Australian Government
Department of Agriculture

A regional proficiency testing program for aquatic animal disease diagnostic laboratories in Asia-Pacific

Final Report

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Contact

Department of Agriculture
Postal address: GPO Box 858
Canberra ACT 2601
Australia
Web: agriculture.gov.au

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Summary

A Regional Proficiency Testing Program for Aquatic Animal Disease Diagnostic Laboratories in Asia-Pacific (the 'regional PT program') was developed in 2011 to strengthen diagnostic capability across Asia—a region that produces most of the world's aquatic animal products. This capability was identified as a requirement to facilitate the sanitary safety of trade in aquatic animal products and to assist countries to improve accurate detection of potentially damaging trans-boundary diseases. The need for improved diagnostic capabilities across Asia was widely agreed and documented prior to developing the regional PT program, however few previous activities had made significant or lasting impacts at the regional level.

The regional PT program was developed as an initiative of the Australian Government Department of Agriculture and funded through its International Agricultural Cooperation Program (IACP). Collaborative partners in the program included the CSIRO Australian Animal Health Laboratory (CSIRO AAHL), the Victorian Government Department of Economic Development, Jobs, Transport and Resources–Australian National Quality Assurance Program (ANQAP) and the Network of Aquaculture Centres in Asia-Pacific (NACA). A regional PT program Steering Committee, comprising members from each of the collaborating partner organisations, oversaw program development and implementation. Roles and responsibilities were well-defined for project collaborators to ensure the effective contribution of expertise necessary to manage a proficiency testing program of this scale.

NACA promoted the regional PT program to potential participants and hosted a preliminary workshop in July 2012 (funded by Australian Government Department of Agriculture). The workshop provided training on diagnostic standards, proficiency testing procedures and laboratory accreditation and gave participants the opportunity to reach agreement on diseases to be included in the program. Experts from the Australian Government Department of Agriculture, ANQAP and CSIRO AAHL provided instruction at the workshop.

Following the workshop, 41 laboratories from 12 NACA member countries participated in proficiency testing for 10 prioritised aquatic animal pathogens of crustaceans and finfish. Outbreaks of disease caused by these pathogens have impacted the aquaculture industry in Asia significantly and rapid and accurate diagnosis is critical for mitigating their impacts. The pathogens selected were (in order of agreed priority): White spot syndrome virus (WSSV), Yellowhead virus (YHV), Taura syndrome virus (TSV), Infectious myonecrosis virus (IMNV), Infectious hypodermal and haematopoietic necrosis virus (IHHNV), megalocytiviruses (e.g. RSIV, ISKNV, GIV), Nervous necrosis viruses (NNV), Koi herpesvirus (KHV), *Macrobrachium rosenbergii* nodavirus (*Mr*NV and XSV) and Spring viraemia of carp virus (SVCV). Mollusc pathogens were considered at the workshop but were not selected amongst the 10 priority pathogens.

Preparation for the four proficiency testing rounds began with the development of non-infectious viral test materials for the 10 priority pathogens. CSIRO AAHL obtained prawn viruses from homogenised infected prawn tissues and finfish viruses from infected cell cultures. Samples were inactivated and fixed in ethanol then diluted into concentrations likely to be encountered in naturally infected animals. Quality controlled test materials were provided to ANQAP to prepare test panels and coordinate their distribution to participating laboratories. As part of the National Association of Testing Authorities (NATA) quality assurance procedures, ANQAP sent blind samples to CSIRO AAHL for homogeneity and stability testing to ensure that the aliquots of small samples were a homogeneous preparation from the bulk stock and expected results would be achieved if the PCR tests were carried out correctly by the participating laboratories. Stability testing confirmed that the sample content had not degraded over time. NACA then coordinated distribution of test kits to participant laboratories.

Test kits were prepared with two negative samples and four positive samples (high, medium and low positives). One of the positive concentrations was provided in duplicate (exception was for KHV as this was developed prior to the request for duplicate positive samples) to assess repeatability. Participant laboratories therefore reported six test results per round for each pathogen.

Laboratories selected which pathogens to test for and were encouraged to use their standard in-house diagnostic methods used in routine testing. Laboratories submitted their test results to ANQAP who presented the de-identified test data in a form suitable for discussion by the regional PT Steering Committee. Detailed reports were sent to individual laboratories after each round to enable comparison of performance between rounds and identify possible areas for improvement. Reports included the results of all laboratories testing for

a particular pathogen; however laboratories could only identify their individual test results through a unique code number, all other laboratories' results remained unidentifiable.

Participation in the regional PT program was consistent across the four rounds of testing with 88% of laboratories returning results for all enrolled tests. Over the two years of proficiency testing, a few issues were recognised affecting laboratory participation and reporting. Some of these issues included in-country logistical challenges with specimen transport, inadequate staffing levels or understanding of required procedures, poor access to diagnostic kits and reagents, and suspected transcriptional errors in data recording. Any future Asia-Pacific laboratory proficiency testing program should aim to accommodate such issues or plan to minimise their impacts to ensure program outcomes are reliable and of value (e.g. stability testing to account for the potential temperature effects on test samples during transport).

After four rounds of proficiency testing, 3564 correct results (86% correct) were reported from a total of 4144 possible correct results. The program outcomes are described by the relative improvements in average scores across all laboratories, incorporating all scores between 0 and 6 for all tests; and are also described by the relative improvement in laboratories reporting all tests correctly; for example, laboratories obtained a score of 6 if all samples within a test panel had a correct result.

The relative improvement in average test results varied between 7.2% and 32% for the 10 priority pathogens. Average improvement was greatest for NNV and IMNV (greater than 20% improvement in scores). Average results improved by between 10% and 20% for IHHNV, *Mr*NV, SVCV, RSIV WSSV, while improvement for YHV, TSV and KHV was between 7% and 10% after the four testing rounds.

The improvement in laboratories reporting all test results correctly followed a similar pattern with an increase for each pathogen of between 11% and 64% between round one and round four. The greatest increase in laboratories reporting all results correctly was for IMNV and NNV (greater than 50% improvement). The number of laboratories reporting all results correctly for *Mr*NV, WSSV, IHHNV, and YHV increased by 25%, while TSV, RSIV, SVCV and KHV improved by 11% to 25%.

