Asia-Pacific Laboratory Proficiency Testing Program for Aquatic Animal Diseases

13th OIE Seminar, 19th WAVLD Symposium, Chiang Mai (Thailand), 19–22 June 2019

AUSTRALIAN ANIMAL HEALTH LABORATORY (AAHL)
www.csiro.au

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1. Why have a regional PT Program
2. Project overview
3. Sample preparation and quality assurance
4. Program update

1. **Why have a regional PT Program**
2. **Project overview**
3. **Sample preparation and quality assurance**
4. **Program update**

Asia-Pacific Laboratory Proficiency Testing Program for Aquatic Animal Diseases: 2012 - 2022

- *Ad hoc* proficiency testing programs have been run (for a limited selection of diseases and countries) but there is limited or no access to ongoing laboratory proficiency testing programs" - NACA Regional Advisory Group for Aquatic Animal Health 2010.
- Requests have been made to the Australian Government by trading partners for assistance in strengthening laboratory capability
- Enquiries regarding participation in Australia's national aquatic animal disease laboratory PT program
- A proposal for a regional PT project was proposed under DAFF's International Agricultural Cooperation Program – and it was successful!

What is proficiency testing (PT)?

- External and independent assessment of laboratory capability to conduct specific diagnostic tests
- Ensures method validation and internal quality control for within-laboratory procedures are working satisfactorily.
- Involves laboratories performing tests on the same samples and comparing results.
- Used to monitor laboratories' continuing performance.

Why have proficiency testing (PT)?

- Mandated by accreditation bodies to participate in external PT programs, for the types of analyses undertaken in that laboratory.
- ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories.
- Essential element of a laboratory's QA program.
- A regional PT program was seen as a way to assist competent authority laboratories to build their capabilities

Benefits of proficiency testing (PT)?

Provides Confidence in Results;

- Test methods are being followed
  - detect any difficulties a laboratory may have with analyses
- Test results are accurate and precise
  - demonstrate repeatability and reproducibility
  - and test reliability
- Training is appropriate
  - identify training needs

- Systematic variations are identified
  - provides information that can assist in future planning for equipment upgrades and staff training
- Consistency between other labs (harmonisation vs. standardisation)
- Credibility and compliance

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Asia-Pacific Laboratory Proficiency Testing Program for Aquatic Animal Diseases

- Three year project from July 2012 to April 2015:
  - 45 laboratories from 14 participating countries
  - Four rounds of testing
  - 10 aquatic pathogens included in disease-specific panels of tests
- Two year project from June 2017 to May 2019:
  - Two rounds of testing
  - 10 aquatic pathogens included in disease-specific panels of tests
- Contract extension to a four year project ending May 2022
  - Six additional rounds of testing
  - Move from pathogen-specific panels to host-specific panels
  - Move from ethanol-fixed (70%) to gamma-irradiated (50kGy) material
- Participation is free for laboratories of Network of Aquaculture Centres in Asia-Pacific (NACA) member countries, and countries of trade significance to Australia.

Project Partners and Roles

Organisation	Responsibilities
DoA Australian Government Department of Agriculture	• Funding and Project Coordination
CSIRO AAHL Fish Diseases Laboratory CSIRO Australian Animal Health Laboratory	• Acquisition and preparation of test materials • Quality and utility testing • Provision of samples in "test ready" form • Technical advice on pathogens and tests
CSIRO AAHL Proficiency Testing Group CSIRO Australian Animal Health Laboratory	• Preparation of samples for distribution • Distribution of test panels • Collation of test results • Preparation of test reports for participants
ANQAP (DPI Vic) Australian National Quality Assurance Program	• Preparation of samples for distribution • Distribution of test panels • Collation of test results • Preparation of test reports for participants
NACA Network of Aquaculture Centres in Asia-Pacific	• Liaison with NACA member countries • Organisation of project workshops

International Standards for Proficiency Testing

Accredited by National Association of Testing Authorities (NATA)

