal diseases.

Contents

Final Report: Growing disease testing capability in northern Australia: Molecular diagnostics for aquatic animal diseases.

Award Recipient: Kelly Condon

Address: (At time of project application ARC Research Hub for Advanced Prawn Breeding) now, JCU AquaPATH James Cook University, Townsville. QLD.

Host Organisation: AAHL Fish Diseases Laboratory, Australian Animal Health Laboratory (AFDL) Geelong. Vic.

Activity Undertaken: Training in the implementation of laboratory quality management systems (LQMS) and qPCR to detect White spot Syndrome Virus (WSSV).

Acronyms:

AAHL: Australian Animal Health Laboratory

AFDL: Australian Fish Disease Laboratory

ANZDT: Australian New Zealand Diagnostic Techniques

BQ: Biosecurity Queensland, QLD Department of Fisheries.

Ct: Cycle threshold

DAWR: Department of Water Resources. Australian Government

FRDC AAHS: Fisheries Resource and Development Cooperation. Aquatic Animal Health Sub-program.

JCU: James Cook University

LQMS: Laboratory Quality Management System

NATA: National Association of Testing Authorities

NQC: Network Quality Control

OIE: Office of International Epizootics, World Health Organisation

QLD: Queensland

qPCR: quantitative Polymerase Chain Reaction

SCAAHLS: Sub-Committee of Australian Animal Health Laboratory standards

RSP: Research Support Programs.

WSSV: Whitespot Syndrome Virus

YHV-1: Yellowhead Virus-1

Outcomes Achieved to date:

The provision of training and access to AFDL resources within this project has developed new competencies by JCU staff in testing for whitespot syndrome virus (WSSV and operating a NATA accredited/pathogen diagnostic laboratory. The FRDC funding and associated training provided by AFDL significantly reduced the time to achieve NATA accreditation. Subsequent to NATA accreditation, JCU AquaPATH was provided permission to test for the detection of WSSV and yellowhead virus-1 (YHV-1) by the QLD Chief Veterinary Officer on 27 August 2018.

This project has led to outcomes for multiple stakeholders.

JCU Specific outcomes includes:

- Gain in knowledge of the LQMS documentation required to operate within NATA requirements.
- Gain in technical knowledge of the operation of qPCRs within a laboratory operating under high throughput and high quarantine conditions.
- Improved technical competency in the operation of equipment in the Thermo Fisher-Applied Biosystems platform.
- Significantly reduced timeframe for JCU AquaPATH laboratory to be in a position to seek NATA accreditation.
- Successfully acquiring NATA accreditation (site 20312).
- JCU being in a position to consult with Biosecurity Qld to be request permission to test for WSSV. Permission was provided on 27/8/18.
- Training of Kelly Condon which can be transferred to other JCU staff at the JCU AquaPATH laboratory.
- Establishing links between JCU and to support future collaboration.
- Increased technical capacity and capability to conduct research to support aquaculture development in Northern Australia.
- Continued monitoring of the performance of JCU AquaPATH to detect WSSV by analysis of the NQC 1-4 supplied within this project.

Aquaculture industry sector specific outcomes includes, but are not limited to:

- Regional access to molecular analysis to support the development of biosecurity management systems on farms.
- Regional access to specific pathogen testing which is operating in compliance with the QLD Biosecurity Act 2014.
- Access to regional facilities to for industry to reduce the risk of introduction of pathogens into farms and improved production.

Government specific outcomes to the training being proposed includes:

- Improved prevention of disease outbreaks on farms which has multiple flow on benefits to government including improved resilience of diversified regional economies, reduced regulatory response costs, improved regional employment, reduced risk of environmental damage.
- Established a cost-effective path to continue the transfer of knowledge to improve capacity to detect pathogens in aquaculture species in regional Australia through the collaborative relationship between with JCU AquaPATH.

- Cost effective establishment of Regional capacity and resources that operate under ISO 17025 standards to support government programs in disease response and disease monitoring activities in Northern Australia.
- Establishment of a regional facility that is harmonized or standardized to the protocols present at the National reference laboratory.
- Integration of an approved detection laboratory within a research facility which enables rapid research into emerging disease issues.

Acknowledgements:

Kelly Condon gratefully acknowledges the support of the FRDC training program funding and the James Cook University Research Support Program. Co-operation of technical staff at the AFDL including Mrs. Stacey Valdeter and Mr. John Hoad is gratefully acknowledged. Dr Nick Moody and Dr Mark Crane from AFDL and the FRDC AAHS are gratefully acknowledged as hosts. Support was also provided by the ARC Hub for Advanced Prawn Breeding through Mrs. Kristin Nunn and Professor Dean Jerry. The project application was gratefully supported by the Australian Prawn Farmers Association.

Background:

The report by the Joint Select Committee on Northern Australia (2016 page *ix*) recognized a pressing need for location of a facility in Northern Australia to real-time test for aquaculture diseases. JCU strategically invested to establish the JCU AquaPATH detection laboratory on the JCU Townsville Campus and sought NATA accreditation in the field of Animal Health.

The incursion and continued detection of WSSV in crustaceans in SE QLD is a concern to the prawn farming industry in Northern Australia. The absence of a regional facility to detect WSSV significantly reduces the ability of the northern prawn farming industry to effectively manage or prevent the biosecurity risks posed by WSSV.

In recognition of the need for regional services to detect WSSV this project was undertaken to provide training to Kelly Condon in the implementation of:

- quality systems within an aquatic animal health testing setting and
- qPCR assays to detect WSSV.

Need:

Through the restrictions of the QLD Biosecurity Act 2014, there are only two laboratories in QLD able to provide testing services to detect exotic aquatic pathogens. As highlighted in the report by the Joint Select Committee on Northern Australia (2016) there is a pressing need for location of a facility in northern Australia to real-time test for aquaculture diseases. The report recommends this facility would be best located in a northern university close to industry as it offers added flow-on advantages such as direct linkages to R&D capabilities (pg. *ix*).

A NATA accredited facility in Northern QLD is considered a priority infrastructure requirement by industry and government stakeholders. The absence of a regionally located facility that can conduct testing for WSSV in particular has current prawn farmers operating under the precarious position of either conducting no testing on wild broodstock or conducting on farm testing to detect WSSV which is in breach of the QLD Biosecurity Act 2014.

As well as the immediate needs of the Australian prawn aquaculture industry to screen for WSSV, finfish companies that export fingerlings are require access to NATA accredited facility that can provide testing for the detection of reportable pathogens such as Nervous Necrosis Virus (NNV). Recognising companies may be shipping samples on a monthly basis access to immediate testing within negotiated turnaround times is required. Laboratories which have been required to respond to the WSSV incursion have not be able to provide rapid turnaround on non-priority 1 rated samples. Companies that require non-priority 1 testing face significant uncertainty to businesses continuity.

Testing for exotic pathogens and for health certification requires a demonstration of meeting standard ISO 17025. In Australia, NATA accreditation is used to demonstrate the requirements of ISO 17025 for conducting services in the field of testing and calibration. Achieving accreditation is a complex task that, in the case of the JCU application to support the Northern aquaculture sector, needs to address at a minimum the following documents:

- the NATA application document for the field of Animal Health (formerly termed Veterinary testing)
- OIE Manual of Diagnostic Tests for Aquatic Animals
- Animal Health Committee Formerly Subcommittee for Animal Health Laboratories Standards (formerly SCAAHLS Veterinary Guidelines for Nucleic Acid detection techniques)
- Australian New Zealand Standard Diagnostic Techniques (ANZDT)
- QLD Biosecurity Act 2014

The strategy being implemented to rapidly upskill Kelly Condon in the various requirements of operation within a NATA accredited facility specific to the molecular detection of aquatic pathogens, directly addresses some of the concerns and needs of industry and situate an inclusive aquaculture disease testing capability in northern Australia.

Objectives:

1. JCU obtains NATA accreditation in the field of Animal Health in the scope of molecular detection and identification of viruses.
2. JCU obtains permission from Biosecurity QLD to conduct testing for the detection of WSSV and YHV-1.
3. Diagnostic capacity for testing for pathogens of significance to tropical aquaculture is established in northern Queensland

Methods:

The project occurred as described in the project application, although some variation from the project flow occurred. The variations consisted of:

1. JCU AquaPATH undergoing NATA accreditation application processes during the period of the project rather than at the completion.
2. Extended period of the project.

The variation was due to:

1. Time required to obtain permission from Biosecurity QLD to allow testing for WSSV within the project scope (Permission obtained 27/8/18).
2. A review of ISO 17025 being completed during the project (ISO/IEC 17025:2017).
3. A mechanical issue with the QuantStudio-3 real-time machine at JCU (February 2018).
4. Time delays in performing testing due to work flow priorities of JCU AquaPATH and AFDL.

Non-technical phases of the project occurred as described in the project proposal. Comparison of analysis to detect to WSSV between JCU and AFDL was completed as described below with reference to NATA Technical Note 17 Validation of Assays (refer to Appendix 4 for proposed method developed in the project and Appendix 5 for panel preparation details):

Network Quality Control

AFDL provided four NQC samples with defined Ct value to JCU AquaPATH for the purpose of initial testing and optimization of systems within JCU AquaPATH to detect WSSV (defined as NQC 1 & 2 CSIRO AFDL AAHL method and NQC 3 & 4 OIE method). These plasmid positive controls were generated as part of FRDC Project 2014-002.