The regional PT program provided 41 laboratories across the Asia-Pacific with the opportunity to assess their diagnostic performance for 10 regionally significant aquatic animal pathogens, and to adapt or modify practices where necessary to improve. Through collective participation and improvement, regional capability to diagnose important aquatic animal pathogens has been strengthened.

1 Program objectives and expected outcomes

Laboratory proficiency testing is an important mechanism for laboratories to test and improve diagnostic capabilities, and successful participation in a recognised program can be a requirement for formal laboratory accreditation. A lack of proficiency testing programs for aquatic animal health laboratories in Asia-Pacific was identified as a major capability deficit by the Regional Advisory Group for Aquatic Animal Health (an advisory group to NACA) at their ninth meeting in November 2010 (NACA 2010). The advisory group noted that *ad hoc* proficiency testing programs had been run (for a limited selection of diseases and countries) but that there was limited or no access to ongoing laboratory proficiency testing programs.

In 2011, the Australian Government Department of Agriculture committed funding to a regional proficiency testing program ('regional PT program') for aquatic animal disease diagnostic laboratories in Asia-Pacific.

1.1 Objectives

The three objectives of the regional PT program were:

1 To strengthen Asia's regional capability to diagnose important aquatic animal diseases that impact on trade, industry sustainability and/or productivity
2 To train participating laboratory personnel in diagnostic standards, and proficiency testing procedures, and to provide technical assistance to improve laboratory performance
3 To establish a laboratory proficiency testing program that meets regional needs and which can be accessed following completion of the project (on a fee for service basis).

Four rounds of testing were offered to participating laboratories in years two and three of the project (2013 and 2014). The provision of testing rounds followed National Association of Testing Authorities (NATA) standards for which ANQAP is accredited, and provided each participant with confidential reports on their testing proficiency. CSIRO AAHL offered participants technical guidance to improve their proficiency throughout the testing rounds.

1.2 Expected outcomes

Expected outcomes from the regional PT program included:

1. Improved diagnostic capability for significant aquatic animal diseases throughout the Asian region (this will be measurable during the life of the project based on improvements in aggregated diagnostic proficiency testing results)
2. Increased confidence of trading partners that countries within the region have the ability to certify the disease status of aquatic animal commodity exports, meet quarantine requirements, and thus ensure the sanitary safety of trade through appropriate pre-border measures
3. Improved capability within Asia to detect important trans-boundary diseases that have the potential to devastate industry sustainability and productivity, thereby reducing their spread.

2 Planning and preparation–2012

2.1 Collaborator responsibilities and pre-planning

The regional PT program was overseen by a steering committee comprising representatives from the Australian Government Department of Agriculture, the Network of Aquaculture Centres in Asia-Pacific (NACA), the Commonwealth Scientific and Industrial Research Organisation – Australian Animal Health Laboratory (CSIRO AAHL) and the Victorian Department of Economic Development, Jobs, Transport and Resources Australian National Quality Assurance Program (ANQAP).

NACA was responsible for managing communications with participating member countries and hosting a preparatory workshop. CSIRO AAHL was tasked with obtaining and preparing sample materials, conducting quality checks, providing bulk sample preparations to ANQAP, and providing limited technical advice to participating laboratories. ANQAP was responsible for the aliquoting and preparation of sample materials, organising homogeneity and stability quality assurance testing, distribution of sample panels to participating laboratories, receipt and collation of laboratory test results, preparing de-identified reports for each round of testing, and ensuring testing results remained confidential. The Australian Government Department of Agriculture funded the project through its International Agricultural Cooperation Program (IACP) and provided overall project coordination. Specific roles and responsibilities for each collaborative partner are detailed in Appendix 1.

The regional PT program Steering Committee's responsibilities included:

4 Finalising the regional PT program *Implementation Plan*
5 Planning and conducting the participant workshop
6 Monitoring project progress
7 Reviewing project reports and key communications prior to circulation to participants
8 Consideration of communications and technical support required to encourage participation
9 Consideration of risks to the project implementation and advising on actions to mitigate those risks.

2.2 Requirements for participation

Forty-one laboratories across 12 NACA member countries participated in the four rounds of aquatic animal disease diagnostic proficiency testing. Participating countries included Cambodia, China, Hong Kong, India, Indonesia, Iran, Malaysia, Myanmar, Philippines, Sri Lanka, Thailand and Vietnam. See Appendix 2 for a full list of participating laboratories.

Participation in the regional PT program was offered at no cost to the key national aquatic animal disease diagnostic laboratory of each NACA member country plus two affiliated laboratories actively involved in aquatic animal disease diagnostics. The laboratories were required to possess the capabilities for level III diagnostics (i.e. virology, electron microscopy, molecular biology and immunology). Because some countries could not nominate three laboratories, other countries with larger laboratory networks were invited to nominate additional laboratories (India, Indonesia, Malaysia, Philippines, Sri Lanka, Thailand, and Vietnam). Participating laboratories were required to be responsible for providing aquatic animal disease diagnostic services to the national Competent Authority (CA).

2.3 NACA hosted workshop

The first year of the regional PT project (2012) involved preparatory activities, commencing with a workshop to train all participants in diagnostic standards, proficiency testing procedures, laboratory accreditation, and to reach agreement on the diseases to be included in the program. The workshop was held for two days from 25–26 July 2012 at the Centara Grand Central Hotel, Bangkok, Thailand. Experts from the Australian Government Department of Agriculture, ANQAP and CSIRO AAHL provided instruction at the workshop. Invitations were also extended to intergovernmental organisations with an interest in aquatic animal health, including the World Organisation for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO).

Ten aquatic animal pathogens of highest common priority were selected by workshop participants for inclusion in the program. The priority list included nine OIE-listed diseases of finfish and crustaceans (OIE 2011) (Table 1). NNV was also included in the regional PT program (not OIE listed but included in the Quarterly Aquatic Animal Disease regional reporting program list of reportable diseases since 2005).

