



OIE Reference Laboratory for Acute Hepatopancreatic Necrosis disease, Infectious Hypodermal and Hematopoietic Necrosis, Necrotizing Hepatopancreatitis, Taura Syndrome and White Spot Disease

April 15, 2022

FINAL REPORT

Dr. S. Kandan

Rajiv Gandhi Centre for Aquaculture (RGCA), MPEDA,
Ministry of Commerce & Industry, Govt. of India,
3/197, Poompuhar Road, Karaimedu village, Sattanathapuram post.,
Sirkali, Pincode-609109, Tamil Nadu, India

Email: rgcapatholab@gmail.com



Dear Dr. S. Kandan,

Thank you for your participation in the February 2022 Ring test for PCR laboratories. In the following pages you will find a summary of your results along with those of **28** other participants. As it is customary, the names of the laboratories have been substituted by a code letter. The letter assigned to your laboratory is the letter “**K**”. On this occasion, we had the participation of laboratories from **16** countries as follows: Australia (1), Belize (1), Brunei (1), Colombia (1), Ecuador (3), Guatemala (1), Honduras (1), India (3), Malaysia (4), Mexico (2), Peru (3), Saudi Arabia (1), Singapore (1), Thailand (2), USA (2), and Vietnam (1).

The pathogens representing a panel of samples you received included **WSSV** (white spot syndrome virus), **PvNV** (*Penaeus vannamei* nodavirus), **IHHNV** (infectious hypodermal & hematopoietic necrosis virus), **TSV** (taura syndrome virus), **YHV** (yellow head virus), **NHP-B** (necrotizing hepatopancreatitis bacterium/*Hepatobacter penaei*), **AHPND** (acute hepatopancreatic necrosis disease), **EHP** (*Enterocytozoon hepatopenaei*), & **IMNV** (infectious myonecrosis virus). Specific pathogen-free (**SPF**) shrimp tissue that was free of these pathogens was also included in the sample sets for DNA and RNA extraction. A summary of the results from all of the participants in the ring test is shown in Table 3. If a laboratory did not test for a particular pathogen, the designation “N/A” (Not Analyzed) was used. If a sample was reported correctly, the box appears 1/1 or 2/2 to indicate the correct result. If no pathogens were detected in the sample, the designation 0/1 or 2/3 highlighted in red were used (False negative). If a sample was reported incorrectly, the box appears 2/1, 3/2, or 4/2 highlighted in red, to indicate the incorrect result (False positive). Tables 4 through 12 show summaries of methodology and turnaround times, respectively.

Your laboratory chose to detect the following pathogens: **WSSV**, **IHHNV**, **TSV**, **YHV**, **NHP-B**, **AHPND**, **EHP**, and **IMNV**. As you could see in Table 3, your laboratory reported all of the DNA and RNA samples correctly. **Congratulations!**



COLLEGE OF AGRICULTURE & LIFE SCIENCES
Animal & Comparative
Biomedical Sciences

Aquaculture Pathology Lab

1117 E Lowell Street Room 109

P.O. Box 210090

Tucson, Arizona 85721



Thank you for your participation in the Ring test. We hope that the results will assist you in your diagnostic work and that you found this exercise useful in assessing your detection/diagnostic capabilities. A printed copy of this report will be mailed to your address in **India**.

Sincerely,

Luis Fernando Aranguren, Ph.D.
Assistant research Professor

Arun K. Dhar, PhD
Associate Professor.
Director, Aquaculture Pathology Laboratory

Disclosure: This Ring test is intended only as a means to evaluate proficiency in the detection of shrimp pathogens by RT-PCR/PCR /RT-qPCR/qPCR. This Ring test is not a certification of the participating laboratory (ies) or of the technician(s) performing such tests. Based on the results of this Ring test, a laboratory should be able to make modifications/improvements as needed.

The University of Arizona, Aquaculture Pathology Laboratory is accredited by ISO/IEC 17043:2010 by ANAB, while demonstrating technical competence in the field of PROFICIENCY TESTING PROVIDER. Refer to the accompanying Scope of Accreditation for information regarding the types of activities to which this accreditation applies

The University of Arizona, Aquaculture Pathology Laboratory is an OIE Reference Laboratory for Acute Hepatopancreatic Necrosis Disease, Infectious Hypodermal and Hematopoietic Necrosis, Necrotising Hepatopancreatitis, Taura Syndrome and White Spot Disease.



Table 1. Summary of the Ring test for PCR laboratories. February 2022

Pathogen	No. laboratories tested for this pathogen	No. laboratories tested correctly	% of correct results	# Labs testing with qPCR	# Labs testing with PCR	Lab with False Positive (1 or more)	Lab with False Negative (1 or more)
IHHNV	23	18	78%	20	5	3	4
WSSV	25	24	96%	21	5	1	1
NHP	18	17	94%	16	4	1	1
IMNV	21	20	95%	20	1	1	1
TSV	22	22	100%	19	5	0	0
AHPND	23	22	96%	21	3	0	1
YHV	21	21	100%	20	2	0	0
EHP	20	19	95%	19	2	0	1
PvNV	3	3	100%	2	1	0	0

Table 2. Cycle threshold (CT values)

Ct Values	IHHNV (Sample 1)	YHV	EHP	IMNV	TSV	AHPND	WSSV	IHHNV (Sample 2, if present)	PvNV	NHP
N	18	19	17	18	19	17	20	17	2	13
CT Mean	25.4	21.3	26.6	22.9	21.2	23.2	20.3	20.6	28.7	20.5
Min	20.3	14	18.95	15.1	13	16.5	15.59	15.19	27.6	11.46
Max	31.4	33.6	35.1	33.0	36.3	32.5	28.9	33.6	29.9	30.9
Sdev	2.8	5.1	3.7	4.4	6.0	4.7	3.5	4.1	1.1	4.9

N= Number of samples analyzed by qPCR/ RT-qPCR; CT Mean= Cycle threshold average; Min= Minimum, CT value reported; Max = maximum CT Value Reported; Sdev= Standard deviation.



