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Dr. Rachel Yap

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Dear Dr. Rachel Yap,

Thank you for your participation in the **February 2021** Ring test for PCR laboratories. In the following pages you will find a summary of your results along with those of **22** 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 “**I**”. On this occasion, we had the participation of laboratories from **14** countries as follows: Belize (1), Ecuador (1), Guatemala (1), India (1), Madagascar (1), Malaysia (3), Mexico (4), Panama (1), Peru (3), Philippines (1), Saudi Arabia (1), Thailand (2), USA (1), and Vietnam (2).

The pathogens representing a panel of samples you received included **WSSV** (white spot syndrome virus), **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, 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!**



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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 **Malaysia**.

Sincerely,

Luis Fernando Aranguren, Ph.D.
Research Scientist

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. 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 2021

Pathogen	No. laboratories tested for this pathogen	No. laboratories tested correctly	% Of correct results	No. Laboratories tested by qPCR/RTqPCR	No. Laboratories tested by PCR/RT-PCR	Lab with False Positive (1 or more)	Lab with False Negative (1 or more)
WSSV	23	23	100%	15	9	1	0
YHV	19	19	100%	13	6	0	0
TSV	22	22	100%	15	8	0	0
IHHNV	23	20	87%	14	11	2	2
IMNV	18	18	100%	14	5	0	0
EHP	15	15	100%	13	5	0	1
NHP	19	16	84%	14	6	2	3
AHPND	20	20	100%	14	8	0	0
PvNV	6	6	100%	3	3	0	0

Table 2. Cycle threshold (CT values)

Ct Values	AHPND (Sample 1)	AHPND (Sample 2, if present)	EHP	YHV	WSSV	IHHNV (Sample 1)	IHHNV (sample 2, if present)	IHHNV (Sample 3, if present)	PvNV	NHP	IMNV	TSV
N	15	3	13	14	16	15	5	4	3	13	15	16
CT Mean	15.7	33.2	25.6	21.7	19.9	27.6	21.7	34.0	24.7	19.6	24.2	21.0
Min	10.1	32.1	20.0	15.4	14.4	19.6	19.4	33.4	17.2	14.5	15.0	14.2
Max	20.5	34.4	31.9	32.7	25.8	34.0	24.6	34.9	28.9	34.5	34.5	28.4
Sdev	3.0	1.1	3.5	5.0	3.1	3.5	2.2	0.6	5.3	4.8	4.9	5.3

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 2021:

LAB	WSSV	PvNV	IHHNV	TSV	YHV	NHP	AHPND	EHP	IMNV	Total correct	False positive (+)		False negative (-)	
											N ^o	Details	N	Details
Lab A	1/1	1/1	2/2	1/1	1/1	1/1	1/1	1/1	1/1	10	0		0	
Lab B	2/1	N/A	1/1	1/1	1/1	N/A	1/1	N/A	N/A	5	1	WSSV (1)	0	
Lab C	1/1	1/1	2/2	1/1	1/1	1/1	1/1	1/1	1/1	10	0		0	
Lab D	1/1	N/A	1/1	1/1	N/A	1/1	N/A	N/A	N/A	4	0		0	
Lab E	1/1	N/A	1/1	1/1	N/A	0/1	N/A	N/A	N/A	3	0		1	NHP (1)
Lab F	1/1	N/A	1/1	1/1	N/A	N/A	N/A	N/A	N/A	3	0		0	
Lab G	1/1	1/1	2/2	1/1	1/1	1/1	1/1	1/1	1/1	10	0		0	
Lab H	1/1	N/A	1/1	1/1	1/1	1/1	1/1	1/1	1/1	8	0		0	
Lab I	1/1	N/A	1/1	1/1	1/1	1/1	2/2	1/1	1/1	9	0		0	
Lab J	1/1	N/A	3/3	1/1	1/1	1/1	2/2	1/1	1/1	11	0		0	
Lab K	1/1	N/A	1/1	1/1	1/1	1/1	1/1	1/1	1/1	8	0		0	
Lab L	1/1	1/1	2/2	1/1	1/1	1/1	1/1	N/A	1/1	9	0		0	
Lab M	1/1	N/A	1/1	1/1	1/1	N/A	1/1	1/1	1/1	7	0		0	
Lab N	1/1	N/A	1/1	1/1	1/1	N/A	1/1	1/1	1/1	7	0		0	
Lab P	1/1	1/1	3/3	1/1	1/1	1/1	1/1	1/1	1/1	11	0		0	
Lab Q	1/1	N/A	1/1	1/1	1/1	1/1	1/1	1/1	1/1	8	0		0	
Lab R	1/1	N/A	1/1	1/1	1/1	1/1	1/1	1/1	1/1	8	0		0	
Lab S	1/1	N/A	1/1	1/1	1/1	1/1	1/1	1/1	1/1	8	0		0	
Lab T	1/1	N/A	2/2	1/1	1/1	1/1	2/2	N/A	1/1	8	1	NHP (1)	1	NHP (1)
Lab U	1/1	N/A	5/2	1/1	1/1	1/1	1/1	0/1	1/1	8	3	IHHNV (3)	1	EHP (1)
Lab V	1/1	N/A	1/1	1/1	1/1	1/1	1/1	1/1	1/1	8	0		0	
Lab W	1/1	N/A	1/1	N/A	N/A	1/1	1/1	N/A	N/A	2	2	IHHNV (1), NHP (1)	2	IHHNV (1), NHP (1)
Lab X	1/1	1/1	1/2	1/1	1/1	1/1	1/1	1/1	1/1	9	0		1	IHHNV (1)