Table 1: The 10 priority aquatic animal pathogens included in the regional PT program

Rank	Pathogen	Label	OIE Listed
1	White spot syndrome virus (WSSV)	WSSV	Yes
2	Yellowhead virus (YHV)	YHV	Yes
3	Taura syndrome virus (TSV)	TSV	Yes
4	Infectious myonecrosis virus (IMNV)	IMNV	Yes
5	Infectious hypodermal and haematopoietic necrosis virus (IHHNV)	IHHNV	Yes
6	Megalocytiviruses (RSIV, ISKNV, GIV etc.)	RSIV	Yes
7	Nervous necrosis viruses (NNV)	NNV	No
8	Koi herpesvirus (CyHV-3)	KHV	Yes
9	*Macrobrachium rosenbergii* nodavirus (*Mr*NV and XSV)	MrNV	Yes
10	Spring viraemia of carp virus (SVCV)	SVCV	Yes

3 Program implementation–2013 & 2014

3.1 Sample preparation, inactivation, aliquoting and storage

Following the 2012 workshop, CSIRO AAHL prepared test materials for the program. Pathogen material for the 10 priority diseases was generated and rigorous quality assurance procedures followed to develop test materials. Bulk lots of fixed tissue containing non-viable (non-infectious) virus were produced at three different concentrations for all but one virus. KHV was the sole exception and was provided at four concentrations, because the need of a duplicate positive as a measure of repeatability was recognized. Bulk preparations of the positive and negative virus concentrations underwent preliminary homogeneity testing at CSIRO AAHL and were then provided to ANQAP for aliquoting, quality assurance testing (further homogeneity testing and stability testing of aliquoted samples) and distribution to participating laboratories.

The four finfish viruses were prepared in cell culture and then fixed in a final ethanol concentration of 70% (v/v) at 23-24°C for 24 hours. Following primary inactivation the precipitate was consolidated by centrifugation, the supernatant discarded and the pellet resuspended in fresh 80% (v/v) ethanol to produce the working stock. The stock was further diluted in 80% (v/v) ethanol containing uninfected cell culture supernatant to achieve a range of concentrations expected in naturally infected fish. Negative samples were prepared using uninfected cell cultures.

The six prawn viruses were supplied as non-infectious prawn tissue homogenate, fixed in 80% (v/v) ethanol. One batch of confirmed test-negative uninfected prawn tissue was used as a negative sample for all agents and was also used as the “diluent” for preparing positive samples of varying concentration.

While participant laboratories were free to use whatever test system they used in usual practice, it was recognized that most molecular testing in the region is based on conventional nested PCR. The three concentrations of positive DNA or RNA target were set to high, medium and low concentrations representative of the analytical range (using standard regional techniques) over which one might expect samples from severe to very light infection to occur naturally. The high sample would provide a clear positive band after first step amplification, the medium sample would provide a weak or negative result after one round of amplification and would probably require second step amplification to get a clear positive result and the low sample would be first step negative and require both first and second step amplification to produce a positive result. Real-time PCR (where available) was only used for homogeneity testing, otherwise all subsequent stability testing was based on conventional PCR to ensure the longer DNA targets required by conventional PCR amplification were maintained over the life of the test samples.

Bulk samples were maintained aseptically for a total of 16 weeks at -20°C prior to transfer to ANQAP for aseptic preparation of aliquots. ANQAP prepared 500µL aliquots of each concentration (high, medium, low and negative) for each pathogen. A total of approximately 14,000 aliquots were prepared to cover samples required for the four rounds of proficiency testing for 10 pathogens across the participating laboratory network. Sample vials were capped with a pathogen-specific coloured vial cap, labelled with the test pathogen and coded with a five-digit number linked to the pathogen concentration via an algorithm. All prepared vials were sealed with parafilm to meet International Air Transport Association (IATA) air transport requirements.

3.2 Quality control–Homogeneity testing

ANQAP returned 10 random aliquots of each stock concentration (high, medium, low or negative) for each of the 10 virus preparations to CSIRO AAHL for quality assurance homogeneity testing. Nucleic acid from each sample was prepared by column extraction and analysed by either conventional PCR or real-time PCR. Real-time PCR testing was performed in duplicate and conventional PCR as individual tests. Where available, OIE-recommended primers and probes were used for quality control analysis. For agents where OIE-recommended primers and probes weren’t available then CSIRO AAHL ‘in-house’ real-time PCR assays were used. Any samples with a coefficient of variation of greater than 5% for the real-time PCR tests, or a discrepancy in interpretation for the conventional PCR tests, were not included in the program and were instead reformulated. The process was repeated until each concentration passed homogeneity testing.

3.3 Quality control–Stability testing

ANQAP maintained all sample aliquots at appropriate temperature for the duration of the project. Stability testing was performed in line with NATA protocols before and after each testing round to ensure that the samples had not degraded over time. For stability testing, samples were incubated at 37°C for 24 hours and then maintained at ambient temperature (approx. 24°C) for five days. These stability test samples were then packaged with the samples (baseline) that had been kept at -20°C and posted to CSIRO AAHL for testing. As for the standard dispatch procedure (i.e. to simulate samples transiting through to participant country laboratories), no attempt was made to maintain the samples at -20°C during the period of transit to CSIRO AAHL. Nucleic acid from each sample was prepared by column extraction and analysed by conventional PCR using OIE-recommended primers.

3.4 Sample distribution

The test panel for each pathogen consisted of six samples, with two negative samples and at least one sample from each of the positive dilutions. A duplicate positive sample was included for all tests other than KHV. ANQAP prepared specially designed packages to protect the samples during transit. Participating countries had a predetermined coordinating laboratory that received packaged samples and distributed them to other laboratories within that country, using a transport and distribution method of their choice. The packaged samples were labelled and sent within sealed envelopes addressed to each laboratory and included accompanying information about the specific samples on a specimen advice form. No attempt was made to maintain the samples at -20°C during transport. The same courier company was used wherever possible. Samples for rounds one and two were sent to participants in May 2013, and samples for rounds three and four in May 2014. Laboratories had the opportunity to request different tests in rounds three and four to those initially tested in rounds one and two.

3.5 Testing and reporting

Participating laboratories reported their results directly to ANQAP on a standardised form which asked for information on the laboratory's details, operator identification, testing methods employed, extraction kits, testing results and C_T values where applicable (which included the positive and negative controls run alongside the pathogen tests), and any problems encountered. Laboratories used their standard in-house methods for PCR testing. De-identified aggregate reports (i.e. summarising all laboratory results after each round) were drafted by ANQAP and checked by the regional PT Steering Committee before being provided to participants after each round (see example, Table 2). To maintain confidentiality, each laboratory was identified only by a unique number that was changed after the first two rounds of testing. Only ANQAP personnel had access to laboratory identifiers, consistent with confidentiality requirements for proficiency testing accreditation.