Table 3. Summary of results for Ring Test PCR-based diagnostic laboratories carried out in February 2022:

LAB	WSSV	PvNV	IHNNV	TSV	YHV	NHP	AHPND	EHP	IMNV	Total correct	False positive (+)		False negative (-)	
											N°	Details	N	Details
Lab A	1/1	N/A	2/2	1/1	1/1	1/1	1/1	1/1	1/1	9	0	0	0	
Lab B	Pend	Pend	Pend	Pend	Pend	Pend	Pend	Pend	Pend		0	0	0	
Lab C	1/1	N/A	1/1	1/1	1/1	N/A	1/1	1/1	1/1	7	0	0	0	
Lab D	1/1	N/A	1/2	1/1	1/1	1/1	1/1	1/1	1/1	7	2	IHHNV, IMNV	2	IHHNV, IMNV
Lab E	1/1	N/A	1/2	1/1	1/1	N/A	1/1	1/1	1/1	6	1	WSSV	2	WSSV, IHNNV
Lab F	1/1	1/1	2/2	1/1	1/1	1/1	1/1	1/1	1/1	10	0	0	0	
Lab G	Pend	Pend	Pend	Pend	Pend	Pend	Pend	Pend	Pend		0	0	0	
Lab H	1/1	N/A	2/2	1/1	1/1	1/1	1/1	1/1	1/1	9	0	0	0	
Lab I	1/1	N/A	2/2	1/1	1/1	N/A	1/1	1/1	1/1	8	0	0	0	
Lab J	1/1	N/A	2/2	1/1	1/1	N/A	1/1	1/1	1/1	8	0	0	0	
Lab K	1/1	N/A	2/2	1/1	1/1	1/1	1/1	1/1	1/1	9	0	0	0	
Lab L	1/1	1/1	2/2	1/1	1/1	1/1	1/1	1/1	1/1	10	0	0	0	
Lab M	1/1	N/A	3/2	1/1	N/A	N/A	N/A	N/A	N/A	4	1	IHHNV	0	
Lab N	1/1	N/A	2/2	1/1	1/1	1/1	1/1	1/1	1/1	9	0	0	0	
Lab P	1/1	N/A	2/2	1/1	N/A	1/1	1/1	1/1	N/A	7	0	0	0	
Lab Q	1/1	N/A	1/2	1/1	1/1	1/1	1/1	N/A	1/1	7	0	0	1	IHHNV
Lab R	1/1	N/A	N/A	N/A	1/1	N/A	N/A	N/A	N/A	2	0	0	0	
Lab S	1/1	N/A	2/2	1/1	1/1	1/1	1/1	1/1	1/1	9	0	0	0	
Lab T	1/1	N/A	2/2	1/1	1/1	1/1	1/1	N/A	1/1	8	0	0	0	
Lab U	1/1	N/A	2/2	1/1	1/1	N/A	1/1	1/1	1/1	8	0	0	0	
Lab V	1/1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1	0	0	0	
Lab W	1/1	N/A	2/2	1/1	1/1	1/1	1/1	1/1	1/1	9	0	0	0	
Lab X	1/1	N/A	2/2	1/1	1/1	1/1	1/1	1/1	1/1	9	0	0	0	
Lab Y	1/1	N/A	2/2	1/1	1/1	1/1	1/1	1/1	1/1	9	0	0	0	
Lab Z	1/1	N/A	1/2	N/A	N/A	1/1	1/1	N/A	N/A	2	2	IHHNV, NHP	3	IHHNV (2), NHP
Lab AA	1/1	1/1	2/2	1/1	1/1	1/1	1/1	1/1	1/1	10	0	0	0	
Lab BB	N/A	N/A	N/A	N/A	N/A	1/1	0/1	0/1	N/A	1	0	0	2	AHPND, EHP
Lab CC	1/1	N/A	1/1	1/1	1/1	1/1	1/1	1/1	1/1	8	0	0	0	

50-259-32200
 1117 E Lowell Street Room 109
 Aquaculture Pathology Lab



Table 4. Summary of PCR/qPCR and extraction methods employed by participants in the Ring test for PCR laboratories for AHPND detection.