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/RTqPCR	DNA /RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
PCR	qPCR	DTAB-CTAB	(AP4 F/R) Dangtip et al. 2015	VpPirA-F/R) Han et al., 2015	<i>Maximo taq DNA polymerase (Geneon)/ PowerUp SYBR Green Master Mix (Applied Biosystems)</i>
PCR	-	IQ2000	IQ2000 PCR	-	IQ2000 kit
PCR	-	CTAB/DTAB (Adaptado de Gustincich et al. 1991)	Nested PCR - OIE 2019 (Basado en Dangtip et al., 2015)	-	AP4-F1: 5' ATGAGTAACAATATAAAAACATGAAAC 3'AP4-R1: 5' ACGATTTCGACGTTCCCAA3'AP4-F2: 5' TTGAGAATACGGGACGTGGG 3'AP4-R2: 5' GTTAGTCATGTGAGCACCTTC 3'
-	qPCR	Buffer Lysis	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	IQ 2000 DNA Extraction Kit	Han et al., (2015).	-	MaxDetect kit
PCR	qPCR	RNA/DNA Taco extraction kit	Han et al., (2015).	Han et al (2015).	1. Sensi FAST Probe No-ROX One-Step Kit 2. Thermo scientific phire hot start II PCR Master Mix
-	qPCR	TACO DNA/RNA Extraction kit	OIE, 2019 (Han et al., 2015).	-	FastStart Essential DNA Probes Master (Roche)
-	qPCR	IQ Real (Silica Extraction Kit)	IQ real	-	IQ Real Kit
PCR	-	SpinTech DNA Extraction Kit	SmartReal AHPND Detection Kit	-	Applied biosystems /TaqMan Fast
-	qPCR	Spin Tech DNA/RNA Extraction Kit	SmartReal AHPND Detection Kit	-	Applied biosystems /TaqMan Fast
-	qPCR	MagMAX™ Total Nucleic Acid Isolation Kit (cat no: AM1840)	Han et al., (2015). (OIE 2019)	-	Path-ID™ qPCR Master Mix Catalog number: 4388644 (Thermo Fisher Scientific, USA)
-	qPCR	OIE 2019 – Chapter 2.2.1	OIE 2019 – Chapter 2.2.1	-	Luna® Universal Probe qPCR Master Mix
PCR	-	Fasmac Co., Ltd. GenCheck DNA extraction reagent	Koiwai, K., Kodera, T.,et al., (2017).	SURE Marketing Company, Inc. GenePasQ DNA Pathogen detection kit	Qiagen Multiplex PCR Plus SURE Marketing Company, Inc. GenePasQ DNA Pathogen detection kit
-	qPCR	IQ REAL GT DNA/RNA CO-EXTRACTION KIT	IQ REAL AHPND/EMS TOXIN 1 Quantitative System	-	-
PCR	-	DTAB / CTAB solution extraction (GeneReach)	Dangtip et al., (2015).	-	-
-	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	Kit / IQ Real TM Quantitative System GeneReach Biotechnology Corp.	IQ real	-	Kit / IQ Real TM Quantitative System GeneReach Biotechnology Corp.
PCR	-	CTAB-DTAB	QS Biotech	-	Dream Taq Green PCR Master Mix- Thermo Scientific
-	qPCR	PROMEGA/MAXWELL for DNA and RNA extractions	Han et al. (2015).	-	5ul TaqMan master mix (4x), lul FIR primer mix, 0.5ul TaqMan probe