Australian Animal Health Laboratory - AAHL

Australian National Quality Assurance Program –ANQAP

Covered by ISO standards 17043 and 13528

"Conformity assessment – General requirements for proficiency Testing (ISO/CASCO 17043:2010)"

"Statistical methods for use in proficiency testing by laboratory comparisons (ISO 13528:2005(E))"

Tests used to determine panel composition

Tests described in the OIE *Aquatic Manual*

Includes conventional PCR assays if participating laboratories use conventional PCR

With the invitation to participate, we also collect information about extractions methods, assays used, result interpretation…

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Samples for Proficiency Testing

- Analytical range covers concentrations that might normally be encountered.
  - Unequivocal strong positive
  - Unequivocal weak positive
  - Unequivocal negative
  - The remaining test samples to be selected from any combination of the above categories with consideration given to the inclusion of pairs of related samples to be included for statistical analysis
- Critical key requirement:
  - Samples are homogenous
  - Samples are stable

Source of Test Materials

- Dependant on pathogens
  - Several OIE listed pathogens included in the panels are exotic to Australia
  - Material sourced from laboratories in the region
- Tissues
  - Crustacean & molluscan viruses can only be cultured *in-vivo*
  - Field material from disease outbreaks
  - Tissues from laboratory infected animals
- Laboratory cultured virus
  - Finfish viruses can be cultured *in-vitro*
- Inactivation methods
  - Initially material was fixed in 70% ethanol
  - Moving towards gamma-irradiated (50kGy) materials
    - More representative of samples a laboratory would receive

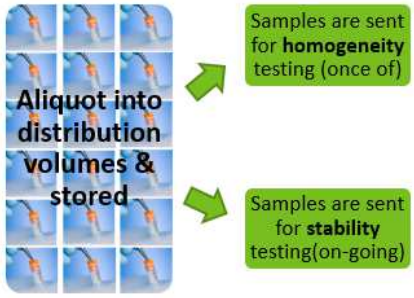
Test sample assessment

- As PT involves a group of laboratories performing the same analyses on the same samples and comparing results, a key requirement is that the samples are ***homogenous*** and ***stable***.

Homogeneity testing

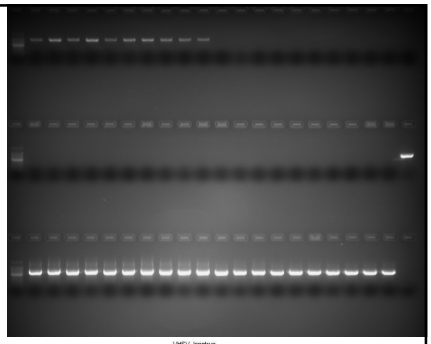
AND

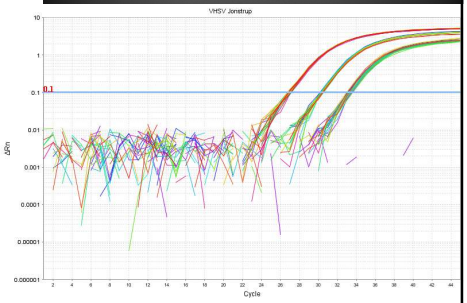
Stability testing



Homogeneity testing

- Prior to distribution of the PT panel
- 10 samples chosen at random for each concentration
- For conventional PCR: assessed visually
- For real-time PCR testing: coefficient of variation ≤ 5%





Homogeneity testing

Homogeneity check (ISO 13528 Annex B)			Example data	
Sample t	value#1, x_{t1}	value#2, x_{t2}	sample average (B.4), x_t	between-test-portion ranges (B.5), w_t
1	33.38	33.35	33.36510086	0.021186829
2	33.16	33.13	33.14491463	0.032772064
3	33.10	33.52	33.31279564	0.417308807
4	33.43	33.45	33.43806267	0.020313263
5	33.22	33.01	33.11329842	0.215885162
6	32.97	33.23	33.10064316	0.253799438
7	33.61	33.43	33.51993179	0.179870605
8	33.23	33.19	33.20585632	0.039009094
9	33.61	33.31	33.4585247	0.305225372
10	33.23	33.14	33.18138885	0.09098053
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
	number of samples g		10	
	general average (B.6) $\bar{x}$		33.2840517	
	STD of sample averages (B.7), s_x		0.154920584	
	within-samples STD (B.8), s_w		0.145330287	
	between-samples STD (B.9), s_s		0.11593076	
Expected standard deviation for proficiency assessme $\hat{\sigma}$				0.6