Technique PCR/RT-PCR	Technique qPCR/RT-qPCR	DNA /RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
-	qPCR	NucleoSpin Tissue (Macherey Nagel)	OIE, 2021	-	GoTaq Probe qPCR Master Mix
-	qPCR	SpinTech DNA Extraction Kit	SmartReal AHPND Detection Kit	-	Applied biosystems /TaqMan Fast
-	qPCR	sbeadex livestock kit, oKtopure automated DNA extraction system	Han et al., 201b	-	2x BHQ Probe Master Mix STD ROX/Dual-Labeled BHQ® Probes
-	qPCR	Nucleic Acid extraction kit (Vivantis)	Aileen see et al., 2021	-	Biolone reagent Ltd /Sensifast SYBR No-ROX kit
-	qPCR	Buffer Lysis	Iq Real	-	Iq Real Kit
-	qPCR	TACO DNA/RNA Extraction kit	OIE 2021, Han et al., 2015.	-	FastStart Essential DNA Probes Master (Roche)
-	qPCR	MagMAX™ CORE Nucleic Acid Purification Kit	ThermoFisher multiplex PCR assay	-	VetMAX™-Plus qPCR Master Mix / VetMAX™ Shrimp EMS/EHP Multiplex Reagents (EMS/EHP)
-	qPCR	SpinTech DNA Extraction Kit	SmartReal AHPND Detection Kit	-	Applied biosystems /TaqMan Fast
-	qPCR	IQ REAL GT DNA/RNA CO-EXTRACTION KIT	IQ REAL AHPND/EMS TOXIN 1 Quantitative System	-	-
PCR (nested)	qPCR	DTAB-CTAB	(TUMSAT-Vp3 F/R) Tinwongger et al., 2014	(VpPirA-F/R) Han et al., 2015	<i>DreamTaq Green (Thermo Scientific)/ PowerUp SYBR Green Master Mix (Applied Biosystems)</i>
-	qPCR	qPCR KIT AccuRive Shrimp DNA Prep Kit	AccuPrid AHPND Dection Kit (EMS)	-	AccuPrid AHPND Dection Kit (EMS)
PCR	-	IQ2000	IQ2000	-	IQ2000 kit
-	qPCR	Taco DNA/RNA Extraction Kit	IQ real	-	IQ real kit
-	qPCR	Zymo Research: D7003 Quick DNA/RNA MiniPrep Plus Kit	Han et al., 2015	-	ThermoFisher: A15299 TaqPath™ 1-Step RT-qPCR Master Mix, CG, Invitrogen: 4308323 TaqMan® Exogenous Internal Positive Control Reagents
-	qPCR	IQ Real (Silica Extraction Kit)	IQ Real	-	Iq Real Kit
-	qPCR	Taco™ Nucleic Acid Extraction Reagent	IQ Real	-	IQ Real Kit
-	qPCR	1. SpinTech DNA/RNA extraction kit 2. MagnaTech DNA/RNA extraction kit	In-house method based on OIE (2019)	SmartReal AHPND Detection Kit	1. Taqman® Fast Advance Master Mix 2. SmartReal AHPND Detection Kit
-	qPCR	DNeasy Qiagen	Han et al., 2015	IQREAL	Qiagen /Quantitect Probe PCR/Commercial kit
-	qPCR	taco™ DNA/RNA Extraction Kit	MaxDetect AHPND/EMS qPCR Detection kit	-	MaxDetect kit
PCR	-	CTAB-DTAB	QS Biotech	QS Biotech	DreamTaq Green PCR Master Mix- Thermo Scientific
-	qPCR	MagMAX™ Total Nucleic Acid Isolation Kit (cat no: AM1840)	Han et al., Aquaculture, 442, 12-15.2015. (OIE 2019)	-	Path-ID™ qPCR Master Mix Catalog number: 4388644 (Thermo Fisher Scientific, USA)
-	qPCR	IQ real	IQ real	-	IQ real kit
-	qPCR	IQ real	IQ real	-	IQ real



Table 5. Summary of PCR/qPCR and extraction methods employed by participants in the Ring test for PCR laboratories for EHP detection.

Technique PCR/RT-PCR	Technique qPCR/RT-qPCR	DNA /RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
-	qPCR	NucleoSpin Tissue (Macherey Nagel)	Liu et al., 2018	-	GoTaq Probe qPCR Master Mix
-	qPCR	SpinTech DNA Extraction Kit	SmartReal EHP Detection Kit	-	Applied biosystems /TaqMan Fast
-	qPCR	sbeadex livestock kit, oKtopure automated DNA extraction system	Patharapol Piamsomboon, Seong-Kyoon Choi, Bambang Hanggono, et al, 2019	-	2x BHQ Probe Master Mix STD ROX/Dual-Labeled BHO® Probes
-	qPCR	Nucleic Acid extraction kit (Vivantis)	Aileen see et al., 2021	-	Bioline reagent Ltd /Sensifast SYBR No-ROX kit
-	qPCR	Buffer Lysis	Iq Real	-	Iq Real Kit
-	qPCR	TACO DNA/RNA Extraction kit	Liu et al., 2018.	-	FastStart Essential DNA Probes Master (Roche)
-	qPCR	MagMAX™ CORE Nucleic Acid Purification Kit	Thermofisher multiplex PCR assay	-	VetMAX™ Plus qPCR Master Mix / VetMAX™ Shrimp EMS/EHP Multiplex Reagents (EMS/EHP)
-	qPCR	SpinTech DNA Extraction Kit	SmartReal EHP Detection Kit	-	Applied biosystems /TaqMan Fast
-	qPCR	IQ REAL GT DNA/RNA CO-EXTRACTION KIT	IQ REAL EHP Quantitative System	-	-
PCR (nested)	qPCR	DTAB-CTAB	(SWP_IF/R y SWP_2F/R) Jaroenlak et al., 2016.	(EHP-bTub-F2/(EHP-bTub-R2) Piamsomboon et al. 2019	<i>DreamTaq Green (Thermo Scientific)/ PowerUp SYBR Green Master Mix (Applied Biosystems)</i>
-	qPCR	KIT AccuRive Shrimp DNA Prep Kit	AccuPid EHP Decion Kit	-	AccuPid EHP Decion Kit
PCR	-	IQ2000	IQ2000	-	IQ2000 kit
-	qPCR	Zymo Research: D7003 Quick DNA/RNA MimiPrep Plus Kit	Liu et al. 2018	-	ThermoFisher: A15299 TaqPath™ 1-Step RT-qPCR Master Mix, CG, Invitrogen: 4308323 TaqMan® Exogenous Internal Positive Control Reagents
-	qPCR	Taco™ Nucleic Acid Extraction Reagent	IQ Real	-	IQ Real Kit
-	qPCR	1. SpinTech DNA/RNA extraction kit 2. MagnaTech DNA/RNA extraction kit	In-house method based on Piamsomboon et al. (2019)	SmartReal EHP Detection Kit	1. Taqman® Fast Advance Master Mix 2. SmartReal EHP Detection Kit
-	qPCR	DNeasy Qiagen	IQREAL	-	Commercial kit
-	qPCR	taco™ DNA/RNA Extraction Kit	MaxDetect EHP qPCR Detection kit	-	MaxDetect kit
-	qPCR	MagMAX™ Total Nucleic Acid Isolation Kit (cat no: AM1840)	Liu et al., 2018, Journal of invertebrate pathology, 151, pp.191-196.	-	Path-ID™ qPCR Master Mix Catalog number: 4388644 (Thermo Fisher Scientific, USA)
-	qPCR	IQ real	IQ real	-	IQ real kit
-	qPCR	IQ real	IQ real	-	IQ real