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/RTqPCR	DNA /RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
PCR	qPCR	DTAB-CTAB	(SWP_1F/R y SWP_2F/R) Jaroenlak et al., 2016.	(EHP-bTub-F2)/(EHP-bTub-R2) Piamsomboon et al. 2019	<i>Maximo taq DNA polymerase (Geneon)/ PowerUp SYBR Green Master Mix (Applied Biosystems)</i>
-	qPCR	Buffer Lysis	IQ Real	-	IQ Real Kit
PCR	-	CTAB/DTAB (Adaptado de Gustincich et al. 1991)	Nested-PCR (Basado en Jaroenlak et al., 2016)	-	SWP_1F: 5' TTGCAGAGTGTGTTAAGGGTTT 3'SWP_1R: 5' CACGATGTGCTTTGCAATTTTC 3'SWP_2F: 5' TTGGCGGCACAATTCTCAAACA 3'SWP_2R: 5' GCTGTTGTCTCCAAGTATTGA 3'
-	qPCR	1.SpinTech DNA/RNA extraction kit 2. MagnaTech DNA/RNA extraction kit	In-house method based on Liu et al. (2018)	SmartReal EHP Detection Kit 1.Taqman® Fast Advance Master Mix 2	1.Taqman® Fast Advance Master Mix 2.SmartReal EHP Detection Kit
-	qPCR	IQ 2000 DNA Extraction Kit	Piamsomboon et al., (2019).	-	MaxDetect kit
-	qPCR	RNA/DNA Taco extraction kit	Liu et al., (2017).	-	Sensi FAST Probe No-ROX One-Step Kit
-	qPCR	TACO DNA/RNA Extraction kit	Liu et al., (2018).	-	FastStart Essential DNA Probes Master (Roche)
PCR	-	SpinTech DNA Extraction Kit	SmartReal EHP Detection Kit	-	Applied biosystems /TaqMan Fast
-	qPCR	Spin Tech DNA/RNA Extraction Kit	SmartReal EHP Detection Kit	-	Applied biosystems /TaqMan Fast
-	qPCR	MagMAX™ Total Nucleic Acid Isolation Kit (cat no: AM1840)	Liu et al., (2018).	-	Path-ID™ qPCR Master Mix Catalog number: 4388644 (Thermo Fisher Scientific, USA)
-	qPCR	TCVN 8710-12:2019 (LFOD-TST-SOP-8854)	TCVN 8710-12:2019 (LFOD-TST-SOP-8854)	-	Luna® Universal Probe qPCR Master Mix
PCR	-	Fasmac Co., Ltd. GenCheek DNA extraction reagent	Koiwai, K., Kodera, T.,et al., (2017).	SURE Marketing Company, Inc. GenePasQ DNA Pathogen detection kit	Qiagen Multiplex PCR Plus SURE Marketing Company, Inc. GenePasQ DNA Pathogen detection kit
-	qPCR	IQ REAL GT DNA/RNA CO-EXTRACTION KIT	IQ Real EHP Quantitative System	-	-
-	qPCR	sbeadex livestock kit, oKtopure automated DNA extraction system	Piamsomboon et al., (2019).	-	2x BHQ Probe Master Mix STD ROX/Dual-Labeled BHQ® Probes
-	qPCR	Kit / IQ Real TM Quantitative System GeneReach Biotechnology Corp.	IQ Real	-	Kit / IQ Real TM Quantitative System GeneReach Biotechnology Corp.
PCR	qPCR	PROMEGA/MAXWELL for DNA and RNA extractions	Jaroenlak et al., (2016).	Liu et al., 2018.	Beads: 50 pmole primers, 25 ul reaction, 1 ul of template