Sample

AFDL provided two panels consisting of thirty "unknown" samples to JCU AquaPATH. One panel was for analysis for the detection of WSSV using the OIE recommended TaqMan assay (Durand and Lightner 2002,. referred to as WSSV-1 within the JCU AquaPATH systems). A second panel was for analysis for the detection of WSSV using the CSIRO AFDL AAHL assay (Sritunyalucksana et al 2006, referred to as WSSV-2 within the JCU AquaPATH systems). For both panels the "unknown" samples consisted of twenty aliquots containing various dilutions of the WSSV positive control plasmid and ten aliquots of negative samples.

The WSSV positives were prepared from synthesized DNA constructs and are used within the AFDL as the source of the Network Quality Control for the detection of WSSV by qPCR (OIE: WSSV NQC 3 & 4) and (CSIRO AFDL AAHL: WSSV NQC 1 & 2).

qPCR analysis

JCU AquaPATH conducted qPCR analysis for the detection of WSSV using the OIE WSSV qPCR and CSIRO AFDL AAHL WSSV qPCR (13-06-002-003 & 13-06-001-002-001). JCU AquaPATH modification of the respective worksheets are attached. Refer to Appendix 7.

Three master mixes were evaluated in the analysis; Applied Biosystems Universal Master Mix, Ag Path-ID One step RT-PCR (4387391 Life Technologies) and Bioline SensiFAST Low Rox (BIO-78005).

qPCR platform

Analysis was conducted on both qPCR platforms present at JCU AquaPATH; Quant Studio 3 and Quant Studio 5 Thermo Fisher real-time PCR machines. Due to the high throughput requirements in the laboratory the QuantStudio 5 machine was used more frequently.

Repeat analysis

Analysis was repeated at least 20 times on different days within the JCU AquaPATH work flow. Ct values were recorded for each sample and matrix combination. The number of replicate tests conducted on each dilution of the equivalence panels are included in Tables 1 and 2. Resulting Ct values were provided to AFDL whereby details of sample preparation and Ct value from CSIRO AFDL analysis were provided to JCU.

Results/Discussion

Consultative and Non-Technical phases of the project:

Briefly, the project progressed through the following process:

1. **Industry consultative and training phase**: Meeting between JCU staff, AFDL and Northern Aquaculture industry representatives to compile industry specific needs (assay targets) of the JCU facility. Kelly Condon conducted a workshop at the APFA annual conference on molecular detection of pathogens (August 2017). Discussions were held with farm staff regarding sample collection methods, interpretation of qPCR results and difficulties faced by industry that relate submission of samples for molecular analysis. Additional discussions were held with other Aquaculture industry partners which indicate a need for other assays to be implemented at JCU AquaPATH. Refer to Appendix 1 for a Summary of outcomes from this phase.

2. **Training phase for Ms. Condon**: Under the direction of staff at AFDL, Ms. Condon attended the AFDL facility for 2 days (11 and 12th October 2017). Refer Appendix 2 for a summary of outcomes from this phase. Briefly, the visit to AFDL consisted of:

    - A tour of AFDL facilities, including introduction to staff, general facility operations, high containment processes, general equipment and laboratory orientations and sample processes from sample receipt through to authorisation of analysis reports.
    - An overview of systems that were implemented to prevent contamination and increase throughput of results was demonstrated.
    - Observation of the quality management systems and relevant SOPs of AFDL. Particularly highlighted were the conditions implemented in response to WSSV testing requirements and the practise by staff to keep documentation as concise as possible.
    - Observation of the AFDL WSSV qPCR assay validation dossier.
    - Observation of the electronic arrangement of the Laboratory Quality Management System.

3. **JCU Preparatory Phase**: JCU staff prepared LQMS documentation with comment provided by Dr Nick Moody. Comparison with the AFDL LQMS ensured appropriate systems were implemented at JCU prior to seeking NATA accreditation.

4. **JCU inspection phase**: Dr Nick Moody visited JCU to conduct a quality management systems observation of the JCU facility. Proposed Method to demonstrate equivalence in detection was prepared by Kelly Condon with guidance from Dr Moody in consultation with NATA Technical Note 17: 2013. (Refer to Appendix 3).

5. **JCU modification phase**: JCU addressed recommendations of Dr Moody's LQMS and NATA technical audit. JCU lodged documentation to NATA. NATA assessment visit 22nd February 2018. NATA accreditation notice within the field of Animal Health received 14 June 2018.

Technical Phase: qPCR analysis to detect WSSV

During the **Final Assessment Phase**, JCU AquaPATH conducted molecular assays on reference panels to demonstrate technical equivalence in detection of WSSV using qPCR with the National Reference Laboratory. Sample preparation and panel label information of the panel are provided in Appendix 5.

Tables 1 and 2 provide a summary of the qPCR Cycle threshold (Ct) values obtained from qPCR analysis conducted by JCU AquaPATH and CSIRO AFDL AAHL using the OIE (Table 1) and CSIRO AFDL AAHL assays (Table 2). The full Ct results are provided in Appendix 8. **Chart 1** illustrates a consistent difference of approximately 1.5-2 Cts between all of the panel sample results obtained at JCU AquaPATH and CSIRO AFDL AAHL using the OIE Assay. The number of replicate tests (n=) of each concentration ranged from 48 to 84 at JCU AquaPATH and 9 to 12 at CSIRO AFDL AAHL.

Table 1 Summary of Ct values obtained at JCU AquaPATH and AFDL AAHL using the OIE Assay								
	Sample Information							
Laboratory	WSSV Copies	10^5	10^4	10^3	$10^{2.5}$	10^2	$10^{1.5}$	10^1
	Sample Id #s	2,16,25	9,29,30	*11,12,21	6,7,24	1,19,22	17,27	*5,13,18
				*NQC-3				*NQC- 4
JCU AquaPATH	Mean Ct	21.6	25.1	28.2	30.6	31.9	33.9	35.0
	St. Dev.	0.3	0.7	0.4	0.4	0.8	0.6	0.6
	n=	72	72	84	72	72	48	84
AFDL	Mean Ct	20.0	23.6	26.8	29.1	30.1	32.6	33.6
	St. Dev.	0.1	0.1	0.1	0.2	0.1	0.1	0.2
	n=	9	9	12	9	9	9	12
Comparison	Diff Av Ct	1.7	1.5	1.5	1.5	1.9	1.4	1.4
	WSSV Copies	10^5	10^4	10^3	$10^{2.5}$	10^2	$10^{1.5}$	10^1
*	Indicates the NQC 3& 4 at AFDL. Acceptance range is +/- 2 Ct.							

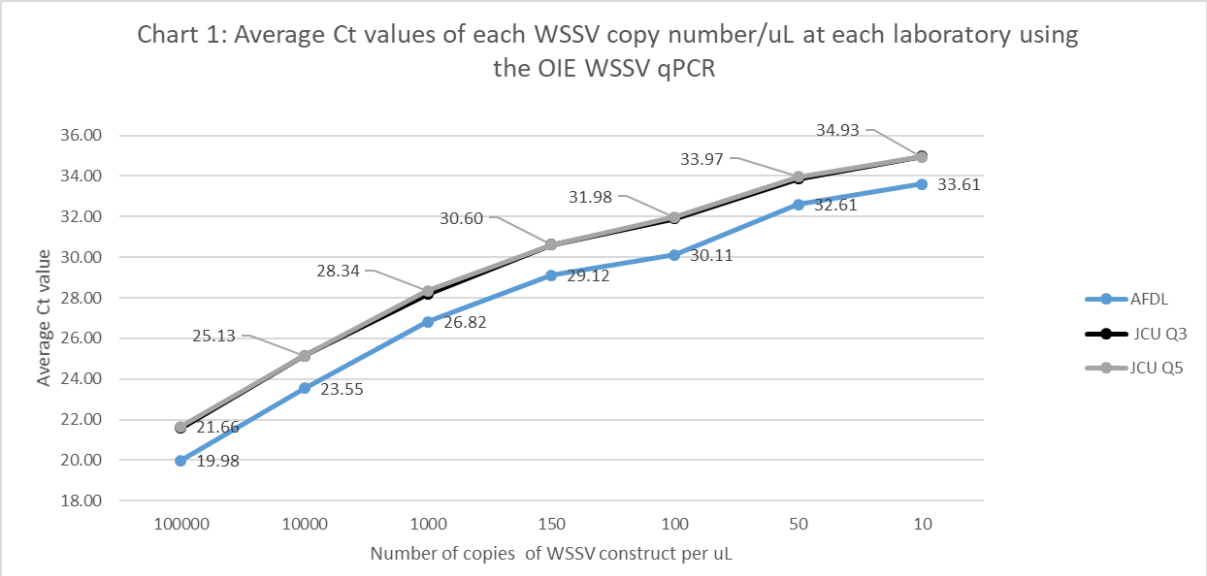
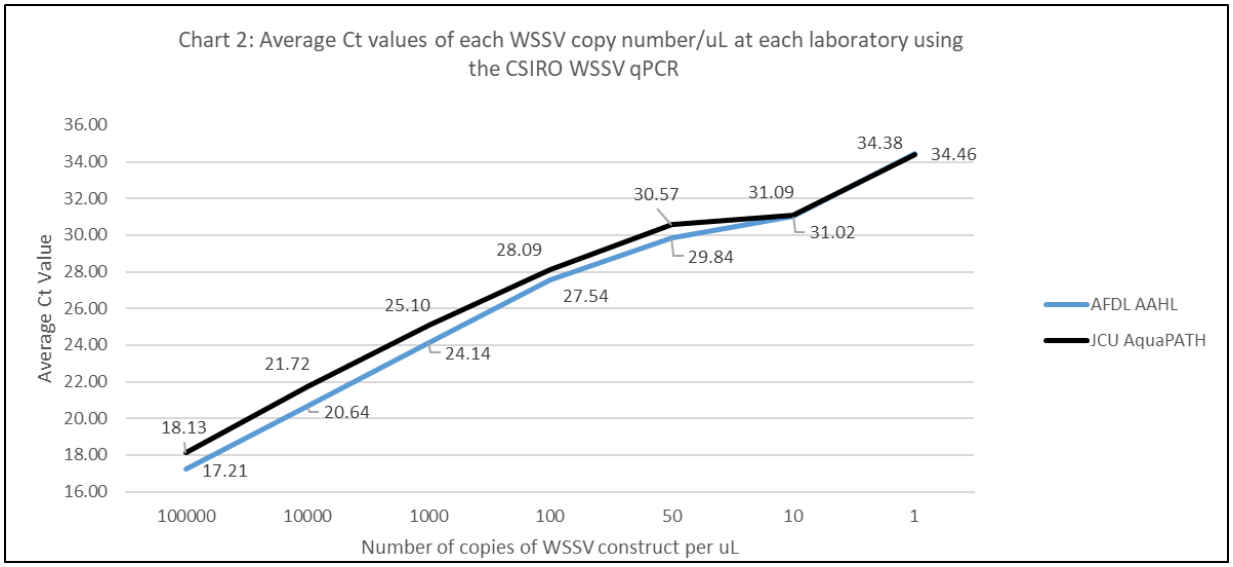