Table 6. Summary of PCR/ qPCR and extraction methods employed by participants in the Ring test for PCR laboratories for IHNV detection.

Technique PCR/RT-PCR	Technique qPCR/RT-qPCR	DNA /RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
-	qPCR	NucleoSpin Tissue (Machery Nagel)	OIE, 2021	-	GoTaq Probe qPCR Master Mix
-	qPCR	SpinTech DNA Extraction Kit	SmartReal IHNV Detection Kit	-	Applied biosystems /TaqMan Fast
-	qPCR	sbeadex livestock kit, oKtopure automated DNA extraction system	Tang & Lightner, 2001.	-	2x BHQ Probe Master Mix STD ROX/Dual-Labeled BHQ® Probes
-	qPCR	Nucleic Acid extraction kit (Vivantis)	Aileen see et al., 2021	-	Bioline reagent Ltd./Sensifast SYBR No-ROX kit
-	qPCR	Buffer Lysis	Iq Real	-	Iq Real Kit
-	qPCR	TACO DNA/RNA Extraction kit	OIE 2021, Tang & Lightner, 2001	-	FastStart Essential DNA Probes Master (Roche)
-	qPCR	MagMAX™ CORE Nucleic Acid Purification Kit	ThermoFisher multiplex PCR assay	-	VetMAX™-Plus qPCR Master Mix / VetMAX™ Shrimp DNA Multiplex Reagents (IHNV, WSSV)
-	qPCR	SpinTech DNA Extraction Kit	SmartReal IHNV Detection Kit	-	Applied biosystems /TaqMan Fast
-	qPCR	IQ REAL GT DNA/RNA CO-EXTRACTION KIT	IQ REAL IHNV Quantitative System	-	-
PCR	qPCR	DTAB-CTAB	(389 F/R) Tang et al., 2007	(VNHH11608F/ VNHH11688R) Tang & Lightner, 2001.	<i>DreamTaq Green (Thermo Scientific)/ PowerUp SYBR Green Master Mix (Applied Biosystems)</i>
PCR	-	DNeasy Blood & Tissue Kits- QIAGEN	OIE 2017 Tang & Lightner, 2001	-	PCR Master Mix (2X) – Thermo Scientific
-	qPCR	KIT AccuRive Shrimp DNA Prep Kit	AccuPid IHNV Dection Kit	-	AccuPid IHNV Dection Kit
PCR	-	IQ2000	IQ2000	-	IQ2000 kit
-	qPCR	Taco DNA/RNA Extraction Kit	IQ real	-	IQ real kit
-	qPCR	Zymo Research: D7003 Quick DNA/RNA MimiPrep Plus Kit	Tang & Lightner 2001	-	ThermoFisher: A15299 TaqPath™ 1-Step RT-qPCR Master Mix, CG, Invitrogen: 4308323 TaqMan® Exogenous Internal Positive Control Reagents
-	qPCR	IQ Real (Silica Extraction Kit)	IQ Real	-	Iq Real Kit
-	qPCR	Taco™ Nucleic Acid Extraction Reagent	IQ Real	-	IQ Real Kit
-	qPCR	1. SpinTech DNA/RNA extraction kit 2. MagnaTech DNA/RNA extraction kit	In-house method based on OIE (2019)	SmartReal IHNV Detection Kit	1. Taqman® Fast Advance Master Mix 2. SmartReal IHNV Detection Kit
PCR	qPCR	DNeasy Qiagen	Krabsetsv et al., 2004	IQ REAL	Promega/Go Taq Flexi/Comercial kit
-	qPCR	taco™ DNA/RNA Extraction Kit	IQ REAL™ IHNV Quantitative System	-	IQ Real kit
PCR	-	Lysis Buffer	QS Biotech	QS Biotech	DreamTaq Green PCR Master Mix- Thermo Scientific
-	qPCR	MagMAX™ Total Nucleic Acid Isolation Kit (cat no: AM1840)	Tang and Lightner Dis Aquat Org 2001, Vol. 44: 79-85. (OIE 2019)	-	Path-ID™ qPCR Master Mix Catalog number: 4388644 (Thermo Fisher Scientific, USA)
-	qPCR	IQ real	IQ real	-	IQ real



Table 7. Summary of RT-PCR/RT-qPCR and extraction methods employed by participants in the Ring test for PCR laboratories for IMNV detection.