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

Technique PCR/RT-PCR	Technique qPCR/RTqPCR	DNA /RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
PCR	qPCR	DTAB-CTAB	(389 F/R) Tang et al., 2007	(VNHHI1608F/VNHHI1688R) Tang & Lightner, 2001.	<i>Maximo taq DNA polymerase (Geneon)/ PowerUp SYBR Green Master Mix (Applied Biosystems)</i>
PCR	-	IQ2000	IQ2000 Nested PCR	-	IQ2000 kit
-	qPCR	Lilif Auto XT	OIE real time	-	Taqman Fast Virus 1-Step – Thermo Fisher
PCR	-	Lysis Buffer (ACTG Kit)	ACTG Molecular solutions Kit	-	ACTG Molecular solutions Kit
PCR	-	DNeasy Blood & Tissue Kits - QIAGEN	OIE 2017 Tang & Lightner, 2001.	-	PCR Master Mix (2X) – Thermo Scientific
-	qPCR	Buffer Lysis	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 IHHNV Detection Kit	1.Taqman® Fast Advance Master Mix 2.SmartReal IHHNV Detection Kit
-	qPCR	IQ 2000 DNA Extraction Kit	IQ Real	-	IQ Real kit
PCR	qPCR	RNA/DNA Taco extraction kit	OIE, 2018 (Tang & Lightner, 2001)	OIE, 2017 (Nunan et al., 2000)	1. Sensi FAST Probe No-ROX One-Step Kit 2. Thermo scientific phire hot start II PCR Master Mix
-	qPCR	TACO DNA/RNA Extraction kit	OIE, 2019 (Tang & Lightner, 2001).	-	FastStart Essential DNA Probes Master (Roche)
-	qPCR	IQ Real (Silica Extraction Kit)	IQ Real	-	IQ Real Kit
PCR	-	SpinTech DNA Extraction Kit	SmartReal IHHNV Detection Kit	-	Applied biosystems /TaqMan Fast
-	qPCR	Spin Tech DNA/RNA Extraction Kit	SmartReal IHHNV Detection Kit	-	Applied biosystems /TaqMan Fast
-	qPCR	MagMAX™ Total Nucleic Acid Isolation Kit (cat no: AM1840)	PvNV Detection kit (GeneReach Boitechnology copr, Tamiwan)	-	Path-ID™ qPCR Master Mix Catalog number: 4388644 (Thermo Fisher Scientific, USA)
-	qPCR	OIE 2019 – Chapter 2.2.4	OIE 2019 – Chapter 2.2.4	-	Luna® Universal Probe qPCR Master Mix
PCR	-	Fasmac Co., Ltd. GenCcheck DNA extraction reagent	Koiwai, K., Kodera, T.,et al., (2017).	SURE Marketing Company, Inc. GenePasQ DNA Pathogen detection kit	Qiagen Multiplex PCR Plus SURE Marketing Company, Inc. GenePasQ DNA Pathogen detection kit
-	qPCR	IQ REAL GT DNA/RNA CO-EXTRACTION KIT	IQ Real IHHNV Quantitative System	-	-
PCR	-	DTAB / CTAB solution extraction (GeneReach)	IQ 2000 IHHNV (nested) detection and Prevention system (GeneReach Corp)	-	-
-	qPCR	sbeadex livestock kit, oKtopure automated DNA extraction system	Tang & Lightner, (2001).	-	2x BHQ Probe Master Mix STD ROX/Dual-Labeled BHQ® Probes
-	qPCR	Kit / IQ Real TM Quantitative System GeneReach Biotechnology Corp.	IQ Real	-	Kit / IQ Real TM Quantitative System GeneReach Biotechnology Corp.
PCR	-	Lysis Buffer	QS Biotech	-	Dream Taq Green PCR Master Mix- Thermo Scientific
PCR	-	PROMEGA/MAXWELL for DNA and RNA extractions	Tang et al. (2006).	-	Beads: 50-pmole primers, 25 ul reaction, 1 ul of template



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/RTqPCR	DNA /RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
RT-PCR	RT-qPCR	Trizol	(4587F/4914R y 4725NF/4863NR) Poulos y Lightner, 2006	IMNV412F/IMNV545. Andrade et al.2007)	<i>RevertAid RT Kit (Thermoscientific) y Maximo taq DNA polymerase (Geneon)/ PowerUp SYBR Green Master Mix (Applied Biosystems)</i>
-	RT-qPCR	Solution de Extraction Iq 2000	OIE	-	Sensi Fast Probe Lo-Rox One Step Mix de Bioline
PCR	-	CTAB/DTAB (Adaptado de Gustinich et al. 1991)	PCR convencional - OIE 2019 (Basado en Tang et al.2007)	-	389F: 5' CGGAACACAACCCGACTTTA 3'389R: 5' GGCCAAGACCAAAAATACGAA 3'
-	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	IQ 2000 DNA Extraction Kit	IQ Real	-	MaxDetect kit
-	RT-qPCR	RNA/DNA Taco extraction kit	OIE, 2018 (Andrade et al., 2007)	-	Sensi FAST Probe No-ROX One-Step Kit
-	RT-qPCR	TACO DNA/RNA Extraction kit	OIE, 2019 (Andrade et al., 2007).	-	LightCycler® Multiplex RNA Virus Master (Roche)
-	RT-qPCR	IQ Real (Silica Extraction Kit)	IQ Real	-	IQ Real Kit
RT-PCR	-	SpinTech RNA Extraction Kit	SmartReal IMNV Detection Kit	-	Applied biosystems /TaqMan Fast
-	RT-qPCR	Spin Tech DNA/RNA Extraction Kit	SmartReal IMNV Detection Kit	-	Applied biosystems /TaqMan Fast
-	RT-qPCR	MagMAX™ Total Nucleic Acid Isolation Kit (cat no: AM1840)	Andrade et al., (2007). (OIE 2019)	-	AgPath-ID™ One-Step RT-PCR Catalog number: 4387424 (Thermo Fisher Scientific, USA)
-	RT-qPCR	OIE 2019 – Chapter 2.2.5	OIE 2019 – Chapter 2.2.6	-	Luna Universal Probe 1-Step RT-qPCR
RT-PCR	-	Fasmac Co., Ltd. GenCheek RNA extraction reagent	Koiwai, K., Kodera, T.,et al., (2017).	SURE Marketing Company, Inc. GenePasQ RNA Pathogen detection kit	Qiagen Multiplex PCR Plus SURE Marketing Company, Inc. GenePasQ DNA Pathogen detection kit
-	RT-qPCR	IQ REAL GT DNA/RNA CO-EXTRACTION KIT	IQ Real IMNV Quantitative System	-	-
RT-PCR	-	RNA solution extraction (GeneReach)	IQ 2000 IMNV detection and Prevention system (GeneReach Corp)	-	-
-	RT-qPCR	sbeadex livestock kit, oKtopure automated RNA extraction system	Poulos and Lightner, (2006); Andrade et al., (2007); Senapin et al., (2007)	-	2x BHQ Probe Master Mix STD ROX/Dual-Labeled BHQ® Probes
-	RT-qPCR	Kit / IQ Real TM Quantitative System GeneReach Biotechnology Corp.	IQ Real	-	Kit / IQ Real TM Quantitative System GeneReach Biotechnology Corp.
-	RT-qPCR	PROMEGA/MAXWELL for DNA and RNA extractions	Andrade et al. (2007).	-	5ul TaqMan master mix (4x), 1 ul FIR primer mix, 0.5ul TaqMan probe