Chart 2 illustrates a consistent difference of less than 1 Ct for each of the panel sample results obtained at JCU AquaPATH and AFDL using the CSIRO AFDL AAHL assay. The number of replicates of each concentration ranged from 56 to 112 at JCU AquaPATH and 9 to 12 at CSIRO AFDL AAHL assay. The standard deviation of each concentration ranged from 0.3 to 1.1 Ct at JCU AquaPATH. The standard deviation of each concentration ranged from less than 0.1 to 0.6 at CSIRO AFDL AAHL assay. All Ct values obtained during JCU AquaPATH analysis were +/- 2 Ct values of the CSIRO AFDL AAHL assay result.

Table 2 Summary of Ct values obtained at JCU AquaPATH and AFDL AAHL using the CSIRO Assay								
	Sample Information							
	WSSV Copies	10^5	10^4	10^3	10^2	$10^{1.5}$	10^1	10^0
	Sample Id #s	26,21,13	28,23,3	*16,6,5	29,4,15	9,10	*2,14,20	12,17,24
Laboratory	Network Controls			*NQC 1			*NQC 2	
JCU AquaPATH	Mean Ct	18.1	21.7	25.1	28.1	30.6	31.1	34.4
	St. Dev.	1.0	0.8	0.9	1.1	1.0	1.1	1.0
	n=	84	84	112	84	56	111	84
AFDL AAHL	Mean Ct	17.2	20.6	24.1	27.5	29.8	31.0	34.5
	St. Dev.	0.1	0.0	0.1	0.1	0.1	0.2	0.6
	n=	9.0	9.0	9.0	9.0	6.0	9.0	9.0
Comparison	Difference Mean Ct	0.92	1.08	0.96	0.56	0.73	0.07	-0.08
	WSSV Copies	10^5	10^4	10^3	10^2	$10^{1.5}$	10^1	10^0
*	Indicates the Network Quality Control 1 & 2 at AFDL. Acceptance range= +/- 2 Ct from average of 24.03 and 31.12 .							



JCU AquaPATH obtained results consistently comparable with that of AFDL using both assays. The difference in Ct values was greatly reduced in the application of the CSIRO AFDL AAHL assay. The difference in performance between the two assays may be a result of improved staff training. Testing with the OIE assay was conducted as the first part of the training program. During this phase JCU AquaPATH were implementing new systems and had not established methods for mixing and thawing the synthetic constructs. Testing with the CSIRO AFDL AAHL assay was conducted after the OIE assay training and significant thawing and mixing of the constructs was completed during template addition. Also, and the use of multichannel, adjustable width, multi-stepper pipettes were implemented for the WSSV AFDL analysis. Monitoring of the performance of JCU AquaPATH to conduct analysis for WSSV by qPCR using the NQCs 1-4 continue beyond the period of the project.

Benefits and Adoption:

JCU AquaPATH has implemented a laboratory quality management system and processes that have been audited and deemed appropriate to meet NATA accreditation in the field of animal health testing. JCU AquaPATH achieved accreditation (site 20312) (Appendix 9).

Within the requirements of the Biosecurity Act 2014, JCU AquaPATH has commenced a step-wise approach to facilitate the inclusion of the detection of WSSV by qPCR into the scope of accreditation. The first step of the approach has been completed within this project, namely the demonstrated equivalence of qPCR to detect a WSSV-like synthetic construct using the WSSV OIE recommended qPCR assay (OIE assay WSSV-1 qPCR) and the additional assay designed by (CSIRO AFDL AAHL assay WSSV-2 qPCR).

The OIE assay was implemented on the Thermo Fisher Quant Studio 3 and 5 platforms using the reagent matrix recommended by the DAWR, equivalent to those employed at CSIRO AFDL AAHL, with additional back up master mixes. The WSSV CSIRO AFDL AAHL assay was applied using a broad reagent matrix which used three Master Mixes and two probe suppliers and both the QuantStudio 3 and 5 PCR machines.

The variation in qPCR results between labs frequently relate to extraction process and master mix interactions with nucleic acid extracts. Due to the absence of an extraction process in this project, no attempt was made to ensure a balanced statistical design to the assay matrix. Further approval from Biosecurity QLD has been provided to conduct analysis on inactivated tissue samples for the detection of WSSV within the Australian National Quality Assurance Program (ANQAP) and on tissue samples collected from areas considered to be free of WSSV. The permission was acquired on the 27/8/18. Continued inclusion of the NQCs 1 to 4 in the WSSV testing protocols will allow JCU AquaPATH to continue to monitor the proficiency of staff and performance of the OIE and CSIRO AFDL AAHL qPCR assays. JCU AquaPATH completed the 2018 ANQAP Proficiency testing for the detection of WSSV. Results from the WSSV analysis have been submitted to ANQAP however inter-laboratory reports have not been issued at the time of this report.

Further Development:

Subject to approvals from Biosecurity QLD further validation will be conducted using deactivated tissue as the sample matrix using multiple master mixes. Presently approvals are for analysis of tissue samples within the ANQAP WSSV PT and for the analysis of samples collected from areas considered WSSV-free for the purpose of supporting translocation of wild broodstock.

Validation of assays to detect additional pathogens of priority industry concern is planned. Adoption of assays to detect agents discovered within partnership projects with JCU AquaPATH has been implemented. JCU AquaPATH has provided research data to AFDL and Biosecurity QLD regarding the detection of Whenzhou Shrimp Virus-2 from research projects.

Further improvements to Quality

Continued application of resources to address items that were recognized in this project and also within NATA accreditation processes (MAN QUAL-004: Risks to quality) are on-going. Refer to Appendix 6 . A new staff member, Mr. Thomas Ackery has been appointed and is undergoing training at JCU AquaPATH. Thomas completed 3 rounds of testing the WSSV CSIRO AFDL AAHL panel, under the supervision of Kelly Condon within his staff training plan. Mr. Ackery also completed analysis on the ANQAP aquatic proficiency panel for the detection of Nervous Necrosis Virus.

Continued investment in infrastructure

JCU continues to invest in facilities at JCU AquaPATH using both operational funds and those obtained from internal funding from JCU Research Support Projects program (RSP) to purchase equipment. (Refer to Appendix 6)

Continued support to Tropical Aquaculture Industries

With collaborative support from industry, JCU AquaPATH is compiling photographic and tissue sample resources that will be applied to improve Biosecurity management systems within tropical aquaculture industries in Northern Australia.

JCU AquaPATH is imbedded within industry research projects including the:

- ARC Research Hub in Advanced Prawn Breeding
- Northern Australia CRC project*: Improved Biosecurity in Prawn Aquaculture* (commenced July 1 2018)
- Two industry linked Innovations Connections Projects monitoring for the presence of endemic viral pathogens in the prawn farming environment.

JCU AquaPATH has permission to conduct testing to support industry prawn translocation requirements and has conducted analysis for 3 hatchery operations in Northern Queensland.

Within the conditions of acquiring permission to test for WSSV JCU AquaPATH must provide quarterly reports of WSSV testing to Biosecurity QLD.