Technique PCR/RT-PCR	Technique qPCR/RT-qPCR	DNA /RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
-	RT-qPCR	TriReagent (Sigma-Aldrich)	OIE, 2021	-	qScript Virus 1-Step ToughMix, Low ROX (2X)
-	RT-qPCR	SpinTech RNA Extraction Kit	SmartReal IMNV Detection Kit	-	Applied biosystems /TaqMan Fast
-	RT-qPCR	sbeadex livestock kit, oktopure automated DNA extraction system	Poulos and Lightner, 2006; Andrade et al., 2007; Senapin et al., 2007	-	One - Step taqProbe qRT-PCR-ROX/Dual-Labeled BHO® Probes
-	RT-qPCR	Nucleic Acid extraction kit (Vivantis)	Aileen see et al., 2021	-	Bioline reagent Ltd /Sensifast SYBR No-ROX kit
-	RT-qPCR	Solution de Extraction Iq 2000	oie	-	Sensi Fast Probe Lo-Rox One Step Mix de Bioline
-	RT-qPCR	TACO DNA/RNA Extraction kit	OIE 2021, Andrade et al., 2007	-	LightCycler® Multiplex RNA Virus Master (Roche)
-	RT-qPCR	MagMAX™ CORE Nucleic Acid Purification Kit	Thermofisher multiplex PCR assay	-	VetMAX™-Plus Multiplex One-Step RT-PCR Kit / VetMAX™ Shrimp RNA Multiplex Reagents (YHV, TSV and IMNV)
-	RT-qPCR	SpinTech RNA Extraction Kit	SmartReal IMNV Detection Kit	-	Applied biosystems /TaqMan Fast
-	RT-qPCR	IQ REAL GT DNA/RNA CO-EXTRACTION KIT	IQ REAL IMNV Quantitative System	-	-
RT-PCR (nested)	RT-qPCR	Trizol	(4587E/4914R y 4725NF/4863NR) Poulos y Lightner, 2006	IMNV412F/IMNV5 45. Andrade et al.2007)	SuperScript III One Step RT-PCR Platinum Taq HiFi (Invitrogen) y DreamTaq Green (Thermo Scientific)/ PowerUp SYBR Green Master Mix (Applied Biosystems)
-	RT-qPCR	KIT AccuRive Shrimp pRNA Prep Kit	AccuPid IMNV Dection Kit AccuLite Reverse Transcription kit A	-	AccuPid IMNV Dection Kit AccuLite Reverse Transcription kit A
-	RT-qPCR	Taco DNA/RNA Extraction Kit	IQ real	-	IQ real kit
-	RT-qPCR	Zymo Research: D7003 Quick DNA/RNA MiniPrep Plus Kit	Andrade et al., 2007	-	ThermoFisher: A15299 TaqPath™ 1-Step RT-qPCR Master Mix, CG, Invitrogen: 4308323 TaqMan® Exogenous Internal Positive Control Reagents
-	RT-qPCR	IQ Real (Silica Extraction Kit)	IQ Real	-	Iq Real Kit
-	RT-qPCR	Taco™ Nucleic Acid Extraction Reagent	IQ Real	-	IQ Real Kit
-	RT-qPCR	1. SpinTech DNA/RNA extraction kit 2. MagnaTech DNA/RNA extraction kit	In-house method based on OIE (2019)	SmartReal IMNV Detection Kit	1. Taqman® Fast Virus 1-step Master Mix 2. SmartReal IMNV Detection Kit
-	RT-qPCR	MagMax Ambion	Andrade et al., 2007	-	Ambion/AgPath
-	RT-qPCR	taco™ DNA/RNA Extraction Kit	IQ REAL™ IMNV Quantitative System	-	IQ Real kit
-	RT-qPCR	MagMAX™ Total Nucleic Acid Isolation Kit (cat no: AM1840)	Andrade et al., 2007 Aquaculture, 264. 9-15 (OIE 2019)	-	AgPath-ID™ One-Step RT-PCR Catalog number: 4387424 (Thermo Fisher Scientific, USA)
-	RT-qPCR	IQ real	IQ real	-	IQ real



Table 8. Summary of PCR/ qPCR and extraction methods employed by participants in the Ring test for PCR laboratories for NHP detection.

Technique PCR/ RT-PCR	Technique qPCR/RT-qPCR	DNA /RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
-	qPCR	NucleoSpin Tissue (Macherey Nagel)	OIE, 2021	-	GoTaq Probe qPCR Master Mix
-	qPCR	sbeadex livestock kit, oKtopure automated DNA extraction system	Aranguren et al., 2010; Vincent & Lotz, 200	-	2x BHQ Probe Master Mix STD ROX/Dual-Labeled BHQ® Probes
-	qPCR	Buffer Lysis	Iq Real	-	Iq Real Kit
-	qPCR	TACO DNA/RNA Extraction kit	OIE 2021, Aranguren et al., 2010.	-	FastStart Essential DNA Probes Master (Roche)
-	qPCR	IQ REAL GT DNA/RNA CO-EXTRACTION KIT	IQ REAL NHPB Quantitative System	-	-
PCR	qPCR	DTAB-CTAB	(NHPF2/NHPR2) Aranguren et al., 2010.	(NHP1300F/NHP1366R) Aranguren et al., 2010	PuReTaq Ready-To-Go PCR beads (General Electric)/ PowerUp SYBR Green Master Mix (Applied Biosystems)
-	qPCR	KIT AccuRive Shrimp DNA Prep Kit	AccuPid NHPB Dection Kit	-	AccuPid NHPB Dection Kit
PCR	-	IQ2000	IQ2000	-	IQ2000 kit
-	qPCR	Taco DNA/RNA Extraction Kit	IQ real	-	<i>IQ real kit</i>
-	qPCR	Zymo Research: D7003 Quick DNA/RNA MimiPrep Plus Kit	Aranguren et al., 2010	-	ThermoFisher: A15299 TaqPath™ 1-Step RT-qPCR Master Mix, CG, Invitrogen: 4308323 TaqMan® Exogenous Internal Positive Control Reagents
-	qPCR	IQ Real (Silica Extraction Kit)	IQ Real	-	Iq Real Kit
-	qPCR	1. SpinTech DNA/RNA extraction kit 2. MagnaTech DNA/RNA extraction kit	In-house method based on OIE (2019)	SmartReal NHPB Detection Kit	1. Taqman® Fast Advance Master Mix 2. SmartReal NHPB Detection Kit
PCR	qPCR	DNeasy Qiagen	Nunan et al., 2008	IQ REAL	GE Healthcare/PureTaq ReadyTo Go PCR Beads/Comercial kit
-	qPCR	taco™ DNA/RNA Extraction Kit	MaxDetect NHPB qPCR Detection kit	-	MaxDetect kit
PCR	-	CTAB-DTAB	QS Biotech	QS Biotech	DreamTaq Green PCR Master Mix- Thermo Scientific
-	qPCR	MagMAX™ Total Nucleic Acid Isolation Kit (cat no: AM1840)	Aranguren et al., Aquaculture. 2010, Vol. 307: 187–192. (OIE 2019).	-	Path-ID™ qPCR Master Mix Catalog number: 4388644 (Thermo Fisher Scientific, USA)
-	qPCR	IQ real	IQ real	-	IQ real kit
-	qPCR	IQ real	IQ real	-	IQ real