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/RTqPCR	DNA /RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
PCR	qPCR	DTAB-CTAB	(NHPP2/NHPR2) Aranguren et al., 2010.	NHP1300F/NHP1366R) Aranguren et al., 2010	<i>Maximo taq DNA polymerase (Geneon)/ PowerUp SYBR Green Master Mix (Applied Biosystems)</i>
-	qPCR	Lilif Auto XT	OIE real time	-	Taqman Fast Virus 1-Step – Thermo Fisher
PCR	-	CTAB/DTAB (Adaptado de Gustincich et al. 1991)	PCR convencional - OIE 2019 (Basado en Aranguren et al., 2010)	-	NHPP2: 5'-CGT-TGG-AGG-TTC-GTC-CTT-CAGT-3' NHPR2: 5'-GCC-ATG-AGG-ACC-TGA-CAT-CAT-C-3'
PCR	-	Lysis Buffer (ACTG Kit)	ACTG Molecular solutions Kit	-	ACTG Molecular solutions Kit
-	qPCR	Buffer Lysis	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
-	qPCR	IQ 2000 DNA Extraction Kit	Aranguren L.F., Tang K.F.J. & Lightner D.V., (2010).	-	MaxDetect kit
-	qPCR	RNA/DNA Taco extraction kit	Aranguren et al., (2010).	-	Sensi FAST Probe No-ROX One-Step Kit
-	qPCR	TACO DNA/RNA Extraction kit	OIE, 2019 (Aranguren et al., 2010).	-	FastStart Essential DNA Probes Master (Roche)
-	qPCR	IQ Real (Silica Extraction Kit)	IQ Real	-	IQ Real Kit
-	qPCR	MagMAX™ Total Nucleic Acid Isolation Kit (cat no: AM1840)	Aranguren et al., (2010). (OIE 2019)	-	Path-ID™ qPCR Master Mix Catalog number: 4388644 (Thermo Fisher Scientific, USA)
-	qPCR	OIE 2019 – Chapter 2.2.3	OIE 2019 – Chapter 2.2.3	-	Luna® Universal Probe qPCR Master Mix
PCR	-	Fasmac Co., Ltd. GenCheek DNA extraction reagent	Koiwai, K., Kodera, T., et al., (2017).	SURE Marketing Company, Inc. GenePasQ DNA Pathogen detection kit	Qiagen Multiplex PCR Plus SURE Marketing Company, Inc. GenePasQ DNA Pathogen detection kit
-	qPCR	IQ REAL GT DNA/RNA CO-EXTRACTION KIT	IQ Real NHPB Quantitative System	-	-
PCR	-	DTAB / CTAB solution extraction (GeneReach)	IQ 2000 NHPB detection and Prevention system (GeneReach Corp)	-	-
-	qPCR	sbeadex livestock kit, oKtopure automated DNA extraction system	Aranguren et al., (2010).	-	2x BHQ Probe Master Mix STD ROX/Dual-Labeled BHQ® Probes
-	qPCR	Kit / IQ Real TM Quantitative System GeneReach Biotechnology Corp.	IQ Real	-	Kit / IQ Real TM Quantitative System GeneReach Biotechnology Corp.
PCR	-	CTAB-DTAB	QS Biotech	-	Dream Taq Green PCR Master Mix- Thermo Scientific
-	qPCR	PROMEGA/MAXWELL for DNA and RNA extractions	Aranguren et al. (2010).	-	5ul TaqMan master mix (4x), lul FIR primer mix, 0.5ul TaqMan probe



