Australian Government
Department of Agriculture and Water Resources

REGIONAL PROFICIENCY TESTING PROGRAM FOR AQUATIC ANIMAL DISEASE DIAGNOSTIC LABORATORIES IN ASIA-PACIFIC

(2018-2022)

WORKSHOP REPORT

Centara Grand Central Ladprao, Bangkok, Thailand

13-14 March 2019

Prepared by the workshop facilitators

1. INTRODUCTION

The Asia-Pacific Laboratory (APL) Proficiency Testing (PT) Program on Aquatic Animal Diseases is a four year long program (2018/19 – 2021/22) designed to strengthen laboratory diagnostic capabilities for aquatic animal diseases of significance across the Asia-Pacific. It is a follow on from a previously funded PT program in 2012-2014 that saw an improvement in the regional capability of Australia's trading partners to detect and diagnose important aquatic animal diseases.

The Australian Government Department of Agriculture and Water Resources (the department) has funded the APL PT program through the Agricultural Competitiveness White Paper. The APL PT program is run by the Commonwealth Scientific and Industrial Research Organisation – Australian Animal Health Laboratory (CSIRO-AAHL) accredited PT scheme provider. The aquatic PT program implemented by the PT scheme provider is accredited under ISO17043 for all agents except *Vibrio parahaemolyticus* (Vp_{AHPND}).

The current program includes 39 laboratories from 14 countries within the Asia-Pacific, including Bangladesh, Brunei, China, Hong Kong, India, Indonesia, Iran, Malaysia, New Caledonia, Philippines, Singapore, Sri Lanka, Thailand and Vietnam.

Ten economically significant finfish and crustacean diseases were agreed upon (Appendix 1), and testing over eight rounds has been offered to each of the laboratories. Throughout the duration of the program, CSIRO-AAHL is available to offer technical advice should laboratories need it.

The Network of Aquaculture Centres in Asia-Pacific[1] (NACA) hosted a workshop on the APL PT program for 33 laboratories from 12 Asia-Pacific countries on 13-14 March 2019 in Bangkok, Thailand. Fifty people in total from 12 participating countries attended the workshop.

The aim of the workshop was to offer direct communication with laboratory representatives from the program to assist in the understanding of diagnostic standards, proficiency testing procedures and laboratory quality assurance management systems. It also allowed participants to discuss any issues they had come across during the first two rounds of completed testing. It is expected that laboratories will gain increased confidence in their

[1] The Network of Aquaculture Centres in Asia-Pacific (NACA) is an intergovernmental organisation of which Australia is a member. The Australian Government Department of Agriculture and Water Resources has delivered a range of aquatic animal health capacity building programs through NACA.

testing capabilities and performance, and increased ability to diagnose and detect aquatic animal diseases of transboundary significance.

2. WORKSHOP STRUCTURE

To gauge any issues and concerns encountered by participating laboratories during the first two rounds of completed proficiency testing in 2018, the Asia-Pacific Proficiency Testing Program Survey was conducted in November 2018. In particular, the survey aimed to gather additional information on regional capacity relating to:

- Laboratory practice and capability, molecular diagnostic assays in general, and diagnosis of aquatic animal diseases, in particular;
- Quality assurance and accreditation; and the
- Implementation and reporting of the APL PT program for the 2018 period.

The structure of the APL PT program workshop was based on the results of this survey and designed to provide participants an opportunity to discuss key issues on the above aspects. The final workshop agenda is included as Appendix 2.

3. PARTICIPATION

The workshop was held for two days from 13 to 14 March 2019 at the Centara Grand Central Ladprao, Bangkok, Thailand. It was co-organised by NACA, CSIRO-AAHL and the department. Fifty participants attended the workshop, representing 33 aquatic animal disease diagnostic laboratories from 12 Asia-Pacific countries, including Brunei, China, Hong Kong, India, Indonesia, Iran, Malaysia, Philippines, Singapore, Sri Lanka, Thailand, and Vietnam (Appendix 3).

The workshop facilitators and resource personnel were experts from the department, CSIRO-AAHL and NACA (Appendix 3).

4. OPENING SESSION AND INTRODUCTION TO THE PROJECT

Dr Derun Yuan (Coordinator Education and Training Programme, NACA) officially opened the workshop on behalf of NACA Director General, Dr Cherdsak Virapat. All of the participants and facilitators were then introduced during the first of three group ‘break-out’ sessions conducted during the course of the workshop (Appendix 2).

As an introduction to the workshop, Dr Eduardo Leano (Coordinator Aquatic Animal Health Programme, NACA) provided an overview on the importance of aquatic animal disease diagnostics in the Asia-Pacific to detect transboundary diseases and facilitate trade.

Dr Yuko Hood (Department of Agriculture and Water Resources) provided a brief overview of the structure of Australia's laboratory network and the wide range of aquatic animal disease surveillance and diagnostic projects currently being led or collaborated on by the department. Of particular interest to attendees was discussion of the difference(s) between active and passive surveillance for the detection of aquatic animal diseases.