Name / Isolate:	VHSV
Test used:	OIE Jonstrup VHSV RT-qPCR
Microstores No:	
Homogeneity SAN:	
Dilution:	
Liquid / Freeze dried:	

These sheets are intended to be used for homogeneity testing of proficiency testing samples according to ISO 13528:2005 (annex B) and the IUPAC "International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories" (Thompson et al., Pure Appl. Chem. 78, 145-196, 2006) respectively by means of duplicate measurements of minimum 10 samples.

Please fill in the name of your samples, your measurement results and the expected standard deviation for proficiency assessment in sheet "13528".

The use of this EXCEL-sheet is free, as long as the copyright statement is not removed.

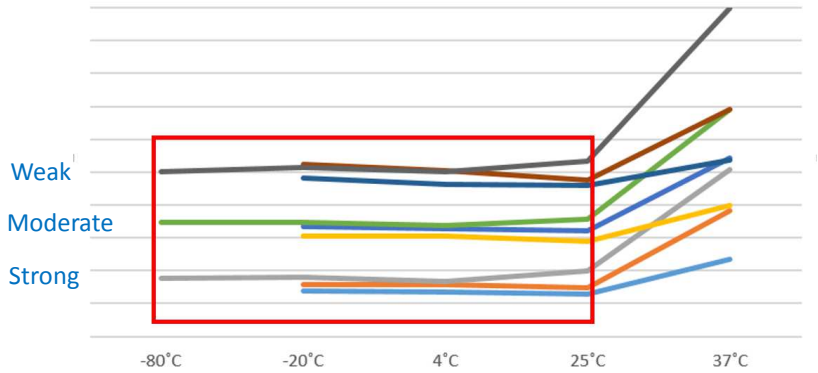
Coefficient of Variation =	0.35%	PASS
For PCR	PASS is <=5%	
For ELISA	PASS is <=15%	

Stability testing

- To demonstrate that test samples will not change significantly over the course of the PT scheme.
- Distinguish between unexpected results and whether they are:
  - due to participant variation
  - instability of the test samples
- Stability testing before and after distribution of PT panels: 3 samples

Stability testing

Spring viraemia of carp virus (SVCV)



Lines for each concentration represent testing at 2, 5 and 10 weeks

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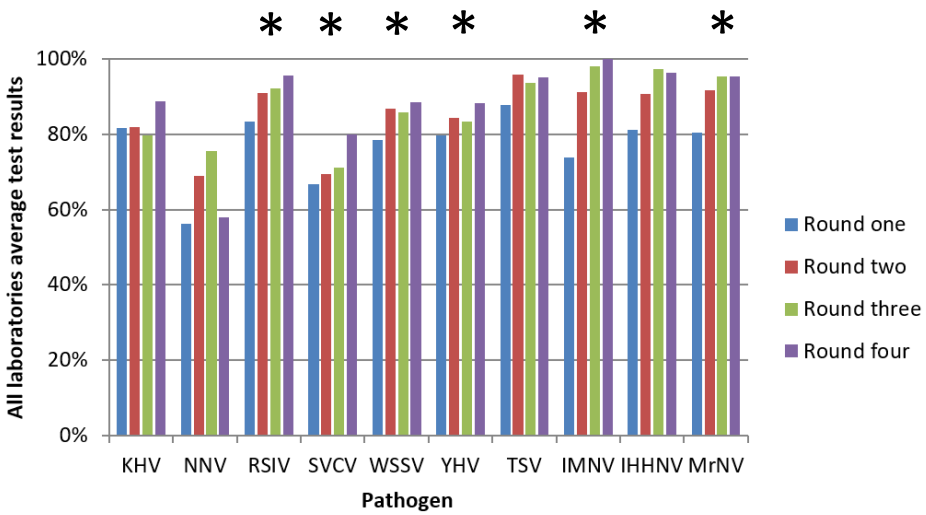
Results from 2012-2015 PT Program