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

Technique PCR/RT-PCR	Technique qPCR/RTqPCR	DNA /RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
RT-PCR	-	Trizol	(PvNV339F/R y PvNV246NF/R) Tang et., al 2007.	-	RevertAid RT Kit (Thermoscientific) y Maximo taq DNA polymerase (Geneon)
-	RT-qPCR	Solution de Extraction Iq 2000	IQ Real	-	IQ Real Kit
RT-PCR	-	Trizol - tiocianato de guanidina fenol (Chomczynski, et al. 1987).	Nested RT-PCR - OIE 2011 (Basado en Tang et al., 2007)	-	PvNV339F: 5' CTG TCT CAC AGG CTG GTT CA 3'PvNV339R: 5' CCG TTT GAA TTT CAG CAA CA 3'PvNV246NF: 5' CAA AAC TGT GCC TTT GAT CG 3'PvNV246NR: 5' GCC TTA TCC ACA CGA ACG TC 3'
RT-PCR	-	IQ 2000 RNA Extraction	IQ 2000	-	IQ 2000 Kit
-	RT-qPCR	MagMAX™ Total Nucleic Acid Isolation Kit (cat no: AM1840)	Tang and Lightner, (2001). (OIE 2019)	-	PvNV Detection kit (GeneReach Boitechnology copr, Tamiwan)
-	RT-qPCR	PROMEGA/MAXWELL for DNA and RNA extractions	PvNV: Real-time PCR protocol developed in this laboratory based on PvNV genome Gen Bank sequence No. EF137180. Unpublished.	-	5ul TaqMan master mix (4x), 1ul FIR primer mix, 0.5ul TaqMan probe



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/RTqPCR	DNA /RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
RT-PCR	RT-qPCR	Trizol	(9992F/9195R) Nunan et al., 1998	(TSV1004F/TSV1075R) Tang et al., 2004	<i>RevertAid RT Kit (Thermoscientific) y Maximo taq DNA polymerase (Geneon)/ PowerUp SYBR Green Master Mix (Applied Biosystems)</i>
RT-PCR	-	IQ2001	Concepto Azul (RT-Nested PCR)	-	Concepto Azul (Kit Taq PROMEGA)
RT-PCR	-	Trizol - tiocianato de guanidina fenol (Chomczynski, et al. 1987).	RT-PCR - OIE 2019 (Basado en Navarro et al., 2009)	-	7171F: 5' CGACAGTTGGACATCTAGTG 3' 7511R: 5' GAGCTTCAGACTGCAACTTC 3'
-	RT-qPCR	Lilif Auto XT	OIE real time	-	Taqman Fast Virus 1-Step – Thermo Fisher
RT-PCR	-	RNA Extraction solution (IQ 2000)	IQ 2000	-	IQ 2000
RT-PCR	-	TRIZOL	OIE 2017, Nunan et al., 1998.	-	SuperScript™ III One-Step RT-PCR System with Platinum™ Taq DNA Polymerase
-	RT-qPCR	Solution de Extraction Iq 2000	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-qPCR	IQ 2000 DNA Extraction Kit	IQ Real	-	IQ Real kit
-	RT-qPCR	RNA/DNA Taco extraction kit	OIE, 2018 (Tang et al, 2004)	-	Sensi FAST Probe No-ROX One-Step Kit
-	RT-qPCR	TACO DNA/RNA Extraction kit	OIE, 2019 (Tang et al., 2004).	-	LightCycler® Multiplex RNA Virus Master (Roche)
-	RT-qPCR	IQ Real (Silica Extraction Kit)	IQ Real	-	IQ Real Kit
RT-PCR	-	SpinTech RNA Extraction Kit	SmartReal TSV Detection Kit	-	Applied biosystems /TaqMan Fast
-	RT-qPCR	Spin Tech DNA/RNA Extraction Kit	SmartReal TSV Detection Kit	-	Applied biosystems /TaqMan Fast
-	RT-qPCR	MagMAX™ Total Nucleic Acid Isolation Kit (cat no: AM1840)	Tang et al., (2004). (OIE 2019)	-	AgPath-ID™ One-Step RT-PCR Catalog number: 4387424 (Thermo Fisher Scientific, USA)
-	RT-qPCR	OIE 2019 – Chapter 2.2.7	OIE 2019 – Chapter 2.2.7	-	Luna Universal Probe 1-Step RT-qPCR
RT-PCR	-	Fasmac Co., Ltd. GenCheek RNA extraction reagent	Koiwai, K., Kodera, T., et al., (2017).	SURE Marketing Company, Inc. GenePasQ RNA Pathogen detection kit	Qiagen Multiplex PCR Plus SURE Marketing Company, Inc. GenePasQ DNA Pathogen detection kit
-	RT-qPCR	IQ REAL GT DNA/RNA CO-EXTRACTION KIT	IQ Real TSV Quantitative System	-	-
RT-PCR	-	RNA solution extraction (GeneReach)	IQ 2000 TSV detection and Prevention system (GeneReach Corp)	-	-
-	RT-qPCR	sbeadex livestock kit, oKtopure automated RNA extraction system	Tang et al., (2004).	-	2x BHQ Probe Master Mix STD ROX/Dual-Labeled BHQ® Probes
-	RT-qPCR	Kit / IQ Real TM Quantitative System GeneReach Biotechnology Corp.	IQ Real	-	Kit / IQ Real TM Quantitative System GeneReach Biotechnology Corp.
-	RT-qPCR	PROMEGA/MAXWELL for DNA and RNA extractions	Tang et al., (2004).	-	5ul TaqMan master mix (4x), lu!F/Rprimer mix, 0.5ul TaqMan probe