Table 9. Summary of RT-PCR/ RT-qPCR and extraction methods employed by participants in the Ring test for PCR laboratories for PpNV detection.

Technique PCR/RT-PCR	Technique qPCR/RT-qPCR	DNA /RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
- RT-PCR (nested)	RT-qPCR -	Solution de Extraction Iq 2000 Trizol	Iq Real (PvNV339F/R y PvNV246NF/R) Tang et al., 2007.	-	Iq Real Kit SuperScript III One Step RT-PCR Platinum Taq HiFi (Invitrogen) y DreamTaq Green (Thermo Scientific)
-	RT-qPCR	MagMAX™ Total Nucleic Acid Isolation Kit (cat no: AM1840)	PvNV Detection kit (GeneReach Boitechnology copr, Tamiwan)	-	PvNV Detection kit (GeneReach Boitechnology copr, Tamiwan)



Table 10. Summary of RT-PCR/RT-qPCR and extraction methods employed by participants in the Ring test for PCR laboratories for TSV detection.

Technique PCR/RT-PCR	Technique qPCR/RT-qPCR	DNA/RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
-	RT-qPCR	TriReagent (Sigma-Aldrich)	OIE, 2021	-	qScript Virus 1-Step ToughMix, Low ROX (2X)
-	RT-qPCR	SpinTech RNA Extraction Kit	SmartReal TSV Detection Kit	-	Applied biosystems /TaqMan Fast
RT-PCR	-	sbeadex livestock kit, oKtopure automated DNA extraction system	Tang et al., 2004.	-	One – Step taqProbe qRT-PCR-ROX/Dual-Labeled BHQ® Probes
-	RT-qPCR	Nucleic Acid extraction kit (Vivantis)	Aileen see et al., 2021	-	Bioline reagent Ltd /Sensifast SYBR No-ROX kit
-	RT-qPCR	Solution de Extraction Iq 2000	Iq Real	-	Iq Real Kit
-	RT-qPCR	TACO DNA/RNA Extraction kit	OIE 2021, Tang et al., 2004.	-	LightCycler® Multiplex RNA Virus Master (Roche)
-	RT-qPCR	MagMAX™ CORE Nucleic Acid Purification Kit	ThermoFisher multiplex PCR assay	-	VetMAX™ Plus Multiplex One-Step RT-PCR Kit / VetMAX™ Shrimp RNA Multiplex Reagents (YHV, TSV and IMNV)
-	RT-qPCR	SpinTech RNA Extraction Kit	SmartReal TSV Detection Kit	-	Applied biosystems /TaqMan Fast
-	RT-qPCR	IQ REAL GT DNA/RNA CO-EXTRACTION KIT	IQ REAL TSV Quantitative System	-	-
RT-PCR	RT-qPCR	Trizol	(9992F/9195R) Nunan et al., 1998	(TSV1004F/TSV1075R) Tang et al., 2004	SuperScript III One Step RT-PCR Platinum Taq HiFi (Invitrogen)/ PowerUp SYBR Green Master Mix (Applied Biosystems)
RT-PCR	-	TRIZOL	OIE 2017, Nunan et al., 1998	-	SuperScript™ III One-Step RT-PCR System with Platinum™ Taq DNA Polymerase
-	RT-qPCR	KIT AccuRive Shrimp pRNA Prep Kit	AccuPId TSV Decion Kit AccuLite Reverse Transcription kit A	-	AccuPId TSV Decion Kit AccuLite Reverse Transcription kit A
RT-PCR	-	IQ2000	IQ2000	-	IQ2000 kit
-	RT-qPCR	Taco DNA/RNA Extraction Kit	IQ real	-	IQ real kit
-	RT-qPCR	Zymo Research: D7003 Quick DNA/RNA MimiPrep Plus Kit	Tang et al. 2004	-	ThermoFisher: A15299 TaqPath™ 1-Step RT-qPCR Master Mix, CG, Invitrogen: 4308323 TaqMan® Exogenous Internal Positive Control Reagents
-	RT-qPCR	IQ Real (Silica Extraction Kit)	IQ Real	-	Iq Real Kit
-	RT-qPCR	Taco™ Nucleic Acid Extraction Reagent	IQ Real	-	IQ Real Kit
-	RT-qPCR	1. SpinTech DNA/RNA extraction kit 2. MagnaTech DNA/RNA extraction kit	In-house method based on OIE (2019)	SmartReal TSV Detection Kit	1. Taqman® Fast Virus 1-step Master Mix 2. SmartReal TSV Detection Kit
RT-PCR	RT-qPCR	MagMax Ambion	Nunan et al., 1998	IQ REAL	Invitrogen/SuperScriptIII/Commercial kit
-	RT-qPCR	taco™ DNA/RNA Extraction Kit	IQ REAL™ TSV Quantitative System	-	IQ Real kit
-	RT-qPCR	MagMAX™ Total Nucleic Acid Isolation Kit (cat no: AM1840)	Tang et al., 2004 Journal of Virological Methods 115, 109–114. (OIE 2019)	-	AgPath-ID™ One-Step RT-PCR Catalog number: 4387424 (Thermo Fisher Scientific, USA)
-	RT-qPCR	IQ real	IQ real	-	IQ real



Table 11. Summary of PCR/ qPCR and extraction methods employed by participants in the Ring test for PCR laboratories for WSSV detection.