3.6 Results analysis

As test results were returned to ANQAP they were tabulated and discordances identified (Table 2). Each laboratory received a score out of six for each pathogen panel, based on the number of samples correctly identified as either positive or negative. A score of six was obtained if all six samples were correctly identified. Statistical analysis could not be performed on results submitted by individual laboratories as fewer than five laboratories returned quantitative results (i.e. C_T-values) in any one round.

3.7 Development samples

At the 2012 workshop, some participants requested that known positive samples for the 10 priority diseases be made available for test development. While this was not part of the original project, a series of additional "development samples" were prepared. Samples for 11 aquatic animal pathogens, including the 10 priority pathogens as well as the finfish pathogen, Viral haemorrhagic septicaemia virus (VSHV) were prepared and made available. While VHSV was not identified as a priority pathogen, there was sufficient interest from participants engaged in export testing to justify the supply of a development sample for this pathogen through the program.

Development samples were supplied as a courtesy for laboratories to develop their current diagnostic capability. These samples were prepared from the same inactivated material as the test panels and were provided upon request and distributed with the test panels in May 2013 and May 2014. It was expected that development samples would only be available for participants not receiving test panels for that particular pathogen. Further to this, it was expected that a request for a development sample would be followed by participation in proficiency testing for that pathogen in the following year.

Table 2: Example of a results summary table

Laboratory No.	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Round 4 Results	R4 Score	R3 Score	R2 Score	R1 Score
Expected Interpretation	Moderate pos	Negative	High pos	Low pos	Moderate pos	Negative		Maximum score = 6	Maximum score = 6	Maximum score = 6	Maximum score = 6
31	Pos	Neg	Pos	Pos	Pos	Neg	PASS	6	6	6	6
32	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	NR	-	-	-	-
36	Pos	Neg	Pos	Pos	Pos	Neg	PASS	6	6	-	-
37	Pos	Neg	Pos	Pos	Pos	Neg	PASS	6	6	6	6
42	Pos	Neg	Pos	Pos	Pos	Neg	PASS	6	6	6	3
43	Pos	Neg	Pos	Pos	Pos	Neg	PASS	6	6	4	2
48	Pos	Neg	Pos	Pos	Pos	Neg	PASS	6	3	6	6
49	Pos	Neg	Pos	Pos	Pos	Neg	PASS	6	6	-	-
55	Pos	Neg	Pos	Neg	Neg	Neg	V	4	3	6	6
57	Pos (Ct 27.75)	Neg (Ct >45)	Pos (Ct 23.37)	Pos (Ct 29.99)	Pos (Ct 26.60)	Neg (Ct >45)	PASS	6	6	6	6
58	Pos	Neg	Neg	Neg	Pos	Neg	V	4	5	6	6
59	Pos	Neg	Pos	Pos	Pos	Neg	PASS	6	6	-	-
60	Pos	Neg	Pos	Pos	Pos	Neg	PASS	6	6	-	-
62	Pos	Neg	Pos	Pos	Pos	Neg	PASS	6	6	6	6
64	Pos	Neg	Pos	Pos	Pos	Neg	PASS	6	6	6	6
66	Pos	Neg	Pos	Pos	Pos	Neg	PASS	6	6	2	2
Expected Interpretation	Moderate pos	Negative	High pos	Low pos	Moderate pos	Negative					

Results were not received from laboratory 32.

PASS All results for test round correct

V Variation in expected result for at least one sample. Review and possible corrective action recommended.

NR No results submitted.

Results for samples A-F provided laboratories with a score out of six. Laboratories were identifiable only through a number (far left column) and could compare results of the current round (in this example round 4) to previous rounds (far right columns).

4 Program results

4.1 Sample homogeneity and stability

Sample homogeneity and stability testing was critically important to ensure that results reported over the four testing rounds were meaningful. This aspect of the program was managed by ANQAP as per NATA requirements and blind samples were sent to CSIRO AAHL for testing.

Quality control measures (see section 3.2 and 3.3) ensured that samples received by participating laboratories were stable and suitable for testing despite the unavoidable logistical issues in reaching laboratories (e.g. transport delays and temperature fluctuations due to a lack of, or inconsistent, refrigeration during sample shipment).

The use of ethanol-fixation appeared to generate material that could be used to provide homogenous sample sets that remained stable for 20 months when stored at -20°C. This was true for both the tissue-derived prawn viruses and the cell culture-derived finfish viruses, irrespective of genome type (ssDNA, dsDNA, ssRNA or dsRNA) or the presence of a viral envelope.

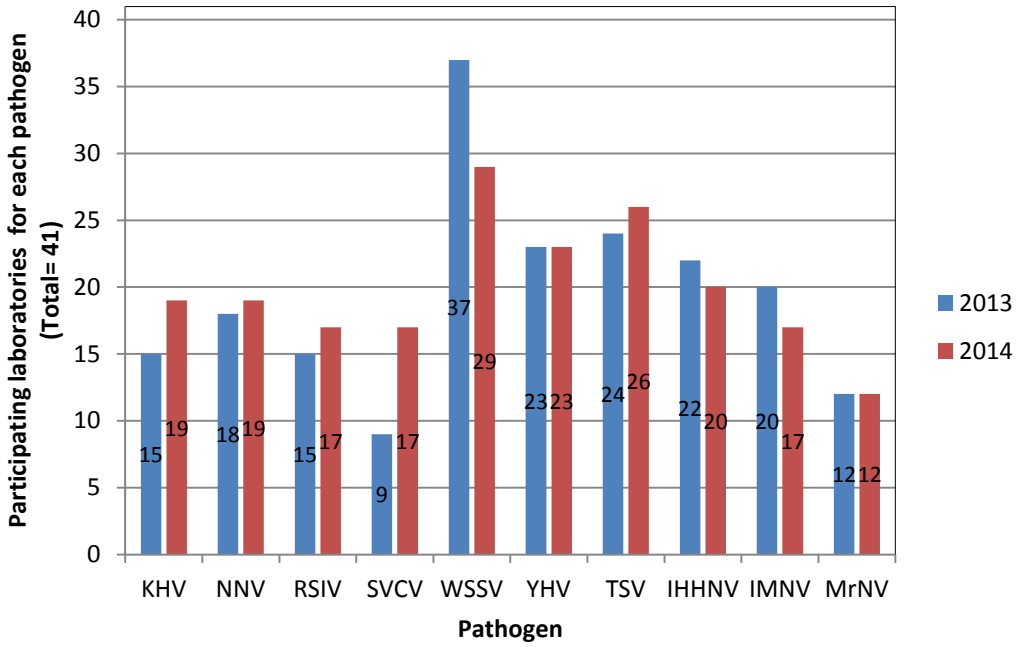
All samples passed stability testing in rounds one, two and three, and at the beginning of round four. However, the NNV low-positive samples failed stability testing at the conclusion of round four. Baseline and stability (temperature treated) samples produced equivocal results when tested by conventional PCR. In light of this, the Steering Committee elected to withdraw this sample from the final round of testing and calculated the NNV scores for this round out of four (the unstable low positive NNV sample had been provided in duplicate), rather than six.