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 qPCR/RTqPCR	DNA /RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
PCR	qPCR	DTAB-CTAB	(146_1F/1R y 146 2F/2R) Lo et al., 1996.	(WSS1011F/ WSS1079R) Durand y Lightner, 2002.	Maximo taq DNA polymerase (Geneon)/ PowerUp SYBR Green Master Mix (Applied Biosystems)
PCR	-	IQ2000	IQ2000 Nested PCR	-	IQ2000 kit
PCR	-	CTAB/DTAB (Adaptado de Gustincich et al. 1991)	Nested PCR - OIE 2019 (Basado en Lo et al., 1996 y Lo et al., 1997)	-	146F1: 5' ACTACTAACTCAGCCTATCTAG 3'146R1: 5' TAATGCGGGTGTAATGTTCTTACGA 3'146F2: 5' GTAAGTCCCCCTCCATCTCCA 3'146R2: 5' TACGGCAGCTGCTGCACCTTGT3
-	qPCR	Lilif Auto XT	OIE real time	-	Taqman Fast Virus 1-Step – Thermo Fisher
PCR	-	Lysis Buffer (IQ 2000)	IQ 2000	-	IQ 2000
PCR	-	DNeasy Blood & Tissue Kits - QIAGEN	OIE Manual Lo et al., 1998.	-	PCR Master Mix (2X) – Thermo Scientific
-	qPCR	Buffer Lysis	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	IQ 2000 DNA Extraction Kit	Durand S.V. & Lightner D.V. (2002).	-	MaxDetect kit
-	qPCR	RNA/DNA Taco extraction kit	OIE , 2017 (Lightner, 2002)	-	Sensi FAST Probe No-ROX One-Step Kit
-	qPCR	TACO DNA/RNA Extraction kit	OIE, 2019 (Durand & Lightner, 2002).	-	FastStart Essential DNA Probes Master (Roche)
-	qPCR	IQ Real (Silica Extraction Kit)	IQ Real	-	IQ Real Kit
PCR	-	SpinTech DNA Extraction Kit	SmartReal WSSV Detection Kit	-	Applied biosystems /TaqMan Fast
-	qPCR	Spin Tech DNA/RNA Extraction Kit	SmartReal WSSV Detection Kit	-	Applied biosystems /TaqMan Fast
-	qPCR	MagMAX™ Total Nucleic Acid Isolation Kit (cat no: AM1840)	Durand and Lightner, (2002). (OIE 2019)	-	Path-ID™ qPCR Master Mix Catalog number: 4388644 (Thermo Fisher Scientific, USA)
-	qPCR	OIE 2019 – Chapter 2.2.8	OIE 2019 – Chapter 2.2.8	-	Luna® Universal Probe qPCR Master Mix
PCR	-	Fasmac Co., Ltd. GenCheek DNA extraction reagent	Koiwai, K., Kodera, T.,et al., (2017).	SURE Marketing Company, Inc. GenePasQ DNA Pathogen detection kit	Qiagen Multiplex PCR Plus SURE Marketing Company, Inc. GenePasQ DNA Pathogen detection kit
-	qPCR	IQ REAL GT DNA/RNA CO-EXTRACTION KIT	IQ Real WSSV Quantitative System	-	-
PCR	-	DTAB / CTAB solution extraction (GeneReach)	IQ 2000 WSSV detection and Prevention system (GeneReach Corp)	-	-
-	qPCR	sbeadex livestock kit, oKtopure automated DNA extraction system	Durand & Lightner, (2002).	-	2x BHQ Probe Master Mix STD ROX/Dual-Labeled BHQ® Probes
-	qPCR	Kit / IQ Real TM Quantitative System GeneReach Biotechnology Corp.	IQ Real	-	Kit / IQ Real TM Quantitative System GeneReach Biotechnology Corp.
PCR	-	Lysis Buffer	QS Biotech	-	Dream Taq Green PCR Master Mix- Thermo Scientific
-	qPCR	PROMEGA/MAXWELL for DNA and RNA extractions	Durand and Lightner, (2002).	-	5ul TaqMan master mix (4x), 11.11 FIR primer mix, 0.5ul TaqMan probe