5. THE WORKSHOP

5.1 Day 1

Dr Caryll Waugh (Proficiency testing team, CSIRO-AAHL) opened the body of the workshop with a presentation on the results of the APL PT program for 2018 (i.e. rounds 1 and 2). A brief overview of the assessment process and statistical analyses used in the report were provided.

Dr Waugh then presented on the use and importance of quality controls in diagnostic laboratory quality assurance programs. The different types of quality controls that can be used to confirm staff competency and training, and assay, extraction and instrumentation performance were discussed. An overview of the use of internal quality controls and network quality controls was also provided.

Dr Nick Moody (Australian Fish Diseases Laboratory (AFDL), CSIRO-AAHL) provided an overview of minimum requirements for PCR laboratory design and PCR workflow standard operating procedures. The workflows used at the AFDL were provided as an example. The need to maintain Australia's national veterinary laboratory guidelines was also highlighted.

Dr John Hoad (AFDL, CSIRO-AAHL) spoke on the importance of sample preparation and extraction. A number of factors can affect the value of samples for testing. Poor or denatured samples will provide PCR results that are difficult to interpret, and sample collection and processing methods should minimise chances for cross contamination. Techniques for nucleic acid extraction should also be evaluated for their effectiveness in a wide range of sample types.

Workshop participants were divided into 3 separate groups for the second 'break-out' session, to discuss in more detail:

PCR workflow and design

Sample processing and extraction

The use of quality controls

5.2 Day 2

Day 2 of the workshop opened with a series of presentations by Dr Moody on the roles and responsibilities of AFDL for aquatic animal diseases, the international OIE standards for validation of diagnostic tests for aquatic animals and an overview of the OIE Aquatic Manual update. Dr Moody noted that each chapter of the Manual will be updated using a new structure to focus more on the validated diagnostic method and surveillance objectives. Test validation was noted to be a time consuming, expensive and onerous task, with many tests in the OIE Aquatic Manual not yet validated to the OIE standards. It was suggested validation be broken down into stages to simplify the process, and laboratories can focus on an equivalence assessment to the methods that are already validated.

Dr Hoad provided an overview of the steps required to evaluate and implement a new or existing PCR test within a laboratory. Primers, probes, procedures and positive controls should all be evaluated when implementing a new test. Equivalence testing may also be required when using new machines, extraction kits, assays, methods (real-time vs. conventional PCR) and/or new brands or batches of master mix, primers or probe.

Dr Gemma Carlile (Proficiency testing team, CSIRO-AAHL) presented on the laboratory accreditation process under ISO/IEC 17025 and the steps required to establish a laboratory quality assurance system. ISO/IEC 17025 is a management system standard specific to laboratories and is applicable to any type or size of laboratory. Dr Carlile also provided an overview of the quality management system used at AAHL.

For the third and final ‘break-out’ session participants discussed:

- SOPs and bench notes – using WSSV as a case study;
- Quality assurance management; and,
- Internal audits and improvement.

Again a summative mind map was produced and a brief overview was provided to the entire group.

Dr Waugh outlined recent changes to the APL PT program testing panel format for 2019. The program is moving away from using pathogen specific panels. Host specific panels will now be used, as this format more closely resembles the diagnostic setting. Participants were advised that greater instruction on how to prepare samples for nucleic acid extraction will be included with the panels. Samples will also be identified as either DNA or RNA viruses and all of the sample once processed according to the sample instructions, should be used for the extraction.

Dr Moody and Dr Hoad provided an overview of several research activities being conducted at the CSIRO-AAHL Fish Disease Laboratory for white spot syndrome virus (WSSV), yellowhead virus (YHV) and megalocytivirus (MCV). The importance of assessing a PCR tests' diagnostic sensitivity and specificity was highlighted. The Fish Disease Laboratory is validating a number of qPCR assays for detection in apparently healthy animals. These tests will be published and the OIE Aquatic Manual updated. Validation materials were sourced from a variety of investigations including disease outbreaks, infectivity trials and imported ornamental fish. The biggest challenge is finding adequate numbers of known-infected apparently healthy animals to determine diagnostic sensitivity.

Dr Moody then gave a presentation on the Australian WSSV outbreak from the perspective of CSIRO-AAHL. The required rapidity for the confirmatory testing was highlighted, with the samples received at AAHL at 8:32 PM on the 30 November 2016 and WSSV confirmed at 9:00 AM on 1 December 2016. The numerous logistical challenges faced by AAHL were also discussed. With approximately 1000 samples to process and test each day, extra staff and large volumes of reagents were required.

The final presentation was given by Dr Hood and focused on Australia's National Wild Surveillance Program for WSSV to confirm freedom. The National Wild Surveillance Program for WSSV was agreed by Australia's National Aquatic Consultative Committee on Emergency Animal Diseases and aims to demonstrate white spot disease (WSD) freedom in areas outside of the infected zone and provide evidence of national freedom from WSD. The survey confirmed that Australia's crustacean populations outside of the infected zone are free of WSD. It is uncertain whether the virus will persist or die out in the infected zone. Surveillance efforts will continue within and outside of the infected zone for at least 2 consecutive years.