On-going staff development though collaborative research arrangements and NATA training programs and industry programs are considered a priority in staff development plans.

References:

Durand S., and Lightner D., (2002) Quantitative real time PCR for the measurement of white spot syndrome virus in shrimp. J. Aquat. Animal Health, 12 128-135 in OIE Aquatic Manual On-line http://www.oie.int/index.php?id=2439&L=0&htmfile=chapitre_wsd.htm

NATA Technical Note 17 (2013) Guidelines for the validation and verification of quantitative and qualitative test methods. Scaling Up: Inquiry into Opportunities for Expanding Aquaculture in Northern Australia. Joint Select Committee on Northern Australia. Commonwealth of Australia 2016. ISBN 978-1-74366-378-3 (printed).

Sritunyalucksana K, Srisala J, McColl K, Nielsen L, Flegel TW. 2006. Comparison of PCR methods for white spot syndrome virus (WSSV) infections in penaeid shrimp. Aquaculture 255**,** 95-104.

Intellectual Property:

No Intellectual Property claims are made within this project.

Appendices:

Appendix 1. Summary of Industry Impediments/Needs prepared from meetings with Industry representatives at APFA Symposium 2017.

Item discussed	Issues raised	Outcome at present
Difficulty in sending samples to the lab	Tubes and buffers required, courier inconsistency in service quality and availability; which samples to collect.	Clients are required to register with JCU AquaPATH. (FORM FRD 002 Client registration). Registered clients are provided with items required for sample submission. Various transport carriers were investigated and optimal couriers determined for the individual farms and urgency of testing requirements. Further investigations are on-going in collaboration with providers of scientific consumables to simplify the collection of samples process. Training in sample collection and transport has been provided to registered clients. Farm Released Documents (FRDs) are prepared within the LQMS.
Application of LAMP assays	Weaknesses in these assays discussed. Lack of validation discussed.	Being investigated by BQ and AFDL.
Clinical signs of disease	Not many pathognomic clinical signs present for prawn viruses.	On-going work at JCU AquaPATH is photographing of clinical signs to align with analysis results. Targeting of samples with specific clinical signs e.g. Red stripe, deformed tails, blisters in brachiostagial membranes and red gills.
Target Assays required	Fish farms require health certification from NATA accredited laboratory for freedom from NNV and RSIV. Prawn farmers suspect presence of presently unidentified agent.	NNV is included in the NATA scope of accreditation. Topic of on-going research and a topic to be investigated in the Northern Australia CRC funded Biosecurity Project. (commenced 1 July 2018).
Example of how results of qPCR can be integrated into management processes	Different farms have different protocols. Assays at JCU AquaPATH won't be validated within NATA scope for provision of quantitative results.	Non-NATA assays can have results presented any way provided is within the NATA rules. This will require: • On-going collection of data from assays to support validation. • Provision of multiple report types to clients. • Northern Australia CRC Biosecurity project will investigate the application of swabs as a sample collection method for farmers.

Appendix 2: Summary of Items identified for attention at JCU AquaPATH from AFDL visit November 2017

Item discussed	Detail	Outcome
NQC monitoring	Identified the need for continued collaboration between labs to monitor inter-laboratory performance of the NQC. The details collected to monitor the NQC.	Agreement to extend the scope of the current MTA between JCU AquaPATH and AFDL. JCU AquaPATH to continue to provide NQC data to AFDL beyond the project. Within the provision of NATA accreditation and BQ permission to test. AFDL NQC register template acquired. Table has been updated to include operator and Master Mix in response to NATA audit and to support validation and monitoring of Metrological traceability of the qPCR assays.
Increased throughput	Kingfisher robot and Tissue lysis machines Increase staff numbers Additional QuantStudio-5 machine.	Kingfisher Extraction Robot acquired at JCU AquaPATH (April 2018). ViaFlow Liquid dispensing system acquired at JCU AquaPATH (June 2018). Employed Mr. Thomas Ackery April 2018. Currently scoping additional staffing options. Additional QuantStudio-5 purchase is in progress. Purchase order placed 30/8/18.
Harmonisation	Types of Mixes and consumables used	JCU AquaPATH has adopted AFDL components where is economically possible but also employs additional reagents recognizing potential supply issues if emergency response is required. JCU AquaPATH continues to, conduct comparative matrix analysis of qPCR components within assays with externally monitored Network controls (e.g. ANQAP samples or WSSV or Pir-A (AFDL implementation panel)

Appendix 3: Summary of items identified from Dr Nick Moody visit to JCU AquaPATH

Item discussed	Detail	Outcome
Laboratory Quality Management Documentation	Write protocols as simply as possible. Requirement for some protocols that were not at JCU AquaPATH.	A number of AFDL protocols adapted for JCU AquaPATH: Staff training and competency checklist WSSV NQC register WSSV worksheets AFDL WSSV qPCR validation dossier (*provided in confidence within MTA not included in this report*)
Staff training	Access to on-going training and working within the Aquatic Animal Health Network	JCU supported travel for Julie Goldsbury to attend the Aquatic Animal Health Technical Forum 2018.
Increased throughput in the laboratory	KingFisher Robot. Increase staff	Addressed in Table 5
Proficiency Panel	General lack of PT panels for the molecular detection of prawn pathogens is recognized as a risk to quality management both at JCU AquaPATH and across the laboratory network.	Agreed to continue MTA with Pir-A like gene detection and possible general inclusion of other pathogens (YHV-7, HPV and other research outcomes). Transfer of some High Load YHV-7 tissues to AFDL. Transfer of information regarding detection of Wenzhou Shrimp Virus-2.
Laboratory Standards and Protocols.	PCR hoods in shared space in MEEL. Although considered "pre-PCR" is in the same room as thermal cyclers. Containment of pathogens within the laboratory.	JCU AquaPATH did not move until response from NATA audit raised same issue. PCR hoods are now in isolated "Pre-PCR suite". PCR consists of 3 hoods divided into Master Mix Preparation; Master Mix dispensing; Template addition. Samples received in deactivating agent. Implementation of automatic Ethanol hand sanitizer dispenser at all laboratory exits and adoption of other protocols within JCU Biosafety approvals process.
Dr Moody: demonstration of data to support validation of CSIRO AFDL AAHL assay to detect WSSV.	Demonstration of data and discussion of OIE and NATA validation path and noted assay characteristics to be investigated with equivalence panel.	Preparation of protocol/process for WSSV panels at JCU AquaPATH. Completion of analysis on WSSV OIE and CSIRO AFDL AAHL implementation panels.

Appendix 4: Proposed Project Method.

Proposed testing schedule for WSSV equivalence at JCU AquaPATH

Background: JCU AquaPATH is participating in an FRDC training program, part of which involves training for the detection of White spot Syndrome Virus (WSSV) by qPCR. JCU AquaPATH is also seeking NATA accreditation in the field of Veterinary testing through the application of tests within the class of detection of nucleic acid. JCU AquaPATH is seeking to include WSSV in the scope of application based on demonstration of equivalence in detection with. CSIRO AFDL AAHL.

The Biosecurity Act in QLD requires JCU AquaPATH to request permission to conduct analysis for the detection of WSSV. Permission has been provided (with the following conditions:

- The Positive control material is a plasmid and not viable or complete WSSV.
- BQ is provided with updates of test results and project reports.

(Correspondence previously provided to Dr Nick Moody).

Aim: To conduct qPCR analysis of positive control constructs to demonstrate equivalence between JCU AquaPATH and CSIRO AFDL AAHL in the detection of White spot syndrome virus (WSSV).

Method:

Network Quality Control

will provide NQC sample with defined Ct value to JCU AquaPATH for the purpose of initial testing and optimization of systems within JCU AquaPATH to detect WSSV. When systems are optimized JCU AquaPATH will conduct repeat end point analysis on the NQC to monitor Limit of detection and provide additional data to calculate measurement of uncertainty in detection.

Sample

provided two panels consisting of thirty "unknown" samples to JCU AquaPATH. One panel was for analysis for the detection of WSSV using the OIE recommended TaqMan assay (Durand and Lightner 2002 in the OIE Aquatic Manual Online Accessed August 2018) The second panel was for analysis for the detection of WSSV using the CSIRO AFDL AAHL designed assay. For both panels the "unknown" samples consisted of twenty aliquots of WSSV positive control plasmid and ten aliquots of Negative or No template samples.
WSSV positive control constructs were prepared from those used within the AFDL as the source of the Network Quality Control for the detection of WSSV by qPCR (OIE: WSSV NQC 3 & 4) and (CSIRO AFDL AAHL: WSSV NQC 1 & 2).

qPCR analysis

JCU AquaPATH will conduct qPCR analysis for the detection of WSSV using the OIE WSSV qPCR and CSIRO AFDL AAHL WSSV qPCR (13-06-002-003 & 13-06-001-002-001). JCU AquaPATH modification of the respective worksheets are attached.

Within the Australian veterinary laboratory network AFDL and EMAI both conduct high throughput analysis for to detect WSSV by qPCR. To establish some resilience in the ability to compare results between high throughput laboratories the Master Mixes used by both laboratories were included in the analysis namely Applied Biosystems Universal Master Mix () and Ag Path ().