Technique PCR/RT-PCR	Technique PCR/RT-qPCR	DNA/RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
-	qPCR	NucleoSpin Tissue (Macherey Nagel)	OIE, 2021	-	GoTaq Probe qPCR Master Mix
-	qPCR	SpinTech DNA Extraction Kit	SmartReal WSSV Detection Kit	-	Applied biosystems /TaqMan Fast
-	qPCR	sbeadex livestock kit, oktopure automated DNA extraction system	Durand & Lightner, 2002.	-	2x BHQ Probe Master Mix STD ROX/Dual-Labeled BHO Probes
PCR	-	Nucleic Acid extraction kit (Vivantis)	Aileen see et al., 2021	-	Bioline reagent Ltd /Sensifast SYBR No-ROX kit
-	qPCR	Buffer Lysis	Iq Real	-	Iq Real Kit
-	qPCR	TACO DNA/RNA Extraction kit	OIE 2021, Durand & Lightner, 2002.	-	FastStart Essential DNA Probes Master (Roche)
-	qPCR	MagMAX™ CORE Nucleic Acid Purification Kit	Thermofisher multiplex PCR assay	-	VetMAX™-Plus qPCR Master Mix / VetMAX™ Shrimp DNA Multiplex Reagents (IHHNV, WSSV)
-	qPCR	SpinTech DNA Extraction Kit	SmartReal WSSV Detection Kit	-	Applied biosystems /TaqMan Fast
-	qPCR	IQ REAL GT DNA/RNA CO-EXTRACTION KIT	IQ REAL WSSV Quantitative System	-	-
PCR (nested)	qPCR	DTAB-CTAB	(146_1F/1R y 146_2F/2R) Lo et al., 1996	(WSS1011F/ WSS1079R) Durand y Lightner, 2002	DreamTaq Green (Thermo Scientific)/ PowerUp SYBR Green Master Mix (Applied Biosystems)
PCR (nested)	-	DNeasy Blood & Tissue Kits-QIAGEN	OIE MANUAL Lo et al 1998	-	PCR Master Mix (2X) – Thermo Scientific
-	qPCR	KIT AccuRive Shrimp DNA Prep Kit	AccuPId WSSV Decion Kit	-	AccuPId WSSV Decion Kit
PCR	-	IQ2000	IQ2000	-	IQ2000 kit
-	qPCR	Taco DNA/RNA Extraction Kit	IQ real	-	IQ real kit
-	qPCR	QIAGEN DNA extraction	Tinwongger et al., 2014	Real Time PCR-WSSV	Maximo DNA polymerase GeneOn
-	qPCR	Zymo Research: D7003 Quick DNA/RNA MiniPrep Plus Kit	Durand & Lightner, 2002	-	ThermoFisher: A15299 TaqPath™ 1-Step RT-qPCR Master Mix, CG, Invitrogen: 4308323 TaqMan® Exogenous Internal Positive Control Reagents
-	qPCR	IQ Real (Silica Extraction Kit)	IQ Real	-	Iq Real Kit
-	qPCR	Taco™ Nucleic Acid Extraction Reagent	IQ Real	-	IQ Real Kit
-	qPCR	E.Z.N.A. TISSUE DNA KIT	IQ REAL	-	IQ REAL KIT
-	qPCR	1. SpinTech DNA/RNA extraction kit 2. MagnaTech DNA/RNA extraction kit	In-house method based on OIE (2019)	SmartReal WSSV Detection Kit	1. Taqman® Fast Advance Master Mix 2. SmartReal WSSV Detection Kit
-	qPCR	DNeasy Qiagen	Durand et al., 2002	IQ REAL	Qiagen /Quantitect Probe PCR/Commercial kit
-	qPCR	taco™ DNA/RNA Extraction Kit	MaxDetect WSSV qPCR Detection kit	-	MaxDetect kit
PCR	-	Lysis Buffer	QS Biotech	QS Biotech	DreamTaq Green PCR Master Mix- Thermo Scientific
-	qPCR	MagMAX™ Total Nucleic Acid Isolation Kit (cat no: AM1840)	Durand and Lightner Journal of Fish Diseases 2002, 25, 381–389. (OIE 2019)	-	Path-ID™ qPCR Master Mix Catalog number: 4388644 (Thermo Fisher Scientific, USA)
-	qPCR	IQ real	IQ real	-	IQ real



Table 12. Summary of RT-PCR/RT-qPCR and extraction methods employed by participants in the Ring test for PCR laboratories for YHV detection.