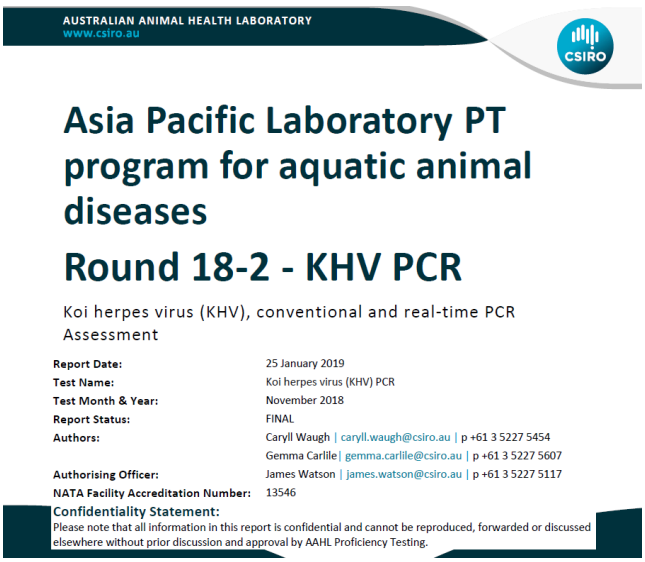
Asia-Pacific Laboratory Proficiency Testing Program for Aquatic Animal Diseases

Current PT Program

- 2018: 34 participating labs from 13 countries.
- 2019: 39 labs participated Round 1, 42 labs enrolled in Round 2, from 14 countries.
- Pathogens to include decided after consultation with participants:

Crustacean	Finfish
Infectious hypodermal and haematopoietic necrosis virus (IHHNV)	Megalocytivirus (RSIV)
Taura syndrome virus (TSV)	Nervous necrosis virus (NNV)
White spot syndrome virus (WSSV)	Koi herpesvirus (KHV)
Infectious myonecrosis virus (IMNV)	Spring viraemia of carp virus (SVCV)
Yellow head virus genotype 1 (YHV1)	
Acute hepatopancreatic necrosis disease (AHPND)	

Current PT Program – report detail



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Asia Pacific Laboratory PT program for aquatic animal diseases

Round 18-2 - KHV PCR

Koi herpes virus (KHV), conventional and real-time PCR Assessment

Report Date:	25 January 2019
Test Name:	Koi herpes virus (KHV) PCR
Test Month & Year:	November 2018
Report Status:	FINAL
Authors:	Caryll Waugh | caryll.waugh@csiro.au | p +61 3 5227 5454
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NATA Facility Accreditation Number:	13546

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Current PT Program – report detail

- Coded, anonymous report
- Collect and report extraction methods and PCR protocols (real-time and conventional) used so laboratories can compare their protocols and results with what other labroratories do

Current PT Program – report detail

Table 3a Comparison based on the calculated mean of the reported Ct values for KHV real time PCR results

Sample	Expected	H	K	O	Q	S	V	W	Median
1	moderate Positive	31.1	-	30.0	30.9	28.2	26.4	26.4	29.1
2	weak Positive	36.5	-	35.9	37.0	36.2	33.2	33.4	36.0
3	moderate Positive	31.4	-	29.0	30.8	27.6	26.9	26.9	28.3
4	moderate Positive	34.5	-	32.1	33.4	36.2	31.4	31.5	32.8
5	strong Positive	26.3	33	22.4	23.4	20.2	20.5	21.3	22.4
6	Negative	-	-	-	-	-	-	-	Negative

Table 3b Comparison based on qualitative interpretation of KHV real time PCR results