Any future outbreak of WSSV into prawn farms in Australia would likely require high throughput testing. As a contingency back up Master Mix for qPCR detection of WSSV the Bioline SensiFAST Low

ROX Master Mix was also included in the analysis. This Master Mix is used within QML, human pathology and is held in reserve stocks within Australia. Beyond this project, further validation of the Master Mixes will be required on Total Nucleic Acid Extracts from tissues.

qPCR platform

Analysis will be conducted on both qPCR platforms present at JCU AquaPATH namely, Quant-Studio 3 and Quant-Studio 5 Applied Biosystems real-time thermal cyclers.

Repeat analysis

Analysis will be repeated at least 20 times on different days within the JCU AquaPATH work flow.

qPCR Master Mix

As a preparatory measure, considering some instances of delayed supply to Townsville with scientific consumables, JCU AquaPATH will conduct repeat analysis using the Applied Biosystems TaqMan Universal PCR Master Mix and the Bioline SensiFAST Probe low Rox Mix. The Bioline Master Mix is considered a back-up Master Mix which is available to JCU AquaPATH with rapid consistent supply to Townsville.

Results

Results will be provided as Ct value of all assays including the detection of the internal contamination sequence. Appropriate statistical analysis will be conducted on the Ct values to:

- Demonstrate limit of detection.
- Sensitivity of detection (positives correctly detected)
- Specificity of detection (negatives correctly detected)
- Reproducibility of detection (of both assays vs AFDL results)
- Measurement of Uncertainty (variation in Ct value of each sample with repeat testing at JCU AquaPATH)

OIE WSSV qPCR PANEL									
PANEL SAMPLE	Rep 1 C_T	Rep 2 C_T	Rep 3 C_T	MEAN C_T	SD	Av all same	SD all same	RESULT INTERPRETATION	SAMPLE DETAILS
5	33.74	33.31	33.76	**33.60**	0.26			POSITIVE	pWSSV_OIE_qPCR 10^1 copies/µL diluted in TE + yeast tRNA (50ng/µL) This is the dilution used for the AFDL NQC-4
13	33.80	34.02	33.65	**33.82**	0.18			POSITIVE	pWSSV_OIE_qPCR 10^1 copies/µL diluted in TE + yeast tRNA (50ng/µL) This is the dilution used for the AFDL NQC-4
18	33.74	33.34	33.16	**33.41**	0.29	33.61	0.262744	POSITIVE	pWSSV_OIE_qPCR 10^1 copies/µL diluted in TE + yeast tRNA (50ng/µL) This is the dilution used for the AFDL NQC-4
19	30.27	30.26	30.28	**30.27**	0.01			POSITIVE	pWSSV_OIE_qPCR 10^2 copies/µL diluted in TE + yeast tRNA (50ng/µL)
22	29.66	30.26	30.07	**29.99**	0.31			POSITIVE	pWSSV_OIE_qPCR 10^2 copies/µL diluted in TE + yeast tRNA (50ng/µL)
1	29.98	30.11	30.10	**30.06**	0.07	30.11	0.188597	POSITIVE	pWSSV_OIE_qPCR 10^2 copies/µL diluted in TE + yeast tRNA (50ng/µL)
21	26.75	26.62	26.76	**26.71**	0.08			POSITIVE	pWSSV_OIE_qPCR 10^3 copies/µL diluted in TE + yeast tRNA (50ng/µL) This is the dilution used for the AFDL NQC-3
11	26.88	26.73	26.88	**26.83**	0.09			POSITIVE	pWSSV_OIE_qPCR 10^3 copies/µL diluted in TE + yeast tRNA (50ng/µL) This is the dilution used for the AFDL NQC-3
12	26.84	26.77	26.85	**26.82**	0.04	26.79	0.07784	POSITIVE	pWSSV_OIE_qPCR 10^3 copies/µL diluted in TE + yeast tRNA (50ng/µL) This is the dilution used for the AFDL NQC-3
30	23.51	23.57	23.52	**23.53**	0.03			POSITIVE	pWSSV_OIE_qPCR 10^4 copies/µL diluted in TE + yeast tRNA (50ng/µL)
9	23.56	23.41	23.56	**23.46**	0.08			POSITIVE	pWSSV_OIE_qPCR 10^4 copies/µL diluted in TE + yeast tRNA (50ng/µL)
29	23.61	23.70	23.70	**23.67**	0.05	23.57	0.086345	POSITIVE	pWSSV_OIE_qPCR 10^4 copies/µL diluted in TE + yeast tRNA (50ng/µL)
2	20.04	20.01	19.93	**19.99**	0.06			POSITIVE	pWSSV_OIE_qPCR 10^5 copies/µL diluted in TE + yeast tRNA (50ng/µL)
25	20.10	20.05	20.03	**20.06**	0.03			POSITIVE	pWSSV_OIE_qPCR 10^5 copies/µL diluted in TE + yeast tRNA (50ng/µL)
16	19.97	19.89	19.84	**19.90**	0.07	19.98	0.079647	POSITIVE	pWSSV_OIE_qPCR 10^5 copies/µL diluted in TE + yeast tRNA (50ng/µL)
24	29.33	29.02	29.03	**29.12**	0.17			POSITIVE	pWSSV_OIE_qPCR $10^{1.5}$ copies/µL diluted in TE + yeast tRNA (50ng/µL)
6	29.26	29.16	28.96	**29.13**	0.16			POSITIVE	pWSSV_OIE_qPCR $10^{1.5}$ copies/µL diluted in TE + yeast tRNA (50ng/µL)
7	29.24	29.08	29.01	**29.11**	0.12	29.12	0.124543	POSITIVE	pWSSV_OIE_qPCR $10^{1.5}$ copies/µL diluted in TE + yeast tRNA (50ng/µL)
27	32.59	32.75	32.74	**32.60**	0.09			POSITIVE	pWSSV_OIE_qPCR $10^{2.5}$ copies/µL diluted in TE + yeast tRNA (50ng/µL)
17	32.61	32.71	32.53	**32.62**	0.09	32.66	0.083081	POSITIVE	pWSSV_OIE_qPCR $10^{2.5}$ copies/µL diluted in TE + yeast tRNA (50ng/µL)
3	und	und	und	**und**	n/a			Negative	TE + tRNA (50ng/µL)
4	und	und	und	**und**	n/a			Negative	TE + tRNA (50ng/µL)
8	und	und	und	**und**	n/a			Negative	TE + tRNA (50ng/µL)
10	und	und	und	**und**	n/a			Negative	TE + tRNA (50ng/µL)
14	und	und	und	**und**	n/a			Negative	TE + tRNA (50ng/µL)
15	und	und	und	**und**	n/a			Negative	TE + tRNA (50ng/µL)
20	und	und	und	**und**	n/a			Negative	TE + tRNA (50ng/µL)
23	und	und	und	**und**	n/a			Negative	TE + tRNA (50ng/µL)
26	und	und	und	**und**	n/a			Negative	TE + tRNA (50ng/µL)
28	und	und	und	**und**	n/a			Negative	TE + tRNA (50ng/µL)