The failure of the low positive NNV sample in the final round of stability testing may have been due solely to its extremely low concentration. However, the identification of this issue highlights the importance of quality control procedures for reliable interpretation of proficiency testing outcomes.

4.2 Laboratory testing

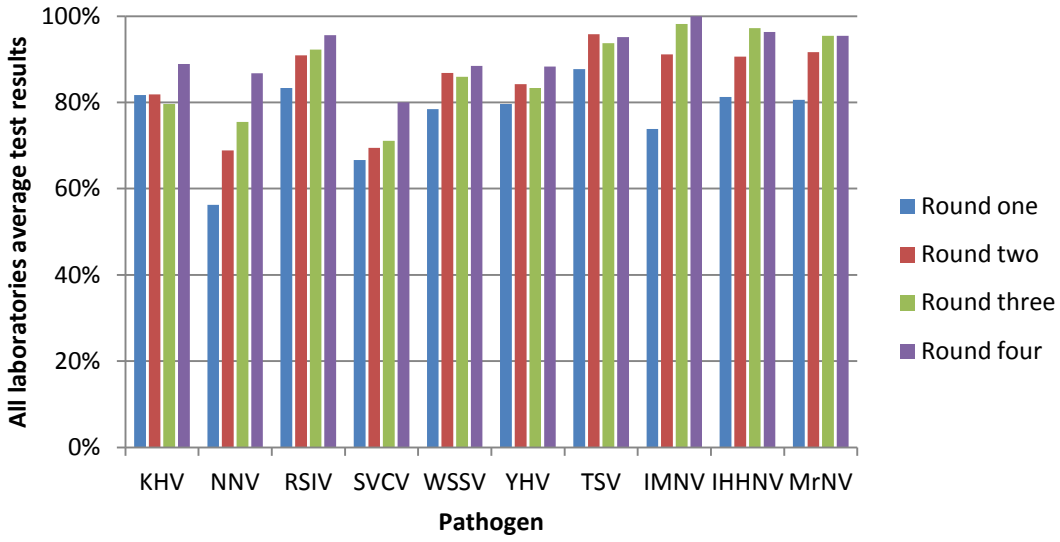
Across all four rounds of testing, 88% of laboratories returned results for all enrolled tests. While many laboratories changed their enrolments from 2013 (rounds 1 and 2) to 2014 (rounds 3 and 4), there were similar numbers of laboratories enrolled for each pathogen (Figure 1), with the exception of a large reduction in the number of laboratories testing for WSSV in 2014 and an increase in SVCV participation for 2014. Proficiency testing for WSSV attracted the greatest number of laboratories in each round.

Figure 1 Number of laboratories participating in proficiency testing for each pathogen in 2013 and 2014



Over four rounds of testing, the 41 participant laboratories reported 3564 correct results (86% correct) from a total of 4144 possible correct results. The average correct diagnostic test results for all participating laboratories, for each pathogen in each round, are provided in **Error! Reference source not found.**. For xample, the average test results across all laboratories testing for KHV in round one was 82%, whereas for round four, the average test result was 89% (noting that 100% means all samples were diagnosed correctly, by all laboratories). Six test results were reported by each participating laboratory for each pathogen in each round (except NNV in round 4 which had 4 test results).

Figure 2 Correct results reported by all laboratories as a percentage of total number of test results reported



The relative change in diagnostic performance between rounds for each pathogen (across all laboratories testing for a specific pathogen each round), is provided in

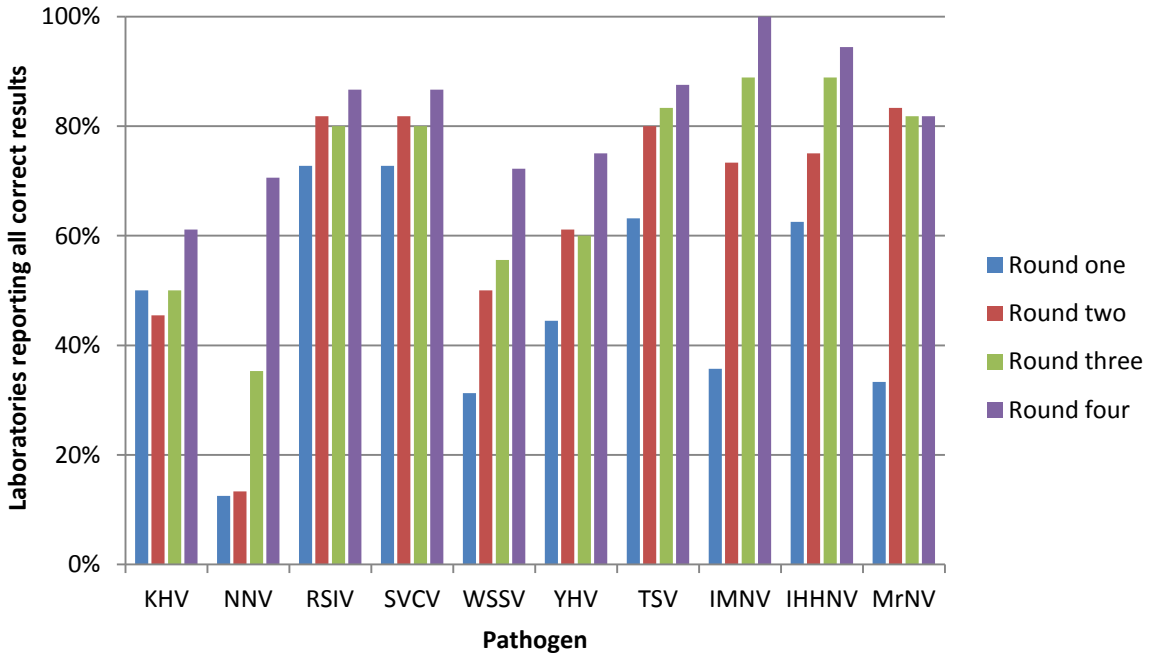
Table 3. The greatest improvement in diagnostic performance between round one and round four was seen for NNV and IMNV. This table provides a measure of the relative improvement in diagnostic performance for all laboratories combined, and takes into consideration all test result outcomes (i.e. each laboratory's score between 0 and 6 for each pathogen in each round).