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/RTqPCR	DNA /RNA extraction method	PCR method 1	PCR method 2	Enzymes/Reagents
RT-PCR	-	Trizol	(GY1,2,4, Y3 y G6) Mohr et al., 2015,	-	<i>RevertAid RT Kit (Thermoscientific) y Maximo taq DNA polymerase (Geneon)</i>
RT-PCR	-	IQ2002	IQ2000 (RT-PCR)	-	IQ2000 kit
RT-PCR	-	Trizol - tiocianato de guanidina fenol (Chomczynski, et al. 1987).	RT-PCR - OIE 2019 (Basado en Mohr et al., 2015)	-	10F: 5' CCGCTAATTTCAAAACTACG 3'144R: 5' AAGGTGTTATGTCGAGGAAGT 3'
-	RT-qPCR	Solution de Extraction Iq 2000	OIE	-	Sensi Fast Probe Lo-Rox One Step Mix de Bioline
-	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-qPCR	IQ 2000 DNA Extraction Kit	Cowley, J.A., et al., (2015).	-	MaxDetect kit
-	RT-qPCR	RNA/DNA Taco extraction kit	Tang & Lightner, (2001).	-	Sensi FAST Probe No-ROX One-Step Kit
-	RT-qPCR	TACO DNA/RNA Extraction kit	Aranguren et al., (2012).	-	LightCycler® Multiplex RNA Virus Master (Roche)
-	RT-qPCR	IQ Real (Silica Extraction Kit)	IQ Real	-	IQ Real Kit
RT-PCR	-	SpinTech RNA Extraction Kit	SmartReal YHV Detection Kit	-	Applied biosystems /TaqMan Fast
-	RT-qPCR	Spin Tech DNA/RNA Extraction Kit	SmartReal YHV Detection Kit	-	Applied biosystems /TaqMan Fast
-	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-DLD.pdf	-	AgPath-ID™ One-Step RT-PCR Catalog number: 4387424 (Thermo Fisher Scientific, USA)
-	RT-qPCR	TCVN 8710-4:2019 (LFOD-TST-SOP-8854)	TCVN 8710-4:2019 (LFOD-TST-SOP-8854)	-	Luna Universal Probe 1-Step RT-qPCR
RT-PCR	-	Fasmac Co., Ltd. GenCheek RNA extraction reagent	Koiwai, K., Kodera, T., et al., (2017).	SURE Marketing Company, Inc. GenePasQ RNA Pathogen detection kit	Qiagen Multiplex PCR Plus SURE Marketing Company, Inc. GenePasQ DNA Pathogen detection kit
-	RT-qPCR	IQ REAL GT DNA/RNA CO-EXTRACTION KIT	IQ Real YHV Quantitative System	-	-
RT-PCR	-	RNA solution extraction (GeneReach)	IQ 2000 YHV / GAV detection and Prevention system (GeneReach Corp)	-	-
-	RT-qPCR	sbeadex livestock kit, oKtopure automated RNA extraction system	Tang et al., (2004).	-	2x BHQ Probe Master Mix STD ROX/Dual-Labeled BHQ® Probes
-	RT-qPCR	Kit / IQ Real TM Quantitative System GeneReach Biotechnology Corp.	IQ Real	-	Kit / IQ Real TM Quantitative System GeneReach Biotechnology Corp.
-	RT-qPCR	PROMEGA/MAXWELL for DNA and RNA extractions	Aranguren, et al., (2012).	-	5ul TaqMan master mix (4x), lul FIR primer mix, 0.5ul TaqMan probe



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

GROUP	DATE DELIVERED	RECEIVED BY LAB (DATE)	FORMAT FOR REPORT SENT (DATE)	WORKING DAYS (M-F)/(S/T)
A	02/15/21	03/02/21	03/09/21	5
B	02/15/21	03/05/21	03/16/21	7
C	02/15/21	04/05/21	04/08/21	10
D	02/15/21	02/22/21	03/01/21	6
E	02/15/21	02/26/21	03/08/21	7
F	02/15/21	03/12/21	03/20/21	5
G	02/15/21	02/22/21	03/31/21	27
H	02/15/21	02/22/21	02/03/21	6
I	02/15/21	02/22/21	02/03/21	7
J	02/15/21	02/26/21	03/08/21	7
K	02/15/21	02/22/21	03/01/21	7
L	02/15/21	03/09/21	03/17/21	8
M	02/15/21	02/23/21	03/02/21	7
N	02/15/21	02/26/21	03/09/21	10
P	02/15/21	02/23/21	02/25/21	2
Q	02/15/21	02/22/21	03/01/21	7
R	02/15/21	02/19/21	03/03/21	6
S	02/15/21	03/10/21	03/18/21	7
T	02/15/21	03/18/21	03/02/21	7
U	02/15/21	03/11/21	03/18/21	8
V	02/15/21	03/02/21	03/12/21	8
W	02/15/21	03/19/21	03/22/21	3
X	02/15/21	2/16/2021	3/29/2021	6