CSIRO WSSV qPCR PANEL

ID	PANEL SAMPLE NUMBER	Rep 1 C_T	Rep 2 C_T	Rep 3 C_T	MEAN C_T	SD	RESULT INTERPRETATION	SAMPLE DETAILS	Sample Preparation	DATE PREPARED	DATE TESTED
A	2	30.76	31.10	30.79	**30.88**	0.19	POSITIVE	pWSSV_CSIRO_qPCR 10^1 copies/µL diluted i	16.2 mL diluent + 1.8 mL 10^2 copies/µL (1708-11-1012). Aliquoted 24 x 500 µL tubes.	12/02/2018	12/02/2018
B	14	30.96	30.97	31.20	**31.05**	0.13	POSITIVE	pWSSV_CSIRO_qPCR 10^1 copies/µL diluted i		12/02/2018	12/02/2018
C	20	31.21	31.10	31.07	**31.13**	0.07	POSITIVE	pWSSV_CSIRO_qPCR 10^1 copies/µL diluted i		12/02/2018	12/02/2018
D	15	27.51	27.48	27.47	**27.48**	0.02	POSITIVE	pWSSV_CSIRO_qPCR 10^2 copies/µL diluted i	16.2 mL diluent + 1.8 mL 10^3 copies/µL (below prep). Aliquoted 24 x 500 µL tubes.	12/02/2018	12/02/2018
E	29	27.51	27.62	27.60	**27.58**	0.06	POSITIVE	pWSSV_CSIRO_qPCR 10^2 copies/µL diluted i		12/02/2018	12/02/2018
F	4	27.48	27.60	27.56	**27.55**	0.06	POSITIVE	pWSSV_CSIRO_qPCR 10^2 copies/µL diluted i		12/02/2018	12/02/2018
G	5	24.19	24.19	24.40	**24.26**	0.12	POSITIVE	pWSSV_CSIRO_qPCR 10^3 copies/µL diluted i	16.2 mL diluent + 1.8 mL 10^4 copies/µL (1708-11-1014) Aliquoted 24 x 500 µL tubes.	12/02/2018	12/02/2018
H	16	24.09	24.07	23.95	**24.04**	0.08	POSITIVE	pWSSV_CSIRO_qPCR 10^3 copies/µL diluted i		12/02/2018	12/02/2018
I	6	24.09	24.21	24.08	**24.13**	0.07	POSITIVE	pWSSV_CSIRO_qPCR 10^3 copies/µL diluted i		12/02/2018	12/02/2018
J	23	20.56	20.64	20.64	**20.62**	0.05	POSITIVE	pWSSV_CSIRO_qPCR 10^4 copies/µL diluted i	13.5 mL diluent + 1.5 mL 10^5 copies/µL (below prep) Aliquoted 24 x 500 µL tubes.	12/02/2018	12/02/2018
K	28	20.69	20.71	20.60	**20.67**	0.06	POSITIVE	pWSSV_CSIRO_qPCR 10^4 copies/µL diluted i		12/02/2018	12/02/2018
L	3	20.63	20.64	20.65	**20.64**	0.01	POSITIVE	pWSSV_CSIRO_qPCR 10^4 copies/µL diluted i		12/02/2018	12/02/2018
M	21	17.25	17.38	17.15	**17.26**	0.12	POSITIVE	pWSSV_CSIRO_qPCR 10^5 copies/µL diluted i	13.5 mL diluent + 1.5 mL 10^6 copies/µL (prepared from 10^7 1708-14-1007) Aliquoted 24 x 500 µL tubes.	12/02/2018	12/02/2018
N	13	17.23	17.18	17.11	**17.17**	0.06	POSITIVE	pWSSV_CSIRO_qPCR 10^5 copies/µL diluted i		12/02/2018	12/02/2018
O	26	17.22	17.20	17.17	**17.20**	0.03	POSITIVE	pWSSV_CSIRO_qPCR 10^5 copies/µL diluted i		12/02/2018	12/02/2018
P	12	35.49	35.30	34.16	**34.98**	0.72	POSITIVE	pWSSV_CSIRO_qPCR 10^0 copies/µL diluted i	11.7 mL diluent + 1.3 mL 10^1 copies/µL (above prep). Aliquoted 24 x 500 µL tubes.	12/02/2018	12/02/2018
Q	24	34.59	33.83	33.82	**34.08**	0.44	POSITIVE	pWSSV_CSIRO_qPCR 10^0 copies/µL diluted i		12/02/2018	12/02/2018
R	17	33.86	34.59	34.49	**34.31**	0.40	POSITIVE	pWSSV_CSIRO_qPCR 10^0 copies/µL diluted i		12/02/2018	12/02/2018
S	9	29.74	29.74	29.85	**29.78**	0.06	POSITIVE	pWSSV_CSIRO_qPCR $10^{1.5}$ copies/µL diluted	8 mL diluent + 2 mL 10^2 copies/µL (above prep) Aliquoted 16 x 500 µL tubes.	12/02/2018	12/02/2018
T	10	29.99	29.74	29.97	**29.90**	0.14	POSITIVE	pWSSV_CSIRO_qPCR $10^{1.5}$ copies/µL diluted		12/02/2018	12/02/2018
NEG A	1	und	und	und	**und**	n/a	Negative	TE + tRNA (50ng/µL)	40 mL of TE Buffer + 40 µL of yeast tRNA (final conc. 50ng/µL) Aliquoted 80 x 500 µL tubes.	12/02/2018	12/02/2018
NEG B	7	und	und	und	**und**	n/a	Negative	TE + tRNA (50ng/µL)		12/02/2018	12/02/2018
NEG C	8	und	und	und	**und**	n/a	Negative	TE + tRNA (50ng/µL)		12/02/2018	12/02/2018
NEG D	11	und	und	und	**und**	n/a	Negative	TE + tRNA (50ng/µL)		12/02/2018	12/02/2018
NEG E	18	und	und	und	**und**	n/a	Negative	TE + tRNA (50ng/µL)		12/02/2018	12/02/2018
NEG F	19	und	und	und	**und**	n/a	Negative	TE + tRNA (50ng/µL)		12/02/2018	12/02/2018
NEG G	22	und	und	und	**und**	n/a	Negative	TE + tRNA (50ng/µL)		12/02/2018	12/02/2018
NEG H	25	und	und	und	**und**	n/a	Negative	TE + tRNA (50ng/µL)		12/02/2018	12/02/2018
NEG I	27	und	und	und	**und**	n/a	Negative	TE + tRNA (50ng/µL)		12/02/2018	12/02/2018
NEG J	30	und	und	und	**und**	n/a	Negative	TE + tRNA (50ng/µL)		12/02/2018	12/02/2018

Appendix 6 Equipment acquisition to address laboratory issues identified during the project

Equipment Name	Equipment task	Implemented due to	Date introduced to Laboratory	Approximate Purchase cost ($)
ViaFlow liquid dispensing robot	High throughput reagent dispensation	Increase throughput.	June 2018	*$150 000* total. *Quotes are provided in confidence from the suppliers.*
UV PCR hoods x 2	Master Mix preparation and dispensation	Dr Moody recommendation and NATA. Reduce risk of contamination	June 2018	
Electronic pipettes x 3	Integrate with the Viaflow and across multiple tube matrix.	Increase throughput, increase precision.	June 2018	
Plate spinners	Spin qPCR plates	NATA: separation of JCU AquaPATH into separate lab.	June 2018	
QuantStudio-5	qPCR analysis	Provide a back-up machine and increase throughput	September 2018	
Kingfisher-96 Extraction robot	High throughput extraction	Increase throughput and standardization of extraction. Harmonisation with labs in the national veterinary network	April 2018	

Appendix 7 JCU AquaPATH worksheets:

WSSV EQUIVALENCE TESTING WORKSHEET

Sample Identification and Test details

Accession Number/s:		**WSSV Panel**		Plate No.	**N/A**
Total Reactions:	30				

Batch/Expiry	Components	Dilution Factor	Quantity µl/well	Total	Tick After Adding
81805A	Bioline SensiFAST	1	10	300	
	Primer F (20pmol)	0.2	1	6	
	Primer R (20pmol)	0.2	1	6	
	Probe (5pmol)	0.05	1	1.5	
	Template	n/a	2.5	n/a	
717112A	Water	1	4.5	211.5	
		Total	20		

Dispense 17.5 ul of Master Mix into each well of the reaction plate		**Cycler used:**	Quant-5
Thermal Cycle: 95°C x 3 mins; 45 x 95°C x10s, 60°C x 30s.			

WSSV-1	Positive Control ID	NQC-3 & 4	Batch No.	Volume	Tick after added
Primer F	WSSV-1 qF (OIE)-TGG TCC CGT CCT CAT CTC AG			6	
Primer R	WSSV-1 qR (OIE)-GCT GCC TTG CCG GAA ATT A			6	
Probe:	WSSV-1 qProbe (OIE)-AGC CAT GAA GAA TGC CGT CTA TCA CA			1.5	
Tested by:	Kelly		Date tested:		
Reference:	Durand and Lightner		Run File:		Comp. Ct

WSSV-2	Positive Control ID	NQC-1 & 2	Batch No.	Volume	Tick after added
Primer F	WSSV-2 qF(CSIRO)-CCG ACG CCA AGG GAA CT			6	
Primer R	WSSV-2 qR(CSIRO)-TTC AGA TTC GTT ACC GTT TCC A			6	
Probe	WSSV-2 qProbe (CSIRO)-6FAM CGC TTC AGC CAT GCC AGC CG			1.5	
Tested by:	Kelly		Date tested:		
Reference:	MTA no reference. Publication in preparation		Run File:		Comp. Ct
			Tested by	Kelly	

AquaPath Detection Laboratory
Molecular Assay Worksheet

FORM **MOL -010**
qPCR Applied Biosystems

WSSV EQUIVALENCE PANEL WORKSHEET

Sample Identification and Test details

Accession Number/s: **csiro AND oie** Plate No. **n/a**

Total Reactions: 30

Batch/Expiry	Components	Dilution Factor	Quantity µl/well	Total	Tick After Adding
	Applied Biosystems Universal Mix	1	10	300	
	Primer F (20pmol)	0.2	1	6	
	Primer R (20pmol)	0.2	1	6	
	Probe (5pmol)	0.05	1	1.5	
	Template (cDNA)	n/a	2.5	n/a	
	Water	1	4.5	211.5	
		Total	20		

Dispense 17.5 ul of Master Mix into each well of the reaction plate **Cycler used:** Quant-5

Thermal Cycle: 95°C x 10mins; 45 x 95°C x15s, 60°C x 45s.

wssv-1	Positive Control ID	NQC-3 & 4	Batch No.	Volume	Tick after added
Forward Primer:	WSSV-1 qF (OIE)-TGG TCC CGT CCT CAT CTC AG			6	
Reverse Primer:	WSSV-1 qR (OIE)-GCT GCC TTG CCG GAA ATT A			6	
Probe:	WSSV-1 qProbe (OIE)-AGC CAT GAA GAA TGC CGT CTA TCA CAC A			1.5	
Tested by:			Date tested:		
Reference:	Durand and Lightner		Run File:		

wssv-2	Positive Control ID	NQC-1 & 2	Batch No.	Volume	Tick after added
Forward Primer:	WSSV-2 qF(CSIRO)-CCG ACG CCA AGG GAA CT			6	
Reverse Primer:	WSSV-2 qR(CSIRO)-TTC AGA TTC GTT ACC GTT TCC A			6	
Probe:	WSSV-2 qProbe (CSIRO)-6FAM CGC TTC AGC CAT GCC AGC CG TAMRA			1.5	
Tested by:			Date tested:		
Reference:			Run File:		

Tested by Kelly

Appendix 8 Full Ct values of each assay.