Table 3: Percentage change reported for each pathogen between testing rounds

Pathogen	R1 to R2	R2 to R3	R3 to R4	Overall: R1 to R4
NNV	12.6	6.6	11.3	30.5
IMNV	17.3	7.0	1.9	26.2
IHHNV	9.4	6.6	-0.9	15.0
MrNV	11.1	3.8	0.0	14.9
SVCV	2.8	1.7	8.9	13.3
RSIV	7.6	1.3	3.3	12.2
WSSV	8.3	-0.9	2.6	10.0
YHV	4.6	-0.9	5.0	8.7
TSV	8.1	-2.1	1.4	7.4
KHV	0.2	-2.2	9.3	7.2

The percentage of laboratories scoring perfect test results for each pathogen is provided in **Error! Reference ource not found.** below (i.e. laboratories reporting correct results for all six samples in a pathogen panel, with the exception of NNV in round four which had a maximum score of four). For example, in round one, 36% of laboratories testing for IMNV reported all results correctly. In round four, 100% of laboratories reported all IMNV results correctly.

Figure 3 Percentage of laboratories reporting perfect test results for each pathogen in each round.



The relative change in the laboratories scoring perfect results per round for each pathogen is provided in

Table 4. For example, between round one and round four, there was a 64% increase in the number of laboratories correctly reporting IMNV results (i.e. diagnosing all samples correctly as either positive or negative).

Table 4: Relative change in the proportion of laboratories reporting perfect results for a pathogen panel between each round of testing

Pathogen	R1 to R2	R2 to R3	R3 to R4	Overall: R1 to R4
IMNV	37.6	15.6	11.1	64.3
NNV	0.8	22.0	35.3	58.1
MrNV	50.0	-1.5	0.0	48.5
WSSV	18.8	5.6	16.7	41.0
IHHNV	12.5	13.9	5.6	31.9
YHV	16.7	-1.1	15.0	30.6
TSV	16.8	3.3	4.2	24.3
RSIV	9.1	-1.8	6.7	13.9
SVCV	9.1	-1.8	6.7	13.9
KHV	-4.5	4.5	11.1	11.1

4.3 Laboratory issues and feedback

The regional PT program was well planned and managed, providing participants with a valuable opportunity to test their laboratory's capabilities. A few issues were identified over the course of the program which may inform any future PT program. Issues included:

- *Delayed and non-reporting* – two laboratories failed to submit any results reports for the program citing financial reasons. Delayed testing, delayed reporting and non-reporting was associated with in-country logistical issues with couriers, postal companies and customs.
- *Laboratory processing* – minor complications were reported by some laboratories affecting their ability to process test samples, such as difficulties in accessing diagnostic kits and reagents, inadequate staffing levels and equipment downtime. One laboratory could not operate electrical equipment for a long period due to severe storms.
- *Diagnostic practices* – a few laboratories appeared to lack understanding of best practice diagnostic methodology, apparently failing to run positive and negative controls at the same time as the tests. Fundamental understanding of PCR was critical to this program. C_T values were rarely provided with diagnostic reports, preventing a more detailed analysis of results.
- *Data recording errors* – a frequent error found in other proficiency testing studies has been simple errors when transcribing data (Jenny & Jackson-Tarentino 2000, Tholen 2002). The regional PT program required laboratories to transcribe a five-digit specimen number unique to each sample. Transcription error is considered a likely cause of some incorrectly reported results.

Feedback from participating laboratories was generally positive. All laboratories appreciated the opportunity to test their proficiency within a confidential program. Laboratories failing to correctly identify pathogens in test samples were advised to re-examine their in-house testing protocols and consider improvements that could be made. For example, laboratories failing to identify the low-positive samples may need to examine the sensitivity of their selected diagnostic tests. False-positive results may require laboratories to re-examine their handling protocols and methods to identify possible opportunities for sample contamination.

4.4 Logistics

A program of this size required efficient, well-planned and well-executed logistics to ensure timely shipping of test samples to the participant laboratories across 12 countries.

Preparation of the homogenous sample material for 10 proficiency testing panels was resource intensive. Prawn viruses, unlike the finfish viruses, cannot be propagated *in-vitro*. As most of the prawn pathogens and some of the natural host species are exotic to Australia, access to sufficient quantities of infected material was limited. This required both the sourcing of infected material from OIE reference laboratories and the experimental propagation of some viruses *in-vivo* in countries where the natural hosts were found. CSIRO AAHL staff, in collaboration with laboratories in selected countries, experimentally infected prawns for the purpose of generating infected material for use in this program. Both the material sourced from reference laboratories and material generated experimentally were inactivated in ethanol (70% or greater) prior to importation into Australia. The inactivated infected material was required to be homogenised to a fine suspension so that the material could be divided and distributed with precision. This process was particularly challenging and significant effort was required to refine suitable methods.

5 Assessment against objectives and outcomes

This regional aquatic animal disease laboratory proficiency testing program delivered high quality (stable and homogenous) proficiency testing materials to 41 aquatic animal disease diagnostic laboratories in the Asia-Pacific region.

5.1 Statement against the program objectives

The regional PT program was established with three objectives. A statement of achievement against each of the objectives is provided below:

1 *To strengthen Asia's regional capability to diagnose important aquatic animal diseases that impact on trade, industry sustainability and/or productivity.*

Achieved

Ten aquatic animal pathogens of importance to the Asia-Pacific region were included in the regional PT program. The 41 participating laboratories prioritised these based on their importance for trade, as trans-boundary diseases or their production impacts. The regional PT program demonstrated improvement (aggregated data averaged across all participant laboratories) in diagnostic performance for every pathogen offered, ranging from 7% to 32% improvement. Laboratories reporting all tests correctly improved from between 11% for KHV to 64% improvement for IMNV.

The benefits of this improved diagnostic performance cannot be determined for each pathogen. However, even small improvements in diagnostic capability for high risk diseases (i.e. high likelihood of spread and high consequence) may have profound implications for preventing impacts of trans-boundary diseases, supporting industry productivity and facilitating safe trade in aquatic animal commodities.

2 *To train participating laboratory personnel in diagnostic standards, and proficiency testing procedures, and to provide technical assistance to improve laboratory performance.*

Achieved

At the completion of each testing round, participant laboratories were provided with their individual test results. These would either affirm their capability to correctly diagnose the selected pathogens, or highlight deficiencies which might then require further investigation or action (e.g. to re-evaluate diagnostic protocols, increase staff training in diagnostic protocols and procedures or invest in improved technologies). Technical assistance and training in proficiency testing procedures was provided at the 2012 workshop.

3 *To establish a laboratory proficiency testing program that meets regional needs and which can be accessed following completion of the project (on a fee–for-service basis).*

Achieved in part

The regional proficiency testing program met regional needs for aquatic animal disease diagnostic testing and was accessed by 12 NACA member countries. After four rounds of testing, all participant laboratories are now familiar with the procedures involved in a proficiency testing program.

There is no ongoing regional proficiency testing program for aquatic animal disease diagnostic laboratories. However, several regional laboratories have requested participation in Australia's National Laboratory Proficiency Testing Program for Aquatic Animal Diseases. Access to this program is limited and provided on a fee-for-service basis.

5.2 Assessment against the expected outcomes

1 *'Improved diagnostic capability for significant aquatic animal diseases throughout the Asian region (this will be measurable during the life of the project based on improvements in aggregated diagnostic proficiency testing results)'.*

This outcome has been demonstrated by the increase in average test scores across all laboratories and the increase in laboratories correctly diagnosing all pathogens between rounds one and four.

2 *'Increased confidence of trading partners that countries within the region have the ability to certify the disease status of aquatic animal commodity exports, meet quarantine requirements, and thus ensure the sanitary safety of trade through appropriate pre-border measures'.*

This outcome is a likely consequence of the improved diagnostic performance achieved through the regional PT program. It is clear that many participants are continuing to pursue opportunities for improved quality assurance.

3 *'Improved capability within Asia to detect important trans-boundary diseases that have the potential to devastate industry sustainability and productivity, thereby reducing their spread'.*

This outcome has been demonstrated by the increase in average test scores across all laboratories and the increase in laboratories correctly diagnosing all pathogens between rounds one and four.

6 Recommendations for future proficiency testing programs

6.1 Ensure participant familiarity with proficiency testing processes

The 2012 workshop was a valuable component of the program, training participating laboratories in proficiency testing processes and preparing them for testing rounds. Section 4.3 of this report identifies some issues that influenced laboratory participation and reporting—some issues are considered unavoidable and represent ongoing challenges for certain countries but others can be mitigated. This program has demonstrated the benefit of a well-planned preparatory workshop to discuss collective priorities; to communicate proficiency testing processes and; to alert participants to any necessary preparations (e.g. securing funding, servicing equipment or training staff).

6.2 Ensure diseases are of highest priority to participants

Participants at the 2012 workshop were asked to nominate pathogens of highest priority. This approach ensured that only diseases of highest common priority to aquatic animal disease diagnostic laboratories in the region were included in the program. This approach is recommended for any future program.

6.3 Ensure the highest standards of quality assurance

It is fundamentally important that any proficiency testing program has rigorous quality assurance measures in place to ensure that test materials reliably produce expected results and that all processes are sound. The project team for this program included extensive experience in provision of proficiency testing, two laboratories with accreditation as proficiency testing providers, expertise in the pathogens being tested, and accredited laboratory quality assurance systems. Further, the project steering committee provided a high level of coordination and oversight.

6.4 Encourage testing continuity for all proficiency testing rounds

A small decrease in average results and correctly reported test results was seen between round two and round three for KHV, TSV, YHV and WSSV. This is likely a result of some laboratories ceasing testing after two rounds and several new laboratories electing to test for a pathogen for the first time in round three. These new laboratories only had the opportunity to refine diagnostic techniques or procedures for round four. To obtain the greatest benefit, participating laboratories should be encouraged to commit to participation in proficiency testing as a routine and ongoing activity.

6.5 Maintain confidentiality

Feedback from participants indicates that confidentiality of laboratory results is a key factor for participation. To ensure strong participation, proficiency testing programs must be designed to provide clear benefits to individual laboratories (detailed confidential feedback and, where possible, assistance to improve) while seeking to achieve the wider objective of stronger regional diagnostic capability.

7 Conclusions

The 41 aquatic animal disease diagnostic laboratories that participated in this regional PT program provide important services to underpin their countries' competent authority declarations of sanitary safety to trade in aquatic animal products; supporting industry productivity and sustainability; and helping to prevent the spread of significant trans-boundary diseases. These laboratories must maintain a consistently high level of diagnostic competency to provide these services. The 2012-2014 regional PT program for aquatic animal disease laboratories in Asia-Pacific has contributed to strengthening this capability.

Participation in proficiency testing as part of a broader laboratory quality management system is important to assure that a sufficiently high level of diagnostic competency is being maintained for priority aquatic animal pathogens. It may also provide confidence in the proficiency of individual laboratories to industry stakeholders and trading partners.

8 Acknowledgments

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The collaborating partners NACA, CSIRO AAHL, and ANQAP are acknowledged for their contributions to the planning, implementation and management of the program.

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OIE Reference Laboratory for IHHNV
Yellow Sea Fisheries Research Institute
Chinese Academy of Fishery Sciences
Qingdao, Shandong
PR China

Coastal Aquatic Animal Health Research Institute
Department of Fisheries
Pawong, Muang, Songkla
Thailand

Ministry of Marine Affairs and Fisheries
Center for Brackishwater Aquaculture Development
Jl. Cik Lanang, Jepara
Central Java
Indonesia

Inland Aquatic Animal Health Research Institute
Department of Fisheries, Kasetsart Campus
Jatujak, Bangkok
Thailand

OIE Reference Laboratory for WTD
C. Abdul Hakeem College
Melvisharam - Vellore District
Tamil Nadu
India

College of Aquaculture and Fisheries
Can Tho University
Can Tho City
Vietnam

All 41 laboratories representing the 12 NACA member countries are acknowledged for their enthusiastic participation in the program.

9 References

Jenny, R.W. and K.Y. Jackson-Tarentino 2000, 'Causes of unsatisfactory performance in proficiency testing'. *Clinical Chemistry*, vol. 46, no. 1, pp. 89–99.

NACA 2010, *Report of the Ninth Meeting of the Asia Regional Advisory Group on Aquatic Animal Health 2010*, Network of Aquaculture Centres in Asia-Pacific: Bangkok, Thailand, available at library.enaca.org/Health/Publication/agm-9-2010-final-report.pdf

OIE 2011, *Aquatic Animal Health Code (2011)*, World Organisation for Animal Health (OIE), available at (current version at time of publication, 2014) oie.int/international-standard-setting/aquatic-code/access-online/

Tholen, D.W 2002, 'Improvements in performance in medical diagnostics tests documented by interlaboratory comparison programs'. *Accreditation and Quality Assurance,* vol 7, no. 4, pp. 146–152.

10 Appendix 1–Regional PT program partner responsibilities

Project partners include the Department of Agriculture, CSIRO AAHL, ANQAP (through the Victorian Government Department of Economic Development, Jobs, Transport and Resources) and NACA.

Table 5: Collaborator responsibilities

Organisation	Responsibilities
Australian Government Department of Agriculture	- Overall project coordination - Chair project steering committee - Development of contracts with project partners - Project reporting to funding scheme - Participate in project workshop
Commonwealth Scientific Industrial Research Organisation Australian Animal Health Laboratory (CSIRO AAHL)	- Participate in project steering committee - Participate in project workshop - Obtain sample materials - Prepare sample materials and conduct quality assurance - Provision of “test ready” materials to ANQAP - Provision of limited technical advice to participating laboratories—as agreed - Reporting on contractual obligations
Australian National Quality Assurance Program (ANQAP)	- Participate in project steering committee - Participate in project workshop - Obtain sample materials from CSIRO AAHL - Prepare sample materials for distribution - Distribute samples to participating laboratories - Receive and collate laboratory test results - Draft de-identified reports for each testing round - Ensure confidentiality of testing results is maintained - Reporting on contractual obligations
Network of Aquaculture Centres (NACA)	- Participate in project steering committee - Participate in project workshop - Communication and liaison with participating NACA member countries - Host a participant workshop; including arranging a venue, catering and travel - Reporting on contractual obligations

11 Appendix 2–Participant laboratories

Participant laboratories in the regional PT program (total = 41).

Cambodia (2)

- Marine Aquaculture Research and Development Center, Fisheries Administration, MAFF.
- Inland Fisheries Research and Development Institute, Fisheries Administration.

China (3)

- The Laboratory of Aquatic Animal Diseases, Shenzhen Exit & Entry Inspection and Quarantine Bureau, Animal and Plant Inspection and Technology Centre AQSIQ.
- Mariculture Organism Disease Control and Molecular Pathology Laboratory, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences.
- Fish Disease Laboratory, Chinese Academy of Fishery Sciences, Zhejiang Institute of Freshwater Fisheries.

Hong Kong (1)

- Aberdeen Molecular Biology Laboratory, Fisheries Branch, Agriculture, Fisheries and Conservation Department, HKSAR.

India (6)

- Aquatic Animal Health and Environment Division, Central Institute of Brackishwater Aquaculture.
- Central Institute of Fisheries Technology.
- National Bureau of Fish Genetic Resources Indian Council for Agricultural Research NBFGR Kochi Unit.
- Central Institute of Freshwater Aquaculture.
- Marine Biotechnology Division Central Marine Fisheries Research Institute.
- Department of Fish Pathology and Health Management Tamil Nadu Fisheries University.

Indonesia (4)

- Fish Health Laboratory for Freshwater Diseases, Main Center Freshwater Aquaculture Development.
- Fish Health and Environment Laboratory Main Centre for Brackishwater Aquaculture Development.
- Testing Laboratory, Fish Health and Environment Brackishwater Aquaculture Development Centre.
- Biologic Molecular Laboratory, Centre of Fish Disease and Environment Investigation.

Iran (3)

- Central Veterinary Laboratory.
- Gillan Fish Diseases Laboratory.
- Boushehr Shrimp Diseases Laboratory.

Malaysia (4)

- Selangor Fisheries Biosecurity Centre, Quarantine Complex.
- Fisheries Biosecurity Laboratory.
- Kedah Fisheries Biosecurity Centre, Department of Fisheries.
- Sarawak Regional Fisheries Biosecurity Centre.

Myanmar (1)

- Aquatic Animal Health and Disease Control Section, Department of Fisheries, Fishery Insepction & Quality Control Lab.

Philippines (5)

- Central Fish Health Laboratory, Bureau of Fisheries and Aquatic Resources (BFAR).
- Regional Fish Health Laboratory, BFAR Regional Office III.
- Regional Fish Health Laboratory, BFAR Regional Office VII.
- Fish Health Section Diagnostic Services, SEAFDEC AQD.
- Negros Prawn Producers Marketing Cooperative, Inc. Laboratory.

Sri Lanka (4)

- Centre for Aquatic Animal Disease Diagnosis and Research, Department of Veterinary Pathobiology Faculty of Veterinary Medicine and Animal Science, University of Peradeniya.
- Brackishwater Fish Health Management and Environmental Monitoring Laboratory Shrimp Farm Monitoring and Extension Unit – NAQDA.
- Inland Aquatic Resources and Research Division National Aquatic Resources Research and Development Agency (NARA).
- Central Veterinary Investigation Center (CVIC), Peradeniya Department of Animal Production and Health, Veterinary Research Institute.

Thailand (4)

- Molecular Biology Laboratory, Coastal Aquatic Animal Health Research Institute.
- Virology Laboratory, Inland Aquatic Animal Health Research Institute, Department of Fisheries.
- Molecular Biology Laboratory, Phuket Coastal Fisheries Research and Development Centre.
- Molecular Biology Laboratory, Inland Aquatic Animal Health Research Institute, Department of Fisheries.

Vietnam (4)

- Department of Animal Health of Vietnam, Regional Animal Health Office No. 6 (RAHO6).
- Center for Environment and Disease Monitoring in Aquaculture at Northern Vietnam, Research Institute for Aquaculture No. 1.
- Southern Monitoring Center for Aquaculture Environment and Epidemic Aquatic Animal Diseases.
- Regional Animal Health Office No. 7, Department of Animal Health.