Technique PCR/RT-PCR	Technique qPCR/RT-qPCR	DNA /RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
-	RT-qPCR	TriReagent (Sigma-Aldrich)	Aranguren et al., 2012	-	qScript Virus 1-Step ToughMix, Low ROX (2X)
-	RT-qPCR	SpinTech RNA Extraction Kit	SmartReal YHV Detection Kit	-	Applied biosystems /TaqMan Fast
-	RT-qPCR	sbeadex livestock kit, oKtopure automated DNA extraction system	Kathy F J Tang 1, Jun Wang, Donald V Lightner. 2004	-	One - Step taqProbe qRT-PCR-ROX/Dual-Labeled BHO® Probes
-	RT-qPCR	Nucleic Acid extraction kit (Vivantis)	Aileen see et al., 2021	-	Bioline reagent LId /Sensifast SYBR No-ROX kit
-	RT-qPCR	Solution de Extraction Iq 2000	oie	-	Sensi Fast Probe Lo-Rox One Step Mix de Bioline
-	RT-qPCR	TACO DNA/RNA Extraction kit	Aranguren et al., 2012.	-	LightCycler® Multiplex RNA Virus Master (Roche)
-	RT-qPCR	MagMAX™ CORE Nucleic Acid Purification Kit	ThermoFisher multiplex PCR assay	-	VetMAX™-Plus Multiplex One-Step RT-PCR Kit / VetMAX™ Shrimp RNA Multiplex Reagents (YHV, TSV and IMNV)
-	RT-qPCR	SpinTech RNA Extraction Kit	SmartReal YHV Detection Kit	-	Applied biosystems /TaqMan Fast
-	RT-qPCR	IQ REAL GT DNA/RNA CO-EXTRACTION KIT	IQ REAL YHV Quantitative System	-	-
RT-PCR (nested)	-	Trizol	(GY1,2,4, Y3 y G6) Mohr et al., 2015,	-	Superscript III One Step RT-PCR Platinum Taq HiFi (Invitrogen) y DreamTaq Green (Thermo Scientific)
-	RT-qPCR	KIT AccuRive Shrimp pRNA Prep Kit	AccuPId YHV Decion Kit AccuLite Reverse Transcription kit A	-	AccuPId YHV Decion Kit AccuLite Reverse Transcription kit A
-	RT-qPCR	Taco DNA/RNA Extraction Kit	IQ real	-	IQ real kit
-	RT-qPCR	QIAGEN DNA extraction	Han et al., 2015	Real Time PCR-YHV	TaqmanUniversal Applied biosystems 4324018
-	RT-qPCR	Zymo Research: D7003 Quick DNA/RNA MiniPrep Plus Kit	Ochoa-Meza et al. 2013	-	ThermoFisher: A15299 TaqPath™ 1-Step RT-qPCR Master Mix, CG, Invitrogen: 4308323 TaqMan® Exogenous Internal Positive Control Reagents
-	RT-qPCR	IQ Real (Silica Extraction Kit)	IQ Real	-	Iq Real Kit
-	RT-qPCR	Taco™ Nucleic Acid Extraction Reagent	IQ Real	-	IQ Real Kit
-	RT-qPCR	1. SpinTech DNA/RNA extraction kit 2. MagnaTech DNA/RNA extraction kit	In-house method based on FRDC, Australia (2015)	SmartReal YHV Detection Kit	1. Taqman® Fast Virus 1-step Master Mix 2. SmartReal YHV Detection Kit
RT-PCR	RT-qPCR	MagMax Ambion	Tang et al., 1999	IQ REAL	Invitrogen/SuperScriptIII/Commercial kit
-	RT-qPCR	taco™ DNA/RNA Extraction Kit	MaxDetect YHV qPCR Detection kit	-	MaxDetect kit
-	RT-qPCR	MagMAX™ Total Nucleic Acid Isolation Kit (cat no: AM1840)	AFDL unpublished, Cowley et al., 2015. FRDC Project No 2013/036 https://www.frdc.com.au/Archived-Reports/FRDC%20Projects/2013-036-DL.D.pdf	-	AgPath-ID™ One-Step RT-PCR Catalog number: 4387424 (Thermo Fisher Scientific, USA)
-	RT-qPCR	IQ real	IQ real	-	IQ real



Table 13. Summary of turnaround times. Ring test for PCR laboratories.

GROUP	DATE DELIVERED	RECEIVED BY LAB (DATE)	REPORT SENT	WORKING DAYS (M-F)/(S/T)
A	2/15/2022	2/25/2022	3/2/2022	3
B	2/15/2022			
C	2/15/2022	2/22/2022	3/2/2022	8
D	2/15/2022	3/18/2022	3/24/2022	7
E	2/15/2022	2/22/2022	3/4/2022	8
F	2/15/2022		3/15/2022	
G	2/15/2022			
H	2/15/2022	3/14/2022	3/18/2022	5
I	2/15/2022	3/1/2022	3/9/2022	7
J	2/15/2022	2/28/2022	3/4/2022	4
K	2/15/2022			
L	2/15/2022	2/21/2022	3/1/2022	5
M	2/15/2022	3/7/2022	3/14/2022	6
N	2/15/2022	2/24/2022	2/28/2022	5
P	2/15/2022	3/11/2022	3/17/2022	5
Q	2/15/2022	3/18/2022	3/28/2022	5
R	2/15/2022	2/21/2022	3/28/2022	5
S	2/15/2022	2/16/2022	2/18/2022	2
T	2/15/2022	3/18/2022	3/28/2022	7
U	2/15/2022	3/3/2022	3/14/2022	7
V	2/15/2022	2/23/2022	3/9/2022	5
W	2/15/2022	3/7/2022	3/15/2022	6
X	2/15/2022	2/18/2022	3/10/2022	15
Y	2/15/2022	2/21/2022	1/3/2022	6
Z	2/15/2022	3/11/2022	3/21/2022	5
AA	2/15/2022	2/27/2022	3/1/2022	2
BB	2/15/2022	2/17/2022	2/28/2022	7
CC	3/8/2022	3/30/2022	4/6/2022	5