CT Value of OIE WSSV Equivalence Panel at JCU and CSIRO AFDL																																
			Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
Machine	NQC-3	NQC-4	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
AFDL	26.91	33.79	30.06	19.99	und	und	33.60	29.13	29.11	und	23.46	und	26.83	26.82	33.82	und	und	19.90	32.62	33.41	30.27	und	26.71	29.99	und	29.12	20.06	und	32.60	und	23.67	23.53
Q3-JCU			31.78	21.55	nd	nd	35.10	30.38	30.95	nd	24.90	nd	28.38	28.70	36.01	nd	nd	21.70	33.33	36.84	32.44	nd	27.89	32.20	nd	30.89	21.62	nd	33.92	nd	25.68	23.80
Q5-JCU			32.70	22.04	nd	nd	34.68	30.76	31.97	nd	26.08	nd	28.43	28.33	35.22	nd	nd	23.06	33.94	36.16	34.45	nd	30.91	32.33	nd	30.86	21.30	nd	34.09	nd	25.83	23.81
Q3-JCU			34.17	21.51	nd	nd	35.53	30.92	30.18	nd	27.07	nd	28.40	28.17	35.43	nd	nd	22.35	34.79	35.56	35.22	nd	28.66	31.99	nd	30.87	21.80	nd	33.25	nd	25.79	23.46
Q5-JCU			32.87	21.38	nd	nd	35.16	30.84	30.46	nd	25.81	nd	28.44	28.14	35.49	nd	nd	21.97	33.80	34.67	32.29	nd	28.18	32.23	nd	30.76	21.45	nd	33.17	nd	25.69	23.08
Q3-JCU			31.58	21.67	nd	nd	34.61	30.82	30.92	nd	25.02	nd	28.44	28.17	34.92	nd	nd	21.88	33.52	34.22	32.24	nd	27.75	32.16	nd	30.89	21.47	nd	35.16	nd	25.84	24.47
Q5-JCU			31.45	21.63	nd	nd	34.51	30.80	31.13	nd	25.04	nd	28.41	28.08	35.23	nd	nd	21.96	33.26	34.05	31.94	nd	27.72	31.94	nd	30.87	21.28	nd	34.13	nd	25.84	24.13
Q3-JCU			31.51	21.51	nd	nd	34.69	30.88	31.00	nd	25.28	nd	28.63	28.05	34.01	nd	nd	22.10	32.89	33.32	31.94	nd	27.39	32.04	nd	30.92	21.40	nd	33.34	nd	25.93	24.14
Q5-JCU			31.55	21.55	nd	nd	35.42	30.74	30.93	nd	24.76	nd	28.41	27.81	35.46	nd	nd	22.16	34.21	35.09	32.05	nd	28.10	32.37	nd	30.90	21.27	nd	32.90	nd	25.74	23.82
Q3-JCU			31.09	21.50	nd	nd	35.28	30.84	30.84	nd	25.11	nd	28.43	27.98	35.14	nd	nd	22.00	34.07	35.24	32.39	nd	28.36	31.92	nd	31.92	21.29	nd	32.92	nd	25.67	24.09
Q5-JCU			31.80	21.56	nd	nd	35.12	30.75	31.01	nd	25.08	nd	28.46	27.92	34.62	nd	nd	22.01	34.00	35.54	32.49	nd	28.08	31.88	nd	30.75	21.42	nd	33.72	nd	25.68	24.43
Q3-JCU			31.48	21.47	nd	nd	35.27	30.68	30.79	nd	25.19	nd	28.70	28.05	35.24	nd	nd	22.15	33.01	35.15	32.71	nd	27.92	32.40	nd	30.64	21.41	nd	33.62	nd	25.62	24.63
Q5-JCU			33.44	21.71	nd	nd	34.77	30.93	30.70	nd	25.20	nd	28.49	27.77	34.73	nd	nd	22.42	34.01	35.34	32.76	nd	29.65	32.45	nd	30.07	21.74	nd	34.12	nd	25.80	24.77
Q3-JCU	28.28	35.14	31.19	21.41	nd	nd	34.18	30.35	30.12	nd	24.81	nd	26.51	28.21	35.01	nd	nd	21.68	34.11	35.31	31.22	nd	28.17	31.71	nd	30.08	21.12	nd	33.99	nd	25.16	25.76
Q5-JCU	28.30	35.05	31.28	21.66	nd	nd	34.42	30.60	30.87	nd	25.09	nd	28.25	27.99	34.96	nd	nd	21.71	33.87	35.05	31.83	nd	28.08	31.91	nd	30.44	21.77	nd	34.16	nd	24.97	25.79
Q3-JCU	28.20	35.23	31.25	21.65	nd	nd	34.04	30.47	30.49	nd	24.77	nd	28.02	27.95	34.53	nd	nd	21.39	34.16	35.08	31.39	nd	27.94	31.64	nd	30.14	20.96	nd	33.88	nd	24.83	25.75
Q5-JCU	28.33	34.61	31.50	21.62	nd	nd	34.75	30.35	30.33	nd	24.81	nd	28.17	28.60	34.54	nd	nd	21.82	33.80	34.60	31.77	nd	28.58	31.59	nd	30.18	21.72	nd	34.16	nd	24.79	25.72
Q3-JCU	28.65	35.11	31.59	21.47	nd	nd	34.69	30.52	29.94	nd	25.07	nd	28.10	28.31	35.04	nd	nd	21.60	34.55	35.25	31.68	nd	28.03	31.48	nd	30.27	21.74	nd	34.71	nd	25.15	25.64
Q5-JCU	28.79	34.70	31.58	21.44	nd	nd	34.30	30.24	30.03	nd	24.77	nd	28.26	27.86	34.60	nd	nd	21.47	34.31	34.89	31.19	nd	28.19	31.41	nd	30.37	21.49	nd	34.02	nd	25.37	25.63
Q3-JCU	28.52	34.48	31.49	21.63	nd	nd	34.52	31.62	30.22	nd	24.81	nd	28.01	27.99	34.99	nd	nd	21.42	34.23	35.24	31.59	nd	28.46	31.48	nd	30.46	21.47	nd	33.13	nd	25.07	25.54
Q5-JCU	28.38	34.13	31.64	21.51	nd	nd	34.99	30.45	30.22	nd	25.07	nd	28.32	28.11	35.48	nd	nd	21.83	34.55	35.12	31.55	nd	28.52	31.49	nd	30.17	21.78	nd	34.08	nd	25.10	25.64
Q3-JCU	28.49	34.48	31.42	21.44	nd	nd	34.56	30.37	30.00	nd	24.77	nd	28.20	28.15	34.95	nd	nd	21.59	34.09	35.20	31.51	nd	28.26	31.33	nd	30.44	21.66	nd	33.94	nd	24.61	25.67
Q5-JCU	28.41	34.69	31.57	21.56	nd	nd	34.61	30.52	30.28	nd	24.81	nd	28.26	28.10	34.89	nd	nd	21.52	34.00	35.51	31.55	nd	28.24	31.60	nd	30.44	20.11	nd	34.29	nd	25.00	25.97
Q3-JCU	28.48	34.64	31.56	21.66	nd	nd	34.93	30.82	30.53	nd	25.11	nd	28.35	28.05	34.92	nd	nd	21.54	34.15	35.65	31.88	nd	28.37	31.78	nd	30.14	21.54	nd	33.98	nd	24.78	25.79
Q5-JCU	28.45	34.88	31.36	21.36	nd	nd	34.78	30.39	30.11	nd	24.99	nd	28.24	28.03	35.27	nd	nd	21.70	34.28	35.14	31.39	nd	28.60	32.22	nd	30.54	21.60	nd	34.35	nd	24.85	25.87

CT Value of CSIRO WSSV Equivalence Panel and JCU and CSIRO AFDL AAHL.