Sample	Expected	H	K	O	Q	S	V	W	Agreement[1]
1	moderate Positive	Positive	Negative	Moderate	Positive	Positive	Positive	Positive	86%
2	weak Positive	Positive	Negative	Low	Positive	Positive	Positive	Positive	86%
3	moderate Positive	Positive	Negative	Moderate	Positive	Positive	Positive	Positive	86%
4	moderate Positive	Positive	Negative	Moderate	Positive	Positive	Positive	Positive	86%
5	strong Positive	Positive	Positive	Heavy	Positive	Positive	Positive	Positive	100%
6	Negative	Negative	Negative	Negative	Negative	-	Negative	Negative	100%

Table 4 Comparison based on qualitative interpretation of KHV conventional PCR results

Sample	Expected	B	C	D	F	H	K	M	R	T	U	AD	Agreement[1]
1	moderate Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	91%
2	weak Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Very weakly Positive	91%
3	moderate Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	91%
4	moderate Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Weak Positive	91%
5	strong Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Strong Positive	100%
6	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	100%
	BAND, bp:	292	409/292	293/408	409	209 / 402	409/292	320	320 / 550	409	409	409	

Asia-Pacific Laboratory Proficiency Testing Program for Aquatic Animal Diseases

Current PT Program – report detail

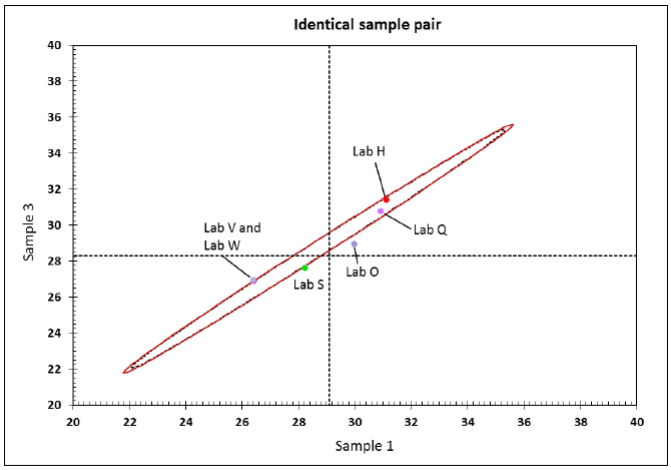

Figure 3: KHV real-time PCR Assay – Youden plot for identical sample pair 1 and 3.

Current PT Program – report detail

3.2 Assessment

Lab Code	Method	Qualitative Assessment[1]	Notes
B	Conventional PCR	Acceptable	Agreement with assigned results.
C	Conventional PCR	Acceptable	Agreement with assigned results.
D	Conventional PCR	Acceptable	Agreement with assigned results.
F	Conventional PCR	Acceptable	Agreement with assigned results.
H	Conventional PCR	Acceptable	Agreement with assigned results.
	Real time PCR	Acceptable	Agreement with assigned results.
K	Conventional PCR	Unacceptable	Results do not agree with assigned values. Failed to detect samples 1-4. Review of procedures is recommended.
	Real time PCR	Unacceptable	Results do not agree with assigned values. Failed to detect samples 1-4. Review of procedures is recommended.
M	Conventional PCR	Acceptable	Agreement with assigned results.
O	Real time PCR	Acceptable with observation	Qualitative results are acceptable. Minor statistical differences are noted but not considered significant. No specific follow-up is recommended.

Conclusions

- Increasing number of participants and increasing requests to participate
- Workshop in Bangkok, Thailand in March 2019 with participant attendance funded. To discuss:
  - the PT Program
  - change in panel composition (from pathogen-specific to host-specific)
  - discuss issues and provide advice on technical aspects of laboratory set-up, test protocols and quality assurance
- Additional workshops planned
- Viewed as a collaboration between the PT Program organisers and participants

Acknowledgements

Australian Government
Department of Agriculture

NACA

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Asia-Pacific Laboratory Proficiency Testing Program for Aquatic Animal Diseases

Thank you

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