6. CLOSING SESSION

Dr Carlile provided the wrap up of the workshop and encouraged all the participants to actively participate in the next six rounds of testing over the next three years. Dr Leano

officially closed the workshop and thanked all the facilitators and participants for sharing their expertise, views and inputs during the workshop, and also reiterated the importance of the APL PT program for all of the participating laboratories.

LABORATORY PROFICIENCY TESTING WORKSHOP

13-14 March 2019

Centara Grand at Central Plaza Ladprao, Bangkok, Thailand

LIST OF AGENTS INCLUDED IN THE PT PROGRAM (2018-2022)

Agent name	Genome	Host target
White spot syndrome	dsDNA	Diseases of crustaceans
Yellowhead virus genotype 1	+ssRNA	
Taura syndrome virus	+ssRNA	
Infectious myonecrosis virus	dsRNA	
Infectious hypodermal and haematopoietic necrosis virus	ssDNA	
Vibrio parahaemolyticus (*Vp*$_{AHPND}$).	DNA	
Megalocytivirus	dsDNA	Diseases of Finfish
Nervous necrosis virus	+ssRNA	
Koi herpesvirus (Cyprinid herpesvirus-3)	dsDNA	
Spring viraemia of carp virus	-ssRNA	

APPENDIX 2: Workshop Agenda

Asia-Pacific Laboratory Proficiency Testing Program for Aquatic Animal Diseases

Participant Workshop Agenda

Location: Centara Grand Central Ladprao, Bangkok

13-14 March 2019

Time	Item	Presenter
DAY 1 (8:00 – 10:30)		
8:00-8:45	1. Registration	NACA
8:45-9:00	2. Welcome	DAWR
9:00-9:15	3. NACA Official opening	NACA
9:15-9:30	4. Introduction; purpose of the workshop; expected outcomes	DAWR/AAHL
9:30-10:00	5. Self-introductions for participants and break out to 6 small groups (46 participants up to 60 participants)	All
10:00-10:15	6. Importance of aquatic animal disease diagnostics to facilitate trade and detect transboundary disease for the Asia Pacific region	NACA
10:15-10:30	7. Australia's laboratory network and aquatic animal disease surveillance and diagnostic activities; AQUAPLAN projects	DAWR
Break (10:30 – 11:00)		
11:00-11:45	8. Summary of the proficiency testing program (Overview).	AAHL PT
11:45-12:30	9. Quality Controls (including network quality controls)	AAHL PT
Lunch (12:30 – 1:30)		
1:30-1:45	10. Presentations: PCR laboratory design and PCR laboratory workflows	AAHL
1:45-2:15	11. Presentations: sample preparation and extraction	AAHL
2:15-3:15	12. Group discussion and summary presentation – 3 breakout groups – i) Workflow, ii) sample preparation & extraction; iii) use of positive controls	All
3:15-3:30	13. Session summary	AAHL
Break (3:30 – 3:50)		
3:50-5:00	14. Troubleshooting and open discussion about test performance	AAHL
5:00-5:15	15. Wrap-up for day 1	DAWR/AAHL
WORKSHOP DINNER – EVENING OF 13 MARCH		

APPENDIX 2: Workshop Agenda

Time	Item	Presenter
DAY 2 (8:30 – 10:30)		
8:30-8:50	16. Presentations: Roles and responsibilities of AAHL for aquatic animal diseases	AAHL-AFDL
8:50-9:20	17. Presentations: Method Validation	AAHL-AFDL
9:20-9:40	18. Presentations: OIE Aquatic Manual update	AAHL-AFDL
9:40-10:00	19. Presentations: Method implementations and equivalence testing	AAHL-AFDL
10:00-10:30	20. Discussion	AAHL-AFDL
Break (10:30 – 10:50)		
10:50-11:15	21. Presentations: Quality Assurance and Laboratory accreditation	AAHL PT
11:15-12:00	22. Breakout activity and group discussion: i) SOPS and bench notes – WSSV case study; ii) QA management; iii) internal audits and improvements	All
12:00-12:30	23. Session summary	All
Lunch (12:30 – 1:30)		
1:30-1:45	24. Overview of AAHL's QA management system	AAHL PT
1:45-2:00	25. Presentations: Future operation of the APL PT program (finfish panel and additional pathogens etc)	AAHL PT
2:00-3:00	26. Presentations: WSSV, YHV1 and MCV testing in Australia (including Q and A)	AAHL-AFDL
Break (3:00 – 3:20)		
3:20-3:40	27. Presentations: WSSV Outbreak Overview	AAHL-AFDL
3:40-4:00	28. Presentations: WSSV National Surveillance	DAWR
4:00-4:30	29. Closing session and wrap-up	DAWR/AAHL
END OF WORKSHOP		

APPENDIX 3: List of Participants

LABORATORY PROFICIENCY TESTING WORKSHOP

13-14 March 2019

Centara Grand at Central Plaza Ladprao, Bangkok, Thailand

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