Test Location	NQC-1	NQC-2	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Sample 24	Sample 25	Sample 26	Sample 27	Sample 28	Sample 29	Sample 30
JCU	25.77	30.54	nd	31.62	22.36	28.89	25.61	25.37	nd	nd	31.48	31.79	nd	34.98	18.42	31.84	28.77	25.79	35.78	nd	nd	31.60	18.23	nd	21.81	35.53	nd	18.61	nd	21.89	28.37	nd
JCU	24.53	29.95	nd	29.94	22.31	26.67	25.82	24.06	nd	nd	29.47	29.22	nd	35.75	19.54	30.15	29.17	23.62	34.82	nd	nd	30.10	18.92	nd	21.01	33.41	nd	17.96	nd	20.86	26.51	nd
JCU	24.31	29.74	nd	31.30	21.06	26.64	25.39	24.62	nd	nd	29.26	29.57	nd	34.62	17.45	30.54	27.35	23.17	34.00	nd	nd	30.30	17.80	nd	21.38	33.55	nd	17.80	nd	21.73	26.92	nd
JCU	24.12	29.83	nd	31.21	20.95	27.14	26.67	24.63	nd	nd	30.15	29.10	nd	33.47	17.23	30.12	27.12	23.81	33.83	nd	nd	30.47	18.62	nd	21.48	33.94	nd	17.18	nd	20.86	26.75	nd
JCU	25.32	29.28	nd	31.23	21.15	27.94	24.72	24.76	nd	nd	30.61	30.32	nd	34.19	18.56	31.42	28.49	24.39	35.31	nd	nd	30.51	18.27	nd	21.95	34.78	nd	16.53	nd	21.62	28.73	nd
JCU	24.97	31.86	nd	31.93	21.81	29.00	25.36	24.59	nd	nd	30.79	30.76	nd	33.25	18.30	30.93	27.97	25.35	34.86	nd	nd	32.28	18.87	nd	22.57	33.68	nd	18.59	nd	22.32	28.82	nd
JCU	25.22	31.15	nd	29.31	21.58	28.80	25.85	25.62	nd	nd	32.68	30.33	nd	34.63	18.15	32.95	29.01	26.26	33.30	nd	nd	30.19	18.84	nd	21.83	35.95	nd	16.01	nd	21.26	27.90	nd
JCU	26.13	30.39	nd	32.85	22.52	29.87	26.38	25.93	nd	nd	32.59	31.18	nd	36.23	19.50	32.91	29.54	25.85	34.01	nd	nd	32.27	19.43	nd	22.87	36.29	nd	18.61	nd	22.58	28.32	nd
JCU	24.93	28.75	nd	30.68	21.50	27.39	25.79	24.82	nd	nd	29.80	31.46	nd	33.88	19.55	30.66	29.21	24.15	34.54	nd	nd	30.23	17.87	nd	21.19	33.27	nd	17.49	nd	20.71	26.75	nd
JCU	24.87	28.87	nd	32.74	23.19	28.83	24.46	22.87	nd	nd	31.44	30.32	nd	32.99	18.21	31.56	26.60	24.80	34.67	nd	nd	32.69	18.37	nd	21.36	35.80	nd	17.97	nd	22.56	27.94	nd
JCU	24.02	30.54	nd	32.54	22.30	29.51	25.83	26.20	nd	nd	32.14	31.73	nd	35.62	18.38	31.72	29.28	26.86	35.75	nd	nd	30.64	18.79	nd	22.53	35.39	nd	17.11	nd	21.27	28.57	nd
JCU	25.90	29.30	nd	32.22	22.35	29.74	25.97	25.87	nd	nd	32.22	31.73	nd	35.23	18.18	30.84	29.15	26.56	35.75	nd	nd	31.72	19.20	nd	21.76	35.82	nd	18.40	nd	22.14	28.55	nd
JCU	24.12	29.84	nd	30.73	22.61	27.11	26.11	24.29	nd	nd	30.85	29.36	nd	33.21	17.34	30.25	27.49	24.84	33.25	nd	nd	31.14	16.80	nd	19.95	33.77	nd	17.31	nd	21.14	28.74	nd
JCU	24.82	31.57	nd	30.74	21.55	27.52	26.52	24.12	nd	nd	30.52	29.15	nd	33.44	17.39	30.47	27.52	25.02	33.92	nd	nd	32.59	16.62	nd	21.99	33.95	nd	18.13	nd	21.60	28.75	nd
JCU	25.07	30.01	nd	31.07	21.49	27.22	27.22	25.01	nd	nd	31.28	30.10	nd	34.22	17.24	31.54	27.48	25.26	33.23	nd	nd	32.63	17.41	nd	21.76	33.72	nd	17.73	nd	21.70	28.83	nd
JCU	24.43	29.93	nd	30.76	21.35	26.53	26.53	24.25	nd	nd	29.42	31.06	nd	32.73	16.80	29.47	28.38	24.14	34.71	nd	nd	31.58	20.52	nd	20.47	33.91	nd	17.16	nd	20.39	26.67	nd
JCU	24.31	29.85	nd	30.72	21.01	26.07	26.07	24.62	nd	nd	29.32	28.86	nd	34.26	16.65	31.36	27.70	24.36	34.62	nd	nd	30.97	15.99	nd	20.49	34.02	nd	16.62	nd	20.14	26.66	nd
JCU	24.11	29.14	nd	30.40	21.86	26.99	25.99	23.89	nd	nd	29.95	29.43	nd	33.29	17.13	30.81	28.54	24.43	34.98	nd	nd	30.33	16.87	nd	20.61	32.44	nd	17.88	nd	21.50	27.28	nd
JCU	25.46	29.15	nd	31.42	20.90	27.74	24.72	24.56	nd	nd	30.97	30.07	nd	34.45	17.98	32.07	27.82	24.03	35.64	nd	nd	30.94	18.35	nd	21.34	35.11	nd	17.47	nd	21.98	27.80	nd
JCU	25.01	30.33	nd	31.87	21.10	27.83	25.38	25.07	nd	nd	30.25	30.67	nd	34.08	19.28	32.09	28.57	24.37	35.17	nd	nd	31.54	18.20	nd	22.10	34.95	nd	17.00	nd	21.26	28.26	nd
JCU	25.14	29.34	nd	31.04	21.94	28.57	25.63	25.77	nd	nd	30.79	28.76	nd	32.86	18.36	32.86	28.58	25.48	35.47	nd	nd	31.13	18.68	nd	21.98	34.57	nd	18.36	nd	22.44	28.76	nd
JCU	25.77	31.79	nd	31.13	21.94	28.57	25.63	25.22	nd	nd	30.79	30.73	nd	33.35	18.36	31.86	28.58	25.48	33.87	nd	nd	31.04	18.68	nd	21.98	33.86	nd	18.36	nd	22.20	28.76	nd
JCU	26.67	30.93	nd	33.58	22.52	29.53	25.89	25.85	nd	nd	31.37	31.09	nd	33.32	18.96	32.87	29.06	25.28	33.78	nd	nd	33.30	20.63	nd	23.14	33.77	nd	18.76	nd	22.98	28.98	nd
JCU	24.07	31.14	nd	31.36	22.71	29.98	26.44	24.03	nd	nd	31.41	31.21	nd	34.71	19.35	31.17	29.20	25.01	34.18	nd	nd	31.94	20.15	nd	23.45	32.74	nd	17.74	nd	21.79	28.92	nd
JCU	25.23	29.92	nd	30.93	22.56	29.91	25.42	25.32	nd	nd	30.89	30.84	nd	35.27	19.12	31.14	29.19	24.33	33.16	nd	nd	31.62	18.43	nd	22.33	32.55	nd	17.07	nd	19.90	26.22	nd
JCU	24.70	29.03	nd	30.35	19.81	25.51	25.39	22.57	nd	nd	31.19	29.22	nd	34.41	17.78	32.96	25.65	24.62	34.91	nd	nd	33.28	18.73	nd	22.15	35.74	nd	17.97	nd	22.51	27.43	nd
JCU	24.95	29.29	nd	33.01	21.57	24.61	25.38	24.16	nd	nd	31.68	30.60	nd	35.77	18.63	31.86	29.57	24.98	34.44	nd	nd	33.78	18.01	nd	22.07	35.58	nd	16.52	nd	22.62	27.70	nd
JCU	25.23	29.53	nd	31.26	21.86	28.76	25.22	25.78	nd	nd	30.73	29.39	nd	33.53	18.68	32.16	29.56	24.83	35.59	nd	nd	31.04	19.69	nd	21.36	34.36	nd	19.00	nd	22.20	28.57	nd
AFDL	24.19	31.21	nd	30.76	20.63	27.48	24.19	24.09	nd	nd	29.74	29.99	nd	35.49	17.23	30.96	27.51	24.09	33.86	nd	nd	30.96	17.25	nd	20.56	34.59	nd	17.22	nd	20.69	27.51	nd
AFDL	24.19	31.10	nd	31.10	20.64	27.60	24.19	24.21	nd	nd	29.74	29.74	nd	35.30	17.18	30.97	27.48	24.07	34.59	nd	nd	30.97	17.38	nd	20.64	33.83	nd	17.20	nd	20.71	27.62	nd
AFDL	24.40	31.07	nd	30.79	20.65	27.56	24.40	24.08	nd	nd	29.85	29.97	nd	34.16	17.11	31.20	27.47	23.95	34.49	nd	nd	31.20	17.15	nd	20.64	33.82	nd	17.17	nd	20.60	27.60	nd

Appendix 9. JCU AquaPATH NATA certificate

NATA

NATA ACCREDITED LABORATORY

National Association of Testing Authorities, Australia

(ABN 59 004 379 748)

has accredited

James Cook University AquaPath Detection Laboratory
College of Science and Engineering & College of Public Health, Medical and Veterinary Science

following demonstration of its technical competence to operate in accordance with

ISO/IEC 17025

This facility is accredited for the tests shown on the *Scope of Accreditation* issued by NATA

Jennifer Evans
Chief Executive Officer

Date of issue: 15 June 2018
Date of accreditation:13 June 2018
Accreditation